THE NUTRITIONAL VALUE OF DIETARY FIBRE FOR RAINBOW TROUT (*SALMO GAIRDNERI*)

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ABSTRACT

The nutritional value of dietary fibre for rainbow trout, Salmo gairdneri was investigated using juvenile fish (10-30g) maintained in freshwater at ambient temperatures under a natural photo period. A preliminary experiment was conducted using five purified dietary fibre sources, namely, α-cellulose, lignin, lignosulphonate, galactomannan and chitin which varied in physical, chemical and textural characteristics. A commercially available, powdered polyethylene was also used as an inert control ingredient and all sources of fibre were included at a realistically low level of 5% in separate semi-purified diets. Although there were no significant differences in the growth of fish at the end of the 10-week trial, several nutritional parameters were affected for rainbow trout fed the different experimental treatments. Mean daily food intake was reduced for trout receiving the lignin, chitin and galactomannan diets compared to the polyethylene control. Similarly the food conversion ratios (FCR) and protein efficiency ratios (PER) were relatively inferior for diets containing chitin and galactomannan compared to the lignosulphonate treatment. Maximum net dietary nitrogen utilization was obtained for the polyethylene control ration whilst lower values were again observed with chitin and galactomannan. Apparent dry matter (DM) and nitrogen digestibility coefficients however were in close agreement for each of the dietary treatments except for the lignin diet which was poorly digested. Generally the results implied that the properties of chitin and galactomannan were worthy of further study at higher inclusion levels and in different physical states.

A specific investigation in which 0, 5, 10, 15 and 20% additions of a purified α-cellulose replaced dietary starch in separate experimental diets failed to produce any significant changes in the growth

performance of trout and only slightly influenced nutrient utilization at the higher 15 and 20% inclusion levels. Negative digestibility coefficients for the 'unavailable' carbohydrate fraction of diets calculated from the 'total' and 'available' carbohydrate contents of diet and faecal samples was considered to be evidence of the non-nutritive and bulking qualities of α-cellulose.

Growth and digestibility trials were then undertaken to examine the effects of including different levels (10 and 20%) and particle size ranges (45-500, 500-1000 microns) of chitin (poly-N-acetyl-D glucosamine) as a natural source of dietary fibre for trout. In a similar experiment, graded amounts of galactomannan polysaccharide (0, 10 and 20%) were added to moist pelleted diets to examine the long term effects of feeding a gel-type fibre characteristic of many commercially available binding agents. Negative digestibility coefficients for both chitin and galactomannan based on specific biochemical measurements together with the failure to detect any chitinase activity in stomach and intestinal tissue was confirmation of the inability of rainbow trout to degrade and utilize these materials. Coarse grades of chitin at the 10 and 20% levels impaired food intake, growth performance and nutrient utilization as shown by the poorer FCR, PER, net nitrogen utilization and digestibility coefficients compared to the diets containing finely ground chitin or the α-cellulose control treatment.

Similar findings were obtained with increasing additions of galactomannan and there were associated reductions in the serum glucose and protein concentrations with each increment of dietary galactomannan. The final carcass compositions of fish were also affected by the gel fibre which caused a significant reduction in the tissue lipid content and an inverse trend in moisture content compared to trout receiving an α-cellulose control diet.

Further investigations using a sacrificial method to follow and quantify the passage of food through the gastrointestinal tract revealed that the physical properties of fibre such as particle size composition, water retaining capacity and viscosity were among several factors which modified gastric evacuation and digestion rates in rainbow trout. From the predicted gastric emptying times (GET), it was apparent that coarsely graded chitin (20%) and both 10 and 20% inclusions of galactomannan considerably increased the residence time of the gastrointestinal contents compared to finely ground chitin and a control diet without added fibre. Although an exponential relationship was found to best describe the stomach emptying profiles obtained, linearization of the data was achieved by applying surface area and volume dependent mathematical models which emphasized the importance of these physical factors.

The combined nutrition and physiological studies supported the contention that fibre is the non-nutritive part of the diet, but it was concluded that the level and nature of the fibrous material has important consequences on the processes controlling food intake and the efficiency of digestion, which in turn may affect the assimilation of nutrients and the performance of growing rainbow trout.

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DECLARATION

I declare that the contents of this thesis embodies original research and has not been previously forwarded for any other degree.

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CHAPTER 1

INTRODUCTION

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1.1 INTRODUCTION

Nutritional researchers have successfully identified many nutrients essential to the growth and health of animals and man. However, as each essential dietary component was identified and added to a purified diet, then so the crude fibre or indigestible organic fraction of the diets became reduced. This is particularly the case for man as a consequence of modern milling methods that remove fibre from natural feed ingredients and the increasing consumption of refined foods rich in sugar and fat.

In the commercial formulation and production of chemically defined rations for the intensive production of farm animals, including fish, complicating factors such as dietary fibre were avoided whenever possible. Thus the reduction of the crude fibre content of experimental diets has been the natural consequence in the search for nutritionally balanced diets. Many nutritionists have been concerned about the removal of fibre from these semi-purified diets and believe that an essential dietary ingredient has been omitted (Lang and Briggs, 1976).

As knowledge of the nutrient requirements of farmed fish increases, then so the feeds formulated for fish husbandry will become more and more independent of expensive animal protein sources such as fish meal and will consist mainly of ingredients of plant origin including soyabean meal, sunflower meal, cereal grains, by-products of the milling and brewing industry and novel materials such as single cell protein (SCP) and krillmeal (Tacon, 1981).

However the use of such products in practical fish diets will in turn result in the introduction of varying amounts of dietary fibre and heteropolysaccharides. Unfortunately the evaluation of such substances is made difficult because information on the nutritional value and dietary requirement of fibre for farmed fish is limited (Halver, 1972), and this seems to be particularly true for the Rainbow trout, Salmo gairdneri (Richardson).

It was therefore the main objective of this thesis to first consider the modern concepts and definitions of dietary fibre and to state those chemical and physical characteristics which may be of nutritional and physiological relevance. The literature regarding the role of dietary fibre in the nutrition of experimental animals and man is extensive. Appropriate studies were therefore reviewed to appreciate the many parameters affected by the inclusion of dietary fibre and to serve as a basis for experiments on the nutritional value of dietary fibre for rainbow trout.

1.2 DEFINITIONS OF DIETARY FIBRE

Classically dietary fibre has been defined as the thread-like structure within plant tissues which provides structural support. The term 'crude fibre' has been used to denote the indigestible organic material present within feedstuffs and complete animal feeds (Waite and Gorrod,1959). McCance and Lawrence (1929) defined crude fibre in analytical terms as the organic residue remaining after the dietary material has been treated successively with dilute acid and, alkali and lipid solvent. It was recognised that the organic residue was composed chiefly of cell wall debris, and that the principle polysaccharide components of the fibre fraction was primarily cellulose, pectins and hemicelluloses associated in turn

with aromatic substances such as lignins (McDonald et al., 1977).

The modern definition of crude fibre now emerging is one which includes all of the components of a feed that are indigestible and therefore unavailable to an animal, with the exclusion of acid-insoluble ash (Inorganic residues). Recent advances in crude fibre analysis (Van Soest, 1967; Southgate, 1978), have led to a better understanding of these components. To an extent, the chemical descriptions of plant fibre polysaccharides are artificial, since these are based on fractionation procedures that are dependent on the solubilities of the constituents in different solvents. However in reality, polysaccharides and lignins are part of a heterogenous complex, the properties of which are variable and characteristic of different plant sources.

Trowell (1972) has defined dietary fibre physiologically as the remnants of plant cell wall materials which are not degraded by the enzymes of the human alimentary tract. The Trowell definition is the most widely used and accepted term, but others have suggested names such as 'Plantix' (Spiller, 1976), which incorporates the fact that fibre forms a heterogenous matrix in plant tissues. Whilst others (Kritchevsky and Story, 1974; Godding, 1976; Hellendoorn , 1976 and Trowell and Godding, 1981) have proposed the terms 'non-nutritive fibre', 'unavailable carbohydrate' and 'edible fibre' in attempts to broaden the definition. However such terms may restrict the value of fibre to a residual, indigestible or non-nutritive aspect. The problem of nomenclature is due to the tendency of many investigators to describe mixed substances such as bran or discrete substances such as cellulose as dietary fibre, and the failure to recognise other dietary components and additives that may be included within these categories.

Spiller and Shipley (1976) are of the opinion that the term should also include other associated cell wall factors, namely waxes, cutins, gum compounds and even cell wall bound proteins and minerals. It must also be mentioned that indigestible dietary fractions may also be present in feedstuffs of animal origin, such as krill meal, shrimp meal, crab meal and in yeasts and single cell proteins. Chitin (poly N-acetyl.D.glucosamine) is a structural polysaccharide found in the exoskeletal tissues of insects and crustaceans. Other complex sugar derivatives differing in chemical structure from those present in plant fibres are found in the cell walls of bacteria and fungi. These are mainly polymers based on N-acetyl-muramic acid, N-acetyl-neuraminic acid and other sialic acid derivatives (Lehninger, 1975).

For the purposes of this thesis, crude fibre is therefore defined as that fraction of the diet that is chemically resistant to digestion in rainbow trout, the components of which may be characterized using specific methods of analysis.

1.3 PHYSICAL AND CHEMICAL CHARACTERISTICS OF DIETARY FIBRE

Numerous researchers have emphasized the differing physical and chemical properties of individual plant and animal fibres and their constituents in terms of the biological effect of dietary crude fibre on the nutritional status of animals.

1.3.1 WATER RETAINING AND GEL-FORMING PROPERTIES OF FIBRE

The water holding capacity of a fibre is defined as the extent to which the fibrous material is capable of retaining water within its matrix in the presence of excess water. Stephen and Cummings (1979) measured the in-vitro water holding properties of a number of dietary fibre preparations, including food materials, bulk

laxatives and gel forming polysaccharides, including sodium carboxymethyl cellulose, locust bean gum, Isogel, carrageenan and Allinson's wheat bran. Gel-forming polysaccharides were found in general to retain more water than particulate fibres such as cereal brans and cellulose based substances. Hydrated dietary fibres expand which in turn increases their bulk density. The capacity of different fibres to absorb and retain water has been used to explain their faecal bulking properties in animals (Tainter and Buchanan, 1954; McConnell et al., 1974). Gel-forming polysaccharides including various hemicellulose derivatives are colloidal electrolytes, the solubility of which are greatly affected for example by the pH, and ionic strength of the medium in which they are immersed. Gels and viscous solutions are formed when divalent cations interact with the negatively charged side groups of these polysaccharides resulting in their partial precipitation (Alginate Industries Ltd., Technical Report, 1980).

In contrast, some of the particulate sources of dietary fibre are hydrophobic in nature with a tendency to exclude water from their physical structure. For example, α-cellulose and lignin (Shah et al., 1982) and chitin (Subramanian, 1975). However, water may still be held between coarse particles of these materials whilst not actually being absorbed directly (Fahey et al., 1980).

1.3.2 ION EXCHANGE PROPERTIES OF FIBRE

Most polysaccharides by virtue of their component sugars have a cation exchange capacity. This can be measured in vitro by titration with sodium hydroxide after washing the fibre to neutrality and charging the ionic groups to the H^+ form by treating with excess hydrochloric acid. Usually the Pk or mean dissociation

constant is used as an index of the ion exchange capacity of the fibre (Eastwood et al., 1976). The majority of fibre sources studied in this way act as monofunctional, weak cation-exchange resins, including alkali soluble rice bran hemicelluloses such as arabinogalactan and galactomannan (Mod et al., 1981).

The property of fibre polysaccharides to behave as weak cationic exchange resins may be of considerable importance with respect to their interactions with other nutrient components of the diet. In vitro binding of a number of trace elements has been demonstrated for different types of fibres (Thompson and Weber, 1979). Fernandez and Phillips (1982)[a] showed that there was a significant interaction of the lignin component of fibre with iron which may be an important factor affecting the bioavailability of dietary iron. Purified α-cellulose, however, and various silicate clays are examples of materials without any appreciable ion exchange capacities because of their relatively simple structure. Such materials may be considered to be relatively inert in this respect (Windell, Horak and Wilson, 1972).

1.3.3 ADSORPTION OF ORGANIC MATERIALS TO DIETARY FIBRE

Dietary fibre components, depending on their physical structure and chemical properties may adsorb and retain nutrients and certain metabolites present within the gastro-intestinal tract. The bacterial changes in the physical state of bile acids for example result in the formation of poorly soluble derivatives that are easily adsorbed to fibre residues. However it would appear that the hydrophobic properties of a fibre are more important in this respect.

Pfeffer et al. (1981) reviewed the interaction of fibre

components and bile acid derivatives. From invitro binding studies it has been concluded that the more hydrophobic bile acids have stronger binding affinities to plant fibre residues such as alfalfa, bran, cellulose and wood lignin. However the hydrophobicity correlation has not been strongly supported by other workers. Studies invitro of bile acid adsorption to well characterised cell wall material derived from pectin and lignified tissues indicated that lignin based residues have higher affinities for bile acid association (Eastwood and Hamilton, 1968).

It should be emphasized that invitro measurements of the physico-chemical characteristics of dietary fibre fractions are only useful indications of how they may influence the physical and biochemical processes of the gastrointestinal tract and are not ideal substitutes for actual studies on experimental animals and man.

1.4 FIBRE IN NATURAL-INGREDIENT DIETS

Within commercial animal feed rations, most definitions of fibre refer to the material present that is neither digested nor absorbed by the animal and which provides physical bulk or substance to a ration.

The tendency for nutritionists to use the term fibre instead of 'roughage' or 'bulk' probably arises from the extensive use of traditional methods of 'crude fibre' analysis. The crude fibre component of dietary ingredients has been one of the variables that feedstuff manufacturers carefully monitor and control. Commodities that have a high protein and low crude fibre content such as fishmeal generally have a high economic value whilst materials having a low protein content and usually a high fibre

content including straw and rice hulls are generally regarded as having a low economic value. Table 1.1 shows the crude fibre contents of a selection of commonly used feedstuffs.

Similarly the large variation in the crude fibre content of feedstuffs in turn affects the ability of certain ruminant and non-ruminant farm animals to accept and digest dietary fibre. The majority of monogastric animals including fish have an intestinal microflora that is incapable of degrading dietary fibre to any extent because they do not possess endogenous enzymes that catalyze the hydrolysis of cellulose and other fibrous constituents of the diet (Stickney and Shumway, 1974). Consequently monogastric animals that do not harbour such symbiotic bacteria, including rats, mice, chickens, dogs and primates cannot tolerate high inclusion levels of fibre. It has been reported by Smith (1971) that carnivorous fish such as rainbow trout may be capable of degrading fibre in the form of cellulose to a limited degree depending upon the bacterial population of the intestine.

1.5 SIGNIFICANCE OF FIBRE IN SEMI-PURIFIED DIETS

Typical examples of materials that serve as crude fibre sources within semi-purified diets are primarily cellulose based compounds. These are generally marketed under a variety of trade names such as Alphacell, Solkafloc, Avicell and Cellophane spangles and are promoted as a non-nutritive 'bulk' or fibre.

A material that is generally believed to provide no nutritive value is an important tool in nutrition research. For example, in a well controlled experiment, one could vary the concentration of the test substance at the expense of a non-nutritive component of the diet and consequently hold all other nutrient levels

TABLE 1.1. Crude fibre content (%) of some commonly used feed ingredients. Composition from McDonald et al., 1977 and FAO, ADCP/REP/11 (1980). (All values are expressed on a dry weight basis.)

Feed Ingredient	Crude fibre (%)
Cereals and by products	
Barley	4.5
Barley, brewers grains, dried	15.2
Brewers yeast, dried	2.7
Corn gluten feed	8.1
Maize, flaked	3.5
Millet	8.1
Oats	10.3
Oat husks	33.0
Oat feed	26.6
Wheat flour	1.9
Wheat bran	9.5
Wheat coarse, middlings	6.0
Oilseed by products	
Cottonseed cake (dec)	7.8
Groundnut meal (dec, extr)	7.9
Soyabean meal (extr)	5.1
Sunflower meal (dec, extr)	16.3
Animal by products	
Herring meal	1.0
Crab meal	11.0
Shrimp meal	11.0
Blood meal	2.5
Meat and bone meal	-
Milk, whey	-

constant, thereby eleminating any dilution effects. With animals that do not effectively digest cellulose, it has become common practice to use purified cellulose as the controlled non-nutritive variable (Lang and Briggs, 1976). Figure 1.1 shows the crude fibre contents of some specialized laboratory test diets for a range of animals.

It should be noted that in the process of extraction and purification, most commercial fibres are finely milled powders and much of their original fibrous nature has been destroyed. In theory these materials consist of microcrystallites but still retain their β-glycosidic linkages which make them resistant to enzymatic hydrolysis.

Carbohydrate gums are distinctive, because unlike particulate types of fibre they are soluble in aqueous solutions and have gel-forming properties. Natural and synthetic gums include agar, alginates, carrageenans, carboxymethyl cellulose, guar gum and gum arabic. These substances have a wide application in the food industry as thickening, gelling, emulsifying and binding agents. The chemical nature and degree of substitution of the side groups of these polymers can be controlled to optimize such properties as viscosity, ionic charge, solubility and resistance to enzymatic degradation (Phillips et al., 1981). Because they are soluble in aqueous solutions, the majority of carbohydrate gums and pure non-cellulosic polysaccharides have insignificant levels of crude fibre when measured using standard analytical methods. Nevertheless, these materials should be considered as fibres because their chemical structure makes them resistant to enzymatic hydrolysis and digestion.

For these reasons, analytical methods have been devised which

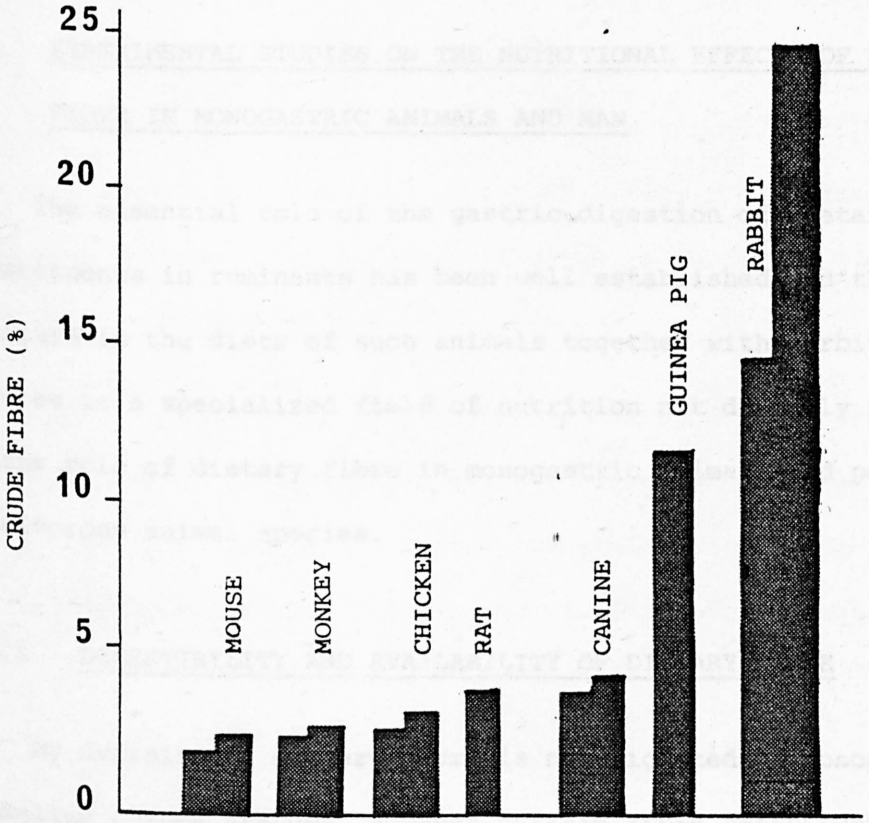


Figure 1.1 Crude-fibre content (%) of Ralston Purina laboratory rations[R]. Multiple listings represent specialized diets, such as weaning rations and high protein formulas for lactation (Reproduced from Lang and Briggs, 1976).

use acid and neutral detergents as solvents coupled with various enzyme preparations to separate soluble polysaccharide fibre components from particulate fractions such as lignin and cellulose (Van Soest and Wine, 1968; Hellendoorn, 1975).

1.6 EXPERIMENTAL STUDIES ON THE NUTRITIONAL EFFECTS OF DIETARY FIBRE IN MONOGASTRIC ANIMALS AND MAN

The essential role of the gastric digestion of dietary fibre constituents in ruminants has been well established and the value of fibre in the diets of such animals together with herbivorous species is a specialized field of nutrition not directly relevant to the role of dietary fibre in monogastric animals and particularly carnivorous animal species.

1.6.1 DIGESTIBILITY AND AVAILABILITY OF DIETARY FIBRE

By definition, dietary fibre is not digested by monogastric mammalian enzyme systems, however certain fibre constituents may be acted upon by the gastrointestinal flora of some species (Ali et al., 1981).

It has been reported by Fetzer et al., (1979) that when human volunteers were fed three purified fibre fractions, the apparent disappearance of cellulose and hemicellulose fractions were 45-46% and 76-90% respectively. Pectinaceous material disappeared completely, although some degradation products were present in the faeces.

The nutritional role of the intestinal microflora in man and other animals in the breakdown of dietary fibre has been demonstrated by comparing the heterogenous carbohydrate content of the

large intestine to that of the lower ileum (Vercellotti et al., 1977). The concentration of sugar residues characteristic of fibrous polysaccharides were all found to be higher in the small intestine than the colonic contents. There are conflicting reports regarding the fate of dietary fibre components when fed to rats. Most studies indicate that cellulose is not digested by the rat to any appreciable extent (Lang and Briggs, 1976). However, according to Yang et al., (1969), rats fed a basal grain diet supplemented with cellulose resulted in increased quantities of volatile organic acids in the caecum.

Although the rabbit is a herbivore, it poorly digests cellulose (16%). Crude fibre digestibility was reported to be 8.5% in healthy rabbits fed a diet containing 15% cellulose powder but was found to be only 3.6% in germ free rabbits (Yoshida et al., 1968).

Cellulose and crude fibre digestibility of various cereal grains vary widely in avian species, ranging from 0-50%. Plantix digestion in chickens was measured by Nakahiro and Isshiki (1975) with mixed ingredients. These studies indicated that the digestibility of non-cellulosic polysaccharides was greater than cellulose. Comparing dietary fibre digestibility of a standard diet (5.3% crude fibre) by leghorn hens and guinea fowl hens, Vogt and Stute (1974) recorded a higher digestion of hemicellulose and lignin by guinea fowl compared with the leghorn hens.

Little is known concerning the digestion and nutritive value of dietary fibre in carnivorous animal species. The crude fibre content of commercial dog foods are generally low, ranging between 1.4 and 6.2%, and between 1.2 and 7.8% within commercial foods

for cats (Wolter, 1978). Trudell and Morris (1975) reported that the apparent digestibility coefficient of a purified wood cellulose was -0.73% in the cat.

The fate of dietary fibre components has also been examined for humans consuming low cellulose and high cellulose diets together with added neutral detergent fibre which represents total cell wall components by Slavin et al., (1981). Hemicellulose digestibility was found to be approximately 70% within a low fibre diet and more than 50% for the crude fibre in diets comprising of fruits, vegetables and refined grains, whilst the apparent digestibility of refined cellulose in humans was found to be minimal.

1.6.2 EFFECTS OF DIETARY FIBRE ON NUTRIENT DIGESTIBILITY AND UTILIZATION

The significance of dietary fibre in nutrition is the subject of wide scientific review. It is generally accepted that the source, level and chemical composition of dietary fibre contributes to nutrition by influencing nutrient bioavailability and modifying the internal environment within the gastrointestinal tract.

1.6.2.1 EFFECT OF FIBRE ON ENERGY AND PROTEIN UTILIZATION

In the pig, the addition of purified cellulose to the diet decreases dry matter and energy digestibility (Henry and Etienne, 1969; Kirchgessner, 1975). Similarly, the increase of dietary crude fibre from 3-9% by the addition of sunflower meal in swine has been shown to lead to a decrease in organic matter and protein digestibility from 72.4 to 60.7% and from 82.7 to 80.9% respectively (Tascento, 1970). On the other hand, in the rabbit,

increasing the dietary crude fibre content from 10 to 26% with purified cellulose was shown to result in a decrease in dry matter and energy digestibility, but did not significantly affect protein digestibility (Lebas, 1975).

Feeding young rats with diets containing various porportions of wheat straw (5-20%) has shown a detrimental effect by crude fibre on protein digestion (Bergner et al., 1975).

It has been suggested that dietary fibre may play a role in sustaining growth and improving the utilization of absorbed nitrogen at marginal intakes of protein and energy in the rat (Nomani et al., 1979)[c]. In contrast, Kiem and Keis (1979) reported decreased weight gain and protein efficiency when mice received increasing intakes of dietary fibre.

Recently, the effects of purified fibre components and wheat brans on several indices of protein utilization were determined for growing rats by Shah et al., (1982). The majority of the fibre sources examined with the exception of cellulose, including pectin, lignin, guar gum and wheat bran caused negative effects on protein utilization as measured by net protein retention, digestibility, and endogenous faecal nitrogen excretion with increased consumption of fibre.

Similar studies have been performed in man. Slavin and Marlett (1980) concluded that the ingestion of solkafloc (and cellulose) at 16 g/day had no detrimental effect on nitrogen utilization, however increases in fat excretion and total faecal energy content in these studies were explained by the adsorption of dietary components to undigested refined cellulose. The observed increase in faecal nitrogen excretion in some studies

is probably due to the enhancement of bacterial activity in the large intestine and an increased requirement for microbial nitrogen. However, mechanical and physico-chemical effects such as the binding of protein to fibre, the promotion of endogenous losses, e.g. desquamated mucosal cells, enzymes, or decreased gastro-intestinal transit time of food may also be responsible for these phenomena (Muztar and Slinger, 1980).

Kiem and Keis (1979) reported that fat excretion was greater in mice fed diets containing 10% hemicellulose and that fat excretion in these groups was significantly higher than for rats receiving cellulose and lignin. Similarly Kennelly and Aherne (1980) observed that the addition of fibre to diets formulated to certain different levels of energy and protein, significantly influences growth and the carcass fat content in swine. Of particular interest has been the relationship between fibre source and level in the diet with blood lipid chemistry, the enterohepatic circulation of bile acids and cholesterol metabolism.

Mathé et al., (1977) showed that dietary fibre sources such as agar agar, cellulose and bran included in basal semi-purified diets to rats had moderate effects on the absorption coefficients of dietary cholesterol. Such changes can greatly affect cholesterol turnover rates in the body. The biosynthesis and metabolism of cholesterol is a highly complex process involving a number of interactions. Excess endogenous cholesterol is transferred into bile acids and various acidic steroids prior to excretion via the intestinal tract (Lehninger, 1975).

The influence of various isolated dietary fibre fractions on faecal output and steroid excretion was studied using growing rats by Nomani et al., (1979)b. The results showed that total bile

acid elimination was markedly affected by the type and level of fibre used. Dietary fibre also influenced the conversion of primary steroids into secondary steroids in the faeces.

Pectins, carragheenan, agar and arabic gum, cellulose and wheat bran at levels of up to 7% in casein-sucrose diets supplemented with cholesterol were fed to rats by Tsei et al., (1976). Of these sources, pectin displayed the greatest hypocholesterolemic effect, carragheenan was inconsistant in lowering serum cholesterol levels but produced an elevation in the carcass and liver cholesterol level. Arabic gum and agar had little effect on the serum cholesterol levels but also increased the amount of cholesterol in the liver and body.

The effect of dietary fibre on the egg yolk, liver and plasma cholesterol concentrations of laying hens was investigated by McNaughton (1978). Egg yolk cholesterol was significantly decreased by feeding alfalfa meal, oats, sunflower meal, rice meal feed or wood shavings as heterogenous sources of fibre. No significant differences were found in plasma cholesterol due to dietary fibre level. However plasma triglycerides decreased and hepatic triglycerides increased with higher inclusion levels of dietary fibre. Akiba and Matsumoto (1977) examined the effects of cellulose on fat absorption in chicks with a balance study using radioactively labelled triolein in the diet. The presence of α cellulose at levels of 4 to 8% in the diet did not affect overall fat absorption, though food passage time through the digestive tract was reduced by feeding cellulose, but a significant depression in liver lipid accummulation was observed. It was suggested by these workers that such depressions were probably due to the effect of cellulose on caloric intake rather than a direct effect on dietary fat absorption.

The previously mentioned studies are of particular relevance to man, since a great deal of research has been recently focused on the role of fibre in preventing certain clinical conditions such as obesity (Evans and Miller, 1975); diabetes mellitus (Crapo et al., 1977) and atherosclerosis and coronary heart disease (Kritchevsky et al., 1977). Many of these diseases have been attributed to the excessive intake of cholesterol and sugar rich foods. The fact that some types of dietary fibre are natural hypocholesterolemic agents thereby reducing serum cholesterol and the arterial deposition of plaques in humans, may be of great significance in the prevention and treatment of these ailments.

Cancer of the large bowel is the most serious of those diseases discussed so far. In North America, Northern Europe and Australasia it is the commonest cause of death from cancer, apart from lung cancer (Burkitt, 1981). Dietary factors appear to play a major role in the aetiology of the disease by promoting tumour growth. Animal experiments suggest that colonic neoplasias and tumours are enhanced by high bile acid levels in the large intestine and particularly secondary bile acids. The action of colonic bacteria on bile acids and steroids can lead to the production of toxins and carcinogens (Cummings, 1980). Since dietary fibre is a complex that acts primarily in the colon, there is a wide consensus of opinion that the faecal bulking properties, ion exchange and adsorptive properties of fibre may be beneficial in diluting and preventing the accummulation of carcinogenic and toxic metabolites.

1.6.2.2 DIETARY FIBRE AND MINERAL NUTRITION

Evidence presented thus far suggests that increased fibre consumption is desirable, but it is important to consider any possible adverse effects of fibre intake. The property of certain fibres to act as cation exchange resins could result in some undesirable effects such as reducing the absorption of several essential mineral elements (Mod et al., 1981). There have been a number of investigations supporting this view.

Stevenson and Unsworth (1978) reported significant reductions in the net availability of Ca, Cu, Zn and P for 18-month old ewes fed diets with varying proportions of dried grass meal, ground straw and barley as sources of roughage. More detailed studies on digestion, absorption and net movement of mineral nutrients in the digestive tracts of young growing pigs were performed by Partridge (1978). Using re-entrant cannulas, the amounts of Ca, P, Mg, Na and K passing through different intestinal sites and faecal concentrations were measured. It was suggested that dietary composition and fibre content is a major factor affecting the bioavailability of trace elements in the pig. Looney and Lei (1977) made a detailed study of the effects of different levels of cellulose on the net availability of Zn at two levels (marginal and abundant) and Cu at two levels (deficient and adequate). After a nine-week experimental period, the copper status of the Cu deficient animals was improved for the high fibre treatment. This suggested that increments of dietary cellulose may have reduced the competitive absorption between Zn and Cu, enabling more Cu to be absorbed.

Fernandez and Phillips (1982[a]) reported that insoluble components of fibre, notably lignin, have a capacity to bind

ferrous iron in invitro studies designed to approximate the conditions of the small intestine post prandially. Some examples of iron deficiency anaemia are thought to be associated with impaired bioavailability of dietary iron. Among the constituents of food that must be considered as potential modifiers of the bioavailability of iron is dietary fibre. Fernandez and Phillips (1982^{b}) extended their earlier invitro studies of iron binding with fibre components by examining the effects of dietary fibre on the absorption of ferrous iron in a canine model. The investigation showed that lignin as a fibre component was a potent inhibitor of iron absorption, pectin less so, and cellulose was without effect in this system. The invivo results correlated well with the invitro studies by the same workers.

In most Western countries, bread is a main source of dietary iron. The largest part of the naturally occurring iron in bread is derived from bran in the flour. In this respect, Björn-Rasmussen (1974) compared iron absorption from a white wheat bread and a bread baked with different amounts of bran. It was concluded from this study that the addition of approximately 7% of bran to bread greatly impaired iron absorption. Reinhold et al., (1976) and Sandberg et al., (1982) similarly reported decreased bioavailability of Ca, Mg Zn and P by humans due to increased fibre consumption as wheat based bread.

It can be appreciated that the influence of dietary fibre on trace element metabolism is a very complicated area of nutritional research owing to the diversity in the ion exchange and adsorptive properties of different fibre components. Investigations are further confounded by the interactions and competitive behaviour of certain elements such as Zn and Cu; Ca and P for different

intestinal binding sites and absorptive mechanisms. However, most studies imply that increased consumption of dietary fibre may prove detrimental by inducing trace element deficiencies when these are present at marginal levels in the diets of animals and man.

1.6.3 THE EFFECT OF DIETARY FIBRE ON GASTROINTESTINAL TRANSIT TIME AND GASTRIC EMPTYING TIME

The efficiency of digestion and absorption is dependent upon the rate at which digesta moves through the gastro-intestinal tract (Clemens and Stevens, 1980). Movement of digesta may be regulated via structural and physiological characteristics of the digestive tract. Its movement is further influenced by physical as well as nutritional characteristics of the diet (Hogan and Weston, 1969). Retention of digesta generally occurs within the stomach and/or hind gut, while passing rapidly through the small intestine. Prolonged retention can aid the digestive process by providing adequate time for host and microbial enzymatic degradation of the ingested materials, as well as enhancing absorption time.

In man, however, prolonged retention of material in the large intestine is undesirable due to the possible accumulation of toxins resulting in disease. Burkitt et al., (1972) surveyed the amount of stools passed by various groups of people and the transit time of food residues through their intestinal tracts in relation to dietary habit. It was found that more refined diets produced smaller, compact stools and a much slower passage time for digesta. By contrast, diets containing ample fibre resulted in soft, bulky stools that traversed the lower gut more rapidly.

It is believed that the excessive straining and high

intra-colonic pressures associated with the passage of the hard stools may damage the submucosal layer of the large intestine. Secondary infections may then develop resulting in such conditions as haemorrhoids, piles, diverticular disease and colitis. Such diseases are rare or absent in underdeveloped countries where the staple diets are rich in natural fibre (Stanway, 1981). Considerable research effort has therefore been directed to study the effectiveness of various dietary fibre sources in reducing intra-luminal pressure and transit time.

Brodribb and Groves (1978) studied the effect of bran particle size on stool weight in human volunteers. The stools resulting from subjects consuming a coarse bran diet were soft, of uniform consistency and bulkier than control diets containing a minimum of fibre, and faecal passage was greatly facilitated with coarse bran. Similar effects were reported by Heller et al., (1980) when a coarse wheat bran was included in the diets of young adult men however no significant differences in the number of defecations per day were noted. These results indicated that coarse dietary bran was effective in retaining faecal water and in promoting rapid transit of digesta through the gut.

On the other hand Cummings et al., (1978) examined the effect of pectin as a gel-forming fibre on large bowel function in humans. These results indicated that pectin was without effect on bowel habit and produced little increase in faecal weight. In contrast, Holt et al., (1979) demonstrated that gel-fibres in the form of guar gum and pectin significantly delayed gastric emptying times in humans but unfortunately, these workers did not report the effects of these fibres on large bowel function.

The effects of fibre and related components on food passage

time has also been reported from numerous animal studies. Akiba and Matsumoto (1977) noted that in chickens, the feeding of pectin as a source of dietary fibre greatly reduced food passage time through the digestive tract. In common with human studies, Kendall (1982) stated that increased consumption of dietary fibre decreased the gastro-intestinal transit times in dogs. When adult beagles were given diets containing, 0, 5, 10 and 20% purified cellulose or with the same levels of wheat bran, wet faecal output was more than doubled at the 20% dietary inclusion levels of both types of fibre. Schemann and Ehrlein (1982) evaluated the effect of a cellulose meal and a low fibre meal on gastroduodenal motility and gastric emptying rates in dogs. High cellulose diets resulted in greater propulsive duodenal contractions and a significantly reduced gastric emptying time.

The absence of fibre in the diets of pigs has also been shown to cause a significant slowing down of food passage time (Canguilhem and Labie, 1977). In these studies a meal substitute fed alone in a liquid form was compared to one supplemented with a bran mixture. Richter and Busch (1970) also observed that the rate of passage of feed for sows was faster with greater inclusion levels of dietary fibre.

In the rat, Sharpalekar et al., (1969) reported that the inclusion of 5-20% cellulose was without effect on gastric emptying time but Brown et al., (1974) showed that a synthetic high viscosity methyl cellulose was very rapidly cleared through the digestive tract.

1.6.4 NUTRITIONAL STUDIES ON THE EFFECTS OF DIETARY FIBRE FOR FISH

The earliest study on the nutritive value of dietary fibre in natural ingredients for fish was that of Bondi et al., (1957). A high degree of digestibility for the dietary fibre present in natural feed ingredients was observed for carp Cyprinus carpio with digestibility coefficients ranging between 25-89% depending on the type of food material and the degree of milling. In general more finely milled cereal grains resulted in higher digestibility. Wood et al., (1954) who studied the effect of sodum carboxymethyl cellulose (CMC) as a binding agent in the preparation of fish diets also observed that the physical state of the feed may be important in determining its digestibility. These investigators also studied the use of various alginate products such as Irish moss, kelgin and kelcoloid as binding agents. It was suggested that these substances might be physically active by preventing nutrients from becoming available.

Buhler and Halver (1961) found that although Chinook salmon fingerlings (Oncorhynchus-tshawytscha) grew satisfactorily on a purified diet alone, the addition of small amounts of cellulose increased growth and the efficiency of protein utilization. When channel catfish Ictalurus punctatus were fed purified diets containing equal amounts of nutrients but with dietary cellulose concentration ranging from 0-51% by the replacement of the basal diet as a whole, fish showed the highest weight gain from the diet containing 21% cellulose (Dupree and Sneed, 1966). In contrast, Leary and Lovell (1975) reported that fibre in the form of α-cellulose was of little nutritive value and produced no significant effect on carcass composition in their studies with

channel catfish. Similarly, Bergot (1981) as part of a digestibility trial involving common carp and rainbow trout showed that dietary concentrations of purified cullulose of up to 20% was not digested by these fish species. Hilton et al., (1983) recently studied the effect of dietary fibre on the growth of rainbow trout. Trout fed for 12 weeks on rations containing 10 and 20% α-floc displayed a significantly lower growth response compared to fish fed the control diet containing no added fibre.

From the limited studies conducted to date, it is evident that no clear picture has emerged on the nutritional value of dietary fibre for fish. Furthermore these studies have tended to concentrate on a single fibre source namely α cellulose. Bearing in mind the wide variety of ingredients that may be included under the broad definition of dietary fibre, it was the main intention to limit experimental studies to a range of purified dietary fibre sources of defined chemical and physical character and selected on the basis of their relevance in practical and experimental diets for fish. These included chitin (poly N-acetyl-glucosamine), lignin, ligno-sulphonate, α-cellulose, galactomannan polysaccharide and polyethylene for the reasons outlined in Chapter 2. The nutritional effects of these dietary fibre sources for rainbow trout were assessed by means of growth and digestion trials, followed by a more detailed examination of the influence of fibre on physiological parameters such as feed intake, gastric evacuation rate and intestinal transit time.

CHAPTER 2

Experiment 1 - A PRELIMINARY STUDY TO EXAMINE THE GROWTH PERFORMANCE OF RAINBOW TROUT FED A RANGE OF PURIFIED DIETARY FIBRE SOURCES SELECTED FOR DIFFERENCES IN PHYSICAL AND CHEMICAL CHARACTER.

CHAPTER 2

Experiment 1 - A preliminary study to examine the growth performance of rainbow trout fed a range of purified dietary fibre sources selected for differences in physical and chemical character.

2.1 INTRODUCTION

A preliminary investigation was undertaken to examine the effects of feeding a selected range of purified fibre sources differing in chemical and physical character. These fibre sources were included in a semi-synthetic diet for trout at a level similar to that normally encountered by fish fed practical and laboratory test diets (c. 5g/100g). The chemical characteristics of the six dietary fibre sources tested, i.e. α cellulose, lignin, lignosulphonate, chitin, galactomannan and polyethylene are shown in Fibure 2.1.

α cellulose was considered because it is the major component of dietary fibre in feed ingredients of plant origin. It is relatively simple in structure being a β(1-4) linked polymer of D-glucose and in its refined commercial form exists as a microcrystalline powder with a low affinity for water.

