DIETARY INTERACTIONS INFLUENCING FEED INTAKE, NUTRIENT UTILISATION AND APPETITE REGULATION IN THE RAINBOW TROUT, *Oncorhynchus mykiss*.

by

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DEDICATION

To my dearest late father, ALI TEKINAY, who always placed so much trust in my career objectives and wished me to be a successful scientist.

Also, to my dearest mother, SAADET TEKINAY, who always supported me, although she was often 4000 km away in my beloved country of Turkey.

And finally to my wife, GULHAN, my daughters, SUMEYYE NUR and SARA NESIBE, who have always been with me in rainy, dark and lonely days at Plymouth between 1995 and today.

ABSTRACT

Dietary interactions influencing feed intake, nutrient utilisation and appetite regulation in the rainbow trout, *Oncorhynchus mykiss.*

Ahmet Adem Tekinay

Dietary factors are one of the most significant considerations in the regulation of appetite in fish since dietary nutrient and energy concentration modulate feed intake in the short and longer term. These interactions may also be important from a commercial aspect, since the objective of aquaculture is to obtain maximum growth, feed efficiency and consumer acceptance of the product.

This thesis addresses the major dietary components which are likely to influence appetite in rainbow trout. These include dietary lipid level, carbohydrate content and degree of complexity as well as energy density and protein/energy ratio. Experimental data is presented which examines the influence of such factors on feed intake, growth performance, nutrient utilisation, gastric evacuation rate, return of appetite and changes with respect to the postprandial level of circulating plasma metabolites.

It is proposed that rainbow trout have the capacity to regulate feed intake within specific constraints. On the other hand, trout become obese when offered high oil diets and fail to control feed intake in the short term, possibly due to the palatability of lipids. Regulation may also appear at a metabolic level following accumulation of lipids in adipose tissue. Gastric evacuation rate was probably the main factor in the short term influencing feed intake. This was irrespective of carbohydrate complexity or level in the diet. However, simple sugars might suppress the appetite of trout in the longer term. The biochemical status of liver via plasma glucose concentration may play a more important role compared to gastric fullness in the long- term regulation of appetite. It was postulated that X-radiography was a paramount technique for the quantification of sequential meals and return of appetite measurements in these investigations.

The above parameters and their interactions were studied in relation to the physiological control of feed intake in order to develop a more defined model for such processes and to improve the optimum feeding regimes for rainbow trout under intensive production conditions. This is discussed within the wider concept of fish nutrition, and the implications for future research in this area are stated.

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CHAPTER I

1. GENERAL REVIEW AND INTRODUCTION

"The daily food consumption of a brown trout, Salmo trutta *L. is affected by a large number of factors which include the size of the fish, the amount of food eaten in a meal, the number of meals in a day, the rate of gastric evacuation, the water temperature, the activity of the fish, the type of food eaten and the availability of food organisms. As there is also interaction between some of these factors, it is not surprising that few workers have studied this complex subject."* J. M. Elliott (1975b)

1.1. Introduction

The principal objective of animal nutrition is to provide an adequate supply of essential nutrients to accomplish the energy requirement of animals under defined production conditions. The significance of voluntary feed intake is paramount in this context; when feed intake is below the optimum, then the proportion utilised for the maintenance and growth requirements becomes unfavourable and weight increment which is the magnitude of growth potential does not occur (Steffens, 1989). Therefore, the aim of nutrition scientists is to balance the quantity and quality of the diet with respect to the nutrient requirements of the specific species under question (Cho, 1990).

In addition, designed feeding strategies must depend on a common knowledge of the modulation of feed intake, since the ultimate aim of aquaculture is to maximise production with a minimum of financial input (Fletcher, 1984). When the diet is offered *ad libitum* this implies

that the composition of the diet should allow fish to obtain sufficient nutrient and energy, but not to overconsume. In practice, this means offering a highly digestible, nutrient-dense diet when maximum production is required but reducing the nutrient density of the feed at other times so as to prevent surplus fat deposition. This approach has widely been used in farm animals (McDonald *et al.*, 1995) however, the practical application of this method in fish nutrition is still not common.

Investigations concerning the regulation of feed intake in animals commenced prior to the 20th century, however much relevant and fundamental scientific study was directed to this area after the 1950's (Forbes, 1995). The theories advanced to explain the central mechanism governing voluntary feed intake can be classified into two main groups; i.e.: homeostatic and non-homeostatic. The homeostatic theories propose that a physiological or biochemical variable is regulated. Two kinds of homeostatic mechanisms can be distinguished; those proposing that specific metabolites in the body are regulated (e.g. fatty acids, glucose and amino acids) and those that propose regulation of energy such as the maintenance of body temperature. The glucostatic theory of Mayer (1955) has proposed that the satiety centre of the hypothalamus contains glucoreceptors sensitive to the concentration of glucose present in the blood. The aminostatic theory that was first advocated by Mellinkoff *et al.* (1956) states that excesses and deficiencies of plasma amino acids are responsible for initiating or inhibiting feed intake in the animal. The alternative lipostatic theory forwarded by Kennedy (1953) suggests that body fat is the regulated variable and that rates of feed intake and energy expenditure are its controls. Finally, the thermostatic theory of Brobeck (1960) states that the heat generated by metabolic fuels either stimulated or inhibited feeding in accordance with the animal's requirement to maintain a constant body temperature. This is particularly relevant to mammals and avian species. Other non-homeostatic systems have been proposed such as ecological, psychological

and computable mechanisms which may also have significance in the feeding of animals (Kissileff & Van Itallie, 1982).

Most theories concerning the control of feed intake include the idea that feed consumption causes changes in the body which are monitored by specific centres within the brain (Figure 1.1) which are used to determine when feeding should terminate (Stricker & Verbalis, 1990). These changes and the routes by which information concerning them is conducted to the brain are referred to as negative feedback signals which are generated in the periphery that are correlated either with short-term (feed consumed) or long-term (energy stored in viscera) regulation (Figlewicz *et al.*, 1996). There is evidence for chemo-receptors for various metabolites and hormones (i.e. amino acids, fatty acids, CCK) in the duodenum but the relative importance of distension, stretch or chemical effects (Stricker & McCann, 1985) probably varies depending on the type of feed encountered by the animal.

Models based on the sequence and connection of outcomes resulting from consumption to ultimate tissue distribution are necessary in order to understand regulation of voluntary feed intake (Novin, 1983; Denbow, 1994).

There is a paucity of scientific investigations concerning the physiological control of appetite in fish, although comprehensive studies have been focused on nutritional requirements of selected cultured species (Fletcher, 1984). The importance of dietary energy level has been studied extensively in relation to voluntary feed intake in fish such as Goldfish, *Carassius auratus* (Rozin & Mayer, 1964), rainbow trout, *Oncorhynchus mykiss* (Lee & Putnam, 1973; Grove *et al.*, 1978), Channel catfish, *Ictalurus punctatus* (Page & Andrews, 1973), turbot, *Scopthalmus maximus* (Bromley, 1980) and plaice, *Pleuronectes platessa* (Jobling, 1980, 1981b). The

olfactory and gustatory (Mackie *et al.*, 1980; Tandler *et al.*, 1982), gastro-intestinal evacuation (Elliott, 1972, 1976, 1991; Fänge & Grove, 1979; Jobling & Davies, 1979; Grove, 1986; Sims *et al.*, 1996) and humoral factors (Harmon & Sheridan, 1992a, 1992b; Himick & Peter, 1995) in relation to the regulation of appetite have all been studied and recently more research seems to be concentrated into this area (Jobling & Miglavs, 1993; Le Bail & Boeuf, 1997; Shearer *et al.*, 1997).

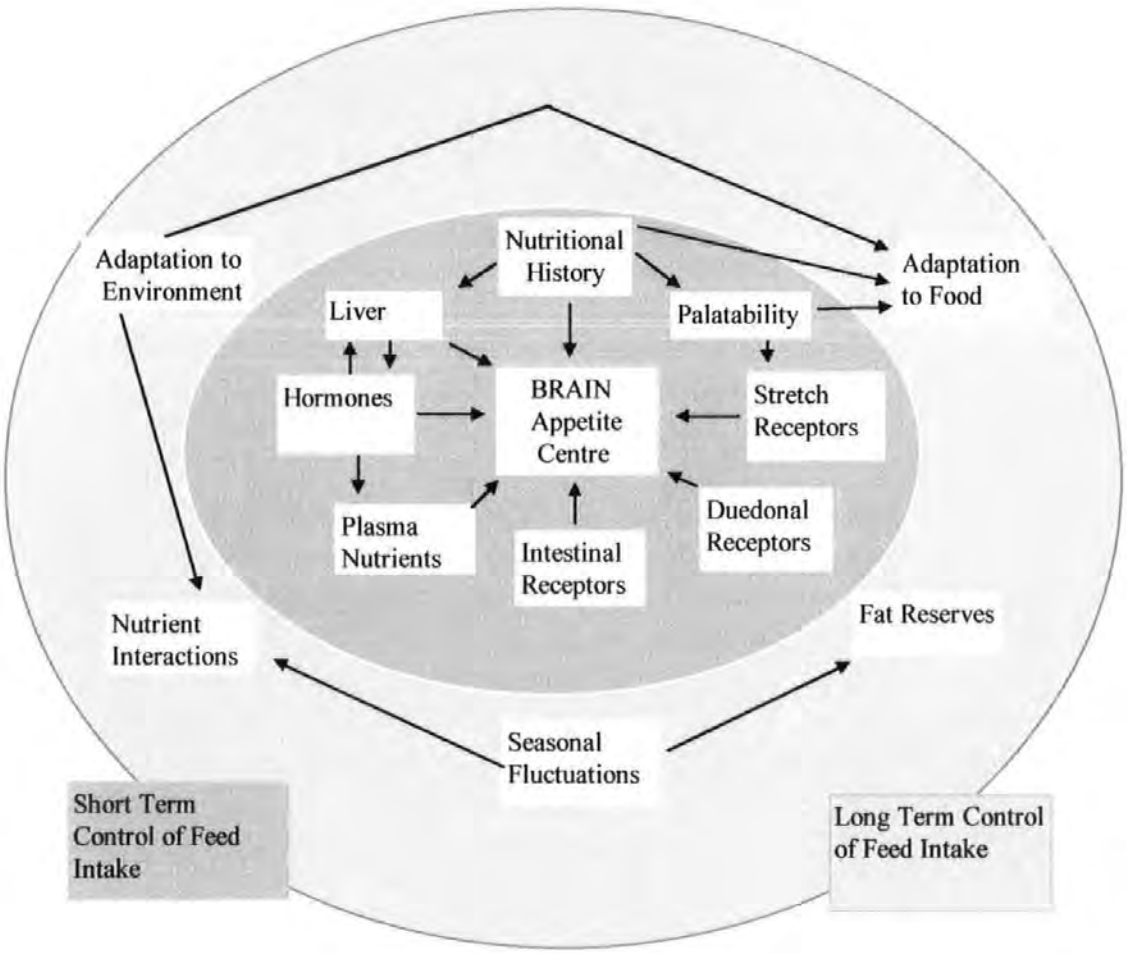


Figure 1.1 Short term and long term factors regulating voluntary feed intake in animals.

Since dietary interactions are one of the most significant considerations in feed intake regulation of fish as well as other animals (Le Magnen & Devos, 1984; Larsen *et al.*, 1991; Baldwin & Sainz, 1995), these complex factors including dietary energy, protein, protein/energy ratio, lipids, carbohydrates and their interactions at the metabolic level are reviewed. In addition, physiological processes such as gastric evacuation rate and systemic effects such as the plasma nutrient levels and all major parameters are reported in the following section in relation to the overall control of appetite in fish.

1.2. Dietary Energy

In common with all vertebrates, fish require energy for all physiological processes including digestion, maintenance of cellular functions and tissue synthesis for growth and replacement (Rozin & Mayer, 1961; Cho & Kaushik, 1985). Even though energy is not a nutrient *per se*, energy composition of a diet is one of the primary consideration in diet formulation for cultured fish (Lovell, 1989; NRC, 1993) as well as farm animals (Rothwell & Stock, 1981; Henry, 1985).

The plane of feeding (i.e. dietary energy and protein intake) influences the metabolic capacity of fish to grow under different production conditions and the energy utilisation involves complex physiological mechanisms after the ingestion of dietary nutrients. Therefore, the fate of energy has been categorised and reviewed by a number of workers from a bioenergetics point of view (Cho *et al.*, 1982; Smith, R., 1989; Cho & Kaushik, 1990; Jobling, 1994; De Silva & Anderson, 1995; Lucas, 1996).

In summary, the difference between the ingested energy (IE) and faecal energy (FE) is termed digestible energy (DE). DE defines the scope for energy utilisation. Metabolizable energy

(ME) displays DE minus energy lost via the branchial route (ZE) and urinary loss (UE). Net energy (NE) is the difference between metabolizable energy and energy dissipated from the heat increment (HiE). Retained energy (RE) which represents metabolizable energy corrected for energy lost as the maintenance energy can be efficiently utilised as growth, reproduction and swimming activity. Fish have low maintenance requirements compared to terrestrial vertebrates (Table 1.1), because fish divert less energy to support body mass due to their central buoyancy, locomotion and body temperature. As poikilotherms, fish do not regulate body temperature, but conform to their surrounding ambient conditions.

Table 1.1 Some typical values for the maintenance and digestible energy and protein requirements of various animal species.

Animal	**Rat**[1]	**Fowl**[2]	**Sheep**[2]	**Pig**[2]	**Cow**[2]	**Fish**[3]
Live Weight (kg)	0.3	2.0	50	70	500	0.15
Maintenance Energy ($MJ/day/kg\ W^{0.75}$)	0.30	0.36	0.23	0.31	0.32	0.03
Digestible Energy ($MJ\ kg^{-1}$ DM)	4.0-5.0	10.9-12.6	9.0-13.0	13.0	10.5	15.0
Crude Protein ($g\ kg^{-1}$ DM)	60.0	145.0-230.0	130.0	160.0	100.0	400.0

1. Estornel et al. *(1995), 2. McDonald* et al. *(1995), 3. Cho (1992)*

The fate of energy from feed ingredients will depend on the physiology of the fish in concern, as well as environmental factors and feeding strategies. From a commercial aspect, dietary digestible (DE) or metabolizable energy (ME) is presumably the most important factor influencing voluntary feed intake in farm animals (Forbes, 1995) and fish (Jobling & Wandsvik, 1983). However, because there are technical difficulties to quantify both gill and urinary loss in

order to determine metabolizable energy (ME) (Cho & Kaushik, 1990), digestible energy (DE) content of the diet is utilised widely in fish nutrition for practical purposes (Jobling, 1983). Therefore, the concentration of nutrients are expressed in terms of digestible nutrient (D_N) per unit digestible energy with the digestible values being measured by using appropriate techniques.