In contrast, the non-cellulosic dietary fibre fractions in plant materials are fairly soluble in nature consisting mainly of hemicelluloses and pectins which include the galactomannans. Structurally the seed galactomannans are pure mannans which carry numerous α-(1-6)D-galacto-pyranosyl side chains and substituents. The essential linear yet highly branched molecular structures of the galactomannans results in properties which are quite different from the unbranched cellulose type polysaccharides. Galactomannans

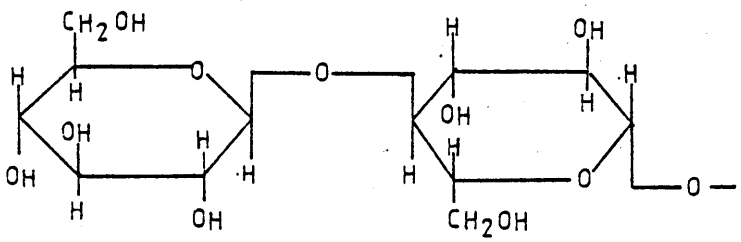


Figure 2.1.1

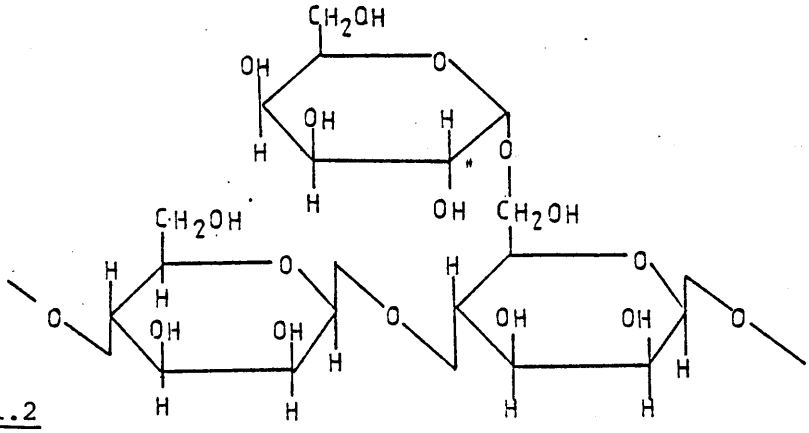


Figure 2.1.2

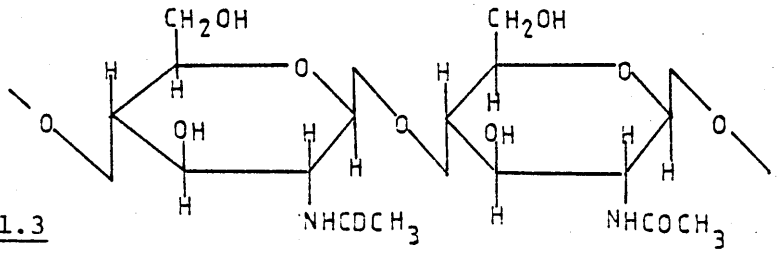


Figure 2.1.3

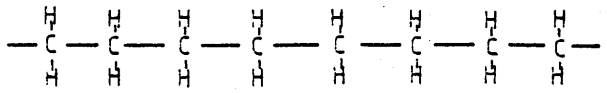


Figure 2.1.4

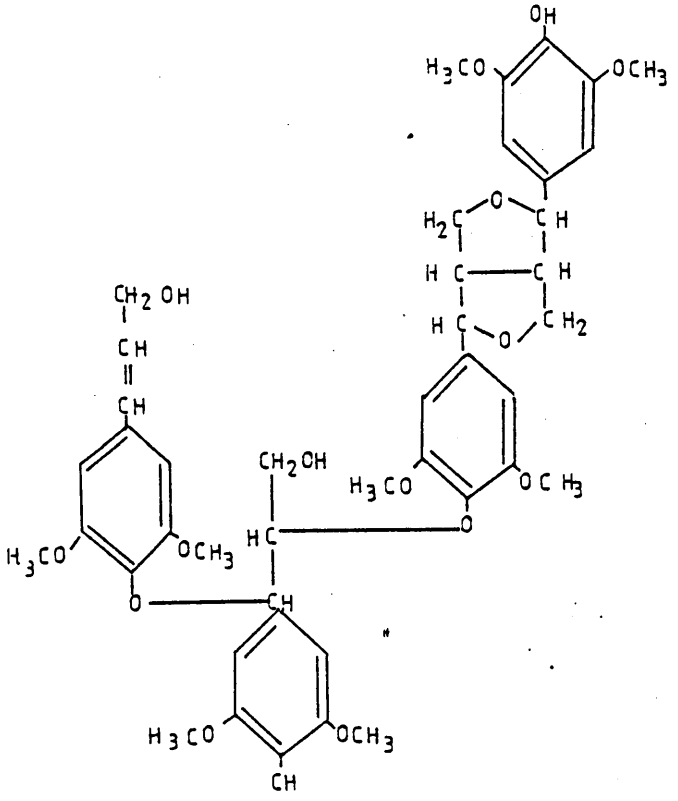


Figure 2.1.5

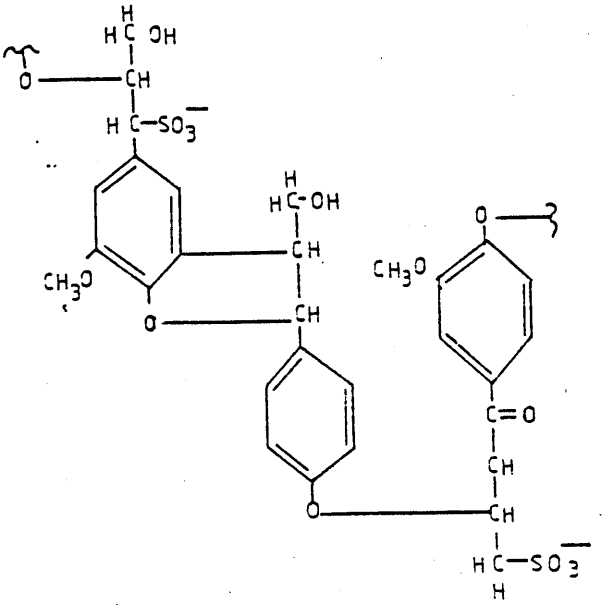


Figure 2.1.6

Figure 2.1 Structural and chemical composition of the dietary fibre sources included in the experimental diets.
Figures 2.1.1, α cellulose, poly-(β1-4)-D-glucose; Fig 2.1.2, Galactomannan, poly-(β1-4)-D-mannose, (α1-6)-D-galactose; Fig 2.1.3 Chitin, poly N-acetyl-D glucosamine (β1-4); Fig 2.1.4 Polyethylene; Fig 2.1.5 Lignin (Kraft Lignin) dehydration polymer of coniferyl and sinapyl alcohols; Fig 2.1.6 Lignosulphonate (Alwatech Ltd.), from Hopwood (1980).

are hydrophillic, producing characteristic viscous gels and are obtained from crushed seeds and beans by hot water extraction (Loewas and Tanner, 1982). They are also very similar in physical character to the alginate binders used in moist diets for fish (Halver, 1972) and have wide applications in the food industry. Lignin which comprises between 17 and 33% of wood is an aromatic polymer of phenolic residues such as coniferyl and sinapyl alcohols. The purified material is hydrophobic and commercially available as a fine powder under the trade name 'Kraft lignin' which is mainly extracted from spruce wood (Kirk, 1980).

A derivative of lignin, lignosulphonate occurs in the sulphite lye as a by-product of the wood pulping industry. Sulphonated lignins vary considerably in molecular weight and degree of sulphonation. Although the product has a number of structural similarities to lignin, the sulphonated side chains provide the polymer with a net negative charge at low pH and a more reactive and hydroscopic property than pure lignin. Lignosulphonates have therefore a number of useful applications such as the treatment of waste water in industrial food plants and the recovery of protein and fat by precipitation. They have also been used as pelleting agents in animal feedstuffs and have been approved as additives by the European Economic Community and United States health authorities (Hopwood, 1980).

Chitin is a major structural component in the skeletal and supporting tissues of many invertebrate species such as annelids (polychaetes and oligochaetes), molluscs, and particularly crustacea such as shrimp, crabs and krill. Chitin is a typical example of a macromolecule and is a β(1-4) polymer of N-acetyl D glucosamine. On hydrolysis it yields a mixture of the monomer

component and the dimer, chitobiose (Zehavi et al., 1971). In many ways the chemical structure is analogous to cellulose except that it is commercially available as coarse flakes that may be ground to different particle size ranges. Since chitin is abundant in the aquatic and marine environment and in the natural foods of many fish, it was considered as a potential source of dietary fibre for rainbow trout since many workers dispute the fact that chitin may be digested by some species of fish (Barrington, 1957; Goodrich and Morita, 1977).

For comparative purposes it was decided that a suitable material should also be included at the same level as the other fibre sources in a separate 'control' diet. Polyethylene was thought to be ideal in this respect since it is widely accepted as being chemically and physically inert and resistant to even strong solvents and acids. The powdered form of the material can be mixed uniformly with other dietary ingredients and would not be expected to present palatability problems or contribute any biological effects when fed to rainbow trout at low levels.

In view of the chemical and physical diversity of the dietary fibre sources used in the present investigation, it was hoped to establish those properties having the greatest influence on the growth performance of rainbow trout at low inclusion levels over a 63-day feeding period which might serve as a basis for further studies.

2.2 MATERIALS AND METHODS

Five fibre sources were chosen on the basis of possible differences in chemical, physical and biological properties:

α cellulose, galactomannan polysaccharide and chitin

(poly-n-acetyl D-glucosamine) were obtained from Sigma Chemicals Ltd., Poole, Dorset. Lignin (Indulin ATR-ATR-Ckl, Westvaco Ltd., S. Carolina, U.S.A.) was provided by the Biodeteriation Information Centre, University of Aston, Birmingham. Lignosulphonate was supplied by Alwatech UK Ltd., High Wycombe, Bucks., and Polyethylene (low density, finely powdered) was obtained from BDH Chemicals, Poole, Dorset.

2.2.1 DIET PREPARATION

Herring meal (70% crude protein, NX6.25, 10% crude lipid) was used as the source of dietary protein and fish oil within all of the experimental diets prepared.

Six rations were formulated to provide 45% crude protein and 12% crude lipid. Chromic oxide and polyethylene were included in all of the diets at 0.5% as inert markers for the determination of digestibility coefficients. All dietary fibre sources were included at 5.0% separately, within the experimental diets. The composition of the experimental diets is shown in Table 2.1 and the particle size distribution of the individual fibre sources is shown in Table 2.2. The diets were prepared by first mixing all of the dry ingredients thoroughly in a Hobart A200 mixer before the addition of the oil and water (containing the mineral premix). The moist diet was then extruded, under pressure, through a 3mm die and the resulting pellets dried carefully by air convection, and stored in air tight containers until fed.

TABLE 2.1 Composition of the experimental diets (% by weight).

Ingredient Diet No	1	2	3	4	5	6
Herring meal	67.70	67.70	67.70	67.70	67.70	67.70
Corn starch	12.68	12.68	12.68	12.68	12.68	12.68
White dextrin	6.34	6.34	6.34	6.34	6.34	6.34
Corn oil	4.28	4.28	4.28	4.28	4.28	4.28
Cellulose	5.00	-	-	-	-	-
Lignin	-	5.00	-	-	-	-
Lignosulphonate	-	-	5.00	-	-	-
Galactomannan	-	-	-	5.00	-	-
Polyethylene	-	-	-	-	5.00	-
Chitin	-	-	-	-	-	5.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00	1.00
Indicator mix[3]	1.00	1.00	1.00	1.00	1.00	1.00
Nutrient content (%)						
Moisture	5.70	5.15	5.88	5.31	4.09	4.60
Crude protein (NX 6.25)	48.89	50.42	49.93	49.57	49.03	50.23
Lipid	10.17	10.50	10.94	9.95	10.88	11.12
Ash	11.26	11.95	12.01	11.17	12.08	10.49

1. Vitamin premix: Supplying per kg of diet; Thiamine HCl 50mg, Riboflavin 50mg, Calcium pantothenate 100mg, Niacin 200mg, Pyridoxine HCl 40mg, Biotin 6mg, Folic acid 15mg, Cyanocobalamin 0.1 mg, Inositol 200mg, Ascorbic acid 1000mg, Choline chloride 4000mg, Menadione 40mg, tocopherol acetate 400mg, p-amino-benzoic acid 50mg, Vitamin A acetate 2000 IU, Vitamin D3 1000 IU (Tacon et al., 1983).

2. Mineral mix: Supplying per Kg diet; $MgSO_4.7H_2O$ 5.10g, NaCl 2.40g, KCl 2.00g, $FeSO_4.7H_2O$ 1.00g, $ZnSO_4.7H_2O$ 0.22g, $CuSO_4.5H_2O$ 0.0314g, $MnSO_4.4H_2O$ 0.1015g, $CoSO_4.7H_2O$ 0.0191g, $CaIO_3.6H_2O$ 0.0118g, $CrCl_3.6H_2O$ 0.0051g (Tacon et al., 1983).

3. Cr_2O_3/polyethylene (1:1).

TABLE 2.2 *Particle size composition of dietary fibre sources (% distribution within the range 45-1000 microns)*

Size range μ	> 1000	790-1000	500-790	250-500	125-250	45-125	< 45.μ
α-cellulose	-	-	49.40	34.41	11.70	3.59	0.90
Lignin	-	-	0.21	87.94	2.39	4.51	4.94
Lignosulphonate	-	-	0.28	96.30	0.76	1.53	1.12
Galactomannan	-	-	0.58	96.23	1.77	1.13	0.29
Polyethylene	-	-	0.76	91.69	5.30	1.92	0.33
Chitin	19.44	26.28	4.13	44.32	2.30	2.43	0.40

2.2.2 ANIMALS AND TANKS

Fish of mean weight 32 grams were obtained from Cloan Hatcheries, Vale of Glendevon, Perthshire, and were randomly distributed at a stocking density of 25 fish per tank. The experimental facilities were located in an outdoor compound and supplied by running city tap water. A schematic illustration is shown in Figure 2.2. Each 40 litre tank was fitted with a diaphragm valve to maintain uniform circulation rates of 4.8L/min. Water drainage from the tanks was maintained via central stand pipes, and so providing an open flow system. The water temperature varied between 4°C and 9°C during the course of the experiment (mean, 6.2°C) and a natural photoperiod was employed.

Twelve fish were sacrificed at the start of the experiment, killed by a sharp blow on the head, and then stored at -15°C for subsequent gross carcass analysis. Fish were fed ad libitum, twice daily (mid-morning and afternoon) and weighed individually under anaesthesia, using 20-30mg/l of Benzocaine (Ethyl p-amino benzoate) (Laird and Oswald, 1975), at biweekly intervals over the 9-week experimental period. On the final day of the experiment, all fish were killed by a sharp blow on the head for similar gross carcass analysis.

Faecal samples were collected by hand stripping according to the methods of Austreng (1978) during the final 2-weeks of the experimental period, pooled, and then dried at 105°C for 24 hours before ground to a fine powder and stored in airtight containers for subsequent chemical analysis.

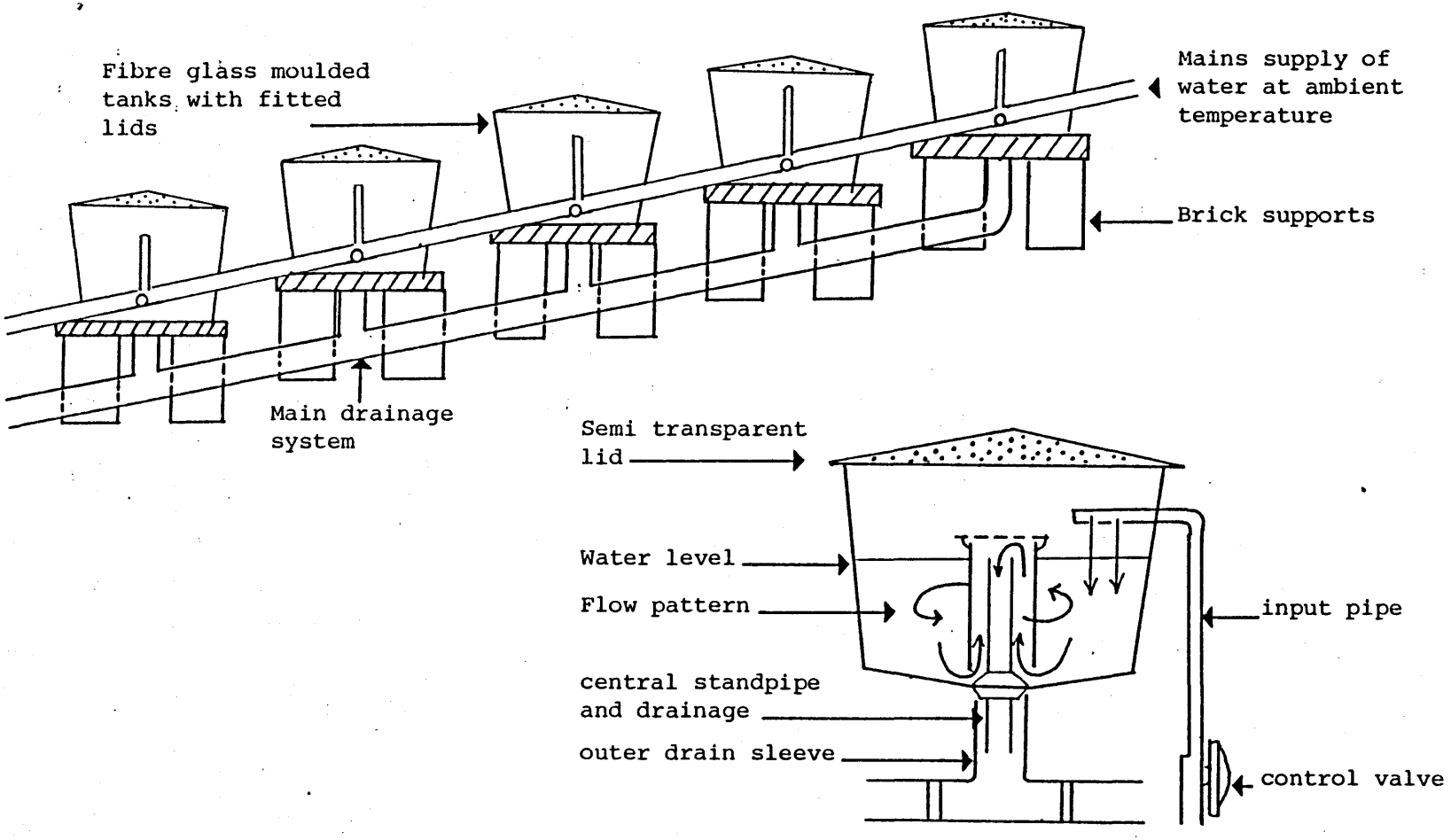


FIGURE 2.2 Arrangement of fish holding facilities designed for the experimental studies.

2.2.3 CHEMICAL METHODS AND SAMPLE PREPARATIONS

Prior to chemical analyses, diets and fish samples from each treatment were oven dried at 105°C for moisture determinations and then finely ground. Total nitrogen, lipid and ash contents of the dried materials were determined using the methods described by the Association of Official Analytical Chemists (AOAC, 1980).

2.2.4 DEFINITIONS OF THE NUTRITIONAL PARAMETERS DETERMINED

i) Specific growth rate (SGR) is defined as the percentage increase in body weight per day.

$$SGR\%/day = \frac{\log_e \text{final body wgt of fish(g)} - \log_e \text{initial body wgt fish(g)}}{\text{Experimental period (days)}} \times 100$$

ii) $$\text{Feed conversion ratio (FCR)} = \frac{\text{mean daily feed intake (g)}}{\text{mean daily live wgt gain (g)}}$$

iii) Protein efficiency ratio (PER) =

$$\frac{\text{mean daily live wgt gain (g)}}{\text{mean daily crude protein intake (g)}}$$

iv) $$\text{Net nitrogen utilization (\%)} = \frac{\text{nitrogen deposition (mg/day)}}{\text{nitrogen intake (mg/day)}} \times 100$$

v) $$\text{Apparent dry matter digestibility (\%)} = 100 \left[1 - \frac{C}{B} \times \frac{1 - B}{1 - C} \right]$$

where B = grams of indicator per gram of faecal dry matter

C = grams of indicator per gram of dietary dry matter

vi) Apparent nitrogen digestibility (%) =

$$100 - 100 \times \frac{\text{\% indicator in diet}}{\text{\% indicator in faeces}} \times \frac{\text{\% nitrogen in faeces}}{\text{\% nitrogen in diet}}$$

2.2.5 STATISTICAL ANALYSIS

Analysis of variance (ANOVA) at the 5% level of significance together with Duncans multiple range test (Duncan , 1955) were applied to the initial and final weights of fish.

2.3 RESULTS

2.3.1 GROWTH

Tables 2.3 and 2.4 show the results of the growth performance, feed utilization and carcass composition for fish over the experimental trial. There was no significant difference in the mean final weights of fish receiving each of the diets ($p>0.05$). The highest specific growth rate (SGR) was obtained for rainbow trout fed the rations containing 5% lignosulphonate and for the diet containing 5% polyethylene (0.71% for each respectively), and the lowest value was obtained for fish fed the galactomannan diet (0.54%).

2.3.2 FOOD UTILIZATION

Mean daily food intake was noticeably better for fish receiving the polyethylene diet compared to the other treatments, although no corresponding trend was obtained for daily live weight gain.

TABLE 2.3 Mean body weights of fish (g) at successive biweekly intervals.

Diet No	1	2	3	4	5	6
Week						
0	32.62	31.61	30.99	33.52	31.02	31.95
2	31.12	30.25	29.97	31.57	29.98	30.80
4	33.24	32.74	33.41	34.29	32.38	32.75
6	38.86	37.42	38.77	38.78	38.60	37.89
9	48.34	46.57	48.32	47.07	48.22	46.31

Food conversion ratio (FCR) was relatively poor for fish fed the galactomannan and chitin diets (1.91; 1.94 respectively) compared to 1.67 for the lignosulphonate diet. The data suggested that protein utilization may also have been affected in fish receiving the galactomannan and chitin fibre sources. The PER values were 1.06 and 1.03 respectively, compared to 1.13 for the polyethylene diet and 1.20 with lignosulphonate.

Maximum net nitrogen utilization was achieved for the polyethylene diet (22.60%) and lower values of 15.92 and 16.96% were obtained for the galactomannan and chitin treatments. The remaining diets containing cellulose, lignin and lignosulphonate showed approximately the same values ranging between 18.40% to 19.59%.

The apparent dry matter (DM) and nitrogen digestibility values were in fairly close agreement except for the lignin diet which resulted in a much lower DM digestibility of 48.28% compared to the mean of approximately 64% for the remaining treatments. However, apparent nitrogen digestibility was only slightly

TABLE 2.4 *Growth, feed utilization and carcass composition of rainbow trout (25 fish, initial weight 32g) after 9-weeks at 4°C to 9°C (mean, 6.2°C).*

Parameter	Diet No. 1	2	3	4	5	6	±SE
Mean initial weight (g)	32.62[a]	31.61[a]	30.99[a]	33.52[a]	31.02[a]	31.95[a]	0.83
Mean final weight (g)	48.34[a]	46.57[a]	48.32[a]	47.07[a]	48.22[a]	46.31[a]	1.47
Specific growth rate (%/day)	0.63	0.62	0.71	0.54	0.71	0.60	
Daily food intake (mg)	446.5	424.0	458.3	410.6	494.7	441.3	
Daily live weight gain (mg)	249.5	237.5	275.1	215.2	273.0	227.9	
Food conversion ratio (FCR)	1.79	1.79	1.67	1.91	1.81	1.94	
Protein efficiency ratio (PER)	1.14	1.11	1.20	1.06	1.13	1.03	
Nitrogen intake (mg/day)	34.9	34.2	36.6	32.6	38.8	35.5	
Nitrogen deposition (mg/day)	6.42	6.51	7.17	5.19	8.77	6.02	
Net nitrogen utilization (%)	18.40	19.04	19.59	15.92	22.60	16.96	
Apparent dry matter digestibility (%)	63.66	48.28	65.85	66.38	60.02	64.33	
Apparent nitrogen digestibility (%)	87.21	80.52	88.00	87.57	86.27	84.95	
Carcass composition (% wet weight)							
	Initial fish						
Moisture	73.84	74.21	73.46	74.43	75.80	72.02	74.61
Crude protein (Nx6.25)	16.21	16.17	16.91	16.24	15.88	17.59	16.30
Lipid	6.69	6.21	7.28	4.52	4.76	7.41	4.24
Ash	2.75	2.21	2.44	2.47	2.51	2.59	2.73

a Mean values with the same superscripts are not significantly different (P>0.05); ± Standard error.

decreased to 80.52% for the lignin diet compared to a mean of approximately 86% for the other diets.

2.3.3 CARCASS COMPOSITION

The carcass composition data suggests that galactomannan, chitin and lignosulphonate may have an adverse effect on body lipid content, since these levels were found to be considerably lower at 4.76%, 4.24% and 4.52% respectively for fish consuming these diets. The carcass protein content of rainbow trout receiving the galactomannan diet was also slightly lower than for those fed the other dietary treatments. It should also be noted that there is an inverse relationship between the carcass moisture and lipid contents of animal tissues (Whittemore and Elsley, 1977). This might explain the slightly higher moisture content observed for rainbow trout receiving galactomannan, chitin and lignosulphonate diets, 74.43%, 75.80% and 74.61% respectively compared to a mean of 73% moisture for the carcasses of fish receiving the other treatments.

2.4 DISCUSSION

The dietary fibre sources were included in the diets at a relatively low level comparable to the crude fibre contents of typical commercial salmonid diets (Edward Baker technical report, 1982). Although the fibres were chosen to differ considerably in physical and chemical character, there was no evidence to suggest that these properties could significantly influence the growth of rainbow trout over a 9-week experimental period at a 5% level of dietary inclusion. Unfortunately the adverse weather conditions and low water temperature prevented the optimum growth of fish

and this together with the variation in individual growth performance may have masked any differences between treatments. Nevertheless, interesting trends emerged which could be related to certain properties of the fibre sources included in the diets tested.

The specific growth rate for trout receiving the lignosulphonate and polyethylene diets appeared to be slightly better than for trout fed galactomannan. The daily food intake for fish consuming the polyethylene diet was marginally greater when compared to the other treatments and particularly better than the diet containing 5% galactomannan. Food conversion ratios and net protein utilization were also shown to be inferior for the galactomannan and chitin diets.

Of the experimental diets, it might be expected that polyethylene would present the least problems in terms of palatability since the material is very fine in texture and without aroma. This inert nature probably explains the slightly superior food intake for fish receiving this diet.

A comparison between the response of rainbow trout fed diets containing lignin and lignosulphonate was interesting in view of the different chemical structures of these two materials. The lignin used in this experiment was a highly refined type extracted from sprucewood by the Kraft lignin process which includes alkali digestion and solubilization of lignin by Na_2S. The 'black liquor' so obtained is progressively acidified by carbon dioxide and sulphuric acid resulting in an acidic lignin fraction called Indulin ART-S. An identical lignin was used in a digestion study involving hamsters by Fahey et al., (1980) at levels of 10, 20 and 30% of the diet. In this investigation, hamsters fed the

different levels of lignin showed fairly similar food intakes but weight loss occurred together with a reduced dry matter digestibility when the animals received 20% and 30% lignin. The results of the present study with trout are in agreement with those of Fahey et al., (1980) in that dry matter digestibility was considerably depressed by the inclusion of 5% lignin, however there was only a slight reduction in the apparent nitrogen digestibility and no effect on nitrogen utilization compared to the polyethylene treatment. According to Stanway (1982), lignin has an anti-laxative effect for humans. In contrast, it was noticed that the faecal material from fish consuming the lignin diet was quite watery in appearance which may have been due to the extremely fine composition and large surface area of the lignin particles resulting in the greater retention of intestinal fluids. Support for the view that lignin may have a laxative effect in fish was provided by Windell et al., (1972)[b]. A Colorado pellet containing a lignin binder was compared with a modified diet containing a bentonite binder and a diet without a binder. The lignin diet was found to pass through the gastrointestinal tract of rainbow trout at a much faster rate compared to the other treatments. A physiological response of this type may account for the reduction in dry matter digestibility and the apparently greater faecal mass observed in the present study with the lignin treatment.

Lignosulphonate differs considerably from lignin due to the presence of negatively charged sulphonated side chains. The material is therefore more hydrophillic and chemically active than lignin. Generally the amine groups of protein molecules carry a net positive charge when the pH value is below the iso-electric point (pH 3.5) for most proteins (Lehninger, 1975). Therefore at a pH of approximately 3, protein molecules and

lignosulphonate carry opposite ionic charges which causes precipitation of the protein. Acidic conditions would also favour the breakdown of the emulsions which hold fats and oils in solution (Hopwood, 1980). There is a possibility that lignosulphonate may also behave in a similar manner in the gastrointestinal tract of trout. The acidic conditions of the stomach may allow the precipitation and concentration of dietary protein and the higher pH of the intestine (pH 5-7) would favour the release of the protein from the protein/lignosulphonate complex. Consequently the better performance of trout with respect to the utilization of nitrogen might be partly due to an increased availability of dietary nitrogen in the ligno-sulphonate treatment.

A comparison of the particle size distributions for the various fibre sources (Table 2.2) shows that except for the ground chitin, most of the sources consisted of particles ranging between 790 microns to below 45 microns in dimension. For lignin, lignosulphonate, galactomannan and polyethylene, 90% of the distribution of particles was between 250 and 500 microns. Therefore these fibre sources were effectively similar in particle size range and so their effects on the performance of rainbow trout ought to be attributable to physical and chemical characteristics other than particle size.

Galactomannan, although of similar particle composition to polyethylene has quite opposite characteristics compared to the other fibre sources. Its ability to form viscous gels in association with aqueous solutions could also have increased the viscosity of the gastrointestinal contents within rainbow trout thereby inhibiting the normal digestive processes. This property may explain the reduced food intake and inferior FCR and nitrogen

utilization observed for trout receiving 5% galactomannan. The effects of chitin however were probably the result of its coarse texture since approximately 45% of its particle size distribution in the present study was above 790 microns. Also, on the basis of this trial, there was no evidence to suggest that chitin is being utilized by rainbow trout. However there is a need to examine this point further with longer term growth and digestibility trials in order to establish good growth and to test the effect of using different particle size ranges of the material.

It should be noted that the fibre sources were chosen to provide well characterized examples of dietary fibre constituents most likely to be encountered by rainbow trout whether in natural or experimental dietary ingredients. On the other hand, the advantages conferred by using semi-purified components of fibre must be balanced against the indirect relationships that these substances may have with natural foods. In particular the processes by which the cellulose, lignin, galactomannan and chitin fractions were prepared could affect their physico-chemical properties.

However the current study was useful in underlining some of these properties and defining the nutritional parameters that may be influenced by including these fibres in the diets of rainbow trout. In this respect, such factors as particle size composition, water holding capacity together with the viscometric properties of certain soluble fibres, may prove to be important considerations for further studies to evaluate their effects on the growth performance and utilization of dietary nutrients in trout.

CHAPTER 3

Experiment 2 - THE EFFECTS OF α-CELLULOSE ON GROWTH AND NUTRIENT UTILIZATION IN RAINBOW TROUT WITH REGARD TO ITS POSSIBLE VALUE AS A NON-NUTRITIVE BULKING INGREDIENT.

CHAPTER 3

Experiment 2 - The effects of α-cellulose on growth and nutrient utilization in rainbow trout with regard to its possible value as a non-nutritive bulking ingredient.

3.1 INTRODUCTION

It has been mentioned that most of the studies involving the effects of dietary fibre on the performance of fish, were based on the use of α-cellulose at different inclusion levels. It was also pointed out that in a number of studies, investigators varied the level of fibre at the expense of the diet as a whole (Hilton et al., 1983) or by the substitution of some other nutrient component of the diet, usually starch (Dupree and Sneed, 1966; Leary and Lovell, 1975).

In view of the conflicting results obtained by some of these workers, it was decided that an experiment designed to evaluate the effects of graded levels of α-cellulose on the performance of rainbow trout was worthy of consideration. It was also the intention of the investigation to establish if α-cellulose was sufficiently inert to be included in future studies as a control variable that could be substituted with other dietary fibre sources without altering the remaining nutrient balance of the diet as a consequence of dilution effects.

However, for the purposes of the present study, α-cellulose was included at different levels by the replacement of dietary starch. Starch is only chemically different to cellulose with respect to its α-glycosidic structure and is therefore susceptible

to enzyme degradation. It could therefore be argued that the 'available' energy content of the diet will be influenced by the inclusion level of dietary fibre. Singh and Nose (1967) showed that the digestibility of starch in diets for rainbow trout was dependent upon the concentration of starch within the diet. For these reasons, particular attention was also noted for the digestibilities of both the 'available' and 'unavailable' carbohydrate fractions of the experimental diets in the current trial.

3.2 MATERIALS AND METHODS

3.2.1 DIETS

Five experimental diets were formulated as shown in Table 3.1. An Edward Baker Ltd commercial salmon starter premix (59% crude protein, NX6.25; 10% crude lipid) was used as the main basal component.

The diet was modified accordingly by the addition of 10% casein and corn starch. Cellulose inclusion varied from 0-20% by the replacement of corn starch in diets 1-5. Corn oil was added to yield a final proximate composition of 45% crude protein and 10% crude lipid, for each of the experimental diets. The diets were prepared as described previously in section 2.2.1, Chapter 2.

3.2.2 ANIMALS AND TANKS

Rainbow trout of mean weight 24 grams were obtained from the Almond Bank Fish Farm, Perthshire and were randomly distributed, twenty fish per tank. The experimental trial was conducted

TABLE 3.1 Composition of the experimental diets (% by weight).

Component	Diet No. 1	2	3	4	5
Salmon starter premix[1]	60.0	60.0	60.0	60.0	60.0
Casein[2]	10.0	10.0	10.0	10.0	10.0
Corn starch[3]	20.0	15.0	10.0	5.0	0.0
Corn oil	6.0	6.0	6.0	6.0	6.0
α-cellulose[4]	0.0	5.0	10.0	15.0	20.0
Vitamin premix[5]	2.0	2.0	2.0	2.0	2.0
Mineral mix[5]	1.0	1.0	1.0	1.0	1.0
Marker[5]	1.0	1.0	1.0	1.0	1.0
Nutrient content(%)					
Moisture	8.15	6.75	7.31	8.11	7.10
Crude protein	45.73	45.21	41.92	41.03	42.78
Lipid	9.79	10.65	10.00	10.79	11.13
'Available' carbohydrate	19.72	15.15	11.65	7.77	4.49
'Unavailable' carbohydrate	2.41	5.65	10.93	18.41	20.88
Ash	7.76	8.63	7.93	7.84	7.01
Crude fibre	0.15	4.20	7.77	11.03	16.12
Cr_2O_3	0.474	0.499	0.432	0.479	0.463

1. Edward Baker Ltd, Salmon fry starter diet 'O'; composition without added oil, (59% crude protein, 7% crude lipid).

2. Casein (essentially vitamin free from Bovine milk).

3. Corn starch (10.76% moisture; 0.08% ash; 81% total carbohydrate.

4. α-cellulose (5.59% moisture; 73.14% crude fibre; 0.16% ash; 81.76% total carbohydrate).

5. As described in section 2.2.1, Chapter 2.

under similar conditions to those described previously using the same experimental system and fish were fed ad libitum, twice daily, as described previously in section 2.2.2. The mean water temperature during the experimental period varied between 6°C and 10°C (mean 8.4°C). Methods for the collection and treatment of initial and final fish, faecal samples together with the feeding and weighing techniques were similar to those outlined previously in section 2.2.2, Chapter 2. The total length of the feeding trial was 70 days (10 weeks).

3.2.3 CHEMICAL METHODS

Fish and diet samples were oven dried at 105°C for 24 hours for moisture determination and the ground samples subjected to standard AOAC (1980) methods for proximate analysis. However crude fibre determination of diet samples was slightly modified for application to smaller samples as described below. Crude lipid was estimated using monofluorotrichloromethane (MF-Freon, refrigerant-11) as the fat extracting solvent (Korn and Macedo, 1973). Chromic oxide concentration in diets and faecal samples was measured according to the method of Furakawa and Tsukahara (1966).

BIOCHEMICAL ANALYSIS

3.2.4 AVAILABLE CARBOHYDRATE (AS GLUCOSE)

The available carbohydrate content of dietary and faecal samples were determined using a modified procedure based on the methods of Hudson et al., (1976) and Kartchner and Theurer (1981).

Method:-

Replicate portions of samples (20-30mg) were weighed into

glass 25ml screw cap vials. 5mls of acetate buffer (0.2M sodium acetate; pH 5.0) was then added and the samples shaken vigourously to achieve dissolution of particles. All samples were then extracted at 90°C for five hours using a shaking water bath. After this period, the suspensions were allowed to cool and 0.1mls of enzyme preparation (Amylo glucosidase - amylo α1,-4α1-6-glucosidase hydrolase/Rhizopus genus mold E.C. 321.3)(1mg/ml) was added to each vial and samples shaken using a bench whirlimixer. An incubation of 16 hours at 48°C was then initiated, after which time the mixtures were cooled and centrifuged at low speed (x5). 25µl aliquots of the supernatants were removed for glucose analysis using a Sigma Chemicals Ltd enzyme colourimetric method, (Technical kit No. 510, 1982). The concentrations calculated on the basis of the glucose standards and blanks were adjusted for the original extraction and reaction volumes, (5.1mls). Total glucose concentrations were expressed as the percentage of the initial sample weights and termed 'available carbohydrate' comprising total glucose derived from starch and free glucose in both diets and faeces. For each set of determinations, a corn starch standard was subjected to the same treatments for reference and calibration purposes.

3.2.5 TOTAL CARBOHYDRATE

Total carbohydrate which includes the 'unavailable' fraction was determined using the phenol-sulphuric acid method of Dubois et al., (1956). This is a general test for the total hexose content of a sample after complete hydrolysis. Concentrated acids dehydrate free sugars to yield furfurals and these condense with phenols to give characteristic coloured products for colourimetric analysis.

Method:-

Approximately 2-3mg of finely ground, dried diet and faecal samples were weighed in triplicate into heat resistant test tubes and recoveries were run on starch using purified corn starch, and α-cellulose. A series of glucose dilutions from a glucose stock solution (Sigma Ltd; BD-glucose, 100mg/dL in 0.1% Benzoic acid) was prepared in the range 0-1mg/ml to construct a suitable calibration curve. 1ml of deionized water was then added to each of the tubes containing test samples and 1ml of the appropriate standard dilutions to tubes assigned for the calibration purposes. After shaking the mixtures, 1ml of 5% phenol-solution was added to each of the samples and the preparations allowed to stand in ice for 15 minutes in a fume cupboard. After this period, 8mls of concentrated sulphuric acid (ARISTAR) was quickly dispensed to all of the tubes, taking care to avoid contact with the sides. A distinct yellow/brown colour appears immediately and the optical density read at 520nm. The total carbohydrate content was measured from a calibration curve based on glucose and expressed as a percentage of the initial sample weights.

3.2.6 'UNAVAILABLE CARBOHYDRATE'

The 'unavailable' carbohydrate fraction of both the dietary and faecal samples was estimated by the difference of the total carbohydrate and 'available' carbohydrate values, and was assumed to consist primarily of α-cellulose. A similar biochemical fractionation procedure was used by Jeltema and Zabik (1980) as revised methods for quantifying dietary fibre components.

3.2.7 STATISTICAL METHODS

Statistical analysis was performed on the initial and final weights of fish and the carcass composition data using analysis of variance and Duncans multiple range test (Duncan, 1955) at the 5% level of significance as described in section 2.2.5, Chapter 2.

3.3 RESULTS

Table 3.2 shows the growth response of rainbow trout at bi-weekly intervals for each of the experimental diets over the 10 weeks growth period. The growth, feed utilization and carcass composition data is shown in Table 3.3 and the faecal concentration of the various nutrient fractions and the digestibility marker is shown in Table 3.4.

TABLE 3.2 Mean weights of rainbow trout (g) at successive biweekly periods for each dietary treatment.

Diet No.	1	2	3	4	5
Week					
0	21.80	23.69	24.27	25.78	25.47
2	27.98	29.53	28.79	29.15	31.16
4	29.82	26.41	28.47	29.48	29.57
6	36.55	34.25	35.86	39.19	38.00
8	45.49	44.50	43.21	49.76	48.25
10	53.04	54.32	51.22	62.20	57.60

3.3.1 GROWTH

The results show that there was no significant difference ($P>0.05$) between the final mean weights of fish consuming diets

Table 3.3 Growth, feed utilization and carcass composition of rainbow trout (20 fish, initial weight 24g) after 10 weeks at 6°C-10°C (mean 8.4°C).

Parameter	Diet No. 1	2	3	4	5	± SE[1]
Mean Initial wgt (g)	21.80[a]	23.69[a]	24.27[a]	25.78[a]	25.47[a]	1.30
Mean final wgt (g)	53.04[a]	54.32[a]	51.22[a]	62.20[a]	57.60[a]	3.63
Specific growth rate (%)	1.27	1.17	1.07	1.25	1.16	
Food intake (mg/day)	581	648	559	636	560	
Weight gain (mg/day)	445.7	437.6	385.0	520.3	459.0	
Food conversion ratio (FCR)	1.30	1.48	1.45	1.22	1.22	
Protein efficiency ratio (PER)	1.68	1.49	1.64	1.99	1.92	
Nitrogen intake (mg/day)	42.51	46.87	37.49	41.75	38.33	
Nitrogen deposition (mg/day)	13.14	13.62	12.30	16.55	14.70	
Apparant (N)utilization (%)	30.91	29.06	32.81	39.64	38.35	
Apparant dry matter digestibility (%)	55.12	49.05	55.84	50.09	55.14	
Apparant nitrogen digestibility (%)	83.94	82.83	83.50	78.85	84.45	
'Available' carbohydrate digestibility (%)	52.48	51.93	45.80	54.30	92.53	
'Unavailable' carbohydrate digestibility (%)	-210	-155.50	-15.88	-9.77	+33.19	
Carcass composition (% wet wgt)						
	Initial fish					
Moisture	78.25	72.42[a]	73.28[a]	73.02[a]	73.31[a]	74.19[a]	1.58
Crude protein	17.35	17.89[a]	18.54[a]	18.73[a]	20.06[a]	18.49[a]	1.95
Lipid	2.90	7.09[a]	6.80[a]	7.14[a]	6.82[a]	5.37[a]	0.60
Ash	2.42	2.44[ab]	2.37[a]	2.53[ab]	2.60[b]	2.44[ab]	0.06

Mean values with the same superscripts are not significantly different ($p>0.05$) 1± standard error.

TABLE 3.4 Nutrient and marker concentrations of faecal samples from each experimental treatment (% moisture-free basis).

Parameter	Diet No. 1	2	3	4	5
Crude protein (Nx6.25)	17.72	16.26	16.81	18.83	15.88
'Available' carbohydrate	20.76	14.34	14.25	7.08	0.75
'Unavailable' carbohydrate	16.56	28.35	20.75	40.29	41.64
Marker (Cr_2O_3)	1.050	0.980	0.975	0.955	1.035

with different inclusion levels of α-cellulose as the source of fibre. The specific growth rates as an index of performance were also found to be in close agreement with each other for the different dietary treatments.