Fish may compensate for a low dietary energy density by consuming more feed (Hilton *et al.*, 1983; Bromley & Adkins, 1984) similar to higher animals (Hansen *et al.* 1981; Kallogeris *et al.*, 1983). However, there are still contradictory implications regarding to the ability of fish to regulate feed intake according to their energy requirements (Brett & Groves, 1979; Jobling, 1986b; Talbot, 1993, Cho *et al.*, 1994). Therefore there is a need to re-examine the feeding systems associated with high energy diets.

1.3. Dietary Protein and Amino acids

Fish do not have an actual protein requirement *per se*, but have an absolute requirement for accurately-balanced combinations of indispensable and dispensable amino acids (Tacon & Cowey, 1985; Wilson, 1989). Amino acid and protein nutrition in fish have been extensively studied and reviewed by a number of workers (Cowey & Sargent, 1979; Ketola, 1982; Wilson, 1985, 1986; Nose, 1989; Murai, 1992; Cowey, 1994).

Fish are capable of deriving significantly more energy per unit weight of protein than terrestrial animals due to their ability to excrete nitrogenous waste as ammonia (NH_3). Due to the large volumes of water required to excrete ammonia, terrestrial animals have to expend energy on the production of less toxic nitrogen compounds such as urea and uric acid (Beamish &

Thomas, 1984). Consequently, metabolizable energy derived from protein by fish is relatively high compared to other animals.

Proteins are the principal organic material present in fish carcass constituting approximately 65 - 75 % of the dry weight of the fish (Wilson, 1989; Nose, 1989). Dietary protein offers the most efficient source of "building materials". Following hydrolysis and digestion of the protein, free amino acids are released and absorbed from the intestinal tract; then, transported to the tissues and organs by the blood. Amino acids are continuously utilised by the fish in order to synthesise new proteins (i.e. growth, reproduction) or to substitute existing proteins (maintenance). Once amino acid requirements have been met, the main fate of amino acids is catabolism for the provision of energy. A paucity of protein in the fish diets causes an impairment in growth whilst excess protein supply increases nitrogen loss via gills (Kaushik & Cowey, 1991).

The requirements for essential amino acids in different fish species have been studied extensively using semi-purified diets based on crystalline amino acids and casein/gelatine blends to simulate proteins of high biological value (BV). Rainbow trout have the ability to utilise diets containing crystalline amino acids or casein hydrolysates as a sole protein precursor more efficiently than common carp, *Cyprinus carpio* (Aoe *et al.*, 1970, 1974)(cited in Murai, 1992). Much slower rates of intestinal absorption of amino acids by cold water fish such as trout compared to warm water fish such as carp have been suggested by Yamada *et al.* (1981) as the possible explanation for this.

According to Steffens (1989), rainbow trout digest protein to a considerable extent in the stomach, where endopeptidase and hydrochloric acid are particularly active and further protein

degradation proceeds in the intestine and the pyloric caeca. Kitamikado & Tachino (1960) have reported that proteolytic activities in the stomach, intestine, and pyloric caeca of juvenile rainbow trout are not so strong and their activities increase as fish grow. The protease activities in the stomach and intestine appear to reach their highest levels at a size around 90 g live weight. This may be one of the factors responsible for the higher protein requirement of young fish (Murai, 1992) relative to dietary energy.

Growth rate may also increase without raising dietary protein level because of an improved feed efficiency coupled with a higher intake per meal. Dietary protein level can be lowered, without affecting the growth performance, by dietary manipulations such as adjustment of amino acid balance and supplementation of a pertinent energy source such as lipids (Watanabe, 1977; Winfree & Stickney, 1981) and carbohydrates (Garling & Wilson, 1976; Kim & Kaushik, 1992). For instance, Luquet (1971) hypothesised that if high quality protein is employed and supplemented with limiting amino acids, 30 % dietary protein is sufficient and a further increase in protein content does not improve performance. Kim *et al.* (1984) claimed that if dispensable amino acids are provided as an energy source, no more than 25 % protein is necessary for protein synthesis in the body of rainbow trout. It should be noted that in this latter experiment, these authors maintained a fixed protein level equivalent to 40 % Crude Protein (CP) for each test diet. The minimum value of 25 % refers to the casein/gelatine components only. Whilst the remainder was a mixture of non-essential amino acids.

One of the ultimate goals of protein nutrition research in fish is the formulation of high quality feeds at lower costs taking advantage of various protein sources, as alternatives to fishmeal which is expensive and of limited supply. Fishmeals, which are produced from a variety of marine fish species are the main protein sources in practical fish diets because they supply an

adequate balance of essential amino acids (Table 1.2). Essential amino acid requirements are the most basic data needed to utilise alternative protein sources such as plant proteins which are deficient in certain amino acids (Nose, 1989).

Table 1.2 Amino acid composition of fishmeal and quantitative amino acid requirements of rainbow trout (g 100 g^{-1} protein).

Amino acid	Fishmeal (mainly herring, mackerel and capelin) (g 100 g^{-1} protein)[1]	Amino acid requirement of rainbow trout (g 100g^{-1} protein)[2]
Leucine	7.5	4.4
Isoleucine	4.5	2.4
Valine	5.4	3.1
Threonine	4.3	3.4
Phenylalanine	3.9	3.1
Tyrosine	3.1	2.1
Methionine	2.9	1.8
Cysteine	1.0	0.9
Tryptophan	1.2	0.5
Arginine	5.8	3.5
Histidine	2.4	1.6
Lysine	7.7	5.3

1. Miller & De Boer (1988) (cited in Pike et al., 1990), 2. Ogino (1980)

1.4. Dietary Lipids

Dietary lipids play a vital role in the biochemical processes of animal tissues, as both a source of essential fatty acids necessary for membrane structure and functions (Cowey & Sargent, 1979) and as a prime energy source to spare proteins for growth (Watanabe, 1982; Sargent *et al.*, 1989). They also moderate the absorption of fat soluble vitamins (NRC, 1993). From a

feed technology standpoint, lipids are useful in binding up light powdered dietary supplements (e.g. vitamin and mineral premixes) in finished pellets. However, excessive amount of lipid may worsen the physical characteristic of feed by lubricating the die.

Fish oil is used in practical salmonid diets, since it contains all known essential fatty acids, especially the (n-3) HUFA type required by marine and freshwater fish. In some species, medium chain triglycerides might be an alternative feasible energy source because they are readily catabolized and spare dietary protein without excessive fat deposition (Cowey, 1993; Nakagawa & Kimura, 1993).

Regulation of lipid metabolism in poikilothermic vertebrates has been generally reviewed by Greene & Selivonchick (1987) and Sheridan (1994).

The amount of body lipid is dependent on the balance between dietary energy input and the metabolic energy demands of the fish. Whole body moisture is inversely related to whole body lipid and decreases or increases as lipid is stored or utilised (Shearer, 1994). Lipid accumulation is affected by *de novo* lipid synthesis and by lipid deposition from different plasma lipoproteins. Lipid metabolism of poikilotherms is also regulated by pancreatic hormones, but the diversity of life history patterns complicates interpretation of experimental results (Sheridan, 1994). Lipid mobilization is directed by intracellular lipase enzymes, particularly during starvation periods (Sargent *et al.*, 1989).