3.3.2 FOOD UTILIZATION

Although differences were obtained with respect to food intake no definite trend emerged in relation of the increased inclusion of α-cellulose. However, when food conversion ratio (FCR) was calculated for the treatments, fish receiving 15 and 20% cellulose resulted in a slightly better conversion efficiency than those receiving lower levels of fibre. This was also evident in terms of the PER values, which indicated a slightly better nitrogen utilization for higher inclusion levels of cellulose in diets 4 and 5. This view was further supported by the greater daily nitrogen deposition for fish receiving these diets and consequently the improved apparent nitrogen utilization values of 39.64% and 38.35% compared to values ranging between 29.06-32.81% for fish fed diets 1, 2 and 3. By comparison, the dry matter and nitrogen

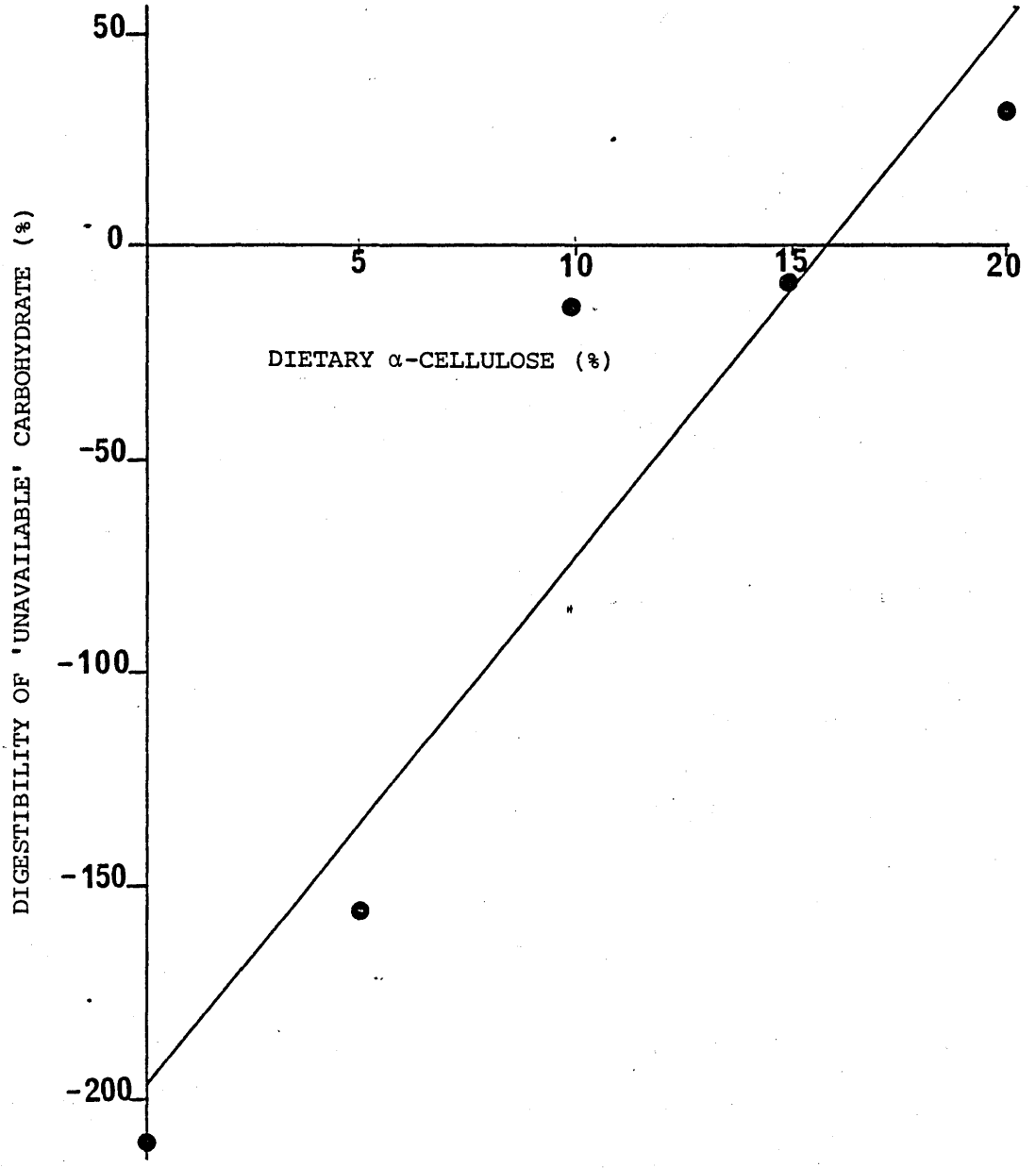


FIGURE 3.1 The relationship between the apparent digestibility of the 'unavailable' carbohydrate fraction of diets and α-cellulose inclusion level.
Y=12.64X-198.01, r =0.95.

digestibility coefficients were within fairly close agreement for the treatments ranging from 49.05-55.84% and 78.85-84.45% respectively.

Coefficients of digestibility for the available carbohydrate fraction were within the range 45.80-54.30% for diets 1-4, but was much higher at 92.53% for diet 5 with 20% α-cellulose and which contained the lowest level of added starch.

A distinct trend was obtained for the apparent digestibility of the 'unavailable' carbohydrate fraction. Although the value was highly negative at -210% for diet 1 containing 0% α-cellulose, there was a progressive decrease in these negative digestibility coefficients with increasing levels of dietary fibre. For diet 5 (20% α-cellulose) the coefficient of digestibility was +33.19%. A significant regression was obtained for the 'unavailable' carbohydrate digestibility and dietary fibre level as shown in Figure 3.1.

3.3.3 CARCASS COMPOSITION

The carcass composition data in Table 3.3 shows that there was no significant difference ($p>0.05$) in content due to the dietary fibre level, although the carcass lipid content of fish receiving 20% α-cellulose appeared to be slightly reduced and the moisture content slightly elevated compared to fish receiving the control diet without α-cellulose.

3.4 DISCUSSION

The results of the study show that adding fibre in the form of α-cellulose to the diets did not induce significant differences in the growth response or carcass composition of rainbow trout. Similar findings were obtained by Leary and Lovell (1975) with channel catfish, although this investigation together with that of Hilton et al., (1983) with rainbow trout were not strictly comparable with the present experiment because these workers substituted the basal diet as a whole with α-cellulose, therefore introducing dilution effects and other variables. Hilton et al., (1983) reported that increased α-cellulose intake at 10 and 20% levels caused a significant depression in growth, and that trout adapted to the fibre by increasing their food consumption. However there was no evidence to suggest that similar effects were operating in the present investigation. The results obtained by other workers may be explained by the fact that dilution of the diet as a whole with fibre would undoubtedly reduce the concentration of major nutrients including protein. It is generally accepted that rainbow trout require considerable amounts of dietary protein for intensive growth (Cowey and Sargent, 1979; Dabrowsky, 1979). Although fish may compensate by increasing feed intake, this may still not be sufficient to sustain good growth at high levels of fibre intake. In the present study, only corn starch was replaced by α-cellulose in the diets and the protein level was not a limiting factor.

Analogous studies of the effects of α-cellulose on growth and protein utilization have been performed with rats (Delorne et al., 1981; Shah et al., 1982). These workers included α-cellulose at increments of between 0 and 35% of the diet either by replacing an

equivalent amount of starch, or the diet as a whole. Generally these studies showed that protein efficiency ratios (PER) decreased and that apparent dry matter and protein digestibilities diminished with increased dietary level of α-cellulose. Shah et al., (1982) reported that compared to other fibre sources tested (i.e. pectin, lignin and guar gum), cellulose had the least effect on net protein retention (NPR). In contrast, Nomani et al., (1979)[c] suggested that at marginal intakes of protein and energy closer to the requirements for growing rats, α-cellulose may result in improved protein utilization by contributing greater intestinal bulk, maximizing the turnover of mucosal cells, inducing enzymic secretions, which in turn might improve the efficiency of protein absorption. The results of the current investigation with rainbow trout seem to support this latter view, since at the 15 and 20% inclusion levels of cellulose, slightly better protein efficiency ratios and net nitrogen utilization values were obtained. However it should be mentioned that the crude protein level within the rations containing high levels of α-cellulose were considerably lower than in the control ration, and this in turn may in part explain the higher PER and NNU values observed.

In this study, 'available' carbohydrate digestibility was seen to be in fairly close agreement (49-56%) for the experimental diets containing up to 15% α-cellulose (5% starch), but was found to be much higher at 92% for the dietary treatment that contained 20% α-cellulose and without added starch. Smith (1971) reported digestibility coefficients for a variety of carbohydrate sources, glucose (79.3%); dextrin (77.4%); cooked starch (51.6%); raw starch (24.0%); and α-cellulose (13.7%). It can be seen that the value for cooked starch was within the range obtained for the

digestibility of the 'available' carbohydrate components of the diets for trout in the present study. Unfortunately most of the values for carbohydrate digestibility in the literature are based on a nitrogen free extract basis (Cho and Slinger, 1977) and are not directly comparable. The results of the present study do not seem to agree with those of Singh and Nose (1967) in which a negative correlation existed between the digestibility of starch and its concentration within the diet. It was also suggested by these workers that rainbow trout only possess a limited degree of amylase activity capable of degrading dietary starch to glucose. It should be noted however that the fish used in their studies were only acclimatized to the experimental rations for two weeks compared to a period of ten weeks in the current investigation. It is well known that the digestive enzymes of fish are able to adapt when the diet substrate intake is altered (Corning, 1980). For instance, when the amount of starch intake increases, the specific activities of a number of enzyme systems involved in the sequential hydrolysis of dietary carbohydrates is also increased thus augmenting the digestion and absorption rates of simple hydrolytic products. Smith (1978) reported that the carbohydrate content of fish feeds is similar in digestibility to raw corn starch (approximately 40%) and that the digestibility of carbohydrate by rainbow trout is highly dependent upon the complexity of the molecule. It was further stated by this author that the apparent digestion coefficients ranged from 86% for pure glucose to 2.8% for α-cellulose in rainbow trout. The very high digestibility for the 'available' carbohydrate component of the diet which contained 20% α-cellulose in the present study was similar to the findings of Inaba et al., (1963). These workers obtained values of 90.0 and 48.2% for the digestibility of α-starch at

dietary levels of 11.5 and 40.2% respectively when bread crumb was fed in conjunction with white fish meal to trout. These findings would suggest that the efficiency of digestion and absorption of 'available' carbohydrate is much greater at very low inclusion levels.

The digestibility coefficients for the 'unavailable' carbohydrate fractions of the experimental diets were all found to be negative except for the treatment containing 20% α-cellulose and all of these values were found to be inversely related to the concentration of α-cellulose in the diet (Figure 3.1). One possibility is that a purified fibre of this type is retained for greater periods in the large intestine due to the increased physical bulk (Leary and Lovell, 1975). An accumulation of dietary fibre from previous meals and a differential flow rate between the various fractions of digesta may account for the negative digestibility values obtained for 'unavailable' carbohydrate. The decreased negativity of the apparent digestibility coefficients with increasing amounts of α-cellulose may possibly reflect the more uniform passage of the dietary components. Smith (1971) noticed a small but significant apparent digestion of purified α-cellulose for rainbow trout which was considered to be surprising due to the small capacity and low temperature of the digestive system for most fish. Presumably if a certain amount of dietary cellulose was converted into free glucose, this would be easily absorbed by trout as a source of energy (Cowey and Sargent, 1979). Unfortunately the method for quantifying 'available' carbohydrate in diets and faecal samples does not distinguish between glucose derived from starch, cellulose or free glucose. Slavin and Marlett (1980) also reported negative

apparent digestibilities for α-cellulose in human subjects which also decreased significantly with increasing levels of dietary fibre. These workers however were unable to offer an explanation for their results. In studies with channel catfish, Leary and Lovell (1975) proposed that cellulose may also increase the residence time for the purified, readily soluble ingredients of the diet in the digestive tract thus allowing an increased uptake of nutrients. This might partly explain the increased efficiency of dietary nitrogen utilization for rainbow trout receiving 15 and 20% α-cellulose. On the other hand, improved nitrogen utilization may have been due to the protein sparing action of the extra available energy from the carbohydrate component of the diet. Other workers have also suggested that α-cellulose is beneficial in improving the utilization of certain nutrients. Buhler and Halver (1961) showed that the addition of 9% cellulose to diets for chinook salmon Oncorhynchus tshawytscha resulted in improved protein utilization compared to a control diet without fibre. However these workers omitted to mention that the substitution of a basal diet with α-cellulose also reduced the protein level of the diet which in turn would also improve protein utilization.

There was no evidence in the present study to suggest that rainbow trout are capable of degrading purified α-cellulose. As mentioned previously, the digestibility coefficients for the 'unavailable' carbohydrate fraction of the diets (which consists mainly of α-cellulose) were negative. Since these values were based upon differences between the 'total' and 'available' carbohydrate contents of diet and faecal material, then it seems likely that the positive digestibility for 'unavailable' carbohydrate obtained for fish fed diet 5 (20% α-cellulose) was an artefact caused by the very high digestibility of 'available'

carbohydrate in this treatment. The possibility of a limited amount of cellulose activity occurring in the digestive tract of trout would have been surprising since these fish are presumed by most workers to lack the ability to produce this enzyme naturally and would only acquire such activity by establishing a suitable bacterial flora within the intestine (Van Es, 1981). This view is also supported by the work of Bergot (1981) in which the digestibility of a pure α-cellulose was measured in diets for rainbow trout and common carp, Cyprinus carpio. It was concluded from these studies that neither species could effectively degrade cellulose and there was no evidence of any associated degradation products in the faecal samples.

The occurance of cellulase activity in the digestive tracts of other freshwater fish was reviewed by Stickney and Shumway (1974) and by Stickney (1975). These workers reported a considerable variation in activity depending on fish species and dietary habit. It was concluded that cellulase activity in fishes is probably produced by microflora in the alimentary tract and that the ingestion of plant detritus, especially by omnivorous and herbivorous species is an important source of such bacteria. It would be unlikely that rainbow trout, a carnivorous species, would have established a similar bacterial population whilst consuming a high protein diet over the relatively short experimental period.

In conclusion, graded levels of purified α-cellulose added at the expense of dietary starch in the present study caused no detrimental effects on the growth performance and utilization of nutrients in trout. It might therefore be possible on the basis of these results to consider α-cellulose as an inert bulking

ingredient for use in experimental diets for rainbow trout. In this way, cellulose may act as a control variable in which it could be substituted by other dietary fibre sources at varying inclusion levels, and without affecting the overall nutrient balance of different dietary treatments.

CHAPTER 4

Experiment 3 - THE EFFECT OF VARYING INCLUSION LEVEL AND PARTICLE SIZE COMPOSITION OF CHITIN (POLY-N-ACETYL-D-GLUCOSAMINE) AS A NATURAL SOURCE OF DIETARY FIBRE.

CHAPTER 4

Experiment 3 - The effect of varying inclusion level and particle size composition of chitin (poly-N acetyl-D-glucosamine) as a natural source of dietary fibre.

4.1 INTRODUCTION

The nutritional value of chitin (poly-N-acetyl-D-glucosamine) in the diets of farmed rainbow trout is open to question. With the present interest in replacing the fishmeal component of fish diets with unconventional protein sources such as shrimp, crab and krillmeal (Tacon, 1981), there is a need to understand the factors which may affect their digestibility. One of the reasons attributed for the poor digestibility of such ingredients within salmonid diets is that they contain approximately 10% crude fibre in the form of chitin (Rehbein, 1981).

Polymeric chitin has many of the characteristics implicit in the definition of crude fibre (section 2.1, chapter 2), but the ability of fish, particularly trout to digest and utilize chitin remains unclear. The results of the preliminary experiment outlined in Chapter 2 suggested that ground chitin obtained from crabshell may have affected the performance of rainbow trout at an inclusion level of 5%, although no significant conclusions could be made. There was also no evidence to indicate that chitin was of any nutritional value to trout.

Given the importance of such physical factors as the amount of fibre present in diets and its particle size composition, a more detailed experiment was designed using a 2x2 factorial arrangement in which a commercially available crabshell chitin similar to that used in the previous study was added to semi-purified diets

for rainbow trout at two separate levels of inclusion and using two distinct particle size ranges. The inclusion of chitin was made at the expense of α-cellulose which ensured the same nutrient concentrations for each of the dietary treatments.

4.2 MATERIALS AND METHODS

4.2.1 DIETS

Polymeric chitin (poly-N-acetyl-D-glucosamine) in the form of flaked crabshell was obtained from Sigma Chemicals Ltd, Poole, Dorset. The material was ground into a fine powder and fractionated into two distinct particle size ranges, 45-500 microns and 500-1000 microns respectively using a series of sieves of appropriate dimensions and a mechanical shaker. (Endecott, test sieve shaker E.F.L. Mk.II)

Five semi-purified diets were formulated (Table 4.1) in which brown fishmeal (70% crude protein, 10% lipid) was used as the main source of dietary protein. All inclusions of chitin were made at the expense of α-cellulose in order to maintain an equal balance of nutrients. Diets were formulated to contain 40% crude protein and 12% lipid. A vitamin and mineral premix was added to satisfy the known requirements of rainbow trout together with an indicator mix (chromic oxide/polyethylene; 1:1) for digestibility determinations. The chemical and physical characteristics of the dietary fibre sources are shown in Table 4.2.

4.2.2 EXPERIMENTAL FISH AND TANKS

Rainbow trout of mean weight 10g were obtained from Cloan Hatcheries, Perthshire, and randomly distributed at a stocking density of 25 fish per tank for each treatment. The experimental

TABLE 4.1 Composition of experimental diets (% by weight)

Ingredient Diet No.	1	2	3	4	5
Herringmeal	56.74	56.74	56.74	56.74	56.74
Corn starch	8.80	8.80	8.80	8.80	8.80
White dextrin	4.15	4.15	4.15	4.15	4.15
Corn oil	6.31	6.31	6.31	6.31	6.31
α-cellulose	20.00	10.00	10.00	-	-
Crabshell chitin (fine)	-	10.00	-	20.00	-
Crabshell chitin (coarse)	-	-	10.00	-	20.00
Vitamin premix[1]	2.00	2.00	2.00	2.00	2.00
Mineral mix[1]	1.00	1.00	1.00	1.00	1.00
Indicator mix[1]	1.00	1.00	1.00	1.00	1.00
Nutrient content (%)					
Moisture	6.46	7.43	5.59	6.02	5.18
Crude protein (NX6.25)	41.16	44.47	45.38	48.81	50.63
Lipid	11.48	9.85	10.43	9.72	10.96
Ash	6.76	7.18	6.99	6.96	7.22
Crude fibre	15.95	15.16	15.46	18.20	17.21
Polymeric chitin	0.00	7.40	6.58	15.10	13.77
'Available' carbohydrate	7.46	8.75	10.15	10.02	9.46
Chromic oxide (Cr_2O_3)	0.456	0.552	0.579	0.505	0.556
Polyethylene	1.26	1.34	1.40	1.66	1.79

1. As shown in Table 2.1; Chapter 2.

TABLE 4.2 Chemical and physical characteristics of the fibre sources.

Parameter	α-cellulose	chitin (fine)	chitin (coarse)
Moisture (%)	5.59	7.65	7.65
Nitrogen (%)	<0.10	5.28	5.97
Ash (%)	0.08	1.53	1.53
Crude fibre (%)	73.14	80.65	81.81
Density (g/cc)	1.24	1.31	1.32
Particle size range (%)			
>1000μ (microns)	-	-	38.76
790-1000μ	-	-	52.41
500-790μ	49.40	-	8.23
250-500μ	34.41	88.37	-
125-250μ	11.70	5.98	-
45-125μ	3.59	4.85	-
<45μ	0.90	0.80	-

system was the same as that described in section 2.2.2. The feeding trial was conducted for 42 days (6 weeks) at an ambient water temperature ranging between 8°C - 12°C (mean 11.1°C) and the fish were fed ad libitum, twice daily, mid morning and mid afternoon. Twelve fish were sacrificed at the start and 25 fish at the end of the experimental growth trial for gross carcass analysis and for the estimation of chitinase activity in the gastro-intestinal tract (section 4.2.4.2). The methods of faecal sample collection, storage and analysis were as described previously (section 2.2.2).

4.2.3 PREPARATION OF SAMPLES, CHEMICAL METHODS

Prior to chemical analysis, diets and fish samples from each treatment were oven dried at 105°C for moisture determination and then ground. Total nitrogen, lipid, ash and crude fibre content of the materials were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 1980). However due to the small sample size of faeces available, faecal nitrogen together with the nitrogen content of the corresponding diets were estimated using a Carlo Erba straumentazione elemental nitrogen analyzer, Model No. 1106.

4.2.4 BIOCHEMICAL METHODS

4.2.4.1 POLYMERIC CHITIN

Sample preparation:-

Samples were prepared for analysis using a method provided by Cowey (pers comm, 1983). 4-5 mg of dietary and faecal material was weighed in triplicate into small plastic reaction vials. All samples were then suspended in 1 ml of 80% methanol and washed for

4 hours whilst subjected to constant agitation in a shaking water bath at 45°C. The resulting suspensions were then centrifuged at a speed setting of X6 for 5 minutes using a bench top centrifuge (MSE 'Minor S'.).

The supernatent was removed and assayed for free N-acetyl-D-glucosamine as described below.

The remaining pellet fraction was dried carefully for a further 4 hours at 45°C and then resuspended in 0.8 mls of 6N HCl and allowed to stand overnight at room temperature. After this period, the vial caps were firmly closed and the samples placed in a boiling water bath for 12 hours with occasional shaking. After centrifugation (X6) for 5 minutes, a 0.2 ml aliquot of each hydrolysate was removed and transferred to new vials and then dried over potassium hydroxide in a vacuum dessicator for 6 hours. The residues were re-suspended in 1 ml of deionized water and mixed thoroughly using a benchtop whirlimixer.

Assay procedure:-

N-acetyl D-glucosamine was assayed using a modified procedure based on that used by Elson and Morgan (1934). Acetylated hexosamines form an oxazole derivative when heated with alkali. This derivative can be condensed with p-dimethyl-amino-benzaldehyde to yield a purple coloured complex. Because the hydrolysis procedure removes the acetyl group, the amino sugar is acetylated with acetyl acetone.

Method:-

0.25 mls of acetyl acetone reagent (1.5 mls diluted to 50 mls with 1.25% N-Na_2CO_3) was mixed with a 0.25 ml aliquot of sample in

a tube sealed with pierced parafilm to allow equalization of pressure. All tubes were then heated at 100°C for 45 minutes using a heating block. After cooling quickly under running water for 5 minutes, 2.5 mls of ethanol and 0.25 mls of Ehrlich reagent was added (1.6 g of p-dimethyl amino benzaldehyde in 30 mls of concentrated HCl and 30 mls of ethanol.). The resulting pink coloured complex was read at 530 nm after standing at room temperature for one hour. Standards of chitin and N-acetyl glucosamine together with a calibration curve based on N-acetyl-glucosamine within the concentration range 0-200 µg/ml accompanied each set of determinations.

4.2.4.2 CHITINASE ACTIVITY

Chitinase activity was determined using the method of Goodrich and Morita (1977)[b].

Samples of stomach, pyloric caecae, small intestine and large intestine were removed by dissection from fish towards the end of the experimental period, weighed on a microbalance and then homogenized in 0.5M sodium phosphate buffer (pH 7.4) using a rotary blender. The contents from each of the intestinal sections were similarly treated. All samples were then further diluted 1:10 (w/v) with the sodium phosphate buffer and a chitin substrate suspension (5 mg/ml) was prepared using the same buffer.

1 ml of each of the sample preparations was added to 1 ml of the substrate solution. In a separate series of control samples, 0.5 mls of toluene was also added to prevent bacterial action during the assay. The samples were then diluted to a final volume of 4 mls with the buffer and incubated at 25°C for 2 hours in a water bath. After incubation, the samples were centrifuged for 5 minutes (X6) and the resulting supernatants removed for assay.

The N-acetyl-D-glucosamine (NAG) assay developed by Morgan and Elson (1934) was employed using 0.5 mls of supernatant. The optical density was read on a Perkin Elmer spectrophotometer at 585 nm. Activities were expressed as μg of NAG released hr^{-1} g $tissue^{-1}$ (wet weight).

4.2.4.3 AVAILABLE CARBOHYDRATE CONTENT OF DIETS AND FAECES

Available dietary carbohydrate was estimated as total glucose using the modified procedure developed by Hudson et al., (1976) as outlined in section 3.2.4 , chapter 3.

4.2.5 DENSITY

The density of the dietary fibre sources were measured using specific gravimetric bottles. Samples of α-cellulose, finely graded chitin and coarse chitin (approximately 2g) were weighed into the bottles, filled with distilled water, sealed with specially ground stoppers and reweighed. The empty weights of the stoppered gravimetric bottles and the weights of the vessels filled with distilled water alone were also recorded. Assuming that the specific gravity of water is equivalent to 1g/cc, it was possible to determine the volume of the weighed samples by displacement using the differences between the weights of water and samples.

$$\text{Density g/cc} = \frac{\text{weight of sample (g)}}{\text{weight of water displaced (g) or (cc)}}$$

4.2.6 DIGESTIBILITY MARKERS

4.2.6.1 CHROMIC OXIDE: (Cr_2O_3) was determined using the method of Furakawa and Tsukahara (1966).

4.2.6.2 POLYETHYLENE: Polyethylene was also used as a marker for comparative purposes and a microquantitative procedure was developed for its estimation in diets and faeces. The method was based on the principle that when certain pure polymers such as polyethylene are heated over their characteristic melting range, there is an associated change in the specific heat capacity of the substance. The release of thermal energy is proportional to the weight of the sample material and can be detected as a peak response using a differential scanning calorimeter.

Method:-

Dietary and faecal samples (20-30 mg) were carefully weighed in duplicate into special thermally calibrated aluminium receptacles and placed into the insulated compartment of a Perkin Elmer type DSC-2 differential scanning calorimeter. All samples were pre-heated for 5 minutes at 400K (127^{o}C) to minimize any interference caused by dietary moisture, and then cooled rapidly to 310K (37^{o}C). After this period, samples were again heated slowly at a rate of 20K/minute and scanned until the temperature of the sample reached 400K. The melting profiles of the polyethylene in the samples were recorded on chart paper and these were calibrated against standard weighed samples of pure polyethylene treated in exactly the same manner. The amount of polyethylene in the diets and faeces were computed from the areas under each peak recorded and expressed as a percentage of the dry weight of the original sample. Typical examples of the melting point profiles for polyethylene in diet, faecal and standard samples are shown in Figure 4.1. It should be noted that the slight distortion of the base line and lower peak was due to the compensatory mechanism applied to minimize recording the energy released from residual moisture and dietary

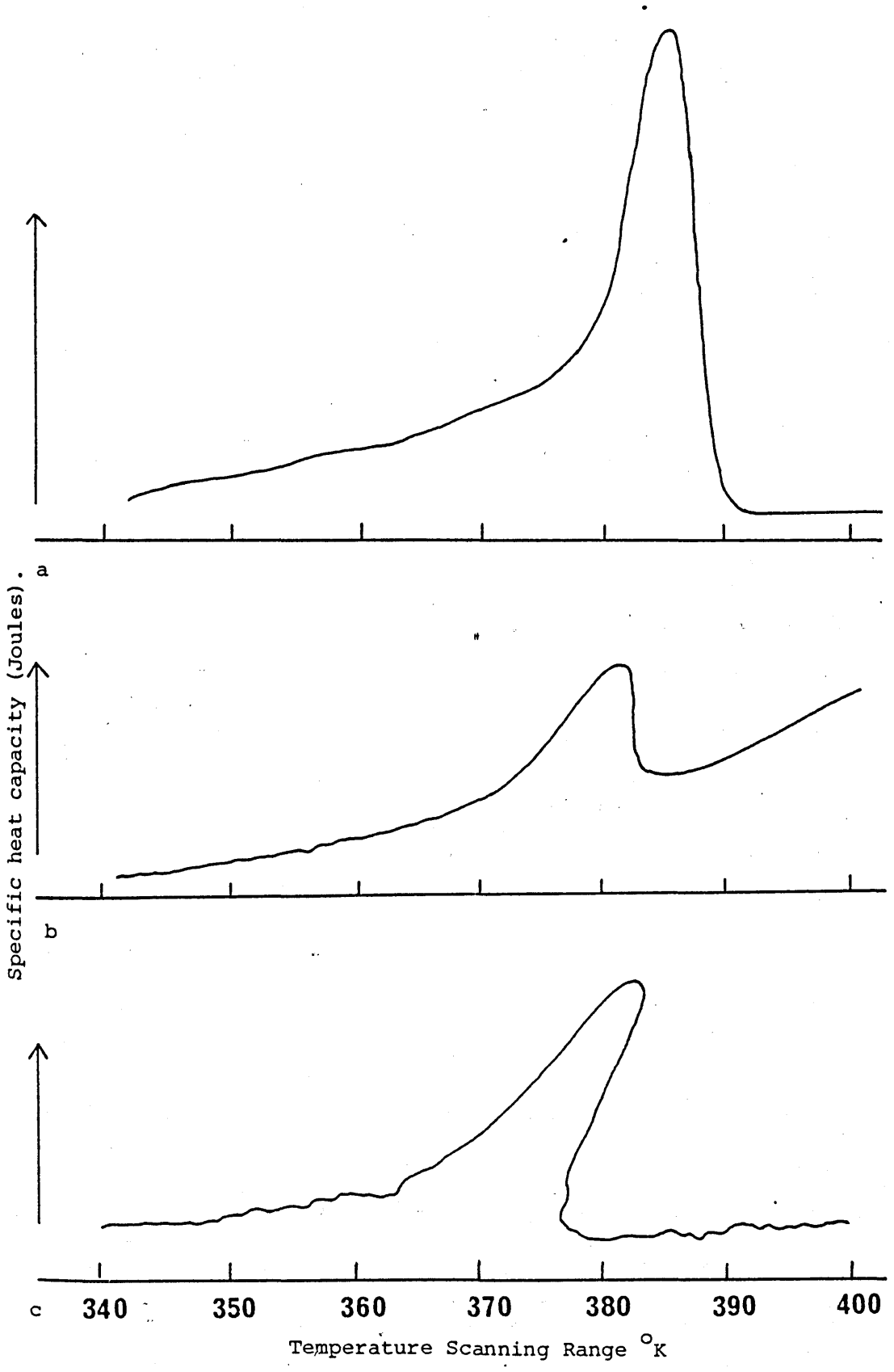


Figure 4.1 Typical melting range profiles of polyethylene measured as the heat output over the range 340-400°K for a) standard sample of purified polyethylene, b) sample of diet, and c) sample of faecal material of approximately equal weight (moisture free basis).

fat. This did not in any way affect the area of the peak response which is specific for polyethylene.

4.2.7 STATISTICAL ANALYSIS

Analysis of variance and Duncans multiple range test was applied to the initial and final weights of fish and the carcass composition data as described in the previous chapters.

4.3 RESULTS

4.3.1 GROWTH

Table 4.3 shows the growth response of rainbow trout at bi-weekly intervals during the 6-week experimental period. The growth, feed utilization and carcass composition data is shown in Table 4.4 and 4.5. Although significant differences were obtained for the initial weights of rainbow trout at the start of the experiment, the final mean weights of the fish showed differences that were not consistent with the earlier trend.

The mean final weights of fish recieving 10% coarse chitin (diet 3) and 20% coarse chitin (diet 5) were not found to be significantly different ($P>0.05$) from each other. Similarly, the final weights of fish fed the 10% fine chitin (diet 2), 10% coarse chitin and 20% fine chitin (diet 4) were not significantly different. However fish fed the 20% coarse chitin diet had a significantly lower ($P<0.05$) final mean weight from those receiving 10% fine chitin and 20% fine chitin and also rainbow trout fed the α-cellulose control ration (diet 1).

The specific growth rate for fish fed the coarse chitin diet

Table 4.3 Mean successive body weights of rainbow trout (g) at bi-weekly intervals over the experimental test period of 6 weeks at 8-12°C (mean, 11.1°C).

Diet No.	1	2	3	4	5
Week					
0	11.86	10.70	9.55	10.86	9.81
2	16.34	16.59	13.79	16.39	12.91
4	22.19	21.63	19.39	22.25	16.77
6	28.35	26.88	23.61	27.09	19.84

was relatively inferior (1.68) to those fed the other dietary treatments which had specific growth rate values ranging between 2.08 to 2.19. The percentage weight gain also showed a marked decrease for fish fed coarse chitin, i.e. 102.2% compared to a range of 139-151% for the other dietary treatments.

4.3.2 NUTRIENT UTILIZATION EFFICIENCY

The mean daily feed intake for rainbow trout showed a decreasing trend with a higher inclusion level of chitin and increased coarseness. Fish fed diet 1 (20% α-cellulose) consumed more food than fish fed diet 2 (10% fine chitin) and similarly, fish fed diet 4 (20% fine chitin) displayed a higher food intake than fish fed diet 5 (20% coarse chitin). Fish fed diets containing different levels of chitin but with the same particle size distribution of material showed the least differences in terms of daily food intake. In contrast fish receiving diets with the same inclusion level of chitin but differing in particle size composition showed the greatest change in food intake.

Table 4.4 Growth, feed utilization and carcass composition of rainbow trout (25 fish, initial weight 10g) fed the experimental diets for 6 weeks at 8-12°C (mean temp 11.1°C).

Parameter — Diet No.	1	2	3	4	5	± SE[1]
Mean initial weight (g)	11.86[b]	10.70[ab]	9.55[a]	10.86[ab]	9.81[a]	± 0.56
Mean final weight (g)	28.35[c]	26.88[bc]	23.61[ab]	27.09[bc]	19.84[a]	± 1.44
Weight gain (%)	139.00	151.2	147.2	149.4	102.2	
Specific growth rate (%/d)	2.075	2.193	2.155	2.176	1.677	
Food intake (g/d)	0.659	0.599	0.492	0.503	0.446	
Weight gain (g/d)	0.393	0.385	0.335	0.386	0.239	
Food conversion ratio (FCR)	1.677	1.556	1.469	1.303	1.866	
Nitrogen intake (mg/d)	43.40	42.60	35.70	39.30	36.10	
Nitrogen deposition (mg/d)	9.52	8.69	8.16	9.52	5.56	
Apparent nitrogen utilization (%)	21.94	20.40	22.86	24.22	15.40	
Apparent dry matter digestibility (%)						
marker:- Cr_2O_3	55.59	52.25	40.30	51.18	25.11	
marker:- polyethylene	55.60	73.64	57.60	62.16	26.51	
Apparent nitrogen digestibility (%)						
marker:- Cr_2O_3	87.50	77.34	75.60	67.64	43.77	
marker:- polyethylene	87.63	87.09	82.41	74.36	44.57	
Available carbohydrate digestibility(%)						
marker:- Cr_2O_3	32.77	58.44	60.08	57.44	18.78	
marker:- polyethylene	33.45	76.34	71.22	66.28	19.93	
Apparent CHITIN digestibility (%)						
marker:- Cr_2O_3	0.00	-2.12	-60.68	-1.53	-143.68	
Carcass composition (% wet wgt)						
	Initial fish					
Moisture	75.92	75.34[a]	76.84[a]	76.52[a]	76.05[a]	76.84[a]	± 0.59
Crude protein (NX6.25)	14.22	14.78[a]	14.12[a]	14.82[a]	14.92[a]	14.37[a]	± 0.52
Lipid	6.60	6.52[a]	5.76[a]	6.12[a]	6.63[a]	5.93[a]	± 0.45
Ash	1.75	2.15[b]	2.08[ab]	2.05[ab]	2.14[b]	2.18[b]	± 0.11

Mean values with common superscripts in each row are not significantly different (P>0.05) [1]SE:- ± standard error of mean.

Chitinase activity µg $NAGhr^{-1}$, gtissue(wet wgt)$^{-1}$ No activity was detected in stomach, pyloric caecae and the

Food conversion ratio (FCR) was found to be superior (1.303) for trout fed diet 4 (20% fine chitin) and inferior (1.866) for diet 5 containing 20% coarse chitin. For the remaining treatments, the FCR values were only marginally higher than for diet 4. Similarly the apparent utilization of nitrogen for the coarse chitin treatment was much lower (15.40%) than for the other diets. However nitrogen utilization in rainbow trout receiving the 20% fine chitin diet was noticeably superior (24.22%).

4.3.3 DIGESTIBILITY OF NUTRIENT COMPONENTS AND CHITIN

Digestibility measurements were based on the use of two marker systems, chromic oxide and polyethylene. The dietary and faecal concentrations of these markers are shown in Table 4.1 and 4.5 respectively together with the concentrations of the respective nutrient fractions. The dry matter (DM) digestibility coefficients for the diets were found to be in general agreement between the two markers for diets 1 and 5 but considerable variation occurred between the markers for the remaining diets. The DM digestibility of diet 5 (20% coarse chitin) and diet 3 (10% coarse chitin) were 25.11; 26.51% and 40.30; 57.60% respectively when based on chromic oxide and polyethylene. These values were much lower than those obtained for the remaining diets, including the α-cellulose control ration.

Similarly trends were evident for apparent nitrogen digestibility. Values based on polyethylene as a marker were generally higher than values based on chromic oxide. It would appear that both dietary markers indicated an inferior apparent nitrogen digestibility for the high chitin inclusion diets, i.e.:- diet 4, 67.64; 74.36%; diet 5, 43.77; 44.56% using chromic oxide and

Table 4.5 Faecal concentration of nutrients, chitin and digestibility markers (% moisture free basis).

Parameter	Diet No. 1	2	3	4	5
Nitrogen (N)	1.19	3.63	3.13	5.48	6.40
'Available' carbohydrate (as glucose)	11.23	7.57	6.76	8.69	10.24
Polymeric chitin	0.00	15.73	17.64	31.24	44.72
Chromic oxide	1.02	1.14	0.96	1.02	0.74
Polyethylene	2.85	4.90	3.24	4.27	2.42

polyethylene respectively. Much higher apparent nitrogen digestibilities were obtained for the remaining treatments i.e. 75.6; 87.5% and 82.4; 87.6% for the two respective markers. The digestibility of the available carbohydrate component of the diet was also affected by the level and coarseness of chitin. The lowest value 18.78 and 19.93% was again noted for the 20% coarse chitin diet (based on chromic oxide and polyethylene). However the control diet with 20% α-cellulose also resulted in a lower digestibility coefficient (32.77; 33.45%) for the available carbohydrate.

The apparent digestibility coefficients for polymeric chitin at each dietary inclusion level and particle size composition were all found to be negative. It can be seen from Table 4.3 that a trend exists for these values. Treatments containing finely ground chitin at both low and high inclusion levels had quite similar negative digestibility values for chitin, i.e.: -2.15 and -1.53% respectively. However the 20% coarse chitin diet resulted in a much greater negative digestibility coefficient of -143.68%

for chitin compared to -60.68% for the 10% coarse chitin diet. It would therefore appear that coarseness of fibre caused an increased negative digestibility at each of the dietary inclusion levels tested.

4.3.4 CARCASS COMPOSITION

Table 4.3 also showed that there was no significant effects ($P>0.05$) on the final carcass composition of rainbow trout fed each of the experimental diets after 6 weeks.

4.4 DISCUSSION

The results showed a marked difference in the growth of fish receiving diets containing 20% inclusion of fine and coarsely ground chitin. The presence of coarse material at the higher level suppressed the growth of rainbow trout. Slight differences, which could not be supported statistically, were also apparent for fish fed fine and coarse grades of chitin at the 10% inclusion level and with respect to the control diet.

Nomani et al., (1979)[c] stated that the non-soluble particulate fibres such as chitin contribute greater particulate bulk than the carbohydrate gums although particulate fibres generally have a lower water retaining capacity. Hedge et al., (1978) fed groups of chicks on a low residue diet with and without supplements of dietary fibre in the form of wheat bran, wheat straw both coarsely and/or finely milled. In these studies, the incorporation of fibre was made at the expense of starch in the basal diet thus altering the energy content of the ration. The results showed that supplementation with coarsely milled wheat straw caused poorer growth in chicks compared to the finely milled wheat straw. It

was suggested that the coarse material probably made the diet unpalatable and difficult to eat, which prevented the animals consuming sufficient food to support normal growth. Most workers agree however that the effect of energy dilution by the addition of dietary fibre generally causes the animal to increase voluntary food consumption. This has been demonstrated for swine by Baird et al., (1975) and Noland and Scott (1960), a similar view is held to be true for fish by Jobling (1980)[a]. It is important therefore to distinguish between the effects due to fibre resulting from a simple dilution of digestible energy and that due to physical bulk which limits palatability (Braude, 1967). In the present study with rainbow trout, the failure of fish fed the diet containing a high inclusion level of coarse chitin to grow as well as fish receiving the other dietary treatments may also be partly attributable to the increased bulk of large dietary fragments. The mean daily food intake for fish fed the 20% coarse chitin diet was noticeably reduced. Vahl (1979) proposed as part of a hypothesis on the control of food intake in fish that satiety depends largely on stomach fullness which is related to the volume, size and spatial distribution of food particles. Swenson and Smith (1973) stated that the particle size of the dietary components was an important factor affecting food consumption, feeding periodicity and gastric digestion in walleye Stizostedion vitreum vitreum. The rate of gastric digestion in these fish was especially affected by particle size. The importance of the fibrous component was also mentioned by McDonald, Edwards and Greenhalgh (1977). These authors stated that voluntary intake is limited by the rate at which food is broken down and removed from the digestive tract. Fibrous materials of low digestibility are broken down slowly. Apart from delaying the access of

enzymes to other food constituents, slower physical comminution leads to a more lengthy retention of food in the stomach with only small dietary particles permitted to pass down into the alimentary tract. There is therefore a relationship between digestibility and the rate of digestion which in turn affects food consumption.

In the present investigation with rainbow trout, the digestibility data implies that polymeric chitin obtained from crabshell conforms to the earlier definitions of a crude fibre. Negative digestibility coefficients were obtained for the chitin component of the diets using a specific biochemical assay. These results suggest that retention of chitin occurs within the intestinal tract to varying degrees. This is borne out by the fact that the negative digestibility values were much higher when the fibrous fraction contained the coarse grade of chitin. A similar effect was noted by Heller et al., (1980) in digestibility experiments with human subjects. Wheat bran was used as the source of dietary fibre and included separately as fine milled (mean particle size 180μ) and coarsely milled (mean particle size 720μ) as part of a controlled diet. It was found that the mean digestibility of the fine bran meal was much greater than the coarse bran diet. Negative digestibility coefficients were obtained when the assessed faecal output was greater than the amount ingested and it was suggested that this might be due either to errors in isolating too much faecal sample during the balance period or the carry over of fibre from a previous meal. In the present study with trout, errors due to collecting faeces may be effectively ruled out since a balance study was not employed and faecal samples were obtained by the hand stripping method of Austreng (1978). Digestibility coefficients were therefore measured indirectly using chromic oxide and polyethylene as the

inert marker. It is likely that greater retention of chitin from previous meals is the major cause of the negative digestibility values obtained (Kionka and Windell, 1972).

The most widely used inert marker in animal and fish studies is chromic oxide (Cr_2O_3). Pappas et al., (1973) described the use of this compound in digestion studies with channel catfish, but some workers have expressed doubt that chromic oxide should be regarded as an ideal marker for investigations with fish. Bowen (1979) argued that whilst the use of Cr_2O_3 provides reliable and accurate information on digestibility when incorporated into dried pelleted diets under pressure, under certain conditions depending upon the texture of the diet, some fish may selectively reject the Cr_2O_3 in the food. To obviate any doubts, a second marker was included in the present study for comparative purposes. Polyethylene was chosen as it is chemically and physically inert and may be mixed uniformly as a fine powder with the other dietary ingredients. It also has a specific gravity similar to the final diet (0.92 g/cc) compared to 5 g/cc for chromic oxide.