In their fresh state, lipids give taste to diets because very short chain fatty acids are fairly volatile and contribute to olfactory and gustatory sensory stimulation. But, in some cases, oxidation of lipids via free-radical chain reactions can create the generation of adverse taste

compounds and reduces the palatability and nutritional value of diets. Consumption of the rancid lipids may produce a toxic effect due to ingestion of free radicals, and consequently this can impair growth in most farmed fish species (Baker, 1996).

Energy storage is important for the support of various physiological, developmental and reproductive events within the organism. However, large lipid reserves may restrict locomotion. Moreover, excessive fat deposition in the abdominal cavity and tissues would adversely affect product and storage quality in farmed fish species (Cho & Kaushik, 1985; Sheridan, 1994). Therefore, lipid levels of salmonid diets were previously recommended to be between 10% to 20 % in practical fish feeds (Smith, R., 1989), however, 30-35 % dietary lipid inclusion in salmonid diets have been well demonstrated today (Kaushik & Medale, 1994) and are standard in most European countries.

1.5. Dietary Carbohydrates

No essential dietary requirement for carbohydrate has been demonstrated in fish; but since carbohydrates are the least expensive form of dietary energy, it is important to provide the appropriate concentration of carbohydrate in the diet of the fish species being cultured (Austreng *et al.*, 1977; Pieper & Pfeffer, 1980; Brauge *et al.*, 1994).

The relative use of dietary carbohydrates by fish depends on the complexity, digestibility and dietary level of the carbohydrate source in question. Earliest work relating to the utilisation of dietary carbohydrate suggested that not more than 20 % carbohydrate should be included in the diet for rainbow trout (Phillips *et al.*, 1948; Luquet, 1971; Cowey *et al.*, 1977a). Hilton & Atkinson (1982) showed that growth performance of rainbow trout was impaired when fed 21 % α- glucose in their diet and they recommended a maximum of 14 % dietary carbohydrate in

the diet for rainbow trout. However, cooked starch and dextrin are better utilised by most fish than simple sugars and improve protein saving capacity of this component (Kaushik & Oliva-Teles, 1985; Kaushik *et al.*, 1989).

Starch is a polymer of D-glucose units with mostly α 1-4 linkages. It consists of three components, amylose, amylopectin and an intermediate material. The proportions of these macromolecules and their structural arrangement in the granule vary according to the origin of starch and to technological treatments (Bergot & Breque, 1983). As in other species, trout may digest treated and modified starches. As the digestive microflora is not sufficiently developed to play a significant role, it seems that starch degradation mostly depends on pancreatic α-amylase secretions. This enzyme seems to be produced in a sufficient amount to hydrolyse starch to the extent that the granule structure has been disorganised. The brush border enzymes which hydrolyse the α-1-6 linkages are not likely to be a limiting factor (Bergot, 1993).

Spannhof & Plantikow (1983) suggested that crude starch reduced amylase activity in the intestinal juices since amylase is absorbed to crude starch, so that starch hydrolysis is effectively inhibited. Crude starch in the diet also accelerates the passage of the chyme through the intestine, thus reducing the time available for absorption. These effects are linked to the poor digestibility of polymerised starch products. Feeding high levels of digestible carbohydrate to salmonids has been reported to result in prolonged hyperglycemia, increased liver size and glycogen content which was proportional to the dietary carbohydrate levels fed (Lee & Putnam, 1973; Arnesen & Krogdahl, 1996). Wilson (1994) hypothesised that the prolonged hyperglycaemia observed in fish following glucose tolerance test and the relative

inability of fish to utilise high levels of simple sugars may be associated to one or a combination of the following;

- low tissue hexokinase activity and lack of an inducible glucokinase enzyme
- glucose being less potent than certain amino acids as a stimulus for insulin release
- the possible inhibition of insulin release by somatostatins secreted due to high blood glucose levels
- relatively low number of insulin receptors in fish as compared to mammals

No study has appeared with reference to the effect of carbohydrate level or carbohydrate source on voluntary feed intake, gastric evacuation and return of appetite in fish. Therefore combined knowledge of nutrient utilisation and physiological factors would help our understanding of the regulation of feed intake with respect to dietary carbohydrate. There is an urgent need to re-evaluate the data on the direct effect of carbohydrates on feed intake in fish.

1.6. Digestible Protein (DP) / Digestible Energy (DE) Ratio

Digestibility provides relatively useful information on how ingested food and nutrient components have been digested and absorbed by the animal (De Silva & Anderson, 1995). In this context, digestible protein, digestible energy and the ratio between these two parameters have been proved to be the most important constituents for consideration in fish nutrition.

Ammonia (NH_3) is only the major end product of protein metabolism in most teleost fish studied to date, comprising between 70-90 % of the nitrogenous catabolites, gills being the main excretory organ. Of the potential pathways of ammonia formation (direct deamination, trans-deamination of amino acids and purine nucleotide cycle), trans-deamination has been recognised as quantitatively the most important in teleost fishes (Kaushik & Cowey, 1991).

Endogenous obligatory nitrogen excretion under fasting conditions is relatively constant for a given species of fish under a given set of environmental conditions. In fed fish, ammonia excretion rates are directly related to levels of protein intake. Hence, in the context of a high dietary concentration of proteins and as a step towards protein sparing, the current tendency is to seek ways of decreasing nitrogenous losses and enhancing nitrogen retention mainly through optimisation of protein-energy ratios in fish diets. Indeed, besides the quantity and quality of dietary protein, an increase in dietary non-protein energy intake has been shown to decrease ammonia production (Cho & Kaushik, 1985). On the contrary, data on the effect of feeding rate and pattern in terms of ammonia excretion under field conditions is relatively scarce.

The importance of protein and lipid deposition depends upon a great number of factors in addition to the maturity of the animal. Large excesses of energy intake and improper balance of protein to energy results in deposition of a greater proportion of the recovered energy as lipid in adipose tissue. In contrast, as the energy intake decreases, the total amount of lipid deposited decreases until a threshold is reached when the consumption of dietary energy is less than that expended as heat. This will result in a net mobilisation of lipid to support protein deposition (Cho & Kaushik, 1990). In the absence of recognition of dietary protein/energy balance and of biological value of proteins in the diets, there are bound to exist large differences in the quantitative assessments of nitrogenous waste output by fish farms. Optimum protein-energy ratio for rainbow trout has been well demonstrated between the rage of 22-24 g DP per kJ DE (Cho, 1992). However, the current commercial trend is far below these limits. Recommended protein-energy ratios for some fish species are presented in Table 1.3.

Kaushik & Medale (1994) pointed out the need to re-evaluate the existing data on essential nutrient requirements of fish taking into account the DE levels of experimental diets used and express the requirement data per unit DE.

Table 1.3. Recommended protein / energy ratio for different fish species

Species	T (°C)	B.Wt (g)	(DP) (%)	(DE) (MJ kg^{-1})	DP / DE	Ref.
Rainbow trout	15.0	90.0	33.0	15.06	22.0	1
Atlantic salmon	10.2	131.0	55.0	22.7	24.2	2
	13.9	1000	36.5	21.6	18.8	3
Brook trout	6.0-17.0	3.0-6.0			32	4
Common carp		20.0	31.5	12.13	25.8	5
Red tilapia	26.3	35.0	30.3	16.0	~19.0	6
Mossambicus tilapia	28.0		30.1	12.6	23.8	7
Channel catfish	27.0	34	28.8	12.85	22.0	8
Sea bass	27.0	3.0-16.0	45.0	16.8	27	9
Hybrid bass		35.0	31.5	11.72	26.8	10
Dentex	20.0	44.0	44.3	18.2	24.3	11

1. *Cho & Kaushik (1985),* **2.** *Grisdale-Helland & Helland (1997),* **3.** *Einen & Roem (1997),* **4.** *Ringrose (1971) (cited in Cho & Kaushik, 1985),* **5.** *Takeuchi* et al. *(1979) (cited in Smith, R., 1989),* **6.** *De Silva* et al. *(1991),* **7.** *El - Dahhar & Lovel (1995),* **8.** *Garling & Wilson (1976),* **9.** *Perez* et al. *(1997),* **10.** *Nematipour* et al. *(1992),* **11.** *Tibaldi* et al. *(1996).*

1.7. Digestion and Gastric Evacuation Rate

The need for estimation of the feed intake of natural fish populations in order to quantify predation and investigation of feeding interactions between species have directed scientists to study gastro-intestinal transit time (Doan, 1973; Jones, 1977) (cited in Fletcher, 1984). Hence, the stomach as a prime regulator of appetite has been the subject of numerous studies on mammals (Snowdon, 1970; Hunt, 1975, 1980; Wirth & McHugh, 1983; Kallogeris *et al.*, 1983; Rayner, 1992; Read, 1992; Mayer, 1994) and a wide range of fish species (Grove *et al.*, 1978; Vahl, 1979; Flowerdew & Grove, 1979; Gwyther & Grove, 1981; Fletcher, 1982; Grove *et al.*, 1985; Singh & Srivastava, 1985; Sims, 1994). These investigations have generally demonstrated that stomach emptying rate and voluntary feed intake are analogous to input rate = output rate (Bromley, 1994). It has been commonly assumed that a major determinant of satiety in animals, and hence the amount of feed consumed for a given meal, is the attainment of a full stomach (Kissileff & Van Itallie, 1982). It was also mentioned by Elliott (1976) and Vahl (1979) that one of the major factors influencing growth in fish is the evacuation rate of the digesta from the stomach. The physiological mechanisms of food emptying and how they might influence gastric emptying profile are central to the understanding of how appetite may be physiologically regulated in fish (Jobling, 1986a).

Gastric evacuation rate as a physiological factor governing appetite revival and regulation is only valid, however, if the variables that may influence the rate are also considered. The time required and the rate at which fish empty their stomachs has been shown to depend on water temperature and the diet quality, which will be affected by the meal and fish size. In addition, the actual stomach emptying phase will be dependent on the degree of distension of the sac-like stomach, the secretory surface area of the stomach and the surface area of the meal (Grove, 1986). These factors have also been demonstrated to influence the secretion of gastric

acid, digestive enzymes and the gut hormones in both fish (Grove & Holmgren, 1992) and mammals. In mammals, complex feedback loops involving gastrointestinal hormones have been implicated in the control of gastric motility and enzyme secretion (Walsh, 1994).

In general, it may be true that animals compensate for changes in the concentration of available energy in the food, unless the physical capacity of stomach restricts intake. However, many investigations provide conflicting results with respect to an energetic basis for physiological control of feed intake in fish.

It is quite reasonable to accept that stomach emptying time or rate plays an important role modulating feed intake, since the relationship between appetite return and stomach emptying has been fairly well documented. However, studies related to this area do not always specify the chemical characteristics and energy partition of feed offered to fish. Therefore physico-chemical composition of diets used for gastric emptying and appetite return aimed to be presented and standardised in this research programme. It is also mentioned that the data on gastric evacuation and appetite revival for specific diet formulations could make considerable progress towards understanding the regulation of feed intake in rainbow trout. Despite an incomplete comprehension of the basic physiology of fish gastro-intestinal functioning, reasonable descriptions and predictions of gastric emptying can be obtained by recording the temperature, fish size, food type, meal size, energy content and particle size for the species under study. Other factors such as reproductive state of the fish, photoperiod, stock density and stress are also likely to affect digestion (Holmgren *et al.*, 1983; Dos Santos & Jobling, 1988).

1.8. Plasma Nutrients and Hormones

The levels of plasma nutrients and hormones in the blood and systemic circulation have been suggested as informing the brain of the animal's metabolic and physiological state and being involved in the control of feeding in higher animals (Forbes, 1995). However very little attention has been focused towards fish in these regards.

Walton & Wilson (1986) stated that there is a positive relationship between dietary and plasma essential amino acid concentration and hepatic amino acid concentration tended to remain more stable throughout the sampling period. It has also been reported that lack of any of the ten essential amino acids suppressed the appetite of fish (Fletcher, 1984).

After the hydrolysis of proteins in the gastro-intestinal tract, amino acids are absorbed and pass along the portal system to the liver. The liver is the principal site of amino acid catabolism in fish, and Krebs (1972) has suggested that a major factor in its control was due to the high K_M values of amino acid catabolizing enzymes relative to the cytosolic amino acid concentrations. However little is known of either amino acid levels in trout liver or how their concentrations are affected by feeding and postprandial nutrient assimilation.

The major role of the liver in avian homeostasis stems largely from two main properties of this organ. Firstly it contains all of the major enzyme systems necessary for synthesis and degradation of glucose, glycogen and triglycerides (McGarry *et al.*, 1987). Secondly, it can switch the direction of carbon flow over key pathways of carbohydrate and lipid metabolism in response to changes in hormonal and nutritional status (Nicholl *et al.*, 1985).

Dietary lipid is absorbed into the lymphatic system rather than into the venous drainage of the intestines in mammals and thus by-passes the hepatic route. However, the liver is an important site of lipid metabolism in mammals and especially in birds (Denbow, 1994). Intravenous infusion of fat emulsion depresses intake, an effect that is not accompanied by changes in plasma or insulin concentrations. As animals fatten, there is a gradual increase in plasma insulin concentration and Woods *et al.* (1986) have reviewed that this is reflected in increased concentrations of insulin in the cerebrospinal fluid which inhibit further feed intake thus acting as a homeostatic mechanism for body fat (Scharrer & Langhans, 1990).

The hormonal regulation of fish metabolism has received increasing attention in the last decade (Sheridan, 1988; Plisetskaya, 1989, 1990; Sundby *et al.*, 1991; Harmon & Sheridan, 1992a, 1992b; MacKenzie *et al.*, 1998) however, there is a paucity of information on hormonal regulation in fish. Most recently, the humoral control of feed intake in fish was overviewed by Le Bail & Boeuf (1997). They suggested that hormones could affect central nervous system centres, align with feed intake behaviour or via vagal afferent neurons and an indirect affect may occur via the gut which slows gastrointestinal transit, thus resulting in stomach distension which activates vagal afferent neurons.

The effects of hormones on protein, lipid and carbohydrate metabolism and of the sparing action of lipids and carbohydrates on protein is obviously related to growth and cannot be easily separated (Matty & Lone, 1985a). These workers also pointed out that plasma amino acids are the set point that regulates energy balance in fish rather than the level of glucose. (Matty & Lone, 1985b). This is readily understandable when one considers that the major component of the feed intake in fish is protein and that carbohydrates constitute a marginal energy source for most fish species. Plasma glucose concentrations are known to be in close

relationship with level of digestible carbohydrate and continue to exert a prolonged (hyperglycaemia) for 24 hours after feeding a carbohydrate rich diet. However, no adequate explanation concerning the effect of high plasma glucose level on voluntary feed intake has been proposed. It could, therefore, be feasible to examine postprandial circulating nutrients which may have a significant contribution in feed intake modulation in fish.