The results showed that considerable variation existed between the digestibility coefficients obtained using the different marker systems for the dietary treatments. By comparing the data presented in Table 4.4, the greatest differences occurred for the intermediate digestibility values and when the digestibility coefficient for a dietary component was relatively high or low, better agreement between the two markers existed. It is difficult to interpret these results, but they do suggest that the question of markers for use in digestibility studies in fish is worthy of further research and that polyethylene could be a feasible alternative to chromic oxide depending upon the nature

and texture of the diet.

The particle size distribution of chitin in the diets appeared to have a marked effect on the digestibility coefficients of the various dietary nutrients. The apparent nitrogen digestibility was considerably reduced for the 20% coarse chitin diet. Although chitin contains an appreciable amount of non-protein nitrogen (6%), this would not account for the decrease obtained because the diet which contained 20% fine chitin only showed a moderate reduction. The apparent nitrogen digestibility for the α-cellulose control diet and 10% chitin diets were relatively higher. The fact that the digestibility of available carbohydrate was also decreased for the 20% coarse chitin treatment but was fairly similar for the other treatments irrespective of chitin level, suggested that the particle size composition of the fibrous fraction of the diet may have an important influence on the digestibility of other nutrient components.

The non-protein nitrogen (NPN) contributed by chitin was assumed to be a source of error in the calculation of net nitrogen utilization. The results showed that a similar trend existed between nitrogen utilization and nitrogen digestibility, with a much lower efficiency being obtained for the 20% coarse chitin diet. The highest value of nitrogen utilization (24.22%) for the diet containing 20% fine chitin compares favourably with the value of 24% obtained by Rumsey and Ketola (1975) for an isolated fish protein fed to Atlantic salmon, Salmo salar at a dietary level of 40%. Similar effects due to particle size have been reported in other animals. For example, Nomani and Stansberry (1982) showed that wheat bran fed in a coarse form lowered the efficiency of nitrogen utilization in young male rats. In the

present study, there was no evidence to indicate that rainbow trout could utilize the NPN content of chitin. However the ability of fish to metabolize and incorporate certain sources of non-protein nitrogen in the synthesis of non-essential amino acids was mentioned by Dabrowski (1979).

The lack of chitinase activity in the gastric and intestinal tissues of trout in the current investigation supports the views of Windell (1966), Kionka and Windell (1972) that chitin is poorly degraded by most freshwater fish species and may persist for a considerable time in the stomach. Rehbein (1981) reported that the chitin extracted from krillmeal resisted invitro pepsin digestion and that this explains the poor digestibility of krill-meal when fed to rainbow trout. It has been suggested that chitinase activity is an inducible response due to the presence of chitinous material in the diet and the consequent establishment of a chitinoclastic bacterial population in the gastro-intestinal tract (Goodrich and Morita, 1977)[a]. In contrast some workers maintain that chitin degradation is the result of true endogenous secretions produced by the digestive system of fish (Dandrifosse, Schoffeniels and Jeuniaux, 1965). These factors were considered for the determination of chitinase activity in the present study. Toluene was added to a separate control group of tissue extracts in order to suppress any activity arising from bacterial action. However chitinase activity was not detectable for treatments with or without added toluene. Barrington (1957) was of the opinion that fish would be unlikely to produce an inducible digestive enzyme in response to substrates frequently encountered in the diet. Few workers are aware that chitinase activity in different tissues and organs of fish may be easily confused with lysozyme and exo-N-acetyl-β D-glucosaminidase enzyme systems. Cornelius and

Dandrifosse (1977) and Lundblad et al., (1979) detected lysozyme activity in the spleen, kidney, leucocytes and lymphomyeloid (haemopoietic) tissues for a number of marine species. Although true chitinase and lysozyme differ slightly in their substrate specificities, several of these workers have demonstrated that some lysozymes are able to hydrolyze chitin and oligosaccharides of N-acetyl glucosamine. The question as to what extent the so-called 'chitinases' of vertebrates are truly specific for chitin or whether the hydrolysis is the result of lysozyme activity is difficult to establish.

In the present investigation with trout, an attempt was made to detect the presence of free N-acetyl glucosamine in the faeces which would have resulted from a limited breakdown of chitin. The failure to detect free N-acetyl glucosamine together with the negative coefficients for chitin digestibility was further evidence that trout lacked the enzyme systems required to degrade crabshell chitin. Prior to the growth trial, the experimental fish were fed entirely on a standard commercial fishmeal based diet. This fact, together with the limited period for which the diets were fed and the relatively aseptic conditions of the experimental system, may have prevented the fish from establishing a suitable intestinal microflora with chitinolytic activity. It should be noted that most of the studies involving chitinase activity in fish have been reported for wild populations taken from the marine environment where chitinoclastic bacteria are abundant, e.g. Okutani and Kimata (1964) with Japanese sea bass, Lateolabrax japonicus; Alliot and Bocquet (1967) for dogfish, Scyliorhinus canicula; Colin and Peres (1971) with scorpion fish, Scorpaena percus and conger eel, Conger conger. It might therefore be unjustifiable

to compare these results with those obtained for rainbow trout under the present experimental conditions.

CHAPTER 5

Experiment 4 - GROWTH RESPONSE AND DIGESTION STUDY WITH RAINBOW TROUT USING GRADED LEVELS OF GALACTOMANNAN AS AN EXAMPLE OF A GEL-TYPE DIETARY FIBRE.

CHAPTER 5

Experiment 4 - Growth response and digestion study with rainbow trout using graded levels of galactomannan as an example of a gel-type dietary fibre.

5.1 INTRODUCTION

The distinctive gel-forming and hydroscopic characteristics of the non-cellulosic polysaccharides have been described in detail in the preceeding chapters.

As part of the growing interest in the nutritional and clinical value of dietary fibre, many of the soluble gel fibres and related polysaccharides have been included either as separate pure fibre fractions or as components in complex mixtures associated with other fibre sources in studies with higher animals and man. Nomani et al., (1979)[c] included pectin as a fibre fraction to study its effects on the growth and protein metabolism of the rat. The influence of various extracted hemicellulose fibres on the growth and utilization of nutrients by swine and hamsters was reported by Fahey et al., (1980). Unfortunately there appear to be few studies involving fish. Bergot (1981) formulated diets for rainbow trout and carp containing 20% of a hemicellulose fraction which was fed to the fish for a period of one month. The digestibility coefficients indicated that hemicelluloses were not appreciably digested by these two species. In a similar study, Morita et al., (1982) fed groups of red sea bream, Chrysophrys major on diets supplemented with levels of up to 12% carboxymethyl cellulose. The author reported that CMC supplementation improved growth and feed efficiency.

The relevance of such studies with fish is apparent if one considers the increasing use of trashfish and ensiled diets for salmonids which are usually compounded with special binder meals consisting of commercial alginates and related gel forming polysaccharides. The use of different viscosity binders to effect textural changes in the diet warrants further investigation as this may also influence overall acceptability and the utilization of nutrients (Meyers and Butler, 1972).

In the first preliminary experiment, galactomannan appeared to have a slight effect on certain nutritional parameters when included at a level of 5%. However it did not affect the growth of rainbow trout. The present investigation was therefore conducted to study the long term effect of including galactomannan in diets for trout at levels of 0, 10 and 20%. Addition of fibre was made at the expense of α-cellulose which ensured an equal nutrient balance for each treatment. In this way, the only variable factor was the ratio of galactomannan/cellulose within the dietary fibre complex. It was also decided that the experimental diets should be fed on a moist basis in order to simulate the practical conditions in which a gel-forming polysaccharide might be used as a binding agent.

5.2 MATERIALS AND METHODS

5.2.1 DIETS

A commercial eel diet was obtained from B.P. Nutrition Ltd., Witham, Essex (the chemical composition of this premix is shown in Table 5.1) and used as the basal diet suitably modified by the addition of varying proportions of α-cellulose and galactomannan

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

Ingredient	Diet No. 1	2	3
B.P. Eel grower diet - (as main basal component)[1]	39.5	39.5	39.5
Casein	30.5	30.5	30.5
Corn oil	9.5	9.5	9.5
α-cellulose	20.0	10.0	0.0
Galactomannan[2]	0.0	10.0	20.0
Indicator (Cr_2O_3)	0.5	0.5	0.5
Nutrient content (% wet basis)			
Moisture	41.94	45.58	42.09
Crude protein (NX6.25)	26.78	25.74	27.12
Lipid	10.32	8.47	8.66
Ash	5.63	5.32	5.67
Nutrient content (% moisture free basis)			
Crude protein (NX6.25)	46.12	47.29	46.83
Lipid	17.77	15.57	14.95
Ash	9.70	9.77	9.79
Galactomannan	0.00	9.00	16.00
Chromic oxide (Cr_2O_3)	0.401	0.399	0.397

1. Composition of basal diet (%):- Crude protein (NX6.25) 39.71; lipid 12.84; crude fibre 1.26; ash 10.69; moisture 8.30.

2. Galactomannan polysaccharide (derived from gum locust bean; Sigma Chemicals Ltd) composition (%):- moisture, 11.79; nitrogen 0.80; crude fibre, 5.50; ash, 0.91.

polysaccharide as the source of dietary fibre.

Three rations were formulated such that on a dry weight (moisture free basis) they provided 45% crude protein NX6.25), 10% crude lipid and three levels of purified galactomannan (from Sigma Chemicals Ltd.) i.e. 0, 10 and 20% of the diet by the replacement of α-cellulose. Chromic oxide was included at 0.5% as the dietary inert marker. The experimental rations were first prepared by mixing all of the ingredients thoroughly using a Hobart A200 mixer with sufficient water to produce a final mixture for each treatment containing approximately 40% moisture by weight. The moist diets were then extruded under pressure as described previously in section 2.2.1, and the resulting pellets stored in airtight containers at -15°C until fed. The formulation and proximate composition of the experimental diets are shown in Table 5.1.

5.2.2 ANIMALS AND EXPERIMENTAL CONDITIONS

Rainbow trout of mean weight 5.5 grams were obtained from Cloan Hatcheries Ltd, Vale of Glendevon, Perthshire in the late autumn and randomly distributed at a stocking density of 25 fish per tank for each of the experimental treatments. The experimental feeding trial was conducted for 112 days (16 weeks) over the winter months until the early spring at ambient water temperatures ranging from 4°C to 5°C (mean 4.3°C) and employing a natural photoperiod.

Twelve fish were sacrificed at the start of the experiment and stored at -15°C for subsequent gross carcass analysis. Partially thawed, moist diets were fed to the fish ad libitum, twice daily using preweighed containers which were then reweighed each week of the growth trial. Fish were weighed individually under anaesthesia at the beginning and end of the experiment as

described for the previous experiments, but were group weighed at biweekly intervals during the intermediate period. Faecal samples were collected over the final 4 weeks of the trial by the method of Austreng (1978), pooled for each treatment, and then oven dried at 105°C for 24 hours before being ground to a fine powder for chemical analysis. All fish were killed at the end of the trial for gross carcass analysis.

5.2.3 BLOOD COLLECTION AND ANALYSIS

Blood samples were collected from fish on the final day of the experiment, extracted from the caudal artery using a surgical syringe. All samples were withdrawn quickly from individually anaesthetized fish at a standard post prandial time interval of 6 hours after a normal feeding period. The blood was pooled from the 25 rainbow trout in each treatment and then permitted to clot in sealed conical plastic vials. Serum fractions were carefully decanted into small plastic, sealed tubes following low speed centrifugation (x 4) of the blood samples for 15 minutes and stored at -18°C. All analyses of the serum were performed within one week of collection.

Total serum protein concentration was determined using the routine colourimetric assay procedure developed for clinical use by Sigma Chemicals Ltd (Technical bulletin No. 540, 1982). Similarly, serum glucose was measured using a Sigma Chemicals Ltd. assay kit based on an enzymatic assay involving the reduction of a chemical dye and the enzymes glucose oxidase and peroxidase (Sigma technical bulletin No. 510, 1982).

5.2.4 CHEMICAL METHODS AND PREPARATIONS

All diets, fish and faecal samples were treated in exactly the same manner as described for the earlier studies. Total nitrogen, lipid and ash contents of the dried materials were determined according to the methods of the Association of Official Analytical Chemists (AOAC, 1980) and chromic oxide by the method of Furakawa and Tsukahara (1966).

DETERMINATION OF GALACTOMANNAN:-

The method used for measuring galactomannan in diet and faecal samples was that used by Southgate (1969). Samples of material (15 mg) and galactomannan (as the control) were extracted with 3 x 5 ml portions of 85% methanol, centrifuged (x 6 for 15 minutes) and the resulting supernate discarded. The precipitates were extracted with 3 x 10 ml volumes of deionized water at 80°C for 30 minutes using a boiling water bath. The precipitates were discarded after further centrifugation (x 6 for 10 minutes) and the combined water supernates treated with 4 volumes of 80% ethanol in order to precipitate galactomannan.

The residues obtained by ethanol fractionation were treated overnight at 30°C with 10 mg of amyloglucosidase (Sigma Chemicals Ltd.) in 2 mls of 0.1M acetate buffer adjusted to a pH of 4.5 with glacial acetic acid and with toluene added as a preservative.

Samples, including an amyloglucosidase blank, were treated with 4 volumes of 80% ethanol and the precipitates taken up in water (25 mls) except for the galactomannan control (50 ml). 1 ml samples of each solution was then analyzed by the anthrone method (Hassid and Newfeld, 1964).

5.2.5 STATISTICAL METHODS

Analysis of variance together with Duncan's multiple range test was applied at the 5% level of significance to the initial and final mean weights of fish and to the carcass composition data.

5.3 RESULTS

The performance and feed utilization parameters for rainbow trout fed each of the experimental diets are shown in Figure 5.1 and Tables 5.2 and 5.3. The final mean weights of the fish were not found to be significantly different, but a noticeable trend was evident throughout the results for the galactomannan diets compared to the α-cellulose control diet. The final mean weights of rainbow trout fed diet 2 (10% galactomannan) and diet 3 (20% galactomannan) were lower than fish receiving diet 1 (0% galactomannan), i.e. 7.79, 8.17 and 9.36 grams respectively. Considering the low temperatures under which the study was performed, a reasonable 70% increase in body weight was achieved for the control diet over the 16 week feeding period.

Specific growth rates, daily food intake and the daily live weight gain were also decreased when fish were fed diets containing increasing amounts of galactomannan polysaccharide. Feed utilization was similarly affected. The food conversion ratio (FCR) for diet 2 (10% galactomannan) was inferior to that of diet 1. FCR for diet 3 (20% galactomannan) was only marginally better than diet 2. As expected, the lower food intakes obtained for diets 2 and 3 resulted in lower daily nitrogen intakes. The efficiency of nitrogen utilization also showed a definite downward trend with increased inclusion of galactomannan, i.e. 12.76, 9.80 and 7.13% for diets 1, 2 and 3 respectively.

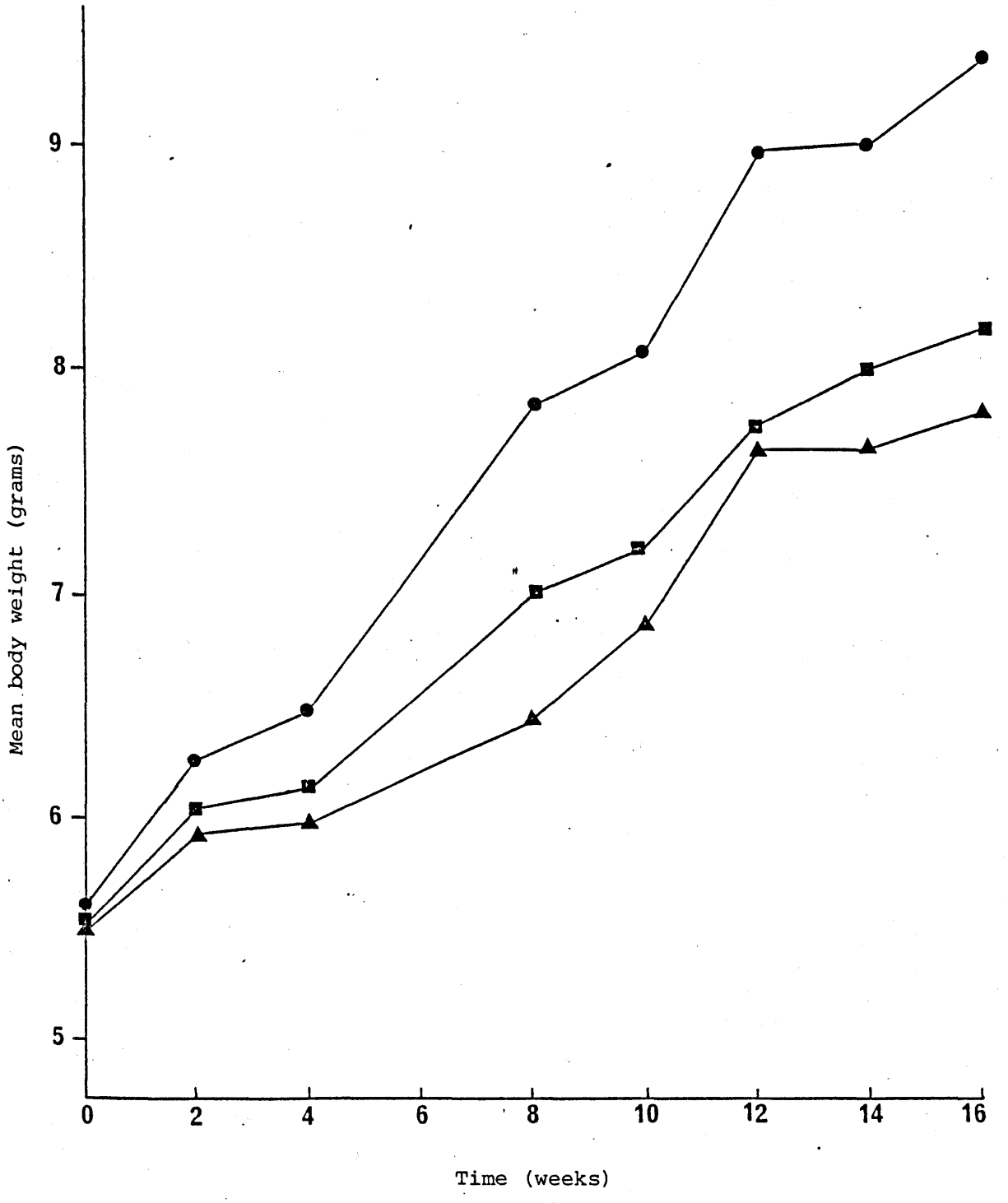


Figure 5.1 Growth response of rainbow trout at 4°C-5°C (mean 4.3°C) for 16 weeks fed a moist diet including ● 0% galactomannan (20% α-cellulose); ▲ 10% galactomannan (10% α-cellulose) and ■ 20% galactomannan (0% α-cellulose).

Table 5.2 Growth, feed utilization, carcass composition and serum nutrient concentrations of rainbow trout fed the three experimental diets for 16 weeks at 4°C-5°C (mean water temperature 4.3°C).

Parameter	Diet No	1	2	3	± SE[1]
Initial mean weight (g)		5.56[a]	5.48[a]	5.52[a]	0.27
Final mean weight (g)		9.36[a]	7.79[a]	8.17[a]	0.67
Specific growth rate (%/day)		0.46	0.31	0.35	
Daily food intake (mg)		77.57	66.14	67.37	
Daily live weight gain (mg)		33.92	20.63	23.66	
Feed conversion ratio (FCR)		2.29	3.21	2.85	
Daily nitrogen intake (mg)		5.72	5.00	5.05	
Daily nitrogen retention (mg)		0.73	0.49	0.36	
Protein efficiency ratio (PER)		0.95	0.66	0.75	
Net nitrogen utilization (%)		12.76	9.80	7.13	
Apparent dry matter digestibility (%)		52.73	53.93	46.24	
Apparent nitrogen digestibility (%)		81.18	82.22	76.81	
Galactomannan digestibility(%)		0.00	-1.68	-0.59	
Total serum protein (g/100 ml)		4.26	4.10	3.59	
Total serum glucose (mg/loo ml)		240.82	64.31	81.63	
Carcass composition (% wet weight)					
	Initial fish				
Moisture	77.52	76.57[a]	76.75[a]	78.71[b]	0.29
Protein (NX6.25)	15.30	14.52[b]	15.15[b]	13.49[a]	0.18
Lipid	6.42	6.47[c]	6.12[b]	5.45[a]	0.06
Ash	2.38	2.51[b]	2.55[c]	2.38[a]	0.01

Mean values in each row with the same superscripts are not significantly different. ($p>0.05$) [1] ±SE = ± standard error.

Table 5.3 Nutrient and marker concentrations within faecal samples for each experimental treatment (% dry weight basis).

Parameter	Diet No. 1	2	3
Crude protein (NX6.25)	17.82	18.00	19.94
Galactomannan	0.00	20.0	30.0
Marker (Cr_2O_3)	0.854	0.872	0.740

It can be seen from Table 5.2 that the apparent dry matter (DM) and nitrogen digestibility coefficients were not appreciably affected for the different treatments except for the very high (20%) galactomannan inclusion level. It was also noted that the apparent digestibility coefficient for galactomannan in the diets at both concentrations were negative, which implies that the polysaccharide was unavailable for digestion and absorption.

A noticeable reduction in the total serum protein concentration was found to occur for fish when fed increased amounts of galactomannan. In the case of diets 2 and 3, there was also a marked reduction in total serum glucose compared to the α-cellulose control (diet 1).

The carcass composition data for the rainbow trout fed the experimental diets is also shown in Table 5.2. Statistical analysis of this data confirms that there was a significant increase in the percentage carcass moisture content and a decrease in protein content for trout fed 20% galactomannan. A more distinct trend was obtained for the carcass lipid content. Fish receiving diets containing graded levels of galactomannan showed a significant reduction ($p<0.05$) in gross lipid content

(6.47%> 6.12%> 5.45%). Although the carcass ash contents also varied with the different treatments, no trend was observed. However the ash content of trout fed 20% galactomannan was significantly lower ($p<0.05$) than for trout receiving the other diets.

5.4 DISCUSSION

When varying levels of galactomannan polysaccharide were fed to rainbow trout as part of a dietary fibre complex with α-cellulose, definite patterns and trends emerged. Unfortunately the low temperature conditions which prevailed during the course of the investigation resulted in suboptimum growth and the differences in growth were not significantly different. However the graphical representation showing the growth response of trout receiving the diets suggests that the diets supplemented with 10 and 20% galactomannan consistently suppressed growth.

It has already been mentioned that most studies related to the effects of hemicellulose and similar polysaccharide fibre sources have been performed with animals other than fish. Few workers have specifically reported the effects of isolated hemicellulose fractions on animal growth. Most of the research has concentrated on the interaction of hemicellulose fractions at the physiological and metabolic level. However Fahey et al., (1976) found that free hemicellulose obtained from sugar cane molasses stimulated the growth of rats when incorporated into the diet at 0.03%. A similar observation was made using acid resistant hemicellulose extracted from corn cob and added at 0.05% in diets for rats (McLaren et al., 1974) or at 0.02% in the diets of lambs (McLaren et al., 1976). It was suggested therefore that by yielding growth factor like substances (efficient at low levels

of incorporation) hemicelluloses may contribute as an energy source to the animal. The influence of pectin as a fibre component on the growth of the rat was investigated by Nomani et al., (1979)[C] at dietary energy levels close to the animals requirement. When pectin was included at 2.1% of the diet, a significant weight gain was achieved for these rats. Fahey et al., (1980) also reported a favourable effect on the growth performance of hamsters receiving up to 30% dietary inclusion levels of corn stalk and soya bean lignin-hemicellulose residues. In the present investigation however, the apparent depression in growth for rainbow trout receiving 10 and 20% inclusions of galactomannan is not in accordance with the previous findings. Most of the research on the effects of dietary fibre on the growth and nutrition of fish fail to take into consideration the broad categories that exist for fibre sources. Consequently most of the results obtained from studies using purified α-cellulose as the sole example of dietary fibre are not comparable with the present study.

Daily food intake for rainbow trout receiving 10 and 20% galactomannan was seen to be slightly lower compared to the 20% α-cellulose control treatment. It has already been mentioned that many non cellulosic polysaccharides swell in aqueous solutions to form gels (Eastwood et al., 1976). The increased bulk resulting from the swelling behaviour of gel-forming polysaccharides such as the galactomannans may help to explain the depressed food intakes observed for rainbow trout. The main factors considered to be important in the control of food intake in fish were outlined in Chapter 4. One of these is the physical bulk and spatial distribution of dietary particles in the stomach compartment and associated with this is the gastric evacuation and digestion rates

(Vahl, 1979). Pure polysaccharides, most of which are gel-forming tend to absorb more water than particulate types of fibre (Stephen and Cummings, 1979) and would therefore be expected to increase the bulk volume of the diet. In the present study with trout, diets were fed on a moist basis with the same level of moisture present in each of the rations (40%). Further, water uptake by the dietary material would be expected to occur in the gastro-intestinal tract due to the natural secretions of digestive fluids. Ali et al., (1981) suggested that water active and gel-forming polysaccharides may modify the viscosity of the gut content and affect gastro-intestinal motility. Since appetite is highly correlated with gastric evacuation (Hoar et al., 1979), it is possible that diets containing high levels of gel-forming fibres may significantly decrease gastric emptying rates in fish as a result of the increased viscosity of digesta. It is unlikely that differences in the food intake for rainbow trout could be attributed to dietary moisture levels as these were fairly similar for each of the experimental diets. However, the relationship of dietary moisture to food intake and gastric evacuation may be important. Poston (1974) implied greater evacuation of moist diets by trout fed pelleted meals of varying moisture content. Similar results were reported by Grove et al., (1978) and by Marais and Kissil (1979). It should be noted, that increasing the moisture content at the expense of another dietary component alters the percent composition and caloric content of the meal, potentially affecting evacuation rate and consequently appetite. In the present study, moisture was not added at the expense of any nutrient component and the main variable factor was the ratio of galactomannan fibre to dietary water content in relation to viscosity. It would therefore be expected that diets containing 0% and 20% galactomannan

would differ considerably in their viscosity. The view that the viscosity of the diet may be an important factor affecting the nutrition and growth of rainbow trout in this experiment is supported by the work of Kawatra et al., (1971) and Viola et al., (1970) in which the effects of feeding guar gum and various pectins and alginates were studied in relation to nutrient digestibility and nitrogen balance in growing male rats. It was found that guar gum produced a decrease in the growth rate and nitrogen absorption whilst alginates and pectin caused a significant reduction in food intake. Shah et al., (1982) also found similar effects when purified fibre components including pectin and guar gum were fed to rats. These workers used fibre levels ranging between 3 and 20% which is comparable to the present study with rainbow trout. For both pectin and guar gum, especially at higher levels, food and hence nitrogen intake was markedly reduced for the rats. Increasing levels of guar gum was found to cause increased excretion of both dietary and endogenous faecal nitrogen per gram of food intake. Only at the 10 and 20% levels of inclusion, did guar gum exert an effect on net protein retention. Guar gum also produced the greatest influence on nitrogen digestibility compared to the other fibre sources tested in the study. In contrast, Spiller (1981) who investigated the effects of graded levels of plant fibres on the faecal output of pig tailed monkeys observed that pectin and locust bean gum had practically no effect on faecal nitrogen excretion as the amount of fibre in the diet increased. However pectin and gum locust bean induced a fairly high faecal moisture content in these animals.

The results of the present investigation involving rainbow trout showed that there was a reduction in the daily nitrogen

retention for fish receiving 10 and 20% galactomannan which appeared to be greater than that which could be accounted by reductions in food intake. Daily nitrogen intakes were quite similar for both galactomannan inclusion levels although a considerable reduction in the daily nitrogen retention was observed for each level of galactomannan against the control diet. As might be expected, a similar pattern emerged for the efficiency of nitrogen utilization for each of these dietary treatments. Since purified galactomannan is very low in non protein nitrogen (Table 5.1) then NPN was not a contributing factor. It was noted that the apparent nitrogen digestibility coefficient was only slightly reduced for the 20% galactomannan treatment. This might imply that factors other than a reduction in nitrogen digestibility resulted in the inferior utilization of dietary nitrogen with increased amounts of galactomannan. It has been suggested that 'assimilation efficiency' and 'apparent digestibility' of dietary nutrients and various other fractions are quite distinct concepts, although closely related (Bowen, 1979). Digestion is strictly the process whereby dietary particles and large molecular weight compounds are broken down into smaller subunits. Assimilation efficiency however depends on the mechanical and chemical passage and removal of nutrients through the gut wall together with their subsequent metabolism. There is evidence from *in vitro* and *in vivo* studies that gel type polysaccharides may physically obstruct nutrient absorption at the gastro-intestinal level, thus impairing utilization. It was suggested by Shah *et al.*, (1982) that the presence of soluble fibre causes the digesta to behave as a gel matrix. The increased viscosity would physically reduce the accessibility of protein molecules held in the matrix to digestive enzymes and the products of digestion from the sites

of absorption. Also, at intestinal pH levels, binding of certain amino acids to the fibre, analagous to the binding of bile acids reported by Eastwood and Hamilton (1968) may occur. Forman and Schneeman (1980) suggested that polysaccharide gums actually coat the absorptive lining of the gut which would directly interfere with the absorption mechanism. Serum protein and glucose levels for rainbow trout in the current study showed a marked depression with increased dietary inclusion of galactomannan, and a graded reduction in serum protein was apparent. It was reported by Jenkins et al., (1978) that certain gelling fibres such as guar gum and pectin cause a dose dependent flattening of the post prandial blood glucose and insulin response in glucose tolerance tests conducted with humans. This was probably attributable to a reduced glucose absorption from the digestive tract as a consequence of the increased viscosity of the gut contents. Similarly, it has been shown that carbohydrates of leguminous origin (high in hemicellulose content) also produce a flattened post prandial glycemic response. Crapo et al., (1981) proposed that the detailed mechanism by which soluble fibres exert their effects is by increasing the viscosity of the 'unstirred' layer between the food and the brush border surface which allows a much slower release of nutrients compared to food devoid of such fibre. Several in vitro model studies supporting these ideas have been conducted by Wong and O'Dea (1983). These workers determined the rate of hydrolysis of starch and the release of the glucose out of dialysis saccs in the presence of different types of fibre. In contrast, the data from these experiments indicated that viscosity was not the critical factor affecting the rate of starch hydrolysis and glucose passage. A greater emphasis was made of the possibility that gel fibres act as a physical barrier,

effectively insulating substances such as starch and presumably protein from hydrolytic attack. A quite different mechanism explaining the actions of gel fibre on nutrient assimilation was proposed by Holt et al., (1979). Implicit in this model is the view that viscous gel fibres slow down the rate of nutrient absorption indirectly by delaying gastric emptying and intestinal passage time. It was also stated by these workers, that gastric emptying is a major determinant of the absorption rate of most substances and that differences in absorption are closely related to changes in the gastric emptying rate. However, Anna et al., (1983) failed to observe any noticeable effects on gastric evacuation rates in swine due to the addition of various gum preparations in the diet, although the maximum inclusion level of fibre in these studies was only 6%, which is much lower than the levels investigated by other workers.

Information on the fate of dietary fibre in the gut is essential in order to evaluate its effects on the gastro-intestinal processes. There have been a number of reports which indicate that certain dietary fibre fractions are extensively degraded in the intestinal tract. Although most studies show that insoluble fibres such as cellulose and lignins are not appreciably degraded by carnivorous species (Lang and Briggs, 1976), there is evidence that the more soluble types of fibre undergo considerable degradation by the action of gut microflora. In studies with humans, the apparent disappearance of a hemicellulose fraction was within the range 76-90% and pectin was found to completely disappear, although some of the degradation products were detected in the faeces (Fetzer et al., 1979). Cummings et al., (1978) also reported that soluble and non-cellulosic polysaccharides may be completely broken down in the human gut. It was stated by Slavin et al.,

(1981) that the extent of such degradation is greatly influenced by the level of fibre in the diet with more complete breakdown occurring at lower concentrations.

In the present study, a fractionation technique was used to detect galactomannan and associated degradation products in the faeces of rainbow trout using the modified procedure of Southgate (1969). The results showed that there was no apparent digestion or utilization of galactomannan and that the fibre survived passage through the gastro-intestinal tract unchanged retaining its original physical and chemical characteristics. This apparent lack of fermentation and breakdown of galactomannan is in agreement with the only other comparable study involving fish (Bergot, 1981). This worker concluded that hemicellulose fractions are not digested by either rainbow trout or carp. It was also mentioned that the intestinal microflora of fish are likely to differ considerably from those present in mammals and that the number and type of bacteria is greatly influenced by water temperature. It is therefore possible that the low temperature conditions during the course of the present growth and digestibility trial may have been a contributing factor which reduced the microbial fermentation capacity of the intestine.

Generally, there was no major change in the gross carcass composition of rainbow trout receiving the different diets at the end of the experimental period. The depression in the carcass protein content of trout fed the diet containing the highest level of galactomannan (20%) is in accordance with the reduced daily nitrogen retention and percent utilization observed for this treatment.

Carcass lipid content however was significantly affected by

the increased amounts of galactomannan. There is a paucity of information concerning the effect of hemicelluloses and other related water soluble carbohydrates on the gross body composition of animals. Evidence that such fibre components may cause a reduction in tissue fat levels was presented by Riccardi and Fahrenbach (1967). Guar gum and pectin given to young male rats as part of a purified ration greatly reduced hepatic lipid levels. When pectin, carragheenan, agar and gum arabic were added at levels of up to 7% in the diets of rats (Tsai et al., 1976), significant hypocholesterolemic effects were noted. Cummings et al., (1979) investigated the effects of pectin on large bowel function in adult men over a 9 week period after which there was a significant elevation in the fat content of stools. Slavin and Marlett (1980) were also able to show that pectinaceous and other gel-type fibres result in an increased fat excretion due to the high degree of methoxylation and lipophilic nature of these materials. These properties may partly explain the results described for rainbow trout. It is probable that the differences in carcass moisture content were associated with the variation in fat levels since an inverse relationship normally exists for these tissue components.

In conclusion, the experimental results confirmed that galactomannan satisfied the previous definitions of a dietary fibre outlined in Chapter 1, and was not degraded or utilized by rainbow trout. Although an increased dietary inclusion of fibre only slightly affected growth performance, more obvious trends were apparent for certain physiological and metabolic parameters. Such effects are likely to be of considerable importance for rainbow trout growing at higher temperatures over a prolonged period.

CHAPTER 6

Experiment 5 - THE INFLUENCE OF DIETARY FIBRE SOURCES OF DIFFERING PHYSICAL AND CHEMICAL CHARACTER ON GASTRIC EVACUATION AND DIGESTION RATES IN RAINBOW TROUT.

CHAPTER 6

Experiment 5. The influence of dietary fibre sources of differing physical and chemical character on gastric evacuation and digestion rates in rainbow trout.

6.1 INTRODUCTION

In the previous chapters, the effects of chitin and galactomannan polysaccharides derived from crabshell and gum locust bean respectively were studied in relation to the growth performance, feed utilization and carcass composition of rainbow trout. Chitin was found to be nutritionally unavailable for rainbow trout and was therefore considered to be a good natural source of particulate fibre. In contrast, purified galactomannan is an example of a soluble gel forming fibre which may impart a relatively high viscosity to the diets and the contents of the gastro-intestinal tract. These properties of fibre were considered to be important factors responsible for the detrimental effects observed in the previous growth trial studies with trout.

Although a number of mechanisms were proposed to account for these effects, no satisfactory explanation could be obtained from nutrition trials alone. The growth of fish is a relatively slow event when compared to the more dynamic processes occurring in the gastro-intestinal tract. In order to obtain substantial effects on growth, considerable changes in dietary composition and texture are required. It is obvious that the ultimate nutritional value of a dietary ingredient largely depends on the fundamental processes of digestion and absorption of nutrients. Physical characteristics of dietary fibres such as particle

size composition, water retaining capacity, gel and viscous properties were considered to have an influence on gastric emptying rate, intestinal transit time and digestion. In order to test this hypothesis for trout, an investigation was conducted to complement and extend the previous studies involving chitin and galactomannan as sources of dietary fibre.

Experimental diets were formulated to include chitin at similar levels and particle size distributions compatible with the investigations described in Chapter 4. Similarly galactomannan was incorporated into a basal diet at graded levels comparable with the study in Chapter 5.

In fish, a number of factors have been shown to affect gastric evacuation rates and digestive functions (Flowerdew and Grove, 1979). These include temperature, meal size, fish size, species in addition to the dietary composition and texture. A combination of these factors probably affect these processes in fish and therefore the influence of dietary fibre cannot be viewed in isolation. It should also be mentioned that many workers have attempted to describe gastric evacuation rate patterns in fish using mathematical concepts such as exponential decay rate equations based on stomach volume changes and surface area effects. The significance of these interpretations are obvious for studies with dietary fibre. Appropriate mathematical models were therefore applied to the stomach evacuation rate data for comparitive purposes and the results discussed in relation to similar work with other fish species and experimental animals.

6.2 MATERIALS AND METHODS

6.2.1 DIETS

Six diets were formulated employing a standard salmon starter premix (Ewos/Baker, Ltd, Bathgate) as the basal component supplying the main nutrients (59% crude protein, 10% crude lipid). Chitin (polymeric N-acetyl-D-glucosamine) was obtained in the form of a flaked crabshell extract and then ground and sieved into two distinct particle size ranges 45-500 microns (fine grade) and 500-1500 microns (coarse grade). The fine and coarse grades of chitin were added separately to the basal diet to produce diets containing 20% by weight of chitin but differing in the particle size composition of fibre.

Galactomannan was added to the basal mixture to produce diets containing 5, 10, and 20% of the gel fibre. All inclusions of chitin and galactomannan were made at the expense of the basal diet, but the control treatment consisted of the salmon starter premix alone without any added fibre. The diets also included 0.5% chromic oxide (Cr_2O_3) as the inert digestibility marker. The composition of the six experimental diets and the chemical and physical characteristics of the diets and dietary fibre sources are shown in Tables 6.1 and 6.2. The experimental diets were prepared by first mixing all of the dry ingredients thoroughly using a Hobart A200 mixer before the addition of water. The moist diets were then extruded, under pressure, through a 3mm die and the resulting pellets dried carefully by air convection, and stored in airtight containers until fed.

TABLE 6.1 Composition of experimental diets (% by weight)

Ingredient / Diet No.	1	2	3	4	5	6
Ewos/Baker Ltd Salmon starter diet premix[1]	79.5	79.5	94.5	89.5	79.5	99.5
Crabshell chitin (fine grade)[2]	20.0	-	-	-	-	-
Crabshell chitin (coarse grade)[2]	-	20.0	-	-	-	-
Galactomannan[2]	-	-	5.0	10.0	20.0	-
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Nutrient content(%)						
Moisture	6.94	8.42	10.24	7.91	9.05	9.82
Crude protein (NX6.25)	61.89	60.48	58.62	57.71	51.87	61.79
Lowry protein	41.45	37.48	39.69	39.99	37.77	38.91
Lipid	6.53	6.54	7.25	7.66	6.96	8.30
Crude fibre	16.85	15.11	1.89	1.66	1.45	1.95
Ash	10.39	9.88	11.02	10.62	9.65	11.92
Density (g/cc)	1.701	2.033	1.526	1.558	1.562	1.355
Water holding capacity (%) (percent water retained after saturation)	58.15	61.67	58.92	66.87	77.11	46.29

1. Ewos-Baker Ltd, Bathgate, Scotland

2. Chitin and galactomannan (Sigma Chemicals Ltd, Poole, Dorset)

TABLE 6.2 Chemical and physical characteristics of diets and sources of fibre.

Parameter	Chitin(fine)	Chitin(coarse)	Galactomannan
Composition (%)			
Moisture	7.65	7.50	11.79
Nitrogen	5.28	5.97	0.80
Crude fibre	80.65	81.81	5.50
Ash	1.53	1.53	0.91
Particle size distribution(%)			
1500-1000 μ(microns)	—	38.76	—
790-1000 μ	—	52.41	—
500-790 μ	—	8.23	0.58
300-500 μ	74.77	—	—
250-300 μ	13.60	—	96.23
125-250 μ	5.98	—	1.77
45-125 μ	4.85	—	1.13
<45	0.80	—	0.29
Density (g/cc)	1.307	1.323	0.828
Water holding capacity (%)	76.48	77.06	90.71

6.2.2 FISH, EXPERIMENTAL CONDITIONS AND COLLECTION OF SAMPLES

Rainbow trout of mean weight 35g were obtained from the College Mill fish farm, Almond Bank, Perthshire and randomly distributed at a stocking density of 100 fish per tank. An acclimatisation period was conducted for three weeks at a mean daily water temperature of 13°C under a natural photoperiod. The fish were fed the experimental diets ad libitum (mid morning and afternoon). Prior to the conclusion of this period however, fish were starved for 72 hours to ensure the complete evacuation of the gastro-intestinal tract.

On the final day, a staggered feeding arrangement was initiated for each treatment beginning at 6 am. Fish were again fed ad libitum but at 1 hour intervals between treatments. Twenty fish were killed immediately following the consumption of food by applying a sharp blow to the head. The viscera of each fish was then exposed by dissection and small metallic hair clips were attached to the lower oesophagus (slightly anterior to the cardiac limb of the stomach), the portion of intestine proximal to the pylorus and to the lower section of the large intestine (above the anal vent). The complete intestine together with contents was then carefully excised and stored frozen at -18°C in sealed polyethylene bags. From this time onwards, 10 fish were removed from each tank corresponding to each of the dietary treatments and subjected to the same procedure. In this way, samples were removed at 6, 12, 24, 36 and 50 hours following the initial consumption of food. The staggered arrangement allowed a continuous comparitive slaughter programme which enabled the gut passage time to be followed for each of the six dietary treatments over a 50-hour period. The mean water temperature varied between 12.9°C - 13.1°C during the course of the experiment.

6.2.3 PREPARATION OF DIGESTA SAMPLES

The frozen gastro-intestinal tracts were partially thawed at room temperature and separated into the stomach and intestinal sections. The clips were removed and the contents from each portion were gently squeezed into preweighed aluminium foil trays and these were reweighed before and after the contents had been oven dried for 24 hours at 105°C in order to calculate the wet and dry weights of the digesta samples. These were then ground into a fine powder and stored in airtight McCartney bottles for subsequent analysis.

6.2.4 PHYSICAL CHARACTERISTICS OF DIETS AND DIETARY FIBRE SOURCES

Water holding capacity

The water holding capacity (WHC) is defined as the weight of water retained per gram of dry material under standard conditions. This was determined for each of the diets and fibre sources (two particle size ranges of chitin and galactomannan) using the method of McConnell *et al.*, (1974).

Samples (approximately 2 grams) of diets were first evenly ground from their pelleted state using a pestle and mortar. Care was taken not to disturb the characteristic particle size 'make up' of the diets as fed to the fish. The materials were oven dried to uniform dryness at 105°C and transferred to airtight containers. Approximately 0.5 grams of sample were weighed in triplicate into plastic vials to which 10 mls of deionized water was added until saturation. The diet and fibre samples were allowed to 'soak' at 20°C for 24 hours before centrifugation at low speed x 4 for 15 minutes using a 'Minor 5' bench top centrifuge. The supernatant was discarded and the resulting pellet weighed. The difference in

weight between the wet and dry material was credited to water holding capacity and was expressed in Tables 6.1 and 6.2 as the final percentage moisture in the diets, fibre and the associated water of the galactomannan suspensions.

Density

The density or specific gravity of the diets and fibre sources was determined as described in section 4.2.5, Chapter 4.

6.2.5 CHEMICAL ANALYSIS

The standard Association of Official Analytical Chemists AOAC (1980) methods of analysis were used as described previously for crude protein, lipid, fibre and ash. Chromic oxide was measured using the method of Furakawa and Tsukahara (1966). A modified Lowry method, (Lowry et al., 1951) was also used for the estimation of protein in diets and corresponding digesta samples at each successive sacrificial period.

Lowry protein

Approximately 20mg of sample material was weighed in triplicate into 25 ml Sterilin plastic containers. 20 ml of IN-sodium hydroxide was then added and the suspensions shaken overnight at room temperature followed by ultrasonic treatment using an MSE Ultrasonicator for 5 minutes, for complete sample dissolution and extraction of protein. Each of the sample mixtures were then transferred to plastic test tubes and centrifuged for 10 minutes at (X8) in order to obtain clear supernatants for protein assay.

100 µl aliquots from each solution was tested by the addition of 2 mls of a reagent C (1 part of reagent B; 0.5% $CuSO4.5H_2O$ in 1% sodium citrate to 50 parts of reagent A; 2% sodium carbonate in

0.1N sodium hydroxide). These mixtures were allowed to stand for 10 minutes at room temperature after which time, 200 µl of reagent D (Folin-Ciocalteau, diluted to 1N, BDH, Ltd) was rapidly pipetted into each tube and mixed thoroughly using a bench top whirlimixer. The reaction yielded a light blue colour and the absorbances were measured at 750 nm after 30 minutes, using a Cecil Instruments standard laboratory spectrophotometer. A series of reagent, sample and pigment blanks together with a range of standard bovine serum albumin dilutions (Sigma chemicals Ltd stock solution 1 mg/ml in 1N NaOH) were treated in the same manner to construct suitable calibration curves for each set of determinations.

6.2.6 MATHEMATICAL AND STATISTICAL INTERPRETATION OF EVACUATION RATE DATA

Three mathematical models describing gastric evacuation rates in fish were considered to be relevant to the present study.

1. VOLUME DEPENDENT MODEL

It is known that distention of the stomach modifies the rate at which food is evacuated. Hopkins (1966) showed that gastric evacuation curves could be linearized by plotting the square root of the volume of material remaining in the stomach against post prandial time. This rational is based on the stomach distention effect, i.e.:- the circumferential tension developed is proportional to stomach radius. Since the radius of a cylinder is proportional to the square root of its volume, the tension developed in the stomach wall is also proportional to the square root of the volume of food material remaining.

Using this model, the instantaneous rate of evacuation is:

$$\frac{dV}{dT} = C^{0.5}$$

where V = volume of stomach contents remaining at time T (hrs), and C = constant.

2. SURFACE AREA DEPENDENT MODEL

The surface area of dietary particles may have a profound influence on the way food is digested and evacuated. Since digestive fluids first attack the outer surfaces of food, it may be expected that the rate of digestion is proportional to the surface area of constituent dietary particles. The surface area of any given food particle increases in proportion to its weight raised to the power of 0.67. Therefore the surface area of a meal will also increase in proportion to meal size to the power of 0.67.

In this case, the instantaneous rate of gastric evacuation would be more suitably described by the relationship

$$\frac{dV}{dT} = C^{0.67}$$

3. EXPONENTIAL CURVE FITTING MODELS

Many studies (Windell, 1966; Kitchell and Windell, 1968; Brett and Higgs, 1970; Tyler, 1970; Elliot, 1972) have shown that the relationship between the amount of food remaining in the stomach with time is best described by a curvilinear rather than a linear relationship and these workers have fitted their data to exponential decay models of the form,

$$y = ab^x,$$

where, y = the amount of stomach contents (g) remaining at x hours, a and b are constants.

The mean weights of the stomach contents on a dry and wet weight basis were re-expressed to the appropriate power functions for the volume and surface area models, i.e.: 0.50 and 0.67 and the regression of these values with time computed. An exponential curve fitting programme was applied to the gastric evacuation rate data of the form $y = ab^x$. The application of the volume and surface area models was an attempt to transform the gastric evacuation rate curves into linear relationships so that the gastric emptying times (GET) could be predicted by extrapolating to the point where the stomach was considered void of material. Similar transformations of data were made by Griffiths (1976), Thorpe (1977), Persson (1979), El-Shamy (1976), Kiørboe (1978), Lane et al., (1979), Grove and Crawford (1980).

6.2.7 DIGESTION RATE

The digestion rates for dry matter and Lowry protein were assessed for each dietary treatment using the method of Windell (1966). This was measured by combining the stomach and intestinal residuum (dry matter and protein) for each successive sacrificial period and expressing these as a percentage of the initial dry matter and protein content of the stomach. Van Soest et al., (1983) suggested that slaughter of the animal is perhaps the only entirely accurate method for determining the pool size of digesta and therefore digestibility.

6.2.8 STATISTICAL METHODS

Analysis of variance and Duncans multiple range test at the 5% level of significance together with linear regression analysis were used where appropriate.

6.3 RESULTS

On examination of the gastro-intestinal tracts, there was a visible difference in the consistency of faecal material resulting from the experimental treatments. Digesta obtained from the chitin diets appeared dry and coarse in texture and particles of undigestible chitin were clearly discernable. However the diet containing the highest level of galactomannan resulted in quite bulky digesta which contained an appreciable amount of fluid.

6.3.1 FOOD INTAKE

TABLE 6.3 Mean weight of stomach contents (g wet wgt basis) at successive time periods for rainbow trout fed diets 1-6

Diet No	1	2	3	4	5	6	± SE
Time (hrs)							
0	2.555^{b}	1.588^{a}	1.741^{ab}	1.845^{ab}	1.380^{a}	1.467^{a}	0.228
6	1.885^{b}	1.239^{a}	1.967^{b}	2.370^{b}	1.872^{b}	1.804^{b}	0.282
12	1.735^{b}	1.065^{a}	1.616^{b}	2.292^{b}	1.812^{b}	1.184^{b}	0.178
24	1.085^{a}	0.826^{a}	0.605^{a}	1.011^{a}	1.316^{a}	0.945^{a}	0.177
36	0.539^{b}	0.472^{b}	0.203^{a}	0.274^{a}	0.715^{b}	0.575^{b}	0.096
50	0.158^{a}	0.377^{a}	0.155^{a}	0.186^{a}	0.405^{a}	0.237^{a}	0.081

TABLE 6.4 Mean weight of stomach contents (g, moisture free basis) at successive time periods for rainbow trout fed diets 1-6.

Diet No	1	2	3	4	5	6	± SE
Time (hrs)							
0	0.979^{b}	0.676^{a}	0.805^{ab}	0.805^{ab}	0.599^{a}	0.725^{a}	0.076
6	0.590^{a}	0.381^{a}	0.560^{a}	0.739^{a}	0.555^{a}	0.483^{a}	0.094
12	0.448^{a}	0.315^{a}	0.361^{a}	0.608^{a}	0.482^{a}	$0.26)^{a}$	0.089
24	0.318^{a}	0.304^{a}	0.110^{a}	0.206^{a}	0.268^{a}	0.178^{a}	0.059
36	0.171^{b}	0.154^{b}	0.029^{a}	0.044^{a}	0.144^{b}	0.102^{a}	0.032
50	0.062^{a}	0.143^{a}	0.011^{a}	0.038^{a}	0.072^{a}	0.047^{a}	0.027

*Mean values with the same superscripts in a row are not significantly different ($P>0.05$) ± Standard error.

Tables 6.3 and 6.4 show the mean weight of food (time 0) and digesta within the stomachs of rainbow trout at successive hourly intervals for each of the experimental diets on a wet and dry weight basis respectively. It can be seen that there was a significant difference ($p<0.05$) in the food intake of fish fed the fine chitin and coarse chitin diets when expressed on either a wet or dry weight basis for the stomach contents at (time 0). However there was no significant difference in the food intakes of trout fed the diets containing graded levels of galactomannan or for the control diet with no added fibre.

On a wet weight basis, the stomach contents of rainbow trout fed the coarse chitin diet remained significantly lower than for the other treatments up to 12 hours following food consumption. This trend was not apparent when the data was expressed on a dry weight basis.

6.3.2 GASTRIC EVACUATION RATE AND STOMACH EMPTYING TIME (GET)

Figures 6.1.1-6 show the curvi-linear gastric (stomach) emptying patterns for digesta on a wet weight basis for each of the 6 experimental diets. From the gastric emptying time data it can be seen that the initial rate for the emptying of diet 1 from the stomach was faster than diet 2 but the profiles for the chitin diets were different to the control diet and those containing graded levels of galactomannan. In these treatments there was an initial increase in the wet weight of the stomach contents before evacuation occurred which created a 'hump effect'. The duration and size of this increase appeared to be a function of the level of galactomannan in the diet.

Since most workers have based their results on the evacuation of stomach material on a dry weight basis, the data obtained in this study was also presented on a dry weight basis in Figures 6.2.1-6. This serves to remove the compounding effects of gastric secretions and dietary moisture which makes the curvi-linear patterns for gastric emptying more apparent. Again diets 1 and 2 containing different particle size distributions of chitin showed a noticeable difference in evacuation rate during the later stages of digestion. The gastric emptying patterns for diets containing galactomannan displayed interesting effects as the level of fibre increased, changing slightly to give an almost sigmoidal shape. This suggested a much slower initial emptying rate, followed by a faster evacuation phase and a slow final release of stomach material. By comparison, the control diet without added fibre gave a normal curvi-linear response.

FIGURES 6.1.1 - 6.1.6 Gastric evacuation rate profiles for rainbow trout fed experimental diets 1 - 6 respectively *ad libitum* at a mean water temperature of 13.1°C. Mean wet weight of stomach contents, g v time (hrs) including 95% confidence limits.

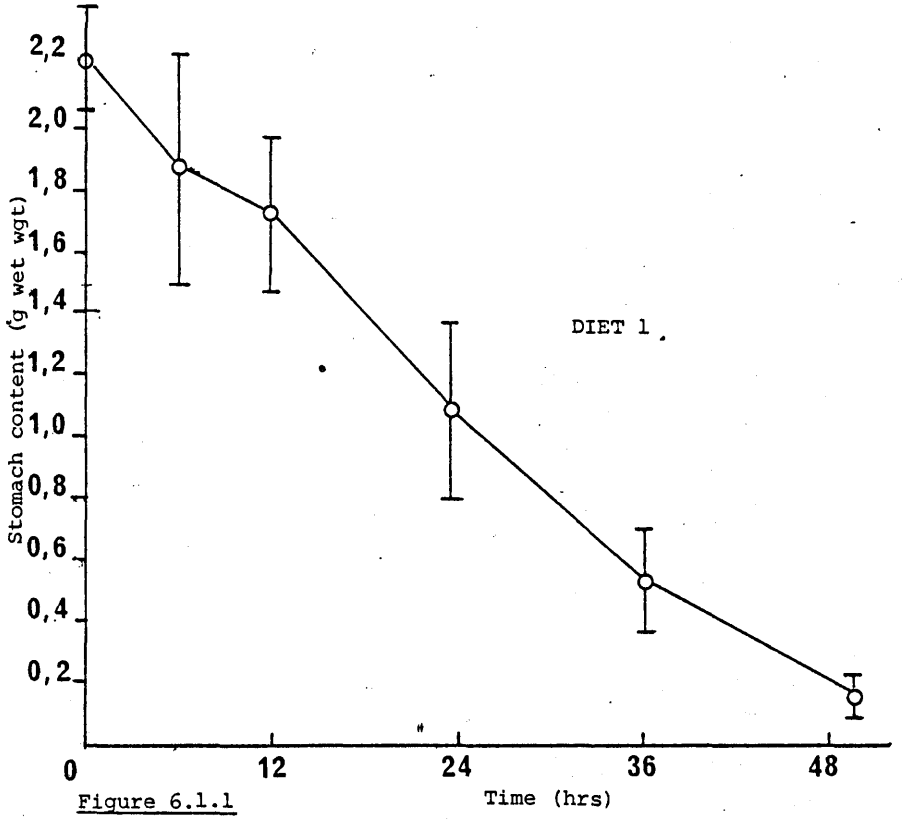


Figure 6.1.1

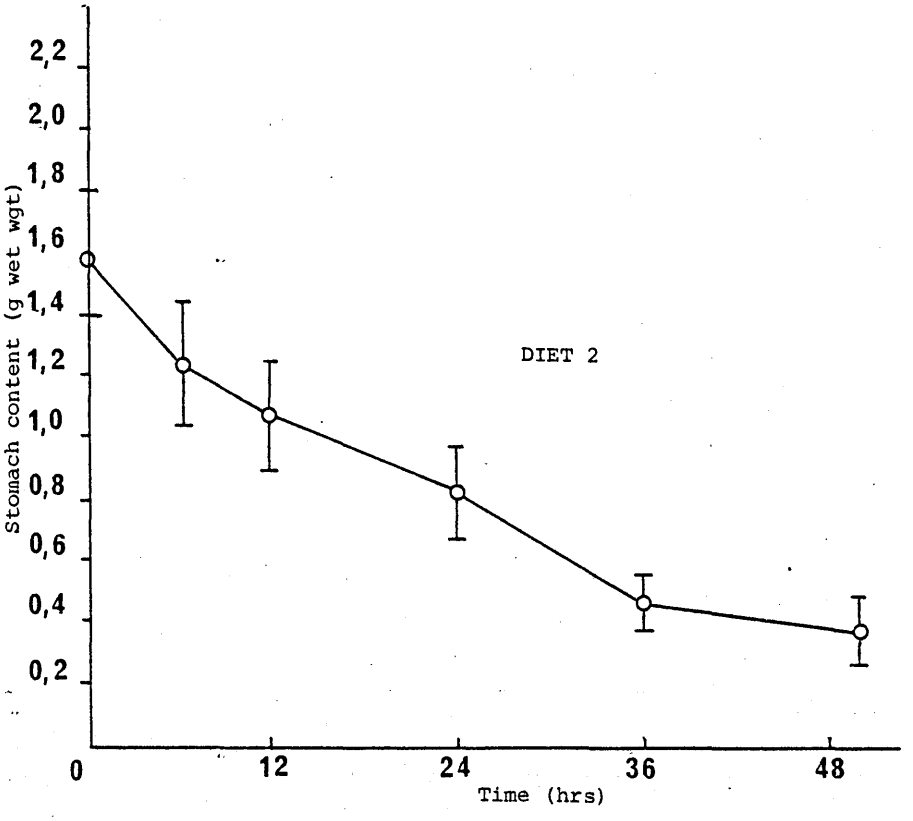


Figure 6.1.2

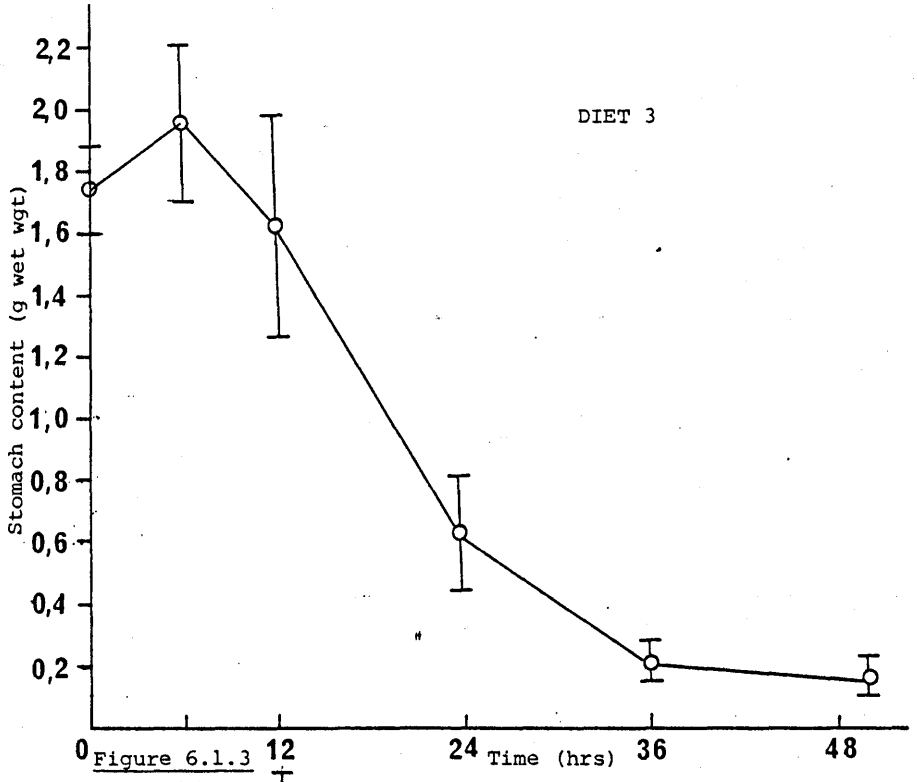


Figure 6.1.3

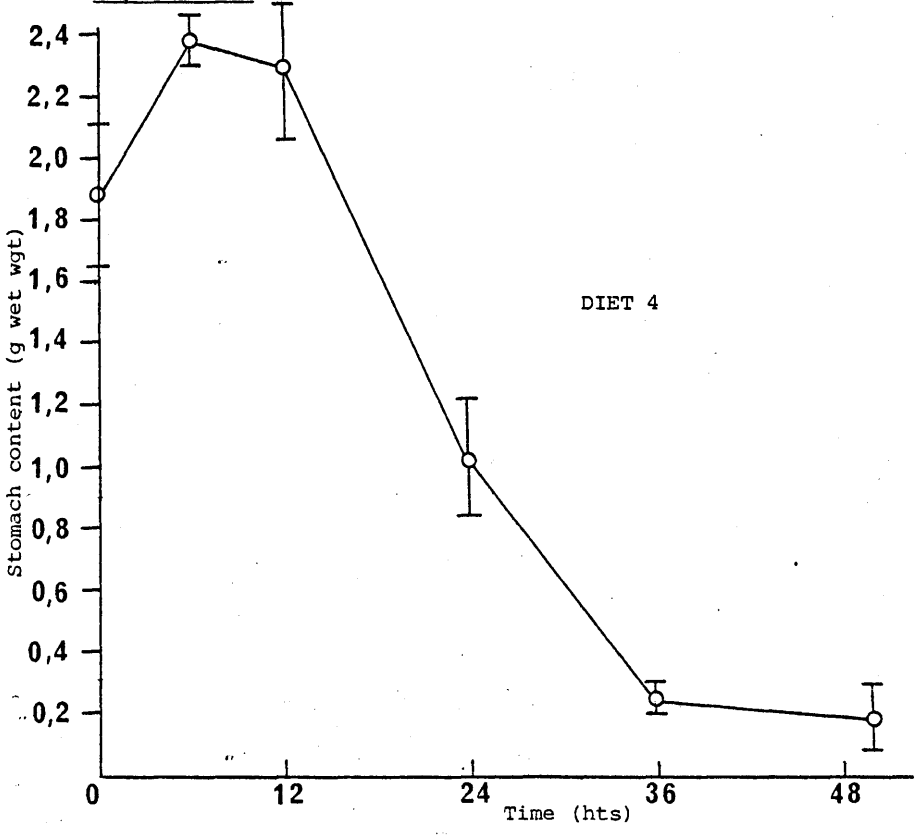
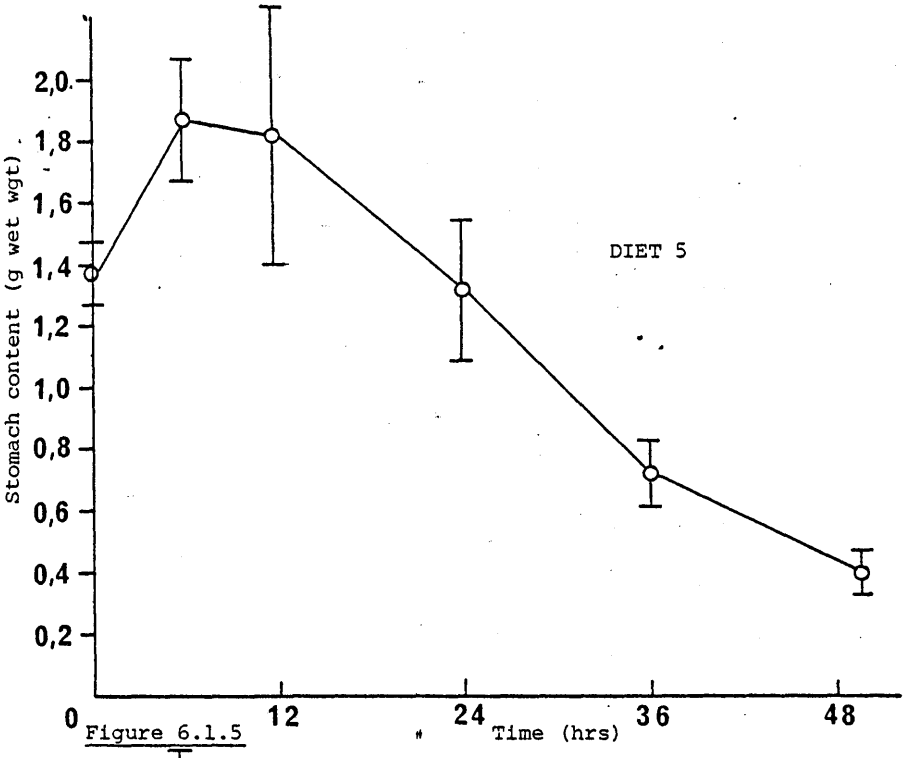


Figure 6.1.4



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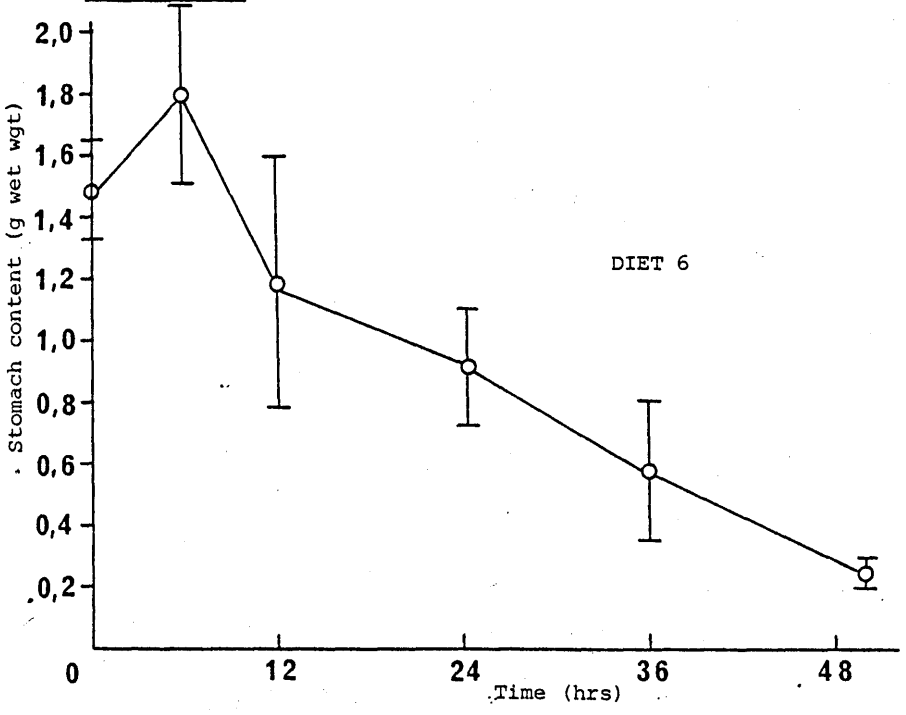


Figure 6.1.6

FIGURES 6.2.1 - 6.2.6 Gastric evacuation rate profiles of rainbow trout fed diets 1 - 6 respectively.

'Mean weight of stomach contents (g, moisture free) v time (hrs) including 95% confidence limits.

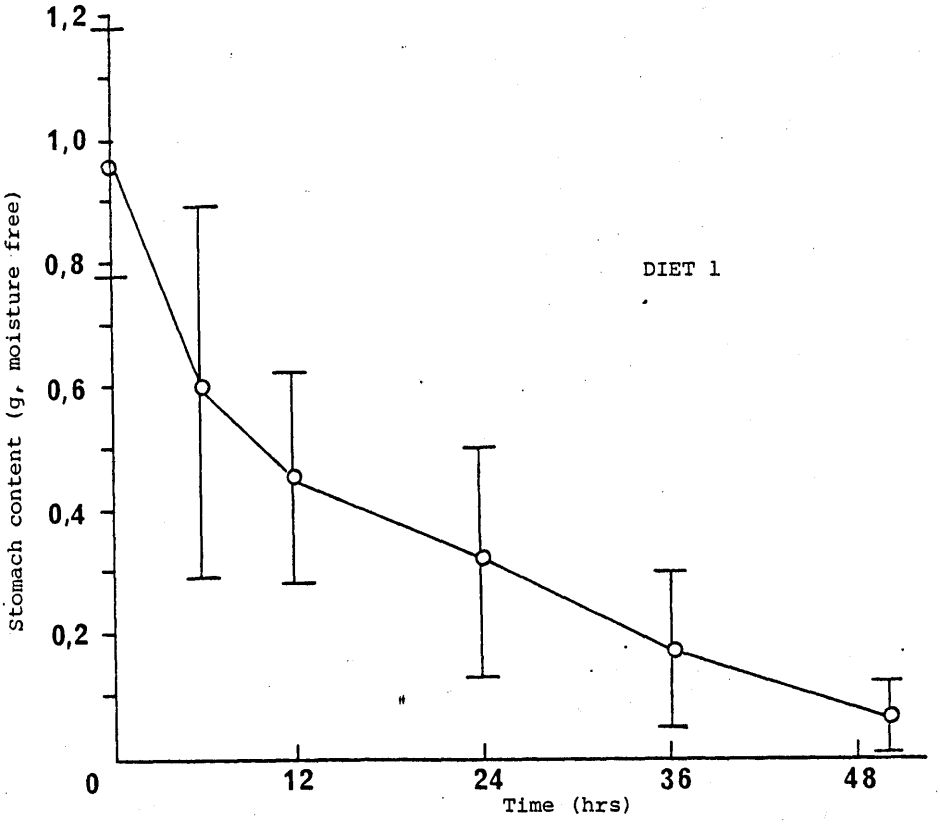


Figure 6.2.1

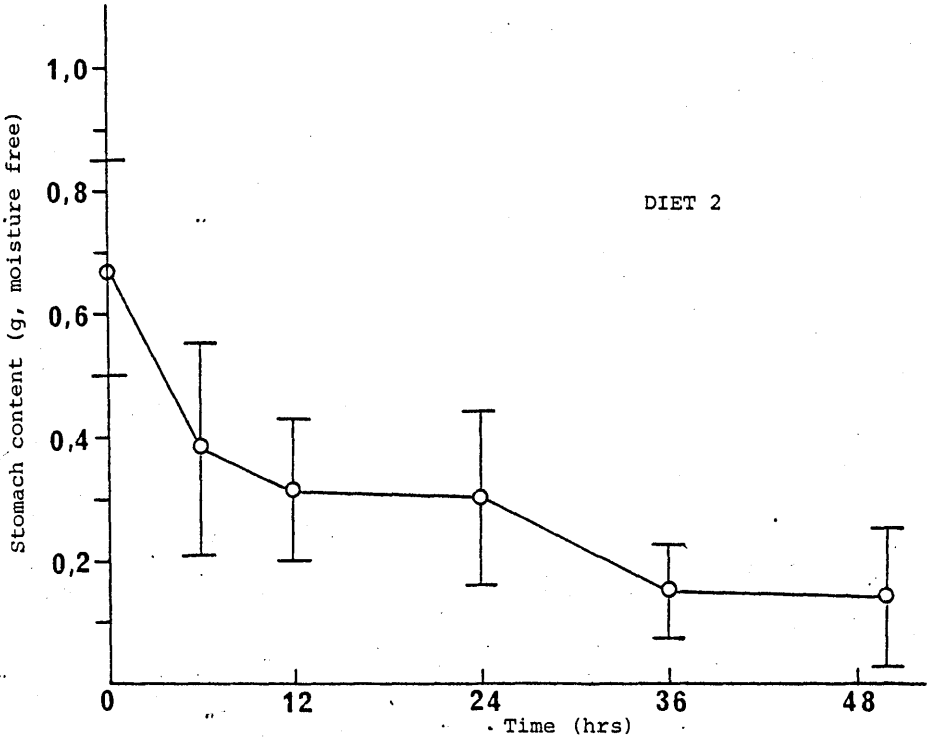


Figure 6.2.2

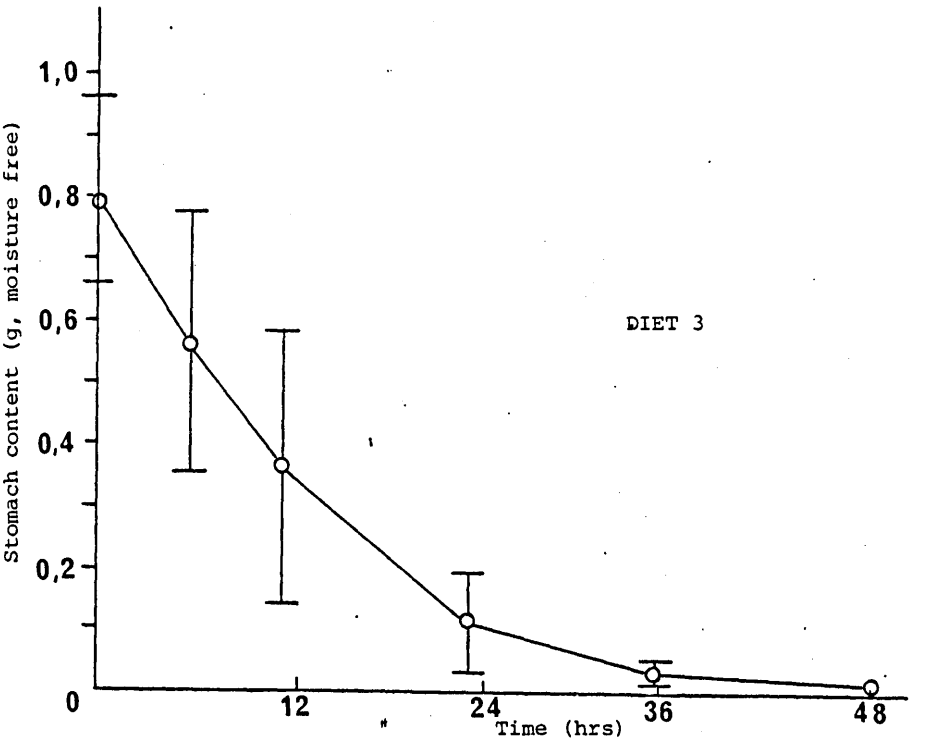


Figure 6.2.3

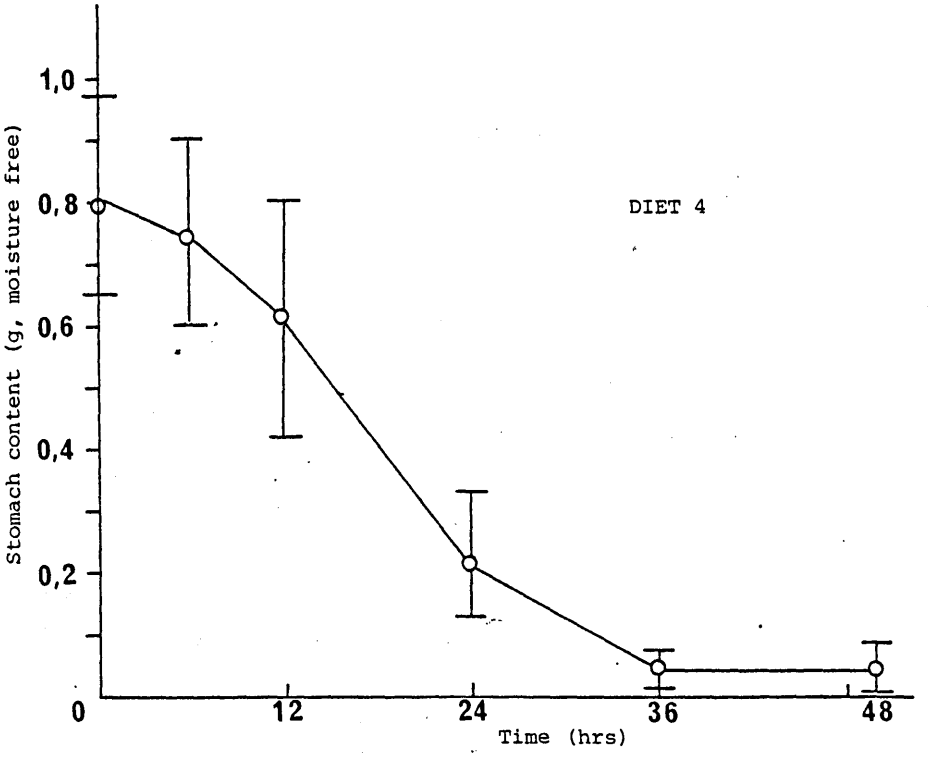


Figure 6.2.4

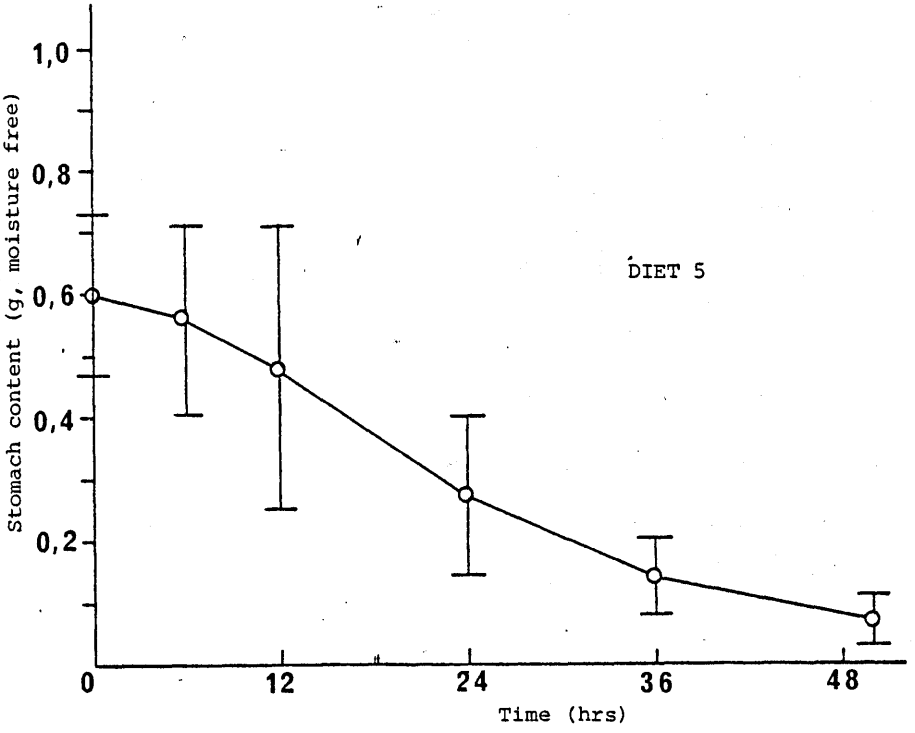


Figure 6.2.5

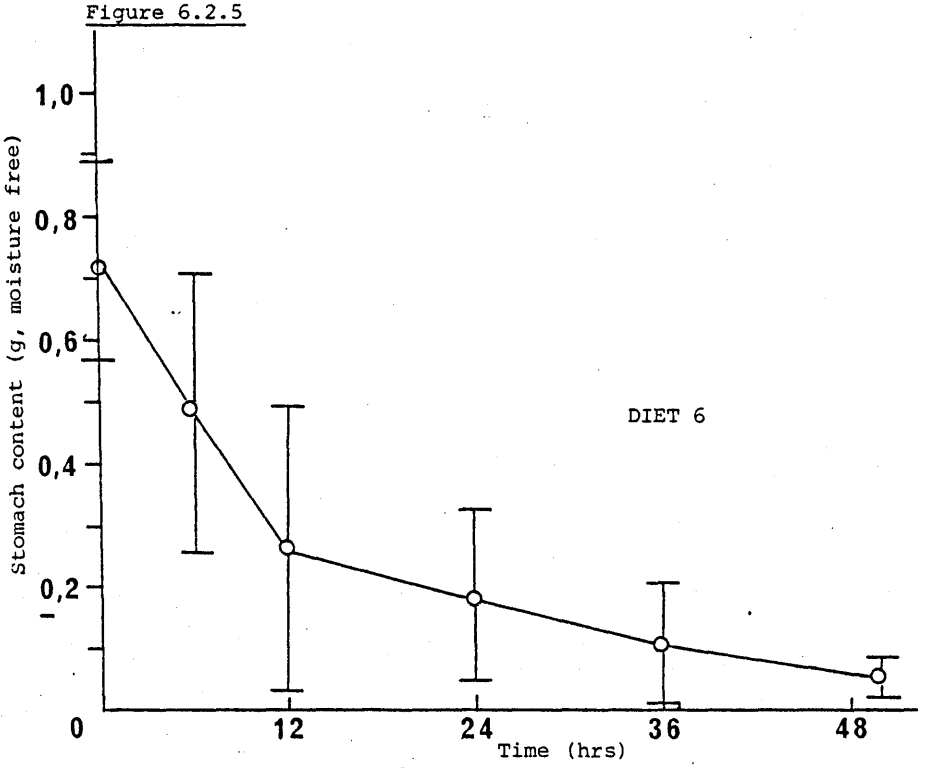


Figure 6.2.6

6.3.3 VOLUME, SURFACE AREA AND EXPONENTIAL TRANSFORMATION OF GASTRIC EVACUATION RATE DATA

From Figures 6.3.1-6 and Figures 6.4.1-6 it can be seen that the gastric evacuation rate data were adequately linearized using the volume (square root) and surface area dependent models. In each case, high regression coefficients were obtained indicating the goodness of fit. The regression equations, coefficients and predicted gastric emptying times calculated on a wet and dry digesta basis are shown in Table 6.5. It was noticed that better correlation coefficients were obtained for the transformation of data to the volume dependent model than the surface area model. As expected, the gastric evacuation rate profiles for all of the dietary treatments conformed to an exponential relationship of the form:

$$y = ab^{x}$$ (Figures 6.5.1-6)

The gastric emptying times were determined for 90% clearance of the stomach contents because for an exponential relationship, 100% evacuation would theoretically require an infinite amount of time. For comparitive purposes, the predicted gastric emptying times for 90% evacuation was also estimated using the volume and surface area dependent relationships.

The presence of coarse chitin compared to the fine grade of material greatly increased the gastric emptying time for the stomach contents on a wet weight basis 83.84, 67.98, 79.43,(Diet 2) and 62.23, 53.94, 45.18 hrs (Diet 1) respectively for the volume, surface area and exponential models. The gradual supplementation of the diet with galactomannan also increased GET. This was confirmed by each of the mathematical relationships applied to the data. An anomaly occurred however for the control diet without added fibre. The predicted GET in rainbow trout fed this diet

FIGURES 6.3.1 - 6.3.6 Linear regression of transformed evacuation rate data using the volume dependent model of

$\sqrt{\text{stomach content, g (moisture free)}}$ v time (hrs) for diets 1 - 6.