1.9. Quantification of Feed Intake in Fish

The consumption, evacuation and absorption of feed are among the most significant parameters measured in laboratory feeding experiments in order to comprehend the information of the physiology of gut and related digestive processes (Talbot, 1985). However, there are still many problems to appreciate in quantification of feed intake in aquatic animals because of the complexity of feeding behaviour which can also differ between species (Kaushik & Medale, 1994).

There are basically two ways of conducting nutritional studies in fish where the aim is to investigate how the amount or the quality of the diet affects growth performance. One involves feeding tanks of fish and measuring the growth rates of separately fed groups of fish with different diets. An alternative method is to quantify the food consumption of the individual fish and to construct from the data an individual animal's food consumption-growth rate relationship for the species (Carter *et al.*, 1995). In some species of fish which can be held individually, e.g. cod, *Gadus morhua*, there is not a problem in determining food consumption and growth rate relationship (McCarthy *et al.*, 1993b). However, in the group feeding of experimental fish, a major problem has been to advance a reliable method to make repeated measurements of feed intake individually. Early efforts involved direct observations of feeding behaviour or the examination of gut contents in order to estimate consumption (Elliott, 1975a;

Elliott & Persson, 1978; Persson, 1979, 1981). These methods have proven inadequate as the techniques involved were time consuming, stressful and periods of pre- or postprandial starvation were necessary in many cases (Talbot, 1985).

In the beginning of 1980's, two non-invasive methods were developed to determine consumption and gastric emptying rates of individual fish, held as groups. Storebakken *et al.* (1981) employed feed labelled with the radioisotope ^{131}I and Talbot & Higgins (1983) introduced a quantitative radiographic technique. These permitted repeated measurements of feed intake rates of fish held as groups without any alteration to the feeding protocol. X-radiography has been the preferred technique for safety purposes. In another invasive method, different coloured feeds were utilised to differentiate sequential feeding rate in rainbow trout (Johnston *et al.*, 1994). Langar & Guillaume (1994) also estimated daily feed intake using radioactive silver iodide (^{124}I Ag) in sea bass, *Dicentrarchus labrax*. New developments in ultrasonic technology may also be an alternative consideration in the near future.

X-radiography has been extensively used in order to produce data on feeding behaviour, digestive physiology and gastro-intestinal mechanisms (Grove, 1986; Jørgensen & Jobling, 1989; Dos Santos & Jobling, 1991; Sims *et al.*, 1996). However, utilisation of the same technique in gastric emptying measurements has been criticised by Jørgensen & Jobling (1988), Dos Santos & Jobling (1991) and Jobling *et al.* (1993) since X-ray dense markers may be retained in the cardiac stomach and consequently result in an overestimation of stomach contents at different time intervals.

X-radiographic methods also have important applications in studies in which quantitative information about the feed intake of individual fish is required. Incorporation of markers of

different sizes into various types of feed also allows the amounts of each type consumed to be determined when feeds are presented either simultaneously or as discrete meals offered within a limited period. Thus, X-radiography could be employed for the quantitative determination of gastrointestinal content of fish under different environmental conditions. In this connection, Jobling *et al.* (1995) recently reviewed the feeding systems with related techniques and suggested a combination of the labelled feed (Brannas & Alanärä, 1992) and on-demand feeding (Alanärä, 1994, 1996) methods using a tagging system in order to analyse differences in feeding behaviour of individual fish and consequent influence of these interactions on feed intake and growth performance.

1.10. Strategies of the Research Program

The objective of this study was to evaluate dietary interactions influencing feed intake, nutrient utilisation and appetite regulation in the rainbow trout, *Oncorhynchus mykiss*. It is apparent that a multi-factorial approach is necessary to comprehend the regulatory mechanisms governing appetite regulation in fish. These complex interactions require separate evaluation in controlled studies before a complete model can be visualised for rainbow trout which is a typical salmonid of major commercial importance in aquaculture. The results from the various feeding and digestibility trials help towards our understanding of feeding regimes commensurate with optimum growth and performance.

Firstly, the importance of the physical and chemical composition of the diet was examined in feeding trials conducted on rainbow trout. Primary experiments focused on the influence of the energy concentration and protein/energy interactions with the aim of establishing the optimum nutrient ratios for growth and feed utilization. An initial purpose of this research programme was to re-evaluate the protein sparing action of dietary lipid as the major energy component in

commercial salmonid feeds. Therefore it is necessary to consider the influence of such diets with respect to growth and feed intake in rainbow trout as a model for future study. In addition, the protein and lipid deposition and growth parameters can be determined by assessing the growth performance, carcass and muscle composition of trout fed under controlled conditions. Furthermore, the use of specialised marker techniques was examined for digestibility measurements. In the case of commercial feeds, endogenous markers such as Acid Insoluble Ash (AIA) were employed compared to the traditional use of chromic oxide in experimental diets.

Secondly, physiological importance of nutrient & energy dense diets and nutrient dilution are taken into consideration, since nutrient density and more especially the protein/energy relationship at varying dietary concentration is likely to be a significant factor regulating feed intake. Therefore, the dietary energy levels were diluted up to 50 % by employing inert materials such as α-cellulose. We are aware of limited studies (Hilton *et al.*, 1983; Bromley & Adkins, 1984) on dilution of dietary energy in rainbow trout but reassessment is necessary due to the contradictory implications. With these experiments, we should be able to understand if the mechanisms governing appetite return in the rainbow trout are related either to stomach fullness or digestibility of the feed, or postprandial plasma nutrients.

Energy dense feeds employed by commercial manufacturers are based on elevated oil levels. The effect of concomitant levels of carbohydrate and filling agents on feed intake in the rainbow trout remain to be elucidated. Therefore the next emphasis was directed towards the assessment of carbohydrate components of typical diets for rainbow trout. As mentioned previously, the level and the type of dietary carbohydrate may greatly influence the availability of digestible energy and is thus worthy of further research. In the first instance, the filler

component (i.e. extruded wheat as the carbohydrate source) is the focus of the investigation. The level and contribution of carbohydrate is chosen to reflect the energy derived from the oil content in commercial feeds used in the initial experiment. The carbohydrate as starch represents the energy fraction of the ration and augments the energy contribution of dietary oil.

The main objective of the further phase of the research program was to incorporate different carbohydrate sources (e.g. D-glucose, maltose, dextrin, native corn starch, native wheat starch and pregelatinized corn starch) in the rainbow trout diets to study the influence of complexity of carbohydrate on feed intake, growth performance, digestibility, stomach evacuation, return of appetite and postprandial plasma nutrients. These studies were designed to test the capability of the rainbow trout to utilise carbohydrates according to their form in practical diets and likely degradation products. Glucose acted as a reference for maximum absorption whilst it expected that the more complex carbohydrates would demonstrate variable digestibility and assimilation rates in the rainbow trout. All the parameters discussed in these trials were determined on fish fed controlled diet formulations produced under laboratory conditions.

For the return of appetite and individual feed consumption studies in rainbow trout, different sizes of radio-opaque marker employing X-ray techniques were applied to measure successive meal consumption under practical feeding conditions.