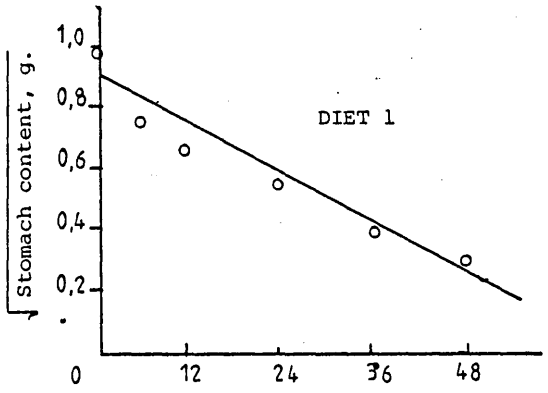


Figure 6.3.1

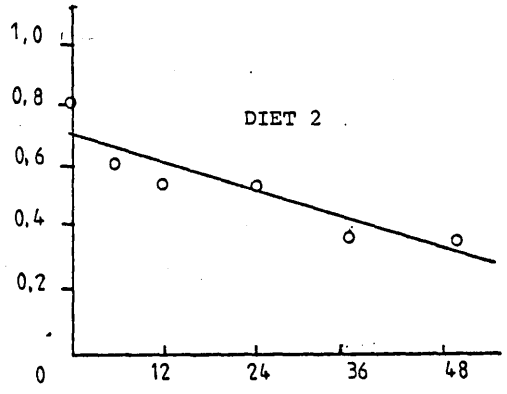


Figure 6.3.2

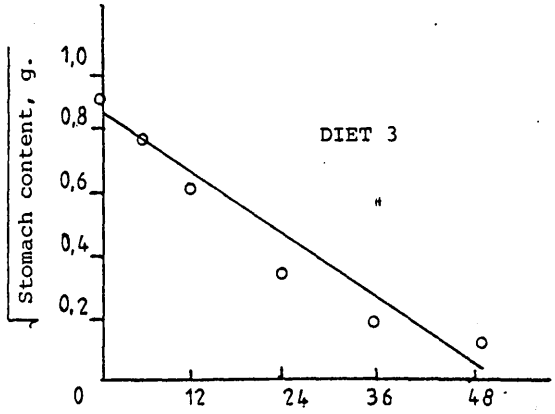


Figure 6.3.3

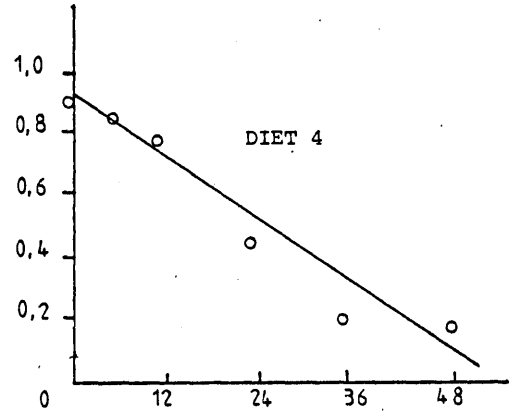
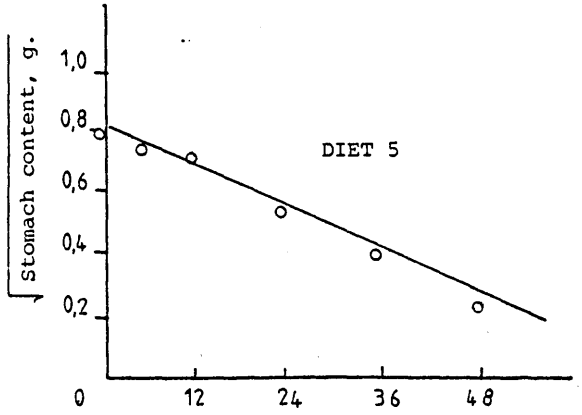


Figure 6.3.4



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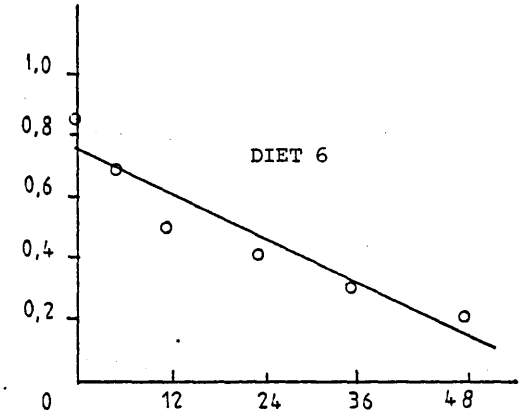


Figure 6.3.6

Time (hrs)

Time (hrs)

FIGURES 6.4.1 - 6.4.6 Linear regression of transformed data using the surface area dependent model (stomach content, g, dry weight)$^{0.67}$ v time (hrs) for diets 1 - 6.

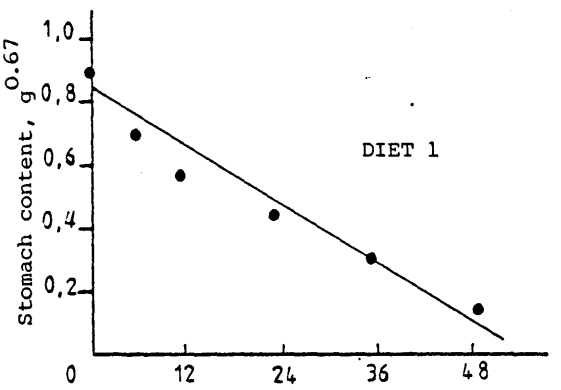


Figure 6.4.1

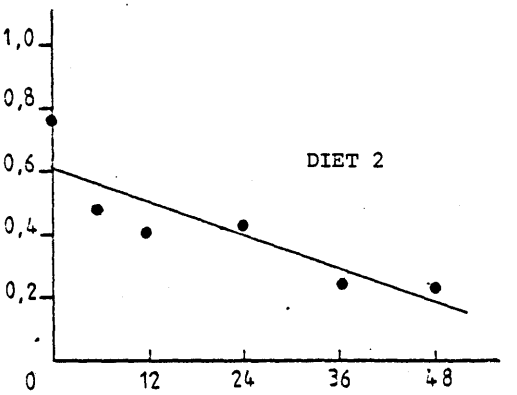
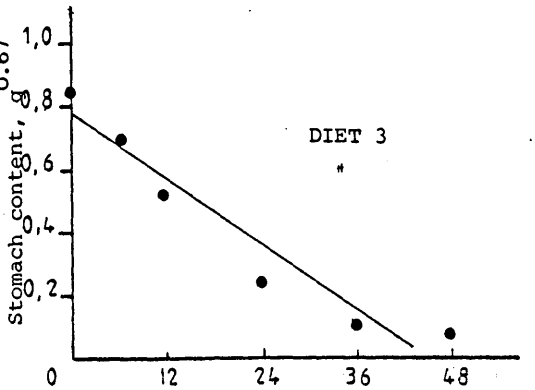


Figure 6.4.2



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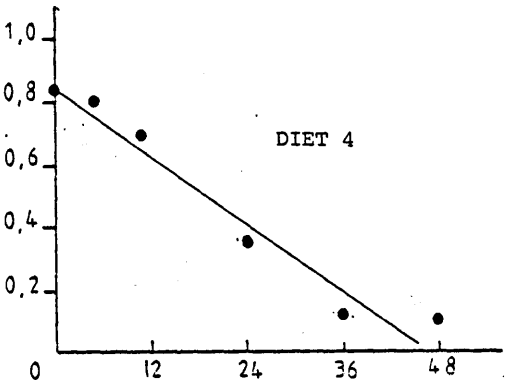

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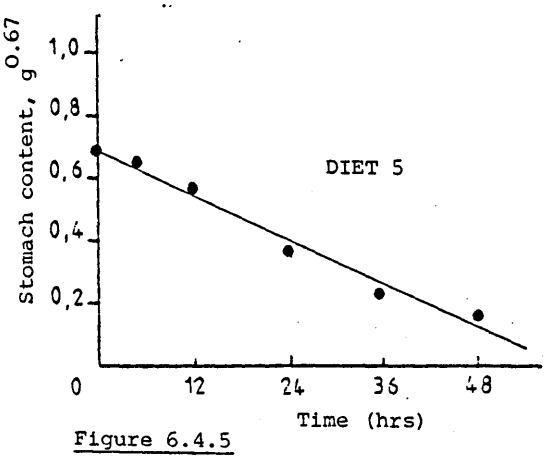

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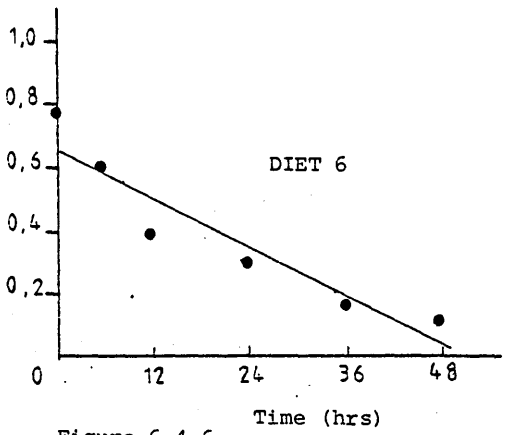


Figure 6.4.6

FIGURES 6.5.1 - 6.5.6 Transformation of gastric evacuation rate data to fit the exponential model $y=ab^x$, for diets 1 - 6.

Where y = stomach content, g dry weight, x = time (hrs) a and b are constants.

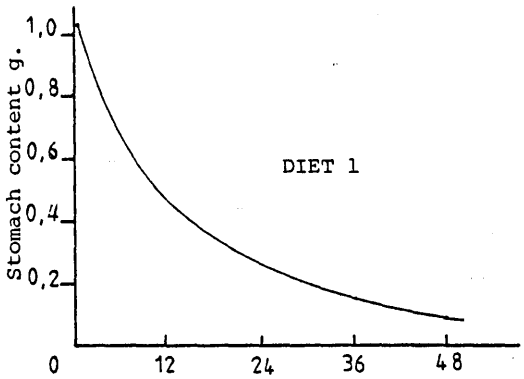
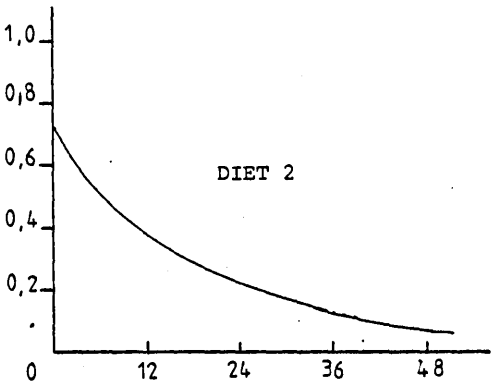


Figure 6.5.1



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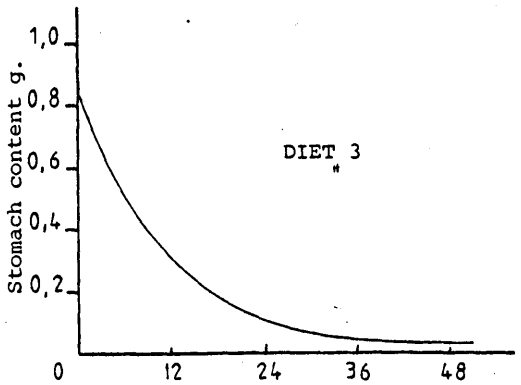

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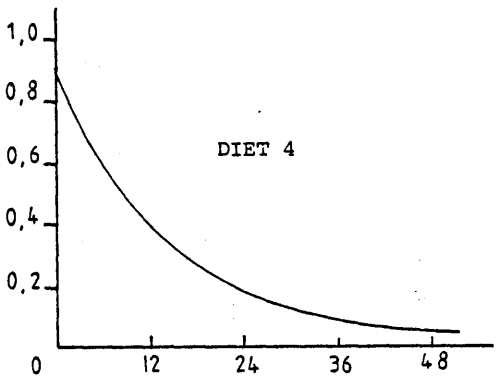


Figure 6.5.4

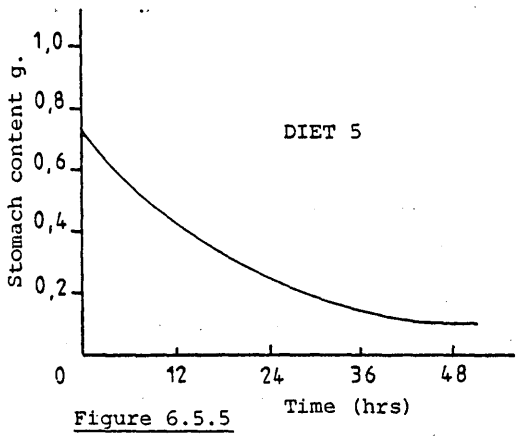


Figure 6.5.5

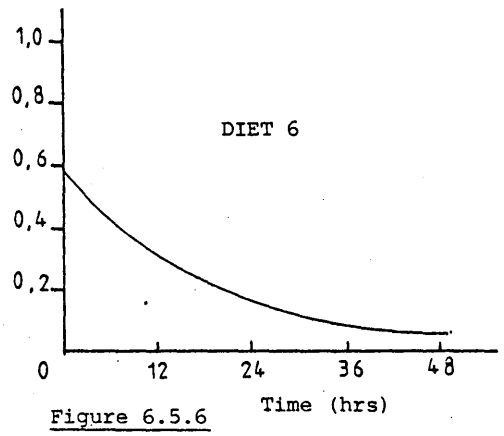


Figure 6.5.6

TABLE 6.5 Regression equations, coefficients and predicted 90% gastric emptying time (GET) of digesta (wet and dry wgt basis) for data fitted to three different evacuation rate models.

		Wet weight basis			Dry weight basis		
		Regression equation	Regression coefficient	Predicted GET	Regression equation	Regression coefficient	Predicted GET
	Diet No			(hrs)			(hrs)
Square root model $\frac{dv}{dt} = CV^{0.5}$	1	y=1.535-0.0222X	0.996	62.23	y=0.895-0.013X	0.977	60.12
	2	y=1.211-0.013X	0.985	83.84	y=0.720-0.008X	0.911	83.04
	3	y=1.423-0.0228X	0.959	56.17	y=0.825-0.016X	0.970	45.25
	4	y=1.577-0.0242X	0.937	58.65	y=0.914-0.016X	0.967	50.47
	5	y=1.378-0.0137X	0.901	90.52	y=0.794-0.011X	0.994	66.20
	6	y=1.315-0.0159X	0.969	74.43	y=0.755-0.012X	0.954	57.57
Surface area model $\frac{dv}{dt} = CV^{0.67}$	1	y=1.738-0.029X	0.998	53.94	y=0.850-0.015X	0.962	51.34
	2	y=1.284-0.017X	0.981	67.98	y=0.643-0.009X	0.896	68.03
	3	y=1.576-0.029X	0.952	48.91	y=0.756-0.017X	0.952	41.00
	4	y=1.806-0.032X	0.929	50.80	y=0.868-0.017X	Q.963	44.91
	5	y=1.521-0.018X	0.896	76.05	y=0.725-0.012X	0.991	56.22
	6	y=1.424-0.020X	0.965	64.08	y=0.680-0.012X	0.934	49.37
Exponential decay model $y = ab^x$	1	$y=2.820(0.950)^x$	0.971	45.18	$y=0.92(0.95)^x$	0.99	45.32
	2	$y=1.533(0.971)^x$	0.991	79.43	$y=0.67(0.96)^x$	0.96	56.10
	3	$y=2.519(0.938)^x$	0.957	35.97	$y=0.91(0.91)^x$	0.99	24.53
	4	$y=2.932(0.946)^x$	0.950	41.41	$y=1.02(0.93)^x$	0.97	31.51
	5	$y=2.028(0.972)^x$	0.911	80.51	$y=0.71(0.96)^x$	0.99	56.34
	6	$y=1.914(0.963)^x$	0.962	61.24	$y=0.63(0.95)^x$	0.99	45.10

was always intermediate to the range of GET values predicted for the galactomannan diets. For 5, 10 and 20% inclusions of galactomannan, the GET's were 56.17, 48.91, 35.97, hrs; 58.65, 50.80, 41.41, hrs; and 90.52, 76.05, 80.51 hrs respectively and 74.43, 64.08, 61.24 hrs for the control diet computed from the same three mathematical models, (volume, surface area and exponential).

The gastric evacuation rate data presented on the dry weight basis was also transformed using the relationships described previously. Similar trends existed for the predicted GET's of digesta on a moisture free basis as for the original wet weight basis. However a slight time lag was apparent for the combined evacuation of particulate material and fluid from the stomach of rainbow trout.

6.3.4 INTESTINAL FLOW RATE

A limited amount of information was obtained from the intestinal flow rate patterns of digesta presented in Figures 6.6.1-6 for diets 1-6 on a wet and dry weight basis. Unfortunately, due to the small sample sizes, material had to be pooled from the small and large intestines and so the profiles are for material passing through the complete intestine rather than separate compartments.

It was observed that when the data was compared on a wet weight basis, there was an initial increase in the intestinal content for each of the experimental diets, followed by a static phase or a slow decrease. However there was a noticeable difference between the flow rate patterns for the chitin diets. Whilst the fine chitin diet resulted in a decreased intestinal content approximately 12 hours following meal consumption, the passage of the coarse chitin diet remained fairly uniform. The sharp decline

FIGURES 6.6.1 - 6.6.6 Variation in the intestinal content (g) v time (hrs) for diets 1 - 6.

● g, (wet weight basis)

o g, (moisture free basis)

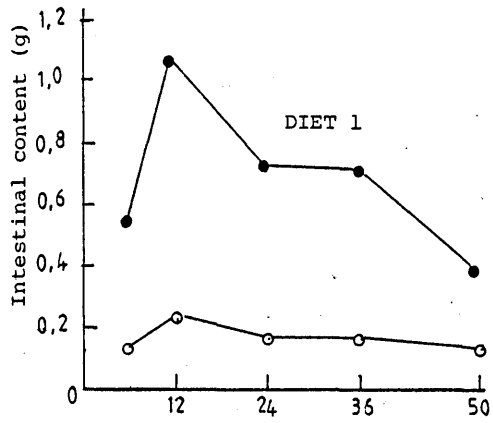


Figure 6.6.1

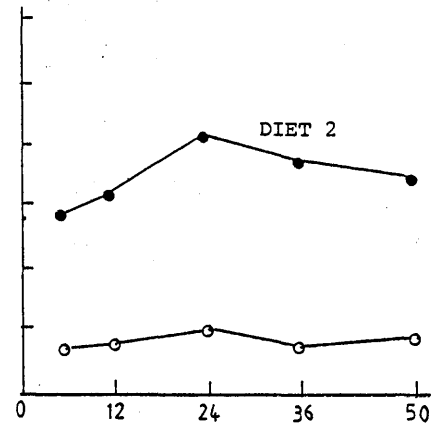


Figure 6.6.2

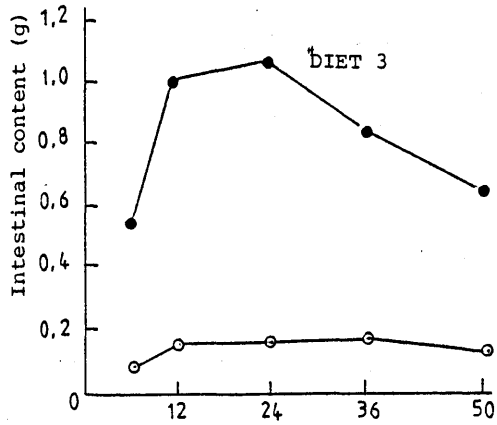
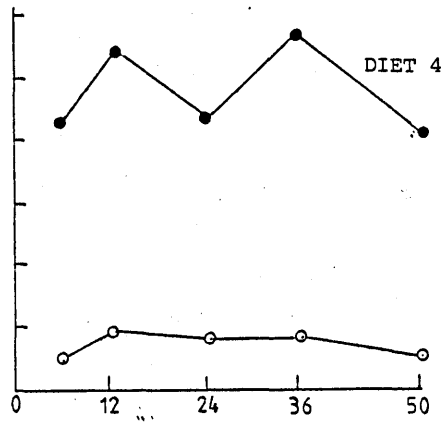


Figure 6.6.3



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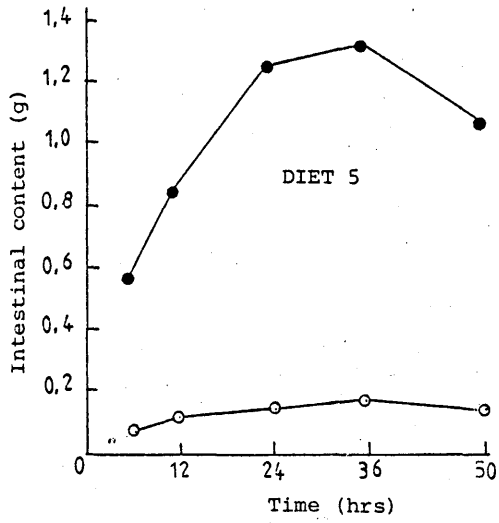


Figure 6.6.5

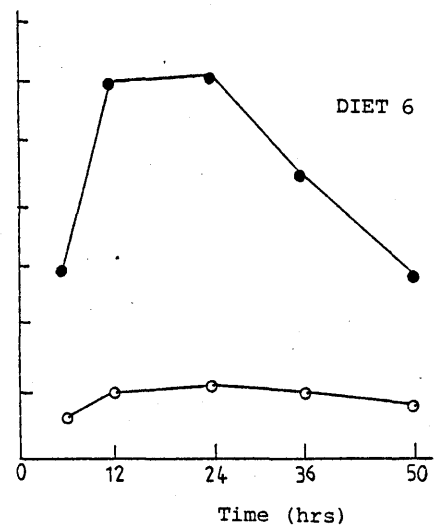


Figure 6.6.6

in the wet weight flow of the intestinal contents was especially noticeable for those diets containing graded levels of galactomannan and the control diet. A comparison of the passage rate of digesta on a dry weight basis showed that there was a uniform flow of material through the intestine at each sacrificial period. One explanation for this could be that the rate of digestion and absorption in the intestine more than compensates for the release of undigested material from the stomach. Alternatively, the uniform passage of digesta through the intestine, whilst the stomach contents were seen to decrease implies that there was a consistent loss of material from the alimentary tract by defecation.

The advantage in measuring the intestinal contents, was that it enabled a complete estimation of the total material remaining in the gastro-intestinal tract during each sacrificial period. If it is assumed that no defecation occurs during this time, then any loss of material represents the state of digestion and absorption or the apparent digestibility of the diet. However the apparent loss of material from the gastro-intestinal tract is more likely to be a combination of digestion, absorption and faecal passage. Nevertheless, digestion rate curves for dry matter and Lowry protein for each experimental diet calculated using the method of Windell (1966) are shown in Figures 6.7.1-6.

In each case, the apparent rate of protein digestion exceeds that of the dry matter and this was quite noticeable during the later stages in the digestion of the coarse chitin diet. It was also observed that the rate of dry matter and protein digestion for this treatment was lower than that of the fine chitin diet 12 hours following the consumption of food, but the initial digestion rates for the two treatments were quite similar.

FIGURES 6.7.1 - 6.7.6 Apparent dry matter and lowry protein digestion rates for diets 1 - 6.

% digestion v time (hrs)

o- - - Lowry protein

o—— Dry matter

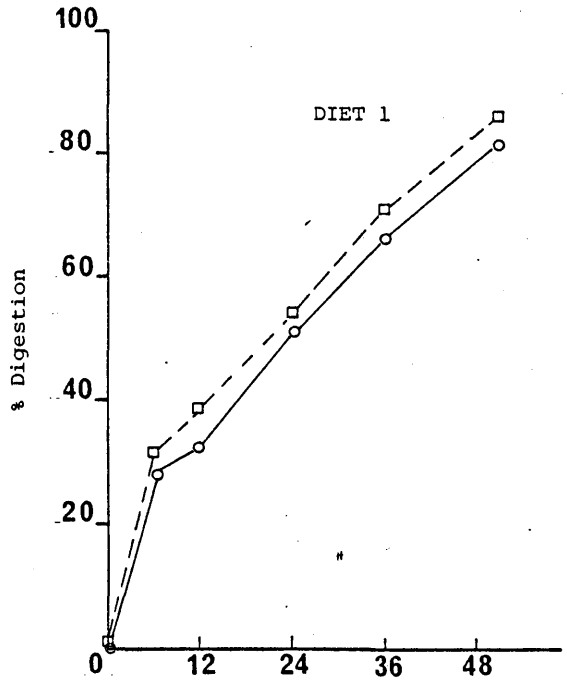


Figure 6.7.1

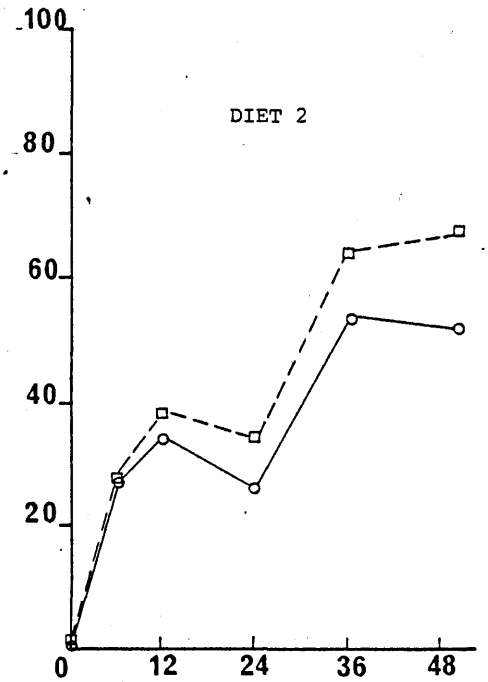


Figure 6.7.2

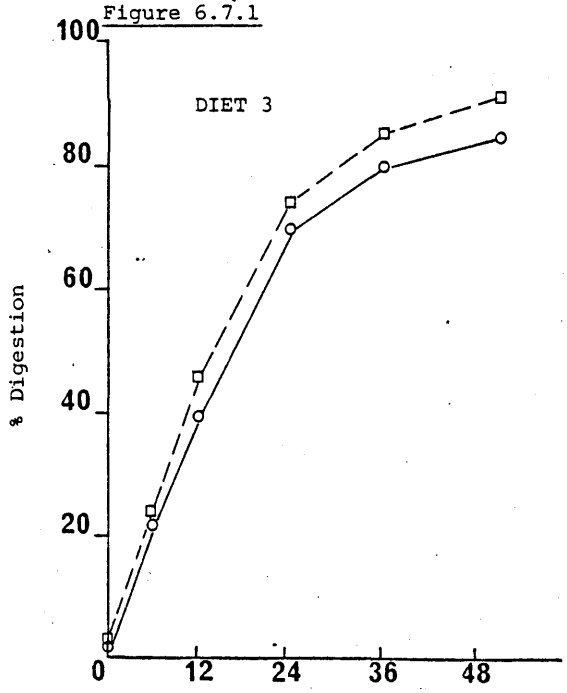


Figure 6.7.3

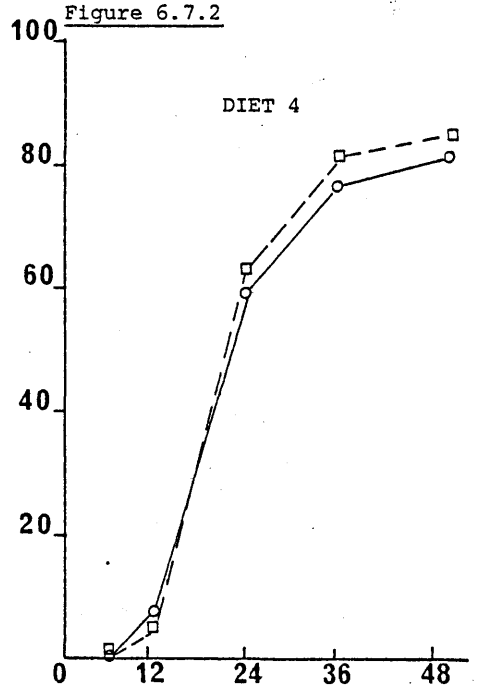


Figure 6.7.4

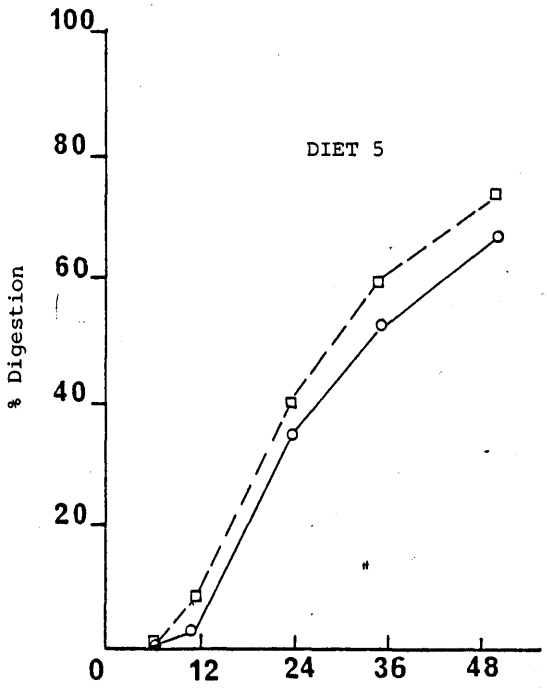


Figure 6.7.5

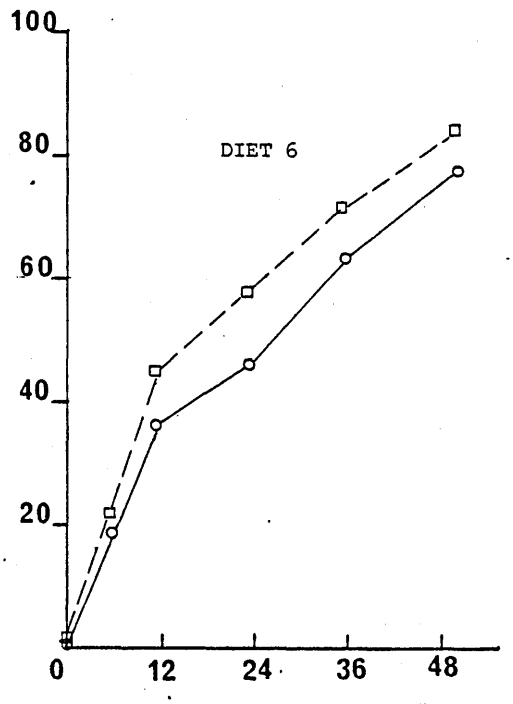


Figure 6.7.6

The level of dietary fibre in the form of galactomannan appeared to change the shape of the digestion rate curves compared to the control diet without added fibre. For diets 4 and 5 containing 10% and 20% galactomannan, a lag phase occurred during the first 12 hours after meal consumption. This lag phase later gave way to a rapid digestion phase which lasted approximately 12-24 hours followed by a final slow phase.

6.3.5 EFFECT OF DIETARY FIBRE ON MOISTURE CONTENT OF STOMACH AND INTESTINAL DIGESTA

The moisture contents of the experimental diets were presented in Table 6.1 and were within the range of 6.94%-10.24%. The statistical analysis of the data in Table 6.6 shows that directly following food consumption (0 hours), the moisture content of stomach digesta resulting from diet 2 (coarse chitin) was significantly higher ($p<0.05$) than in the digesta produced by diet 1 (fine chitin). There was also a significant increase ($p<0.05$) in the moisture levels of stomach digesta for all of the galactomannan and control diets compared to the chitin diets. However for all of the treatments, the moisture content of stomach digesta rapidly increased with time (Figures 6.8.1-6). For both the chitin diets, a plateau effect developed approximately 6 hours after food intake in which the moisture levels in the stomach remained static at about 70% for the remaining 40 hours. In contrast, there was a continued increase in moisture uptake for the remaining dietary treatments which was shown to be statistically significant. The digesta from these diets had a much higher final moisture content (80-90%) but no relationship was shown to exist in the stomach for moisture uptake and galactomannan concentration.

TABLE 6.6 Variation in the moisture content (%) of stomach and intestinal digesta at successive time intervals for rainbow trout fed the experimental diets.

STOMACH

Diet No	1	2	3	4	5	6	± SE
Time (hrs)							
0	55.20^{bc}	57.48^{d}	53.90^{b}	56.52^{c}	56.56^{cd}	50.36^{a}	0.70
6	70.19^{ab}	69.72^{ab}	72.36^{bc}	69.11^{a}	70.51^{ab}	74.26^{c}	0.96
12	73.65^{b}	70.35^{a}	79.85^{c}	73.36^{b}	73.14^{b}	78.61^{c}	1.37
24	69.42^{a}	68.07^{a}	83.05^{b}	81.12^{b}	80.43^{b}	82.96^{b}	1.17
36	68.57^{a}	69.78^{a}	86.89^{c}	85.21^{c}	80.39^{b}	83.67^{bc}	1.32
50	70.57^{a}	67.70^{a}	89.33^{c}	86.04^{b}	80.40^{b}	82.74^{b}	2.10

INTESTINE

Diet No	1	2	3	4	5	6	± SE
Time (hrs)							
6	78.58^{a}	81.73^{b}	85.39^{c}	87.64^{d}	89.15^{e}	82.25^{b}	0.61
12	79.65^{a}	79.72^{a}	86.95^{c}	86.75^{c}	87.37^{c}	83.33^{b}	0.69
24	77.85^{a}	77.15^{a}	86.14^{c}	87.49^{c}	90.16^{d}	82.54^{b}	0.61
36	76.82^{a}	77.44^{a}	85.80^{c}	87.99^{d}	88.86^{d}	81.09^{b}	0.76
50	76.11^{a}	74.25^{a}	82.86^{b}	86.80^{c}	87.85^{c}	80.58^{b}	0.90

Mean values in each row with the same superscripts are not significantly different ($p>0.05$) SE ± standard error.

FIGURES 6.8.1 - 6.8.6 Variation in moisture content (%) of digesta within the stomach v time (hrs) for rainbow trout fed diets 1 - 6 respectively.

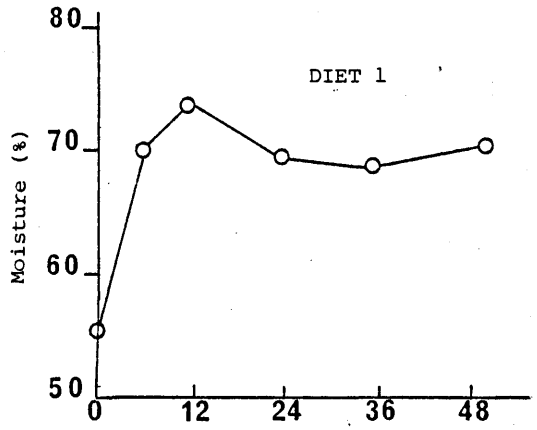


Figure 6.8.1

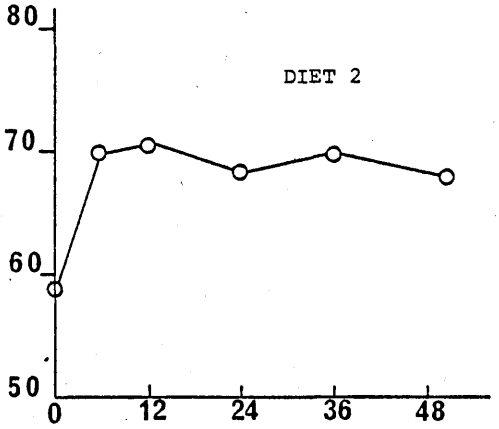


Figure 6.8.2

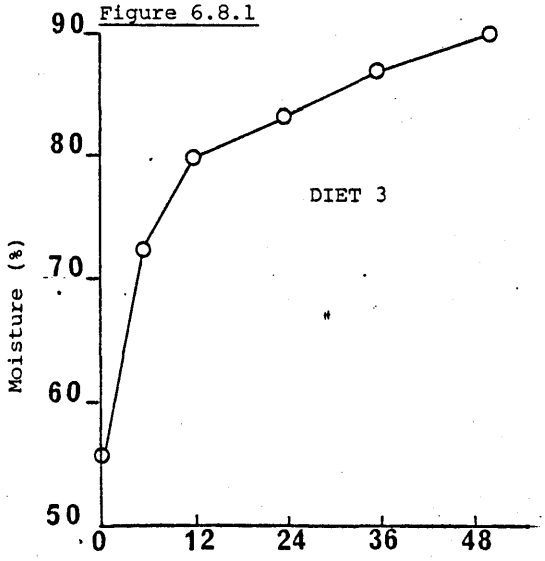
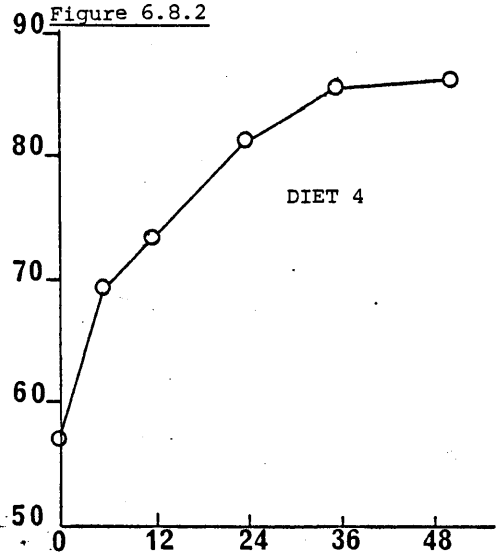


Figure 6.8.3



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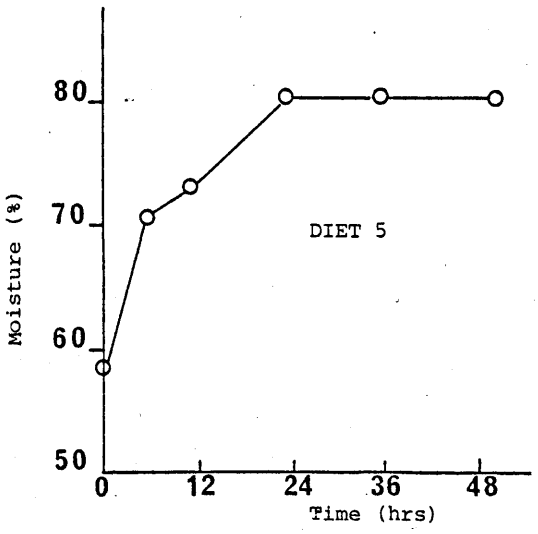

Figure 6.8.5

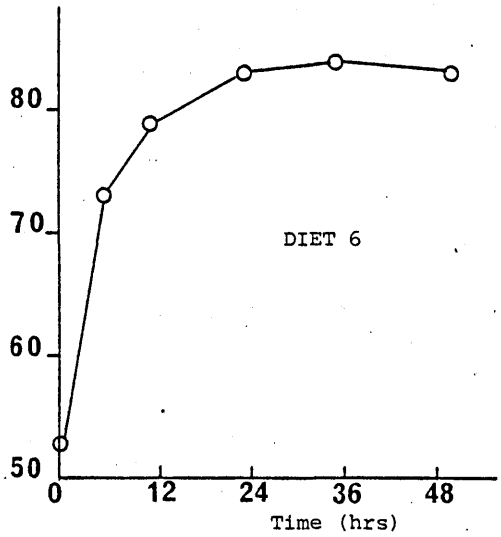

Figure 6.8.6

Table 6.6 also shows the moisture levels of the intestinal digesta for each dietary treatment at successive time intervals. The moisture levels were seen to be significantly different ($p<0.05$) for the various diets and this became more apparent at the 6-hour sacrificial period. At this stage, the coarse chitin digesta had a slightly higher moisture level (81.37%) compared to the fine chitin digesta (78.58%), but there was no significant difference between the coarse chitin treatment and the control diet. The inclusion of graded amounts of galactomannan (5, 10 and 20%) resulted in intestinal moisture levels in digesta of 85.39, 87.64 and 89.14% respectively. These trends continued for the remaining time periods with the galactomannan digesta consistently retaining greater amounts of fluid compared to the chitin and control diets. The data was also displayed in graphical form (Figures 6.9.1-6) to show the marked differences between the pattern of moisture uptake for intestinal digesta compared to the stomach in rainbow trout fed the experimental diets. By comparison, the percentage moisture content of the intestinal digesta remained fairly uniform and these levels were generally higher than the maximum levels attained in stomach digesta.

Tables 6.1 and 6.2 include values obtained from in vitro measurements of the water retaining capacities of the experimental diets and the fibre sources tested. Diet 2 containing coarse chitin retained a greater amount of moisture (61.67%) than diet 1 (fine chitin, (58.15%) when samples were saturated with deionized water. The control (diet 6) and diets 3, 4 and 5 with graded additions of galactomannan retained water in proportion to the amount of fibre. The measurements of water uptake for individual dietary fibre sources showed that there were only slight differences between fine and coarse grades of chitin (76.48% and 77.06%

FIGURES 6.9.1 - 6.9.6 Variation in the moisture content (%) of the intestinal digesta v time (hrs) for rainbow trout fed diets 1 - 6.

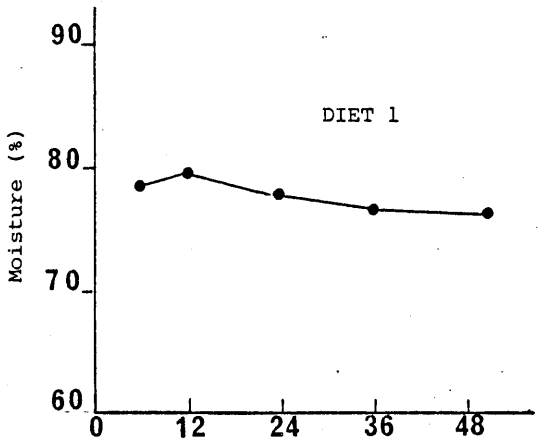

Figure 6.9.1

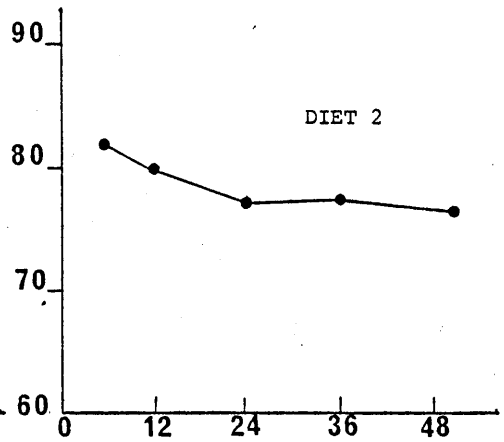

Figure 6.9.2

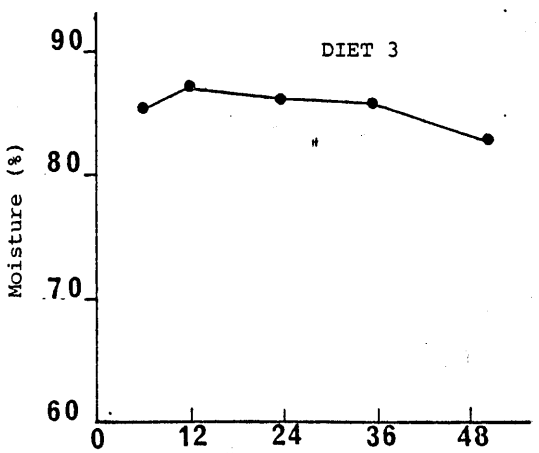

Figure 6.9.3

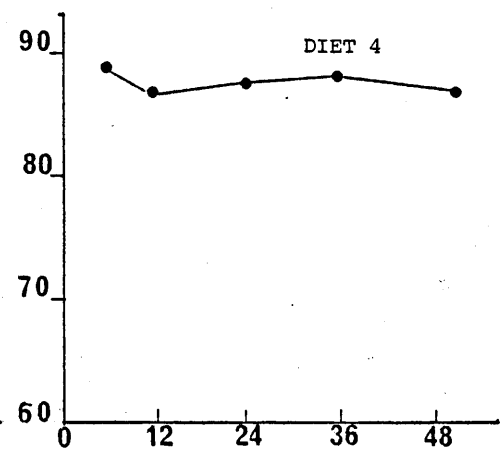

Figure 6.9.4

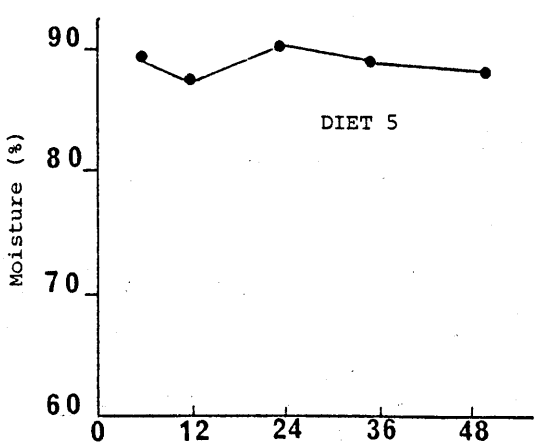

Figure 6.9.5

Time (hrs)

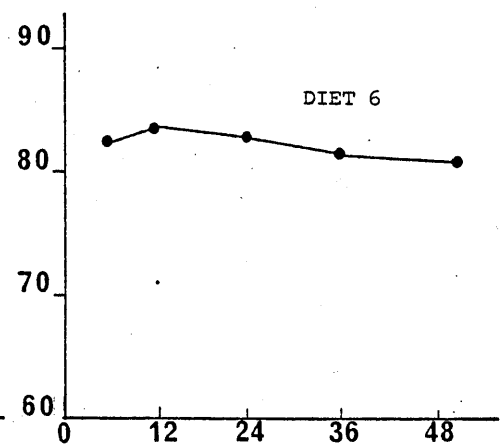

Figure 6.9.6

Time (hrs)

respectively) and the greater water retaining potential of galactomannan was confirmed (90.71%).

Linear regression analysis (Figure 6.10) was performed for the relationship between dietary galactomannan level and the retention of moisture by diets and corresponding intestinal digesta at the 6-hour sacrificial period because at this stage, all of the dietary treatments showed significant differences (see Table 6.6). The correlation coefficients were found to be significant and a lower correlation was observed using the *in vitro* water uptake values. On the basis of this information it would appear that moisture uptake is linearly related to the level of dietary galactomannan and that this capacity may persist within the intestine for a considerable period of time.

6.4 DISCUSSION

It is desirable before considering how the source and level of dietary fibre may influence the gastro-intestinal responses of rainbow trout to briefly review those parameters which are known to generally affect these processes in fish. Amongst the environmental factors, temperature has received the greatest attention. The negative correlation between gastric emptying time (GET) and temperature has been widely reported. (Fange and Grove, 1979; Jobling, Gwyther and Grove, 1977 with turbot, *Scophthalmus maximus* and plaice, *Pleuronectes platessa*). For salmonid species, Brett and Higgs (1970) observed that gastric digestion was accelerated with increasing temperature in studies with fingerling sockeye salmon, *Onchorhynus nerka*. The rate of gastric evacuation and digestion as affected by temperature is especially important in the consideration of the digestion of nutrients by fish. In the

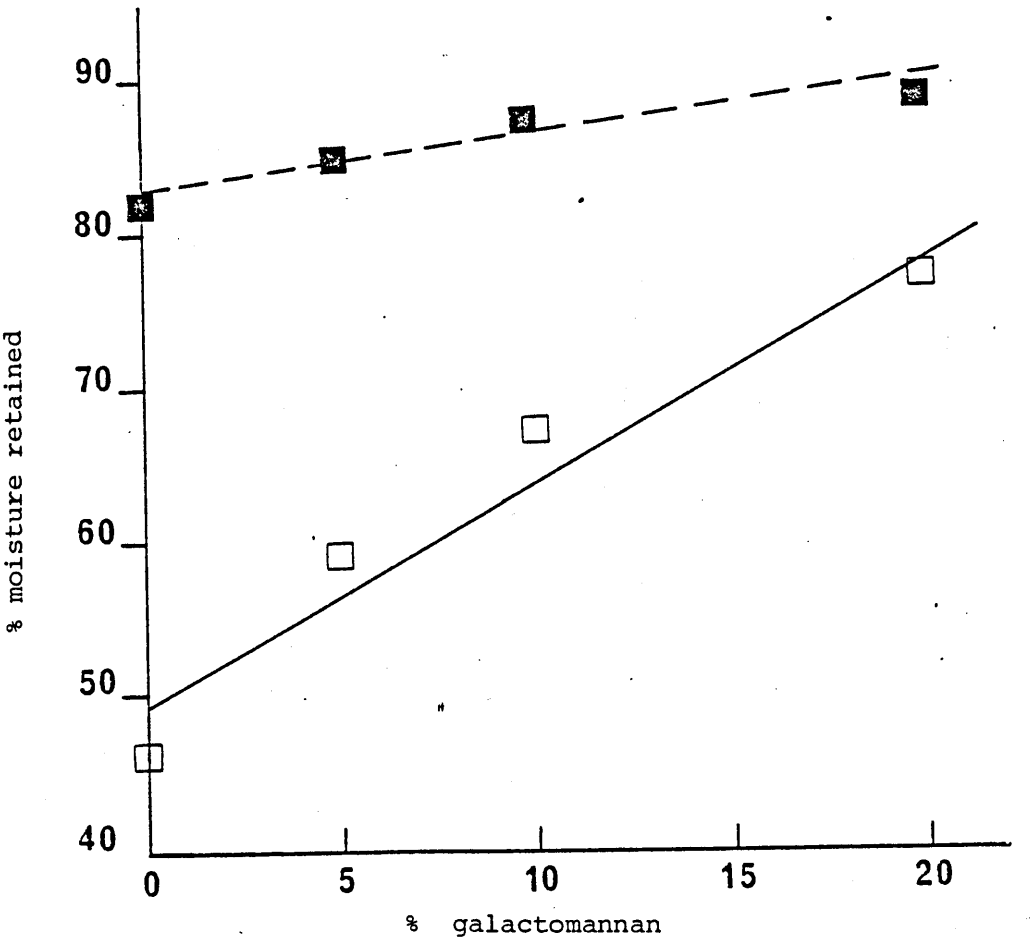

FIGURE 6.10 Relationship between moisture retention (%) in diets and corresponding digesta with increasing levels of galactomannan.

■ Based on the mean moisture levels of intestinal contents at the 6-hour sacrificial period

$Y = 1.49X + 49.29$ $r = 0.98$

□ Based on *in vitro* measurements following saturation of dietary samples with deionized water.

$Y = 0.332X + 83.20$ $r = 0.94$

present investigation with rainbow trout, the effect of temperature variation on food intake and gastro-intestinal function was considered to be minimal because the water temperature remained relatively constant throughout the experimental period at 12.9 - 13.1°C, mean 13.0°C.

One of the most important physical factors affecting gastric emptying rates in fish is the relationship between meal size and the body weight of the animal. The general findings are that for a given sized fish at a stated temperature, an increase in meal size usually prolongs stomach emptying time. The increase in the gastric emptying time is not linearly related to meal size because partial compensation is achieved by an increase in the emptying rate as measured by the weight of material translocated per unit time (Windell and Norris, 1969).

If the size of a single meal is increased, most workers agree that the rate of digestion is also increased when expressed as grams/hr. This increase may mean that a given percentage of a meal present in the stomach is digested in the same time for different meal sizes (Elliot, 1972), although other workers have stated that a larger meal, whilst evacuating at a faster rate, takes longer to reach a given percentage stage of digestion (Beamish, 1972). Therefore most investigators agree that a smaller meal is evacuated from the stomach in a shorter time than a larger meal, but that the instantaneous rate of evacuation is greater for the larger meal. Gastric digestion rate has also been reported to increase with the body size of the fish. Flowerdew and Grove (1979) found that small turbot processed a given ration (expressed as a percentage of the body weight) faster than larger fish. However Jobling (1980)[a] observed that the rate of gastric evacuation in

plaice was relatively unchanged by fish size and Persson 1981; 1982 reported similar findings for perch, Perca fluviatilis and roach, Rutilus rutilus.

For the present study with rainbow trout, a standard fish size (35 g) was used in each of the dietary treatments and the fish were fed the diets ad libitum in order to determine the effects of the different fibre sources and inclusion level on voluntary food intake and the gastrointestinal response.

The amount of food consumed in a meal, the satiation time and the subsequent return of appetite has been extensively reported for salmonids by Brett (1971); Brett and Higgs (1970); with sockeye salmon and by Elliot (1975[ab]) with brown trout. It was demonstrated by these workers that the appetite response in these fish is closely related to stomach fullness. Therefore the amount of food in the stomach at the time of satiation provides a measure of the voluntary food intake of an animal (Miner, 1955). The relationship between the amount of food required to satiate a fish, the frequency of feeding and the gastric emptying rate was investigated for rainbow trout by Grove et al., (1978). Appetite after deprivation as determined by voluntary food intake was found to return in close conjunction with gastric emptying time. Elliot (1975[a,b]) stated that food consumption and evacuation rate in brown trout is greatly affected by the number of meals taken in a day or feeding frequency. The presentation of multiple meals at a defined feeding frequency would probably have complicated the present investigations with rainbow trout in relation to food intake and gastric evacuation rate. Although such an arrangement would have been of more physiological and practical relevance - single meals were fed ad libitum to rainbow trout at the start of the experimental period.

This approach enabled the food intake, gastric evacuation and digestion rates to be followed for a defined quantity of food with time and also allowed a single mathematical treatment of the data.

Much use has been made of various radiographic techniques to follow gastric evacuation rates in fish and to assess feed intake (Jobling et al., 1977; Flowerdew and Grove, 1978; Ross and Jauncey, 1981). These methods often involve the addition of radioopaque markers such as barium sulphate as contrast materials. Grove et al., (1978) reported that the inclusion of barium sulphate in test meals for rainbow trout affected gastric emptying rate. Barium sulphate has also been shown to have an irritant effect in some studies with higher animals (Sun et al., 1959). In addition, most studies which rely on contrast materials in test diets usually necessitate the force feeding of fish due to palatability problems. Recently, Talbot and Higgins (1983) developed a novel method for measuring food intake and gastric evacuation rates in juvenile atlantic salmon, Salmo salar. The technique involves adding a known amount of finely divided iron particles to the diet which can be related to the amount of food material present in different regions of the gastro-intestinal tract. These workers reported that no palatability problems were associated with the use of metallic powder in the diet as a marker which had the advantage of allowing fish to feed normally. Unfortunately, a similar technique could not be applied to the present investigations with rainbow trout because of the lack of suitable x-ray facilities at the time of this study.

Therefore the best alternative was considered to be the use of the comparitive slaughter method and rainbow trout were fed ad libitum in order to assess voluntary food intake.

It should be noted that the fish were deprived of food for a period of 72 hours prior to the commencement of the experiment to ensure complete evacuation of digesta from the previous meals. The 72-hour deprivation period was considered to be adequate, since Grove et al., (1978) reported that trout of a similar size to those used in the present study consumed a maximum amount of food 50 hours after the initial intake of a standard meal at 11-12^{o}C.

The results of the current investigation showed that a significant reduction occurred in the food intake of trout fed a coarse chitin diet compared to fish fed finely ground chitin.

It has been suggested by Vahl (1979) that appetite in fish may be largely dependent on the physical bulk of the stomach contents and the time taken for its removal. Other workers have shown that when a ration is diluted with various bulking materials, fish respond by increasing their feeding frequency in order to maintain a relatively constant energy intake (Lee and Putman, 1973; Grove et al., 1978; and Jobling, 1980[a]). However in this respect, the voluntary food intakes of rainbow trout fed the basal diet substituted with increasing levels of galactomannan were not significantly different. The two chitin treatments and the treatment containing the highest level of galactomannan were all directly comparable as each contained the same theoretical amount of fibre (20%). Although a greater food intake may be expected for the 20% galactomannan diet compared to the basal diet without added fibre, no significant differences were found. This may indicate that it is difficult for trout when presented with single meals to adjust their food intake in relation to dietary energy content, and may only do so by regulating multiple meal consumption over a longer time period.

Jobling et al., 1977; Flowerdew and Grove, 1979; Jobling and Davies, 1979 and Jobling, 1980[a], have all mentioned that the physical and chemical composition of a diet is very important in controlling food intake, the rate of gastric evacuation and digestion. Jobling (1981[a]) suggested that a correlation might exist between gastric evacuation rate and the level of specific nutrients in the diet, for example fat is known to slow gastric emptying rate to a greater extent than either proteins or carbohydrates (Jobling, 1980[b]), and gastric emptying time was found to be much faster in trout on high fibre diets according to Hilton et al., (1983). The differences observed in the food intakes of fish receiving the chitin diets were probably due to the physical differences in the particle size range of the chitin component of these diets. Although only slight differences existed for the crude fibre content of these diets, considerable variation was found in the particle size distributions of chitin. The effect of grinding fibre is to decrease its ability to hold water, presumably by breaking down regions of potential water holding capacity (McConnell et al., 1974). Brodribb and Groves (1978) reported that for rice bran, greater water retention was measured in coarse material than for finer grades. Coarse chitin also showed a characteristic swelling effect due to water uptake in vitro. It was noticed that the initial percentage moisture for the stomach contents of fish receiving the coarse chitin diet was significantly higher than in the fine chitin diet. Possibly the increased bulk associated with this capacity to retain water accounted for the reduction in food intake observed for the coarse chitin diet. Similarly, the treatment which contained the highest inclusion level of galactomannan resulted in the highest in vitro water retaining capacity, and also appeared to be quite viscous in

texture compared to the other diets after treatment with distilled water. This property may also help to explain the slightly reduced feed intake of rainbow trout fed 20% galactomannan.

The gastric evacuation rate data obtained in the experiment was found to conform to an exponential decay model when the passage of material was expressed on a dry weight basis for each of the treatments. This description of the emptying pattern in fish is in agreement with the studies of Tyler (1970); Brett and Higgs (1970); Elliot (1972); Persson (1979); Grove and Crawford (1980) in rainbow trout, plaice, turbot and roach. However the gastric emptying rate data could also be adequately fitted to a square root and surface area based model as described previously in section 6.2.6.

Implicit in the square root model is the assumption that the instantaneous rate of evacuation is dependent upon the amount of food in the stomach and that the pattern of evacuation of a small meal corresponds to the later stages in the evacuation of a larger meal. Therefore regression lines (relating the square root of the weight of food remaining in the stomach to post prandial time) for different meal sizes should theoretically have the same slope. This was not found to be the case however for the two chitin treatments. The slopes for the diets containing graded levels of galactomannan were found to be quite similar to the control diet. Although the square root model was a better fit for the data, the application of the surface area dependent model also resulted in good linearization of the gastric evacuation rate curves. There is very little information in the literature regarding the passage and digestion rates of dietary particles varying in surface area and size. Swenson and Smith (1973) were of the opinion that the

digestion of food particles begins on the outer surface and that a given sized meal composed of small particles would be digested and evacuated more rapidly from the stomach than a single large bolus of food. In contrast, Elliot (1972) found that rates of gastric evacuation in brown trout, Salmo trutta were unaffected by variations in the size and number of different food organisms and their relative surface areas. Of particular relevance to the present study was the particle size distribution and surface area effects of the undigestible fibrous fraction in the form of chitin.

The decreased gastric evacuation rates for the coarse chitin diet was confirmed by each of the models fitted to the data. This finding is also supported by the observations of Kionka and Windell (1972) for rainbow trout. Their data showed that large pieces of undegraded chitin are retained in the stomach after all other visible organic matter is evacuated. The delay in the gastric removal of chitin was attributed to the size of the pieces, and that particles larger than the diameter of the pyloric valve may require softening and further grinding prior to leaving the stomach. Edwards (1971) and Western (1971) also suggested that narrow sphincters and intestinal lumens restrict the passage of large undigestible food components from the stomachs of fish. Windell et al., (1969) believed that the stomach may withhold its contents until a certain degree of liquefaction and digestion is attained before material is released into the intestine. The change in the physical conditions of various food items as digestion proceeds has been reported by Hunt (1960), Western (1971) and Jones (1974). These workers suggested that the greater the percentage or particle size of chitin or shell material present in the diet, then the longer it remains recognisable in the stomach. McDonald and Waiwood (1982) proposed as a result of studies on the digestion rates of different prey items

in ocean pout, Macrozoares americanus; atlantic cod, Gadus morhua; winter flounder, Hippo glosoides platessoides and the american plaice, Pseudo pleuronectes americanus, that the rate of evacuation and digestion will "level off" at a point determined by the percentage of indigestible material within the food, notably chitin. The results of the present study in which the predicted gastric emptying time was greater for the coarsely textured chitin is in agreement with these findings. It was mentioned that the volume and surface area models adequately linearized the data for each of the treatments, but it was interesting that the volume model provided the better fit. Jobling (1980)[a] proposed that the volume of stomach material is the most important determinant of the shape of the gastric emptying curve with the dietary particle surface area possibly acting as a modifying factor on the rate of evacuation. This would confirm that the differences between the gastric emptying profiles of the chitin diets was mainly a function of physical bulk rather than a surface area effect. It was expected that the volume dependent model might be a better fit for the galactomannan and control treatments, as these diets were of similar particle size composition and so large differences in the surface area of particles would not have been an important factor. However, the relatively high water retaining capacity of galactomannan may have affected the volume of digesta in the stomach.

Unfortunately, most of the recent gastric evacuation and digestion rate studies involving fish and the mathematical descriptions applied to the emptying patterns have been reported for species consuming natural food organisms. The pattern of evacuation in the adriatic roach, Rutilus rubio, chub, Leuciscus cephalus and maranec, Pactychilion pictum fed natural prey items was satisfactorily described by exponential curve fitting (Kitchell, Stein and Knezene,

1978). Grove and Crawford (1980) also found that the intestinal evacuation rate in the stomachless blenny, Blennius pholis was basically exponential in character. Persson (1979; 1982) described similar evacuation rate patterns for perch, Perca fluviatilis fed encapsulated chironomids and various zoo plankton organisms. The relationship between the gastric evacuation rate pattern as affected by two types of prey items, squid and bivalves in the winter flounder, Pseudo pleuronectes americanus was examined indirectly by measuring the rate of hunger return (Heubner and Langton, 1982). Again, although a linear model was found to adequately define the decay rate of a bivalve meal, an exponential model gave the best overall fit to the stomach evacuation rate data.

A degree of caution is required before interpreting the results obtained from the present investigation with rainbow trout on the basis of these other findings. Frequently, workers have neglected to specify the size of the fish used in their experiments, the meal size, or dietary composition and have often compared evacuation rate data obtained under different environmental conditions. Sometimes the experimental design may be criticized for influencing the gastric emptying process. For example, Swenson and Smith (1973) in their studies with walleye, Stizostedion vitreum vitreum, found a linear relationship between stomach content and time, but excluded information on gastric evacuation beyond 90% digestion perhaps unaware that digestion rates generally decrease beyond this stage. Jones and Green (1977) force fed dogfish, Scylliorhinus canicula in their experiments, without mentioning the possible bias associated with this method, and it should be noted that indirect estimates of gastric evacuation rates in fish based on the return of appetite, are not realistic substitutes for the accuracy obtained using a sacrificial technique. A number of

workers have reported gastric emptying and digestion rates for fish fed multiple meals on a daily basis. Persson (1982) tested a model for estimating food consumption in roach, Rutilus rutilus L. in relation to the evacuation of gut contents. Hofer et al., (1982) in similar experiments with roach was correct to point out that many investigations have been limited to the study of single rations, administered at the start of gastric evacuation rate studies. Such feeding schedules are different from natural conditions, at least for omnivorous and herbivorous fish with undifferentiated intestinal tracts. Therefore their relevance to fish with well defined stomachs such as trout is questionable. Perhaps of more significance to the present study, is that the physiological demands upon the digestive system of a fish consuming dry pelleted food is likely to be quite different compared to a meal consisting of natural food organisms (Windell et al., 1969).

It was reported by Windell et al., (1978) that the viscosity of the stomach contents may not be a significant factor influencing gastric emptying rates in trout. These workers reasoned that the preground ingredients of commercially available, pelleted diets are easily liquified in water. It was also suggested that artificially prepared diets may require a longer period in the stomach than natural foods, even though they may be of finely ground consistency. The reason is not fully understood but may be related to particle size composition, moisture content and general texture. In this respect, the physiological response of rainbow trout to different processing techniques such as steam pelleting and extrusion of diets was investigated by Hilton et al., (1981). Trout reared on extruded pellets displayed prolonged gastric emptying in comparison with those fed steam pelleted diets. These fish also showed a

reduced daily food intake. It was also observed that prolonged contact with water caused expansion of extruded pellets and it was suggested the resulting increase in bulk was an important contributing factor in explaining the effects shown by this type of diet. The digesta produced by fish consuming the steam pelleted diet appeared fine and watery, whilst that of extruded diets was coarse and lumpy. Similarly, in the present study with rainbow trout the diets containing galactomannan and chitin caused the digesta to differ quite visibly in texture. The high galactomannan (20%) diet resulted in a viscous intestinal content which appeared to be associated with a large amount of mucous, whilst the coarse chitin diet was almost 'gritty' in appearance in the lower intestine of trout. It was stated in the results section, that in vitro measurements concerning the water holding capacity of the various diets produced a good correlation between the percentage water retained by dietary material and the level of galactomannan. In this respect, the diets were considered to be useful models demonstrating possible effects on gastric emptying and digestion rate arising from differences in moisture content and/or viscosity.

A sharp increase was observed in the moisture level of the stomach contents with time for both chitin and galactomannan treatments. This was especially apparent during the first 12 hours associated with the rapid phase of gastric emptying. However, the results showed a significant decline in the rate of moisture uptake for the chitin diets compared to the control and galactomannan treatments after this period. This might be partly explained by the fact that gel-forming polysaccharides such as galactomannan absorb water due to the osmotic pull exerted by counter ions attracted to the charged side groups. Water uptake continues until the gel matrix of the material produces sufficient elastic resistance to balance

the osmotic pressure (Stephen and Cummings, 1979).

A similar increase in moisture uptake and liquifaction of stomach contents has also been shown to occur in other animals (Malagelada, 1977). This investigator attempted to distinguish between gastric emptying patterns for solid and liquid meal components and to quantify the discriminating function of the stomach in allowing differential evacuation of solids and liquids into the intestinal tract. It was also suggested that partial digestion and solubilization decreases the volume of the intra-gastric solids whereas endogenous secretions greatly expand the liquid phase of gastric contents.

The concept of the stomach only emptying the solid particulate component of a meal may therefore have little physiological significance since a) different fractions of a meal can be emptied independently and b) the composition of the meal is constantly modified by gastro-intestinal secretions which expand the liquid phase and reduces the volume of the solid phase during the course of digestion. In order to accurately assess stomach emptying, it is necessary to simultaneously quantify the evacuation pattern of the complete meal and physical components. Most of the gastric evacuation rate studies performed with fish may be criticised for their failure to take into consideration the solid/liquid model to describe the composition of digesta, and investigations have been confined to the evacuation of food material on a dry matter basis. For a more complete understanding of the action of chitin and galactomannan fibre sources on water uptake by digesta and gastric evacuation rate, the original wet weight of the stomach contents remaining with time for each dietary treatment was also fitted to the same mathematical models described previously. As the results show, gastric evacuation rates for the combined solid-liquid phases

of the digesta closely resembled that of the solid phase alone and similar trends were evident for the predicted gastric emptying times. It was noted however, that the gastric evacuation times were generally greater for the combined digesta than those predicted for digesta on a dry weight basis alone. This delay in stomach emptying was assumed to be due to the retention of water and was considered to be a useful index of the water retaining power of the different dietary fibres. The delay in the evacuation of solid/liquid digesta was relatively small for the chitin treatments but was much greater for diets containing galactomannan. The delay in evacuation for the liquid phase of the galactomannan diets may be further evidence of the greater water retaining capacity of this material and would help explain the prolonged "hump effect" noticed for the evacuation rate patterns of the galactomannan diets expressed on the wet weight basis. The extent and duration of the 'hump' was probably a reflection of the increased affinity of the digesta for moisture. A similar pattern for the evacuation of separate solid and liquid components of digesta from the stomach has been reported by Low et al., (1978) in digestion and absorption studies with young pigs. These workers were of the opinion that the amount of gastro-intestinal secretions absorbed by digesta, depends to an extent on the digestibility of the diet and that the moisture content is usually maintained at a level providing uniform osmolarity. Evidence that such a mechanism may operate in the gastro-intestinal tract of rainbow trout was shown by the static phase in the moisture level of stomach digesta for the chitin treatments. The relationship of dietary moisture to evacuation and assimilation of food is important. A meal containing a large amount of digestible organic matter may exhaust the acid secretory capacity of the stomach (Norris et al., 1973) affecting assimilation and growth

efficiency. In a recent study by Garber (1983) there was no significant difference between the digestion rates of a standard diet containing 4.7% moisture and the same diet diluted to 24.1% moisture when fed to yellow perch, Perca flavescens. Flowerdew and Grove (1979) also found that there was no significant difference in evacuation rates in turbot when a fishmeal diet was replaced with water on a percentage basis. Poston (1974) however, implied greater evacuation of moist diets by trout fed pelleted meals of varying moisture content. Similar findings were reported by Grove et al., (1978).

In the current investigation, the moisture of the stomach contents resulting from the galactomannan and control diets were not significantly different from each other. This suggests that some other factor, possibly the viscosity of dietary material is responsible for the delayed gastric emptying observed with increasing levels of galactomannan. It was expected that the control diet (without added fibre) would show a reduced gastric emptying time, given that increasing levels of galactomannan prolonged gastric emptying. However the gastric emptying time for the diet containing 5% galactomannan was less than the control diet. This implies that at low inclusion levels, galactomannan imparts sufficient bulk to promote faster transit of stomach contents but at higher levels (in association with the same amount of water) the viscometric property of the fibre may slow down the passage of food.

A significant linear relationship existed between dietary galactomannan level and the percentage moisture of intestinal digesta hours following meal consumption. Of interest, was the reduced slope of the regression line describing the retention of moisture in vivo compared to the relationship obtained from in vitro

studies (Figure 6.10). This suggests that water uptake by intestinal digesta is controlled and influenced by other factors. Therefore *in vitro* measurements of water retention by diets only serves as a useful index, and cannot be used to accurately predict the *in vivo* effects of the various treatments.

Several investigators have reported that when certain dietary fibres are fed to experimental animals, a substantial part disappears during its passage through the gut (Milton-Thompson and Lewis, 1971). The implication of these observations are twofold; either digestion in the gut totally destroys the capacity of fibre to hold water or secondly the fibre remaining in the intestinal residue continues to exert an effect, but the capacity to do this is dependent upon how much fibre survives digestion. Cummings *et al.*, (1979) stated that dietary fibre acts mainly in the large intestine due to the greater retention and concentration of undigestible matter in that region of the gut. The large intestine is also the main site of water and mineral re-absorption and where faecal material is compacted prior to excretion. In the present study with trout, the flow of digesta in the intestine for the chitin diets on a wet weight basis reflected the gastric emptying patterns. These observations together with the effect of graded levels of galacto-mannan on the moisture content of intestinal digesta support the earlier conclusions in Chapters 4 and 5 that chitin and galacto-mannan resist digestion in rainbow trout.

The rate of digestion is known to be much slower for fish than mammals and the processes of absorption are bound to be influenced by the passage rate of food through the alimentary tract (Possompes *et al.*, 1975). According to Fauconneau *et al.*, (1983), the passage rate of dietary particles through the alimentary tract in

piokilotherms as in homeotherms is a function of digestive balance and is important in determining the availability of nutrients. Garber (1983) mentioned that increased transit would minimize the potential benefit of any increased food intake. These alterations would occur independently of any changes in the synthesis of digestive enzymes and the absorption rate of nutrients. This means that fish might not necessarily show impaired nutrient utilization when transit time is relatively fast. It also follows that a slower passage of digesta does not always imply better utilization of nutrients. It was originally intended in this investigation to assess digestibility in the stomach and intestinal compartments separately for the treatments using chromic oxide as an inert reference marker. Unfortunately, the mixing of partially digested stomach contents with intestinal material and the pooling of samples for analysis prevented the application of this technique. To overcome this problem, the extent of digestion was based on the combined amount of stomach and intestinal residium obtained for each sampling period, expressed as a percentage of the initial weight of food consumed. Van Soest (1983) suggested that slaughter of the animal is perhaps the only entirely accurate method of assessing the pool size of material in each gastro-intestinal compartment. It has the disadvantage however of not being amenable to time studies since an animal is sacrificed at a single point in time of which the data is representative.

A similar method was used by Windell (1966) for studies with blue gill sunfish and by Swenson and Smith (1973) with walleye. These latter workers fitted polynomial regression equations to their data and showed that a linear relationship existed between percentage digestion and time. Karpevitch and Bakoff (1937) described digestion as a process comprised of an 'effection' phase

during which up to 90% of the food is digested rapidly and a 'residual' phase where digestion is relatively slow. The residual phase is variable in duration and is influenced by the level and type of dietary fibre. Magnusson (1969) and Tyler (1970) have also reported that digestion rates decrease during the later stages in fish. For these reasons, no attempt was made to transform the digestion rate data obtained in the present study and the results were presented graphically to show the various stages of digestion (Figures 6.7.1-6). It was noted that considerable digestion had occurred for the chitin diets after approximately 12 hours and this was in agreement with the data of Windell (1966) obtained for blue gill sunfish. Since digestion rates are closely related to gastric emptying and intestinal transit time, it was not surprising that a considerable reduction was observed in the digestion rate of the coarse chitin diet compared to the fine chitin treatment. This effect was probably due to the greater stomach retention of coarse chitin particles which accounted for 20% of the diet.

The initial lag in the digestion rates of diets containing 10 and 20% galactomannan was also probably due to the slower release of stomach contents during this period. Other workers have also reported a lag phase prior to the rapid stage of digestion. It is known that enzyme reactions are exponential in nature and therefore start slowly (Jennings, 1972). In experiments where a starvation period preceeds the experiment, the digestive secretions may not be present when food first enters the stomach (Elliot, 1972). However this is not a satisfactory explanation for the effects seen in the present study since the deprivation times were the same for each treatment. Windell (1967) proposed that the time lag depended mainly upon the resistance of the foods surface and on the ratio of the surface area to volume. One further consideration is that food

may enter the intestine before it is fully digested, the stomach acting as a holding facility and emptying its contents as the intestinal material permits.

This would help explain the uniform concentration of chromic oxide present in the intestinal digesta for each of the treatments at successive time intervals, which made indirect measurements of digestibility difficult, and also accounts for the uniform passage of digesta through the intestine on a dry weight basis.

The protein digestion rate was found to parallel but slightly exceed that of the dry matter for the experimental diets. This was expected, since protein is the major component of the diet and is relatively more accessible than the remaining fractions which included fibre. Windell (1966)" also determined the rate of protein digestion for blue gill sunfish. It was found that 25.1% of the protein intake was either digested, absorbed or passed into the intestine during the first 6 hours following the consumption of a single meal fed ad libitum. Moreover, 44.7, 55.3 and 82.7% digestion of protein was attained after 10, 14, 18 and 22 hours respectively. It was also reported that the rate of protein and organic matter digestibility was parallel and the amount of protein digested per unit time was relatively constant. Only small amounts of protein were found in the intestine which supported an earlier finding that the efficiency of protein absorption for blue-gills is approximately 97%.

An important consideration made by Windell was that the nitrogen content of digesta does not entirely originate from the meal. The amount of nitrogen present in the digestive enzymes or in the bacterial flora is not known; consequently the calculated values for

protein digestion may be slightly low.

Of particular relevance to the investigation with rainbow trout, was the appreciable amount of non-protein nitrogen (NPN) contributed by chitin. Although it was reported in Chapter 4 that rainbow trout cannot utilize this NPN, its presence would have overestimated the crude protein content (NX6.25) of dietary material and digesta. The problem was avoided by using the Lowry protein estimation method which estimates α-amino acid protein directly and does not include non-protein nitrogen. However, a disadvantage of the method is that it is affected by the amino acid profile of proteins and is therefore not ideal for comparing the protein levels of different feed ingredients. The same protein source was used in the experimental diets containing chitin and galactomannan and so the amount of protein in corresponding digesta samples were directly comparable.

In conclusion, these studies have clearly shown that particle size composition, viscosity and the water retaining properties of dietary fibres are important factors which may influence gastric evacuation rates in rainbow trout.

The delay in the passage rates for diets containing coarsely graded chitin and galactomannan was associated with a lowered food intake for rainbow trout fed these diets and affected the apparent digestion rates of dry matter and protein. These results were in accordance with the findings expressed in Chapters 4 and 5, and further help to explain the reduced appetite and utilization of nutrients in rainbow trout receiving coarse chitin and galactomannan as sources of dietary fibre. Finally, the investigation confirmed that dietary fibre affected gastric emptying rate in trout.

CHAPTER 7

GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS.

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The introductory chapter served to review the modern definitions and concepts of dietary fibre in relation to the nutrition and digestive physiology of experimental animals and man. Dietary fibre was shown to be a generic term for a wide variety of natural and synthetic substances present in various feed ingredients and commercial diets. By definition, fibre is not appreciably digested during its passage through the gastro-intestinal tract and the physical and chemical characteristics were considered to be important factors that could influence the processes of digestion and therefore the performance of growing animals. Although the research literature is extensive in this respect, there appeared to be a lack of information for fish, especially carnivorous species such as trout.

The preliminary experiment outlined in Chapter 2 was necessary in order to establish the nutritional parameters most likely to be affected by feeding dietary fibre to rainbow trout. The investigation was useful in directing attention towards sources of dietary fibre other than α-cellulose. A range of purified sources of fibre were tested at a low inclusion level (5%) in semi-purified diets which were formulated to meet the known nutritional requirements for growing rainbow trout. These diets included lignin, lignosulphonate, galactomannan, chitin, polyethylene as well as α-cellulose as examples of dietary fibre.

The aim of this particular study was to show the diversity in the texture, physical and chemical characteristics of these materials

in relation to their effects on growth performance and utilization of nutrients by rainbow trout. Using chitin and galactomannan as examples, it could be seen that dietary fibre may either be particulate in nature: existing in a wide range of particle sizes, or soluble in nature: associating with aqueous solutions to produce viscous gels.

Although only slight differences were noticeable for the growth performance of trout fed the experimental diets after 10 weeks, it was reasoned that more pronounced effects, especially on certain nutritional parameters could be obtained at higher inclusion levels of fibre, using chitin and galactomannan as particulate and gel-forming sources.

Criticism was made of the failure by many workers to consider the wider implications of the term dietary fibre in relation to studies with fish. Some have preferred instead to limit experiments to the use of α-cellulose as the main fibre component. Inclusion of α-cellulose was frequently made at the expense of other nutrient components of diets which introduced other experimental variables. It was therefore necessary to establish whether refined α-cellulose is sufficiently inert to be considered as a non-nutritive bulking material in test diets for trout before embarking upon further studies involving different dietary fibre sources. In this way, α-cellulose could be substituted by these materials, thereby acting as a control variable and ensuring that the overall nutrient balance of dietary treatments was kept constant. The growth and digestibility trial outlined in Chapter 3 was conducted primarily for this purpose and partly as a result of the conflicting reports expressed by other workers regarding the effects of α-cellulose on the growth performance of fish. In

this study, specific biochemical analyses were developed to separately quantify available and non-available carbohydrate fractions in diets and faeces. This fractionation approach was considered to be an improvement on previous methods of crude fibre analysis which fail to discriminate between different dietary fibre components. The results of the experiment in Chapter 3 showed that graded levels of α-cellulose (up to 20% of the diet) resulted in no significant differences in growth and only slightly affected nitrogen utilization at higher levels. Below 15% inclusion, the negative apparent digestibilities for unavailable carbohydrate was taken as evidence that α-cellulose is largely undigested by rainbow trout and is perhaps selectively retained in the gastro-intestinal tract depending upon dietary concentration. Having clarified this point, suitable experiments were designed to study in more detail the effects of particle size composition and the gel-forming characteristics of fibre on the performance of trout.

The experiment involving chitin, showed that the purified material was a natural source of particulate fibre. Again, the negative apparent coefficients of digestibility for chitin based on a specific method of analysis for component sugars in dietary and faecal samples showed that chitin could not be degraded by rainbow trout under the experimental conditions. Although it is accepted that the grinding of feed ingredients improves their digestibility and nutritive value, there is no information in the literature specifically related to the effects of dietary fibre particle size distribution and texture on growth performance and nutrient utilization in fish.

From the study, attention was focused on the significantly reduced food intake of rainbow trout receiving the diet containing a high level of coarsely graded chitin compared to fish fed a diet with the same level of finely ground material. Particle size effects of dietary fibre in the form of chitin were also apparent at the lower inclusion level of 10%, and for all treatments, a definite trend was observed between the dietary levels of coarse chitin and the apparent digestibility of fibre.

Dry matter and nitrogen digestibilities were markedly affected by the high concentrations of coarse chitin which may in turn have explained the reduced dietary nitrogen utilization for this treatment. It was assumed that the non-protein nitrogen content of chitin was unlikely to be a contributing factor in view of the resistance of chitin to degradation in rainbow trout.

Another feature of the experiment, was the comparitive use of two different digestibility markers. Concern has been expressed by a number of workers regarding the validity of digestibility coefficients based on chromic oxide. For this reason, polyethylene was included as an alternative marker with a density closer to that of the experimental diets. Digestibility coefficients based on the two respective markers were found to be in general agreement for dietary components having a low digestibility. However the results deviated from each other in some of the treatments. Although a new technique was developed for determining polyethylene in diet and faecal samples, the chromic oxide method had the advantage of being more amenable for the routine analysis of samples.

In Chapter 5, a purified galactomannan was used as the source of dietary fibre with contrasting physical and chemical

characteristics compared to chitin. Addition of the fibre was made at the expense of α-cellulose to give a dietary fibre complex varying in composition.

Unfortunately the differences obtained in the growth of rainbow trout receiving increased levels of galactomannan were only a long term trend and was not shown to be significantly different. The experiment was of interest, bearing in mind that although galactomannan is an example of a hemicellulose type plant fibre, the gel and water retaining properties are similar to certain binding agents commonly used in moist diet preparations for fish. Therefore the use of moist diets containing galactomannan was appropriate for this experimental investigation and had the advantage of stimulating the appetite of rainbow trout during the cold winter months of the trial. The low water temperature also necessitated an extended growth period of 4-months to ensure reasonable growth and to establish any long-term physiological changes that might have resulted from the dietary treatments. There was no evidence to indicate that galactomannan was degraded by rainbow trout. The negative digestibility coefficients for the fibre based on a fractionation procedure for measuring galactomannan in diets and faeces suggested a certain degree of retention for the fibre in the gastro-intestinal tract.

Since digestion rate and the activity of the gut microflora are greatly affected by temperature, then an obvious extention of this study would be to conduct the same experiment at a higher temperature in accordance with the optimum conditions for growth.

It was interesting to find that the reduced performance of rainbow trout fed increasing amounts of galactomannan should also

be reflected by the lower concentrations of serum protein and glucose. The usefulness of these parameters as an index of nutritional status is debatable due to the variation of these levels in normal fish blood. Care was taken in the study to obtain representative samples of blood at the same post prandial time period for each of the treatments in order to minimize variations in nutrient levels with time. Perhaps in future it might be possible to establish a standard baseline for the systemic levels of major nutrients using a large number of healthy fish fed a specified diet. This would be quite useful for comparitive studies with fish receiving experimental diets.

The reduction in serum protein and glucose concentration supported the views of other workers that gel polysaccharides such as galactomannan may interfere with the processes of digestion and the absorption of nutrients. These effects together with the reduction in daily food intake would explain the reduced digestibilities and poorer utilization of nutrients observed for trout receiving high levels of galactomannan.

It can be argued however that the concentrations of gel polysaccharide used in the present study were much higher than the levels that would be used as binders in production-type diets for trout. However it is reasonable to speculate that certain binding agents may produce similar effects at lower levels when fish are growing at their maximum rate over a considerable period of the production cycle. In this context, it would be interesting to compare the effects of different commercial binders at realistic dietary inclusion levels on the performance of rainbow trout.

An important criticism that can be directed towards the growth and digestibility trials presented in this thesis was the lack of

statistical evidence to support the differences observed for the various nutritional parameters. Greater replication within treatments would have been preferable, but the pooling of fish and other sample materials was often unavoidable due to the scale of the investigations and the limitations imposed by the design of the experimental system. It would have been an advantage had the studies been conducted using a large number of individually tagged fish, grouped into smaller tanks for each treatment. Although this was not practical for the conditions provided, such an arrangement would have allowed better statistical interpretation of the data and facilitated the collection of blood and faecal samples.

The investigation outlined in Chapter 6 was mainly intended to complement and expand on the theme of the previous growth and digestibility trials involving chitin and galactomannan. It was obvious however that these experiments alone could not verify whether the observed effects were due to the increased physical bulk of the stomach contents or to more fundamental processes such as food passage time and digestion rate.

Gastric evacuation and digestion rate studies with chitin confirmed that trout have a capacity to selectively retain different sized particles of fibrous material and that a high level of coarsely ground chitin reduced food intake and overall digestibility. Galactomannan also reduced food intake at the higher inclusion level and this effect was probably due to a combination of the increased bulk of the stomach contents and a slower gastric emptying rate. The experiments showed that the water retaining capacity of dietary fibre is an important factor influencing these parameters and this was especially apparent

for galactomannan.

In general the results were compatible with the findings of the previous experiments and confirmed earlier speculations that the primary effects of dietary fibre were at the gastro-intestinal level in relation to the control of food intake, passage time and consequently, the efficiency of digestion.

Mathematical interpretation of the gastric emptying rate patterns was an obvious consideration in view of the many models proposed by other workers. The linear transformation of the exponential curves using both surface area and volume dependent relationships implied that these physical factors are important and contribute in the shaping of the gastric emptying rate pattern in rainbow trout.

If the particle size composition and physical bulk of undigestible dietary components can influence gastro-intestinal processes to such an extent, then this raises a number of questions regarding the texture and physical state of feed ingredients with a high nutritional value. Many cereal and animal by-products as well as protein concentrates vary widely in texture and physical composition. The relationship between these characteristics and digestion warrants further study in rainbow trout. Such investigations would be of practical use in evaluating the potential of different ingredients and designing the best processing techniques for optimizing their use.

In conclusion, the nutritional value of dietary fibre for rainbow trout is still open to question, since much depends upon the source, nature and the amount of fibre present in the diet. The impaired performance of trout fed certain types of dietary

fibre probably resulted from a combination of factors including changes in the gastric emptying and digestion rates which in turn affected food intake and the efficiency of nutrient utilization.