Most of the experiments centred in this programme comply with a common approach with feed intake and appetite return forming the main component of the study. Growth performance and feed utilisation parameters implicit to the nutritional status of rainbow trout are reported when necessary and an integrative approach is a key objective of the investigations.

In summary, the objective of the experimental programme was directed towards establishing novel techniques and methodology for feed intake measurements. Improved resolution of appetite measurements is fundamental to such investigations and considerable attention is given to the development of X-radiography and other quantitative procedures.

Finally, the interactions between parameters determined and their relative importance in the design of practical feed formulations in relation to the physiological factors regulating voluntary feed intake are discussed:

- Improved feeding strategies to optimise growth and feed conversion
- The possibility of using cereal based carbohydrate sources as a partial replacement for the main energy source in diets.
- Understanding the complex physiological mechanisms associated with the regulation of voluntary feed intake and digestion rates in trout.
- Predictions of digestive efficiency and relationship to faecal output at different levels of feed intake.

CHAPTER 2

2. GENERAL MATERIALS AND METHODS

2.1. Experimental Animals and Holding Facilities

2.1.1. Experimental Fish

The all female (♀) rainbow trout, *Oncorhynchus mykiss* utilised in each of experiments were obtained from a local trout farm (Mill Leat Trout, Ermington, UK) and were allowed to acclimatise to the experimental conditions at the University of Plymouth for a period of at least two weeks before their use within the nutritional trials reported.

2.1.2. Experimental Facilities

All feeding trials were conducted in standard recirculation fresh water systems utilising bio-filtration units and six self-cleaning experimental tanks of 400 litres capacity in the aquarium of the Fish Nutrition Laboratories. This was situated in the basement floor of the Davy Building, Main Campus, University of Plymouth, Plymouth, UK.

Each experimental tank received a parallel input of water at a flow rate of 10 l min^{-1}. The temperature was held constant at 15 °C ± 0.2 °C. Lighting was set to operate on a constant 12 hours light / 12 hours dark cycle using artificial illumination from fluorescent tubes simulating natural photoperiod. The water quality was monitored routinely for dissolved oxygen (DO), ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3). Weekly partial water replenishment was performed in the system to ensure that all water quality parameters were within the known tolerance limits for rainbow trout under similar experimental conditions.

2.1.3 General Feeding

Before commencing any feeding experiments, the fish were acclimated for four weeks during which time they were fed to satiation three times daily at 09.00, 13.00 and 17.00 hrs on a maintenance diet particular to each prospective dietary treatment (Trouw Aquaculture, standard trout pellet, 4mm) (Trouw Aquaculture, UK, Wincham, Cheshire, England, UK).

In the course of the nutrition trials, the animals were fed either as a percentage of the live weight or satiation (until no feed is eaten) three times daily according to the protocol of experiments. The restricted ration size was determined on the basis of bi-monthly weighing and the percentage body weight fed during each of the feeding trials is mentioned where appropriate.

2.2. The Test Diets

2.2.1. Diet Formulation

Over the present series of experiments, different diet formulations were applied which were modified to suit the objective of each nutrition trial. Three commercial diets were utilised only in the first experiment (Chapter 3). Laboratory manufactured diets were formulated by using different feed ingredients to match the nutritional requirement specification of rainbow trout from the literature such as Cho & Cowey (1991).

2.2.2. Diet Materials and Manufacture

The semi-practical type diets were manufactured using Low Temperature (LT 94) Norsea-mink fish meal, extruded wheat feed, poultry meat meal, blood meal, D-glucose, maltose, dextrin, native wheat starch, native corn starch, pregelatinized corn starch, alpha cellulose, vitamin and mineral premix, carboxy-methyl-cellulose as a binder and cod liver oil. All experimental diets

except commercial counterparts were manufactured under the standard condition described below.

The dry powdered ingredients of each single diet were weighed differently and mixed in the bowl of a Hobart A101 food processor (Hobart Manufacturing Company Ltd, London) and the supplementary fish oil (Seven Seas Ltd. Hull, England) was then added. Finally an appropriate volume of distilled water was added during continuous mixing to yield a uniform paste considered sufficiently moist for extrusion.

All diets were processed into suitable pellets. Thus, using a Hobart food processor; the diets were extruded through a series of die holes of diameter 3/16 mm. The practical type diets were then spread thinly onto trays and air dried at 44°C in a fan assisted drying cabinet. The dried diets were then stored in black polyethylene bags within airtight plastic container. In addition, representative samples of all experimental diets were removed directly after manufacture and stored at -20°C prior to analysis for proximate composition and subsequent feeding.

2.3. Analysis of Proximate Composition

2.3.1. Determination of Moisture Content

The moisture content of feed and fish carcass and muscle was determined according to the A.O.A.C. (1990) procedure. In summary, samples of feed materials, entire fish carcasses or muscle were weighed and dried to a constant final weight at 105°C inside a fan assisted Pickerstone E 70F oven (R E Pickerstone Ltd., Thetford, Norfolk). The percentage moisture in the sample was calculated thus:

Moisture (%) = Change in weight (g) / Initial weight (g) x 100

2.3.2. Determination of Crude Protein Content

The protein content of feed, faeces and fish samples was determined by the Kjeldahl method. Typically, after 500 mg of dried material in duplicate was weighed into a borosilicate digestion tube, 2 Kjeldahl catalyst tablets (2 X 3 g K_2SO_4, 105 mg $CuSO_4.5H_2O$ and 105 mg TiO_2, Thompson and Capper Ltd, Runcorn, Cheshire) and 20 cm^3 of concentrated H_2SO_4 (Sp.Gr. 1.84) were added. Digestion was carried out in a Gerhardt Kjeldatherm digestion block (C. Gerhardt Laboratory Instruments, Bonn, Germany) for 30 minutes at 250°C followed by a further 75 minutes at 380°C with the acid fumes collected and neutralised by 15 % NaOH in a Gerhardt Turbosog unit.

After cooling, using a Gerhardt Vapodest 3S distillation unit, the sample was diluted with distilled water and neutralised with 40 % NaOH. The inorganic ammonia in the sample was then collected into 50 cm^3 of saturated orthoboric acid (H_3BO_3) by steam distillation. Using BDH '4.5' indicator, the distillate was titrated against 0.2 M HCl and the percentage protein in the dry sample determined thus:

% Crude Protein = [Titre sample (ml) - Titre blank (ml)] x 0.2 x 14.007 x 6.25 / Weight of sample x 100

where;

0.2 = [HCl] in moles

14.007 = Relative molecular mass of nitrogen

6.25 = Constant describing relationship between nitrogen and protein content of sample.