Of particular importance, was the use of purified dietary fibres of defined character throughout these investigations which was in marked contrast to the limited studies of previous workers. This approach enabled separate evaluations for the different properties of fibre and a control over the number of variables in experimental diets.

Finally, the results showed that there was no nutritional advantage gained by including purified fibres in the diets of rainbow trout. An exception however, might be the addition of α-cellulose as a control variable in experimental investigations.

It is hoped that the findings and ideas presented in this thesis offer a basis for more extensive studies involving trout. It would appear that the nutritional implications of the complex dietary fibre mixtures present in natural feed ingredients and the use of various commercial additives such as binders in production diets offer much scope for further research.

REFERENCES

Akiba, Y. and Matsumoto, T. (1977) Effects of dietary cellulose on fat absorption in chicks. Jap. J. Zootech. Sci., Vol 49, No. 5, p351-356.

Alginate Industries Limited, Technical report (1980). 15 pages. 22 Henrietta St., London.

Ali, R. Staub, H., Coccodrilli, G. Jr., and Schanbacher, L. (1981). Nutritional significance of dietary fibre: Effect on nutrient biovailability and selected gastrointestinal functions. J. Agric. Food Chem., Vol 29, p465-472.

Alliot, E. and Bocquet, J. (1967) Presence d'un systeme enzymatique chitinolytique dans le tube digestif d'un selacien: Scyliorhinus canicula. CR. Soc. Biol., Vol 161, p840-845.

Anna, L., Low, A.G. and Sambrook, I.E. (1983) National Institute for Research in Dairying, Shinfield, Reading RG2 9AT. Lack of effect of guar gum on gastric emptying in pigs. (In press)

A.O.A.C. (1980) Official Methods of Analysis, 13th ed., Association of Official Analytical Chemists, Washington, D.C. 1018pp.

Austreng, E. (1978). Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture, 13, p265-272.

Baird, D.M., McCampbell, H.C. and Allison, J.R. (1975) Effect of level of crude fibre, protein and bulk in diets for finishing hogs. J. Anim. Sci., 41, p1039-1047.

Barrington, E.J.W. (1957) Chitinase activity in marine vertebrates. The Alimentary canal and digestion. The physiology of the fishes, I, p109-161. Ed. by Brown, M.F. Academic press, New York, 1957.

Beamish, F.W.H. (1972) Ration size and digestion in largemouth bass, Micropterus salmoides and Lacepede. Can. J. Zool., 50, p153-164.

Bergner, H., Simon, O., Zimmer, M. (1975) Einfluss des Gehaltes nativer rohfaser in diaten von ratten auf die aminosaurenresorption. Arch. Tierernahr., 25., p95-104.

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

Bjorn-Rasmussen, E. (1974) Iron absorption from wheat bran. Nutr. Metabol., 16, p101-110.

Bondi, A., Spandorf, A. and Calmi, R. (1957) The digestibility of fibre in diets for carp. Bamidgeh., 9, p13.

Bowen, S.H. (1979) Chromic oxide in food assimilation studies. Comments. Trans. Amer. Fish. Soc., 108, p650-652.

Braude, R. (1967) The effects of changes in feeding patterns on the performance of pigs. Proc. Nutr. Soc., 26, p163-181.

Brett, J.R. (1971) Satiation time, appetite and maximum food intake of sockeye salmon Oncorhynchus nerka. J. Fish. Res. Bd. Can., 28, p409-415.

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. Can., 27, p1767-1779.

Brodribb, A.J.M., and Groves, C. (1978) Effect of bran particle size on stool weight. Gut., 19, p60-63.

Brown, W.H., Ramsey, J.C. and Gehring, P.J. (1974) The lack of significant absorption of methyl-cellulose, viscosity 3300cP, from the gastrointestinal tract following single and multiple oral doses to the rat. Food Cosmetics Toxicol., 12, p373-376.

Buhler, D.R., Halver, J.E. (1961) Nutrition of salmonid fishes IX, The carbohydrate requirement of chinook salmon. J. Nutr. 74, (3), p307-318.

Burkitt, D. (1981) Diseases related to bowel content. Chapter 6. p46. In Don't Forget Fibre in your Diet. by Denis Burkitt. Pub. by Martin Dunitz Ltd., London.

Burkitt, D.P., Walker, A.R.P. and Painter, N.S. (1972) Effect of dietary fibre on stools and transit-times, and its role in the causation of disease. The Lancet, December 30th, 1972, p1408-1411.

Canguilhem, R., Labie, C. (1977) Variation de la duree du transit intestinal chez le porc selon la teneur de la ration en cellulose. Revue. Med. Vet., 128, p1669-1681.

Cho, C.Y. and Slinger, S.J. (1977) Significance of digestibility measurement in formulation of feeds for rainbow trout. Proc. 13th Annu. Nutr. Conf. for Feed. Manuf. Toronto, Can., May 4-6, p15-19.

Clemens, E.T. and Stevens, C.E. (1980) A comparison of gastro-intestinal transit time in ten species of mammal. J. Agric. Sci. Camb., 94, p735-737.

Colin, D.A. and Peres, G. (1971) Etude comparee des systemes enzymatiques chitinolytiques du tube digestif de quelques teleosteens marins. Ann. Inst. Michel Pacha., 4, p1-9.

Cornelius, C. and Dandrifosse, G. (1977) Substrate specificity of the β-1,4-N-acetyl glucosaminidases of vertebrates. Biochem. Systematics and Ecology, Vol 5, p53-56.

Corning, T. (1980) The adaptation of digestive enzymes to the diet. Reprod. Nutr. Develop. 20 (4B), p1217-1235.

Cowey, C.B. and Sargent, J.R. (1979) Fish nutrition. (Review) In Fish Physiology, Vol VIII, p1-69. Academic Press, Inc.

Crapo, P.A. Insel, J., Sperling, M. and Kolterman, O.G. (1981) Comparison of serum glucose, insulin and glucagon responses to different types of complex carbohydrate in non-insulin-dependent diabetic patients. Am. J. Clin. Nutr., 34, p184-190.

Crapo, P.A., Reaven, G. and Olefsky, J. (1977) Post prandial plasma glucose and insulin responses to different complex carbohydrates. Diabetes., 26, p1178-1184.

Cummings, J.H. (1980) The Colon. Recent advances in Gastroenterology, Vol. 4, p87-116.

Cummings, J.H., Southgate, D.A.T., Branch, W., Houston, H., Jenkins, D.J.A., James, W.P.T. (1978) Colonic response to dietary fibre from carrot, cabbage, apple, bran and guar gum. Lancet., i, p5-9.

Cummings, J.H., Southgate, D.A.T. and Branch, W.J. and Wiggins, H.S. (1979) The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. Br. J. Nutr., 41, p477-485.

Dabrowski, K. (1979) Feeding requirements of fish with particular attention to common carp. A review. Pol. Arch. Hydrobiol., 26(1/2); 135 - 158.

Dandrifosse, G., Schoffeniels, E., and Jeuniaux, Ch. (1965) Secretion de chitinase par la muqueuse gastrique isolee. Biochimica Biophysica Acta., 94, p153-164.

Delorne, C.B., Wojcik, J., and Gordon, C. (1981) Method of addition of cellulose to experimental diets and its effects on rat growth and protein utilization. J. Nutr., Vol. 111, No. 9, p1522-1527.

Dubois, M., Gilles, J.K., Hamilton, P.A., and Smith, F. (1956) Colorimetric method for determination of sugars and related substances Anal. Chem., 28, p350-356.

Duncan, D.B. (1955) Multiple range and multiple F tests. Biometrics., Vol 11, p1-4.

Dupree, H.K. and Sneed, K.E. (1966) Response of channel catfish to different levels of major nutrients in purified diets. U.S. Dept. Interior, Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife. Technical Paper. No.9, 21pp.

Eastwood, M.A. and Hamilton, D.(1968) Studies on the absorption of bile salts to non-absorbed components of diet. Biochem. Biophys. Acta., 152, p165.

Eastwood, M.A., Martin, A., Mitchell, N.D. (1976) Physical properties of a fibre: a biological evaluation; in Spiller, Amen, Fiber in human nutrition. p109-129. (Plenum Press, New York, 1976).

Edward Baker, Ltd. Animal Feed Manufacturer Technical report (1982), from Edward Baker Ltd., Bathgate, Scotland.

Edwards, D.J. (1971) Effect of temperature on rate of passage of food through the alimentary canal of the plaice, Pleuronectes platessa L. J. Fish. Biol., 3, p433-439.

Elimarn, M.E. and Orskov, E.R. (1983) The effect of diet composition and particle size on the outflow of chromium-mordanted protein supplements from the rumen of high yielding dairy cows. Proc. Nutr. Soc., 42, 31A.

Elliot, J.M. (1972) Rates of gastric evacuation in brown trout, Salmo trutta L. Freshwat. Biol., 2, p1-18.

Elliot, J.M. (1975)[a] Number of meals in a day, maximum weight of food consumed in a day and maximum rate of feeding for brown trout, Salmo trutta L. Freshwat. Biol., Volume 5, p287-303.

Elliot, J.M. (1975)[b] Weight of food and time required to satiate brown trout, Salmo trutta L. Freshwat. Biol., Volume 5, p51-64.

El-Shamy, F.M. (1976) Analyses of gastric emptying in bluegill (Lepomis macrochirus). J. Fish. Res. Bd. Can., 33, p1630-1633.

Elson, L. and Morgan, W. (1934) A colorimetric method for the determination of N-acetyl-glucosamine and N-acetylchondrosamine. Biochem. J., 28, p988-995.

Evans, E. and Miller, D.S. (1975) Bullring agents in the treatment of Obesity. Nutritional Metabolism, 18, p199-203.

Fahey, G.C., Frank, G.R., Jensen, A.H. and Masters, S.S. (1980) Influence of various purified and isolated cell wall fibres on the utilization of certain nutrients by swine and hamsters. J. Food Sci., 45, p1675-1680.

Fahey, G.C., Miller, B.L. and Hadfield, H.W. (1979) Metabolic parameters affected by feeding various types of fibre to guinea pigs. J. Nutr., Vol. 109, No.1, p77-83.

Fahey, G.C., Williams, J.E. and McLaren, G.A. (1976) Influence of molasses lignin-hemicellulose fractions in rat nutrition. J. Nutr., 106, p1447-1451.

Fange, R. and Grove, D.J. (1979) Digestion. In Fish Physiology. (W.S. Hoar, D.J. Randall and J.R. Brett, Eds.), Vol VIII, p161-260. New York and London: Academic Press.

F.A.O. (1980) Fish feed technology. Lectures presented at the F.A.O./U.N.D.P. Training course in fish feed technology held at the College of Fisheries, University of Washington, Seattle, Washington, 9 October - 15 December, 1978. A.D.C.P./80/11.

Fauconneau, B., Choubert, G., Blanc, J., Breque, J. and Luquet, P. (1983) Influence of environmental temperature on flow rate of foodstuffs through the gastrointestinal tract of rainbow trout. Aquaculture, 34, p27-39.

Fernandez, R., and Phillips, S.F. (1982)[a] Components of fiber bind iron in vitro. Amer. J. Clin. Nutr., 35, p100-106.

Fernandez, R., and Phillips, S.F. (1982)[b] Components of fibre impair iron absorption in the dog. Amer. J. Clin. Nutr., 35, p107-112.

Fetzer, S.G., Kies, C., Fox, H.M. (1979) Apparent digestibility of three purified fibre fractions in adolescent boys. Cereal Chem., 50, p34-39.

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. J. Fish. Biol., 14, p229-238.

Foltz, J.W. (1979) Chromic oxide in food assimilation studies. Comments. Trans. Amer. Fish. Soc., 108, p650-652.

Forman, L.P. and Schneeman, B.O. (1980) Effects of dietary pectin and fat on the small intestinal contents and exocrine pancreas of rats. J. Nutr., 110, p1992-1999.

Furakawa, 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 in fish. Bull. Jap. Soc. Sci. Fish., 32, p502-506.

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, p41-49.

Godding, E.W. (1976) Dietary fibre redefined (letter). Lancet, i, p1129.

Goodrich, T.D. and Morita, R.Y. (1977)[a] Bacterial chitinase in the stomachs of marine fishes from Yaquina Bay, Oregon, USA. Mar. Biol., 41, p355-36).

Goodrich, T.D. and Morita, R.Y. (1977)[b] Incidence and estimation of chitinase activity associated with marine fish and other estuarine samples. Mar. Biol., 41, p349-353.

Griffiths, W.E. (1976) Feeding and gastric evacuation in perch, (Perca fluviatilis L.) Mauri. Ora., 4, p19-34.

Grove, D.J. and Crawford, C. (1980) Correlation between digestion rate and feeding frequency in the stomachless teleost, (Blennius pholis L.). J. Fish. Biol., 16, p235-247.

Grove, D.J., Loizides, L.G. and Nott, J. (1978) Satiation amount, frequency of feeding and gastric emptying rate in Salmo gairdneri. J. Fish. Biol., 12, p507-516.

Halver, J.E. (1972) Components of plant and animal foodstuffs other than nutrients. In Fish Nutrition. Academic press, New York, San Francisco, London. p184-186.

Hassid, W.Z., Newfeld, E.F. (1964) Quantitative determination of starch in plant tissue. Methods Carbohydrate Chem., 4, p33-36.

Hedge, S.N., Rolls, B.A., Turvey, A. and Coates, M.E. (1978) The effects on chicks of dietary fibre from different sources: a growth factor in wheat bran. Br. J. Nutr., 40, p63-68.

Hellendoorn, E.W. (1975) Enzymatic determination of the indigestible residue (dietary fibre) content of human food. J. Sci. Fd. Agric., 26, p1461.

Hellendoorn, E.W. (1976) Beneficial physiologic action of beans. J. Am. Diet. Ass., 69, p248-253.

Heller, S.N., Hackler, L.R., Rivert, J.M., Van Soest, P.J., Roe, D.A., Lewis, B.A. and Robertson, J. (1980) Dietary fibre: the effect of particle size of wheat bran on colonic function in young adult men. Am. J. Clin. Nutr., 33, p1734-1744.

Henry, Y. and Etienne, M. (1969) Effets nutritionnels de l'incorporation de cellulose purifiee dans le regime du porc en croissance finition. Annls. Zootech., 18, p337-357.

Hilton, J.W., Atkinson, J.L. and Slinger, S.J. (1983) Effect of increased dietary fibre on the growth of rainbow trout, Salmo gairdneri. Can. J. Fish. Aquat. Sci., Vol 40, p81-84.

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 R. Aquaculture, 25, p185-194.

Hoar, W.S., Randall, D.J. and Brett, J.R. (1979) Fish Physiology. Vol. 8. Academic Press, New York, 786pp.

Hofer, R., Forstner, H. and Rettenwander, R. (1982) Duration of gut passage and its dependence on temperature and food consumption in roach, Rutilus rutilus L: laboratory and field experiments. J. Fish Biol., 20, p289-299.

Hogan, J.P. and Weston, R.H. (1969) The digestion of pasture plants by sheep. III. The digestion of forage oats varying in maturity and in the content of protein and soluble carbohydrate. Austr. J. Agric. Res., 20, p347-363.

Holts, S., Heading, R.C. and Carter, D.C., Prescott, L.F., and Tothill, P. (1979) Effect of gel fibre on gastric emptying and absorption of glucose and paracetamol. The Lancet, March 24th, 1979, p636-639.

Hopkins, A. (1966) The pattern of gastric emptying: a new view of old results. J. Physiol., 182, p144-149.

Hopwood, A.P. (1980) Recovery of protein and fat from food industry waste waters. J. Inst. Wat. Poll. Contr., No 2, p225-232.

Hudson, G.J., John, P.M.V., Bailey, B.S. and Southgate, D.A.T. (1976) The automated determination of carbohydrate. Development of a method for available carbohydrates and its application to foodstuffs. J. Sci. Food Agric. 27, p681-687.

Huebner, J.D. and Langton, R.W. (1982) Rate of gastric evacuation for winter flounder Pseudopleuronectes americanus. Can. J. Fish.

Aquat. Sci., Vol. 39, p356-360.

Hunt, B.P. (1960) Digestion rate and food consumption of Florida gar, warmouth and largemouth bass. Trans. Am. Fish. Soc., 89, p206-211.

Inaba, D.C., Ogino, C., Takamatsu, T.U. and Kurokawa, K. (1963) Digestibility of dietary components in fishes II. Digestibility of dietary protein and starch in rainbow trout. Bull. Jap. Soc. Fish., 29, p242-247.

Ismail-Beigi, F., Reinhold, J.G., Faraji, B. and Abadi, P. (1977). Effects of cellulose added to diets of low and high fibre content upon the metabolism of calcium, magnesium, zinc and phosphorous by man. J. Nutr., Vol 102, p510-518.

Jeltema, M.A. and Zabik, M.E. (1980) Revised method for quantifying dietary fibre components. J. Sci. Food. Agric., 31, p820-829.

Jenkins, D.J.A., Wolever, T.M.S., Leeds, A.R., Gassull, M.A., Haisman, P., Dilawari, J., Goff, D.V., Metz, G.L., Alberti, G.G.M.M. (1978) Dietary fibres, fibre analogues and glucose tolerance: importance of viscosity. British Medical Journal, 1, p1392-1394.

Jennings, J.B. (1972) Feeding, digestion and assimilation in animals. (2nd ed). Pergamon Press, London. 244pp.

Jobling, M. (1980)[a] Gastric evacuation in plaice, Pleuronectes platessa L.: effects of dietary, energy level and food consumption. J. Fish Biol., 17, p187-196.

Jobling, M. (1980)[b] Gastric evacuation in plaice, Pleuronectes platessa. J. Fish Biol., 17, p547-551.

Jobling, M. (1981)[a] Dietary digestibility and the influence of food components on gastric evacuation in plaice, Pleuronectes platessa L. J. Fish Biol., 19, p29-36.

Jobling, M. (1981)[b] Mathematical models of gastric emptying and the estimation of daily rates of food consumption for fish. J. Fish Biol., 19, p245-257.

Jobling, M. and Davies, P.S. (1979) Gastric evacuation in plaice, Pleuronectes platessa L.: effects of temperature and meal size. J. Fish Biol., 14, p539-546.

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). J. Fish Biol., 10, p291-298.

Jones, B.C. and Green, G.H. (1977) Food and feeding of spiny dogfish (Squalus acanthius) in British Columbia waters. J. Fish. Res. Bd. Can., 34, p2067-2078.

Jones, R. (1974) The rate of elimination of food from the stomachs of haddock Melano-grammus aeglefinus, cod Gadus morrhua and whiting Merlangius merlangus. J. Cons. Int. Explor. Mer., 35 (3), p225-243.

Karpevitch, A. and Bakoff, E. (1937) The rate of digestion in marine fishes. Zool. Zhurn., 16, p28-44 (Russian; English summary).

Kartchner, R.J. and Theurer, B. (1981) Comparison of hydrolysis methods used in feed, digesta, and fecal starch analysis. Agric. Food Chem., 29, p8-11.

Kawatra, B.L., Garcha, J.S. and Wagle, D.S. (1971) Effect of feeding guar gum and of guar meal on nitrogen balance and on intestinal absorption of amino acids. J. Res. Punjab. Acric. Univ., 8, p227-231.

Kendall, P.T. (1982) Dietary fibre - a role in the diet of dogs. Pedigree digest, 9, No. 1, p5-7.

Kennelly, J.J. and Aherne, F.X. (1980) The effect of fibre addition to diets formulated to contain different levels of energy and protein on growth and carcass quality of swine. Can. J. Anim. Sci., 60, p385-393.

Kiem, K. and Keis, C. (1979) Effects of dietary fibre on the nutritional status of weanling mice. Cereal chem., 56, p73-78.

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., No 1, p112-115.

Kiorboe, T. (1978) Laboratory measurements of digestion rate in O-group flounder, (Platichthys flesus L.) at two temperatures. Ophelia, 17, p155-161.

Kirchgessner, M. (1975) Zum einfluss von Zellulose - und spurelementenzulage auf die Verdaulichkeit der Rohnahrstoffe beim schwein. Zuch-tungskunde, 47, p96-103.

Kirk, T. Kent. (1980) Lignin biodegradation: Microbiology, chemistry, and potential applications. Vol. 1, C.R.C. Press, p2-20.

Kitchell, J.F., Stein, R.A. and Knezené, B. (1978) Utilization of filamentous algae by fishes in Skadar Lake, Yugoslavia. Proceedings of the International Association of Theoretical and Applied Limnology. 20, p2159-2165.

Kitchell, F.J. and Windell, J.T. (1968) Rate of gastric digestion in pumpkinseed sunfish (Lepomis gibbosus). Trans. Am. Fish. soc., 97, p489-492.

Korn, S. and Macedo, D., (1973) Determination of fat content in fish with a non toxic, non-inflammable solvent. J. Fish. Res. Bd. Can., 30, p1880-1881.

Kritchevsky, D. and Story, J.A. (1974) Binding of bile salts in vitro by non-nutritive fibre. J. Nutr., 104, p458-462.

Kritchevsky, D., Tepper, S.A., Williams, D.E. and Story, J.A. (1977) Experimental atherosclerosis on rabbits fed cholesterol free diets. 7. Interaction of animal or vegetable protein with fibre. Atherosclerosis, 26, p397-403.

Laird, L.M. and Oswald, R.L. (1975) A note on the use of Benzocaine (Ethyl P-aminobenzoate) as a fish anaesthetic. J. Inst. Fish. Mgmt., 6, p92-93.

Lane, E.D., Kingsley, M.C.S. and Thornton, D.E. (1979) Daily feeding and food conversion of the diamond turbot: an analysis based on field data. Trans. Am. Fish. Soc., 108, p530-535.

Lang, J.A. and Briggs, G.M. (1976) The use and function of fibre in diets of monogastric animals. Fibre in Human nutrition. ed. Spiller,

G.A. Plennum press, New York. p151-169.

Leary, D.F. and Lovell, R.T. (1975) Value of fibre in production-type diets for channel catfish. Trans. Amer. Fisheries. Soc. Vol. 104, No. 2, p328-332.

Lebas, F. (1975) Influence de la teneur en energie de l'aliment sur les performances de croissance chez le lapin. Annls Zootech., 24, p281-288.

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, p916-922.

Lehninger, A.L. (1975) Proteins, purification and characterization Chapter 7, p157. Sugars, storage polysaccharides and cell walls Chapter 10, p249. Cholesterol metabolism, Chapter 24, p682. In: Biochemistry, 2nd Ed., Worth publishers Ltd., New York.

Loewus, F.A. and Tanner, W. (1982) Reserve polysaccharides other than starch in higher plants. Plant carbohydrates I, Encyclopedia of Plant physiology, New series, Volume 13A, by Springer-Verlag Berlin Heidelberg. p419-425.

Looney, M.A. and Lei, K.Y. (1977) Dietary fibre, zinc and copper: Effects on serum and liver cholesterol levels in the rat. Nutr. Rep. Int., Vol 17, No. 3, p329-337.

Low, A.G., Partridge, I.G. and Sambrook, I.E. (1978) Studies on digestion and absorption in the intestines of growing pigs. Br. J. Nutr., 39, p515-526.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Determination of protein in biological samples. J. Biol. Chem., 193, p265.

Lundblad, G., Fange, R., Slettengren, K. and Lind, J. (1979) Lysozyme, Chitinase and exo-N-acetyl-β-D-glucosaminidase (NAGase) in lymphomyeloid tissue of marine fishes. Mar. Biol., 53, p311-315.

Magnusson, J.J. (1969) Digestion and food consumption in skipjack tuna. Trans. Am. Fish. Soc., 98, p379-392.

Malagelada, J.R. (1977) Quantification of gastric solid-liquid discrimination during digestion of ordinary meals. Gastroenterology, 72, p1264-1267.

Marais, J.F.K. and Kissil, G.W. (1979) The influence of energy level on the feed intake, growth, food conversion and body composition of Sparus aurata. Aquaculture, 17, p203-219.

Mathe, D., Lutton, C., Rautureau, J., Coste, T., Gouffier, E., Sulpice, J.C. and Chevallier, F. (1977) Effects of dietary fibre and salt mixtures on the cholesterol metabolism of rats. J. Nutr., Vol 107, No. 3, p466-474.

Meyers, S.P. and Butler, D.P. (1972) Alginates as binders for crustacean rations. Prog. Fish Cult., Vol 34, No 1, p9-12.

Milton-Thompson, G.J. and Lewis, B. (1971) The breakdown of dietary cellulose in man. (abstract) Gut, 12, p853-854.

Miner, R.W. (1955) The regulation of hunger and appetite. Ann. N.Y.

Acad. Sci. 63, p1-144.

Mod, R.R., Ory, R.L., Morris, N.M. and Normand, F.L. (1981) Chemical properties and interactions of rice hemicellulose with trace minerals, in vitro. J. Agric. Food Chem., 29, No 3, p450-454.

Morita, K., Furvichi, M. and Yone, Y. (1982) Effect of carboxy-methyl cellulose supplemented to Dextrin-containing diets on the growth and feed efficiency of red sea bream, Chrysophrys major. Bull. Jap. Soc. Sci. Fisheries, 48(11), p1617-1620.

Muztar, A.J. and Slinger, S.J. (1980) The effect of fibre on metabolic and endogenous energy losses in mature cockerels. Nutr. Rep. Int., Vol 22, No 2, p183-186.

McCance, R.A. and Lawrence, R.D. (1929) The carbohydrate content of foods. Med. Res. Council. Spec. Report Ser. No 135, HMSO? London, 1929.

McConnell, A.A., Eastwood, M.A. and Mitchell, W.D. (1974) Physical characteristics of vegetable foodstuffs that could influence bowel function. J. Sci. Food. Agric., 25, p1457-1464.

McDonald, P., Edwards, R.A. and Greenhalgh, J.F.D. (1977) Animal Nutrition, 2nd Ed, 450 pages. Longman press, London and New York.

McDonald, J.S. and Waiwood, K.G. (1982) Rate of digestion of different prey in Atlantic cod (Gadus morhua), Ocean pout (Macrozoarces americanus), Winter flounder (Pseudo pleuronectes americanus) and American plaice (Hippoglossoides platessoides). Can. J. Fish. Aquat. Sci., Vol. 39, p651-658.

McLaren, G.A., Cuppett, S.L., Williams, J.E., Fahey, G.C. and Smith, T.R. (1974) Acid resistant hemicellulose fractions in rat nutrition. Nutr. Rep. int., 10, p163-172.

McLaren, G.A., Williams, J.E., Fahey, G.C., Smith, J.E. and Woolf, H.A. (1976) Influence of acid resistant hemicellulose on urea nitrogen utilization by lambs. J. Anim. Sci., 43, p1072-1076.

McNaughton, J.L. (1978) Effect of dietary fibre on egg yolk, liver, and plasma cholesterol concentrations of the laying hen. J. Nutr., Vol. 108, No 11, p1842-1848.

Nakahiro, Y., Isshiki, Y. (1975) Effect of caecal ligation on digestibility of crude fibre, cellulose and pentosan in chickens. Jap. Poultry Sci., 12, p138-139.

Noland, P.R. and Scott, K.W. (1960) Effect of varying protein and energy intakes on growth and carcass quality of swine. J. Anim. Sci., 19, p67-74.

Nomani, M.Z.A., Albrink, M.J., Davis, G.K., Lai, H.Y.L. and Watne, A.L. (1979)[a] Changes in serum cholesterol with the variable digestibility of fibre diets at low energy intake. Nutr. Rep. Int., Vol 20, No 3, p363-369.

Nomani, M.Z.A., Bradac, C.J., Lai, H.L. and Watne, A.L. (1979)[b] Effect of dietary fibre fractions on the faecal output and steroids in the rat. Nutr. Rep. Int., Vol 20, No 2, p.269-278.

Nomani, M.Z.A., Fashandi, E.F., Davis, G.K. and Bradac, C.J. (1979)[c] Influence of dietary fibre on the growth and protein metabolism of

the rat. J. Food Sci., Vol 44, p745-751.

Nomani, M.Z.A. and Stansberry, S.C. (1982) Effect of dietary fibre fractions on the apparent digestibility of nitrogen and protein efficiency ratio in rats on two feeding plans. Nutr. Rep. Int., Vol. 26, No 4, p695-701.

Norris, J.S., Norris, D.O. and Windell, J.T. (1973) Effect of simulated meal size on gastric acid and pepsin secretory rates in bluegill (Lepomis machrochirus). J. Fish. Res. Bd. Can., 30, p201-204.

Okutani, K. and Kimata, M. (1964) Studies on chitinolytic enzyme in digestive tracts of Japanese sea-bass, (Lateolabrax Japonicus). Bull. Jap. Soc. Sci. Fisheries, Vol. 30, No 3, p262-266.

Owsley, W.F., Knabe, D.A. and Tanksley, T.D. (1981) Effect of sorghum particle size on digestibility of nutrients at the terminal ileum and over the total digestive tract of growing-finishing pigs. J. Animal Sci., Vol. 52, No 3, p557-566.

Pappas, C.J., Tiemeier, O.W. and Deyoe, C.W. (1973) Chromic sesquioxide as an indicator in digestion studies on channel catfish. Prog. Fish Cult., Vol. 35, p97-99.

Partridge, I.G. (1978) Studies on digestion and absorption in the intestines of growing pigs. Net movements of mineral nutrients in the digestive tract. Br. J. Nutr., Vol. 39, p527-537.

Persson, L. (1979) The effects of temperature and different food organisms on the rate of gastric evacuation in perch, Perca fluviatilis.

Freshwat. Biol., Vol. 9, p99-104.

Persson, L. (1981) The effects of temperature and meal size on the rate of gastric evacuation in perch, (Perca fluviatilis) fed on fish larvae. Freshwat. Biol., 11, p131-138.

Persson, L. (1982) Rate of food evacuation in roach (Rutilus rutilus) in relation to temperature, and the application of evacuation rate estimates for studies on the rate of food consumption. Freshwat. Biol., 12, p203-210.

Pfeffer, P.E., Doner, L.W., Hoagland, P.D. and McDonald, G.G. (1981) Molecular interactions with dietary fibre components. Investigation of the possible association of pectin and bile acids. J. Agric. Food Chem., 29, p455-461.

Phillips, G.O., Wedlock, D.J. and Williams, P.A. (1981) Gums and stabilizers for the food industry - interactions of hydrocolloids. Proc. Int. Conf., Clwyd, Wales, 1981. Vol. 6, Nos 1-6.

Possompes, B.P., Bergot, P. and Luquet, P. (1975) Mise au point d'une methode d'etude du transit gastro-intestinal chez la truite arc-en-ciel Salmo gairdneri Richardson: Influence du nombre de repas, des quantites ingerees et de la temperature d'acclimatation. Ann. Hydrobiol., 6, (2), p131-143.

Poston, H.A. (1974) Effect of feeding brown trout (Salmo trutta) a diet pelleted in dry and moist forms. J. Fish. Res. Bd. Can., 31, p1824-1826.

Rehbein, H. (1981) Amino acid composition and pepsin digestibility of krill meal. J. Agric. Food. Chem., 29, p682-684.

Reinhold, J.G., Faradji, B., Abadi, P. and Ismail-Beigi, F. (1976) Decreased absorption of calcium, magnesium, zinc and phosphorous by humans due to increased fibre and phosphorous consumption as wheat bread. J. Nutr., Vol. 106, p493-504.

Riccardi, B.A., Fahrenbach, M.J. (1967) Effect of guar gum and pectin N.F. on serum and liver lipids of cholesterol fed rats. Proc. Soc. exp. Biol. Med., 124, p749-752.

Richter, K. and Busch, B. (1970) Prufung von rationen mit unterschiedlichem energie-rohfaser und wassergehalt an graviden sauen hinsichtlich verdaulichkeit und durch gangszeiten. Arch. Tiernahr., 20, p679-686.

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. Biol., 19, p333-344.

Rumsey, G.L. and Ketola, H.G. (1975) Amino acid supplementation of casein diets of Atlantic salmon (Salmo salar) fry and of soybean meal for rainbow trout (Salmo gairdneri) fingerlings. J. Fish. Res. Bd. Can., 32, p422-426.

Sandberg, A.S., Hasselblad, C. and Hasselblad, K. (1982) The effect of wheat bran on the absorption of minerals in the small intestine. Br. J. Nutr., 48, p185-191.

Schemann, M. and Ehrlein, H.-J. (1982) The utility of cellulose meals for studies on gastrointestinal motility in dogs. Digestion, 25, p194-196.

Shah, N., Atallah, M., Mahoney, R.R. and Pellett, P.L. (1982) Effect of dietary fibre components on fecal nitrogen excretion and protein utilization in growing rats. J. Nutr., Vol 112, No 4, p658-666.

Sharpalekar, K.S., Sundaralli, O.E. and Narayanara, M. (1969) Effect of inclusion of extraneous cellulose on the gastric emptying time in adult albino rats. Can. J. Physiol. Pharmacol., 47, (4), p387.

Singh, R.P. and Nose, T. (1967) Digestibility of carbohydrates in young rainbow trout. Bull. Freshwater. Fish. Lab. Tokyo, Vol. 17, p21-25.

Slavin, J.J., Brauer, P.M. and Marlett, J.A. (1981) Neutral detergent fibre, hemicellulose and cellulose digestibility in human subjects. J. Nutr., Vol. 111, No 2, p287-297.

Slavin, J.L. and Marlett, J.A. (1980) Effect of refined cellulose on apparent energy, fat and nitrogen digestibilities. J. Nutr., Vol. 110, No 10, p2020-2026.

Smith, R.R. (1971) A method for measuring digestibility and metabolizable energy of fish feeds. Prog. Fish Cult., Vol. 33, p132-134.

Smith, R.R. (1979) Methods for determination of digestibility and metabolizable energy of feedstuffs for finfish. Sumposium on fish nutrition and feed technology In Finfish nutrition and fishfeed technology. (J.E. Halver and K. Tiews Eds), Vol. II pp 453-460, Heenemann Verlag, Berlin.

Southgate, D.A.T. (1969) Determination of carbohydrates in foods. 2. Unavailable carbohydrates, J. Food Sci. Agric., 20, p331.

Southgate, D.A.T. (1978) The analysis of dietary fibre - the choices for the analyst. J. Sci. Food. Agric., 29, p979-988.

Spiller, G.A. (1976) A new term for plant fibres in nutrition. Am. J. Clin. Nutr., 29, p934-935.

Spiller, G.A. (1981) Effect of graded dietary levels of plant fibres on fecal output in pig-tailed monkeys. Nutr. Rep. Int., Vol. 23, No 2, p313-320.

Spiller, G.A. and Shipley, E.A. (1976) New perspectives on dietary fibre. Food. Product. Development, October, 1976, p71-73.

Stanway, A. (1981) Taking the rough with the smooth. Pan Books Ltd., London and Sydney. 282 pages.

Stephen, A.M. and Cummings, J.H. (1979) Water-holding by dietary fibre in vitro and its relationship to faecal output in man. Gut, 20, p722-729.

Stevenson, M.H. and Unsworth, E.F. (1978) Studies on the absorption of calcium, phosphorous, magnesium, copper and zinc by sheep fed on roughage-cereal diets. Br. J. Nutr., p491-496.

Stickney, R.R. (1975) Cellulase activity in the stomachs of freshwater fishes from Texas. Proc. 29th Annual. Conf. South Eastern. Assoc. Game and Fish comm., 1975. p282-286.

Stickney, R.R. and Shumway, S.E. (1974) Occurrence of cellulase activity in the stomachs of fishes. J. Fish. Biol., 6, p779-790.

Subramanian, V. (1975) Studies on the physical and chemical properties of chitin and related polymers. Manuscript Reprod. McGill Univ. Montreal. Mar. Sci. Cent., 27, 75 pages.

Sun, D.H.C., Shay, H.A. and Woloshintt, J. (1959) Effect of tricyclamol on gastric emptying and intestinal transit. Am. J. dig., 4, p282-288.

Swenson, W.A. and Smith, L.L. Jr., (1973) Gastric digestion, food consumption, feeding periodicity, and food conversion efficiency in Walleye (Stizostedion vitreum vitreum). J. Fish. Res. Bd. Canada., 30, 9, p1327-1335.

Tacon, A.G.J. (1981) The possible substitution of fish meal in fish diets. Proc. Scott. Mar. Biol. Assoc. and Highlands and Islands Dev. Board, Fish Farm meet., Oban, Scotland. 26-27 Feb. 1981, p46-56.

Tacon, A.G.J., Stafford, E.A. and Edwards, C.A. (1983) A preliminary investigation of the nutritive value of three terrestrial lumbricid

worms for rainbow trout. Aquaculture, 35, p 187-199.

Tainter, M.L. and Buchanan, O.H. (1954) Quantitative comparisons of colloidal laxatives. Annals. of the New York Academy of Sciences, 58, p438-454.

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., Vol. 23, No 2, p211-221.

Tascento, V. (1970) Feeding value of sunflower meal with different contents of cellulose for pigs. Lucr. Stiint. Inst. Cercet. Zootech., 27, p641-649.

Thompson, S.A. and Weber, C.W. (1979) Influence of pH on the binding of copper, zinc, and iron in six fibre sources. J. Food. Sci., Vol. 44, p752-754

Thorpe, J.E. (1977) Daily ration of adult perch, Perca fluviatilis L. during summer in Loch Leven, Scotland, J. Fish. Biol., 11, p55-68.

Trowell, H. (1972) Ischaemic heart disease and dietary fibre. Am. J. Clin. Nutr., 25, p926-936.

Trowell, H. and Godding, E.W. (1981) Fibre: New terminology or new concepts. Am. J. Clin. Nutr., 34, p1163.

Trudell, J.I. and Morris, J.G. (1975) Carbohydrate digestion in the cat. (abstr.) J. Anim. Sci., 41, p329-

Tsei, A.C., Elias, J., Kelley, J.J., Lin, R.C. and Robson, J.R.K. (1976) Influence of certain dietary fibres on serum and tissue cholesterol levels in rats. J. Nutr., 106, p118.

Tsei, R.C.Y. and Lei, K.Y. (1979) Dietary cellulose, zinc and copper: Effects on tissue levels of trace minerals in the rat. J. Nutr., Vol. 109, No 6, p1117-1122.

Tyler, A.V. (1970) Rates of gastric emptying in young cod. J. Fish. Res. Bd. Can., 27, p1177-1189.

Vahl, O. (1979) An hypothesis on the control of food intake in fish. Aquaculture, 17, p221-229.

Van Es, A.J.H. (1981) Feeds rich in cellulose in ruminant and non-ruminant digestion. Agriculture and Environment, 6, p195-204.

Van Soest, P.J. (1967) Non nutritive residues: A system of analysis for the replacement of crude fibre. J. Assoc. Off. Agric. Chem., 49 (3), p546.

Van Soest, P.J., Uden, P. and Wrick, K.F. (1983) Critique and evaluation of markers for use in nutrition of humans and farm and laboratory animals. Nutr. Rep. Int., Vol. 27, No 1, p17-28.

Vam Soest, P.J. and Wine, R.H. (1968) Acid detergent extraction to measure ADF fibre. J. Ass. Off. Agric. Chem., 50, p50.

Vercellotti, J.R., Salyers, A.A., Bullard, W.S., Wilkins, T.D. (1977) The role of the colon microflora in the breakdown of dietary fibre. Can. J. Biochem., 55, p1190.

Viola, S., Zimmerman, G. and Mokadij, S. (1970) Effect of pectin and algin upon protein utilization, digestibility of nutrients and energy in young rats. Nutr. Rep. Int., 1, p367-374.

Vogt, H., Stute, K. (1974) Vergleich der Verdaulichkeit einiger Kohlenhydratfraktionen bei Huhnern und Perlhuhnern. Arch. Geflugelk., 38, p117-118.

Waite, R. and Gorrod, A.R.N. (1959) The structural carbohydrates of grasses. J. Food. Sci. Agr., 10, p308-310.

Western, J.R.H. (1971) Feeding and digestion in two cottid fishes, the freshwater Cottus gobio and the marine Enophrys bubalis. J. Fish. Biol., 3, p225-246.

Whittemore, C.T. and Elsley, F.W.H. (1977) Practical Pig Nutrition, Farming Press Limited, p63-64.

Windell, J.T. (1966) Rate of digestion in the blue gill sunfish, Lepomis machrochirus. Invest. Indiana. Lakes and Streams, Vol. 7, No 6, p185-214.

Windell, J.T. (1967) Rates of digestion in fishes. In: The biological basis of freshwater fish production (Ed. S.D. Gerking). Blackwell Scientific Publications, Oxford and Edinburgh, 495 pages.

Windell, J.T., Foltz, J.W. and Sarakon, J.A. (1978) 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), p613-616.

Windell, J.T., Horak, D.L. and Roth, J. (1972)[a] Gastric evacuation of the Colorado dry diet by hatchery reared rainbow trout, Salmo gairdneri. J. Colorado/Wyoming Acad. Sci., Vol. VII, No 2-3 (28), p91.

Windell, J.T., Horak, D.L. and Wilson, W. (1972)[b] Rate of gastric evacuation of pelleted trout food containing different binders by rainbow trout. J. Colorado/Wyoming Acad. Sci., Vol. VII, No 2-3 (29), p91.

Windell, J.T. and Norris, D.O. (1969) Gastric digestion and evacuation in rainbow trout. Prog. Fish Cult., Vol. 31, p20-26.

Windell, J.T., Norris, D.O., Kitchell, J.F. and Norris, J.S. (1969) Digestive response of rainbow trout, Salmo gairdneri, to pelleted diets. J. Fish. Res. Bd. Can., Vol. 26, No 7, p1801-1812.

Wolter, R. (1978) L'alimentation rationnelle des carnivores domestiques. Revue Med. vet., 129, p7-40.

Wong, S. and O'Dea, K. (1983) Importance of physical form rather than viscosity in determining the rate of starch hydrolysis in legumes. Amer. J. Clin. Nutr., 37, No 1, p66-70.

Wood, E.M., Griffin, P.J. and Snieszki, S.F. (1954) The effect of sodium carboxymethyl cellulose)CMC) as a binding agent in the preparation of diets. Progr. Fish. Cult., 16, p19.

Yang, M.G., Manoharan, K., Young, A.K. (1969) Influence and degradation of dietary cellulose in caecum of rats. J. Nutr., 97, p260-264.

Yoshida, T., Pleasants, J.R., Reddy, B.S., Wostmann, B.S. (1968) Efficiency of digestion in germ free and conventional rabbits. Br. J. Nutr., 22, p723-737.

Zehavi, U., Jeanloz, R.W., Horton, D. and Kerstin, D. (1971) Oligosaccharides derived from chitin. Biochem. Prep., 13, p14-18.