2.3.3. Determination of Total Lipid

Total lipid in the samples of feed, faeces and carcass was determined by either Soxhlet extraction or a method derived from the preparative procedure described by Folch *et al.* (1959). In order to

carry out the Soxhlet extraction 5.0 g of dried sample was weighed into a cellulose extraction thimble (Whatman) which was fitted to a Gerhardt Soxtherm unit. The sample was refluxed with 130 cm^3 of petroleum ether (40-60 fraction) for 40 minutes in the "recovery" mode. This was followed by a further 70 minutes of reflux with the Soxtherm in the "circulation" mode. After this period the Soxtherm was again set for recovery and the remaining solvent removed from the collected lipid residue by evaporation. The change in weight of the collecting vessel was proportional to the lipid content of the sample and hence the percentage of lipid in the dry sample was calculated as follows:

% Lipid = Weight of lipid residue collected (g) / Weight of sample x 100

The alternative method of lipid determination was followed by a gravimetric determination of the lipid content of the solvent extract. Thus, 500 mg of dry material was weighed into a 50 cm^3 erlenmeyer flask to which 10 cm^3 of chloroform: methanol (2:1) was added. The flasks were sealed and left overnight at room temperature. At the end of this period the extract was filtered through a Whatman #2 filter into a test tube and the residue in the Erlenmeyer quantitatively removed using a further 10 cm^3 of chloroform: methanol. Duplicate 5 cm^3 aliquots were transferred to pre-weighed test tubes and the solvent evaporated at 55°C using a water bath. The weight gained by the test tube was proportional to the lipid content of the sample and hence the percentage of lipid in the dry material was calculated thus:

% Lipid = Weight gain of tube (g) / Weight of sample (g) x 100

2.3.4. Determination of Carbohydrate

Carbohydrate in the feed and in the faeces was determined to quantify carbohydrate digestibility in each treatment throughout the research program using a modified method derived from that outlined by Morris (1994).

Using a centrifuge tube calibrated to 10 cm^3, 50 mg of the dry material in triplicate was weighed accurately and 4ml volume of HCl (2 mol l^{-1}) was added. Following vortex mixing, samples were heated in a boiling water bath for 2 hours. At the end of this period, 2ml of hydrolysate was transferred and neutralised with NaOH (0.5 mol l^{-1}) using phenol red as an indicator. The solution was then made up to a final volume of 10 cm^3 and 25 µl was then withdrawn to determine the concentration of glucose in the hydrolysate by the glucose oxidase method as outlined in section 2.7.1. The glucose based carbohydrate content of the feed or faecal materials (g/g wet weight) was then determined thus:

Carbohydrate (%) = [glucose] in hydrolysate (mg ml^{-1}) x 20^a x 0.9^b / Weight of sample x 100

[a] where, 20 is the dilution factor

[b] where, due to the difference in molecular weight, 0.9 is factor allowing the estimation of glycogen from the measured glucose content of the tissue.

2.3.5. Determination of Ash Content

The ash content of the dry material was determined as outlined in the A.O.A.C handbook (1990). Thus, 500 mg of dry sample were weighed into a crucible and heated for 8 hours at 525°C in a Carbolite GLM 11/7 furnace (Carbolite Furnaces Ltd, Bamford, Sheffield). The weight gained by the crucible was proportional to the ash content of the sample and hence the percentage of ash in the sample was calculated thus:

% Ash = Weight gained by Crucible (g) / Weight of sample (g) x 100

2.4. Determination of Energy Content

The energy value of the test diets and faeces were obtained by bomb calorimeter using the standard technique A.O.A.C. (1990). Analysis was carried out using an adiabatic bomb calorimeter (Gallenkamp and Co. Ltd., Loughborough, England).

Approximately 0.5 g of diet or faeces and same amount of benzoic acid were mixed thoroughly and pressed into a pellet. Then the pellet was suspended by gun cotton from a platinum wire connecting the anode and cathode inside the bomb. Absorption of the combustion gases was achieved by inclusion of 1 ml of water in the bomb. The bomb was then filled with pure oxygen to 30 bar and immersed in a water jacket of known temperature. The bomb was fired and the maximum temperature reached by the water jacket was recorded. The energetic value of the benzoic acid standard, diet and faecal samples was calculated using the following formula:

$E_s = \Delta_t S / \Delta_{t/g} B \times E_B / W$

where, 'E_s' represents the energy value of the sample in kJ g^{-1}, '$\Delta_t S$' is the temperature difference in °C due to combustion of the sample, '$\Delta_{t/g} B$' is the temperature change of the combustion of 1g of benzoic acid and 'E_B' is the energy value of 1 g of benzoic acid standard in kJ g^{-1}. 'W' is the weight of the sample.

2.5. Digestibility Trials

2.5.1 Faeces Collection

Following growth trials, manual removal of faeces from the experimental fish was performed according to Austreng (1978) (see for discussion, Rodrigues, 1994). Fish were starved one day for complete evacuation of gastro-intestinal tract of rainbow trout. Groups of fish were fed with respective diets in the morning (9.00 am) until all fish are satiated (approximately 45 minutes). Uneaten feed (if any) was cleaned from the bottom of the tanks. Next morning each group of fish was evacuated in a portable tank. Every individual fish was immersed in an anaesthesia solution ethyl p-amino benzoate (Benzocaine, Sigma Chemical Co. Ltd, Poole, UK; 1g dissolved in 100ml of ethanol, added to freshwater at a concentration of 5ml l^{-1}) for two to three minutes and dried to prevent water mixing in to the faeces collection dish. Faeces then was stripped by

squeezing gently from abdominal part to the anus of the fish until faeces in the last part of the intestine was evacuated in labelled aluminium dishes. No blood contamination was observed during this procedure. Stripped fish were returned to their experimental tank following recovery. The same procedure was repeated for all fish and collected faecal material was freeze-dried and frozen at –70 °C until nutrient and energy analysis was later performed. After completion of faeces collection, fish were recovered by feeding three days with respective diets and again starved for 72 h for the next stripping phase. No mortality occurred during the course of faeces collection. Each digestibility trial lasted until sufficient amount of faeces (approximately 5 g) was collected for further proximate analysis and inert marker determination.

2.5.2 Determination of Chromic oxide

The chromic oxide (Cr_2O_3) content of both the test diets and the faecal material was determined by the analysis for chromium in samples using flame atomic absorption. Due to the inert nature of the chromic oxide this could only be carried out after the samples had undergone a form of the wet acid digestion first described by Furukawa & Tsukahara (1966).

Triplicate 50-100 mg samples of the test diets and the corresponding faecal materials were weighed out into dry 250 ml. borosilicate digestion tubes. 5 ml of concentrated nitric acid (HNO_3) was added to each tube prior to their being heated to 120 °C for 75 minutes in the digestion block (Gerhardt - Kjeldatherm KT-20). After digestion, all of the organic matter was seen to have disappeared, the tubes containing clear solution and varying amounts of green precipitate. Once cool, 3 ml of concentrated sulphuric acid (H_2SO_4) and 2 ml Perchloric acid were added to each tube and all tubes were reheated to 200 °C for another 75 minutes, at which point a yellow, orange solution was obtained. After cooling, 30 ml of

deionised water was added to each tube. The resulting solutions were then filtered through Waterman "Fast Flow" hardened ashless paper and made up to 50 ml in a volumetric flask. The solutions were stored in plastic bottles in the darkness and refrigerated at 2 °C until required for chromium analysis. The samples were analyzed for chromium using a Varian AA-975 series Flame Atomic Absorbance Spectrophotometer. This was fitted with a chromium lamp set at a wavelength of 357.9 nm. The spectral band pass setting was 0.2 nm and the lamp current 7mA.

2.5.3. Calculation of Apparent Digestibility Coefficients

Percentage apparent dry matter and nutrient digestibility were calculated using the following formulas:

Apparent dry matter digestibility (%) =

100 - (100 x (% Cr_2O_3 in feed / % Cr_2O_3 in faeces))

Apparent nutrient digestibility (%) =

100 - (100 x (% Cr_2O_3 in feed / % Cr_2O_3 in faeces) x (% Nutrient in faeces / % Nutrient in Feed))

2.6. Determination of Blood Nutrients

2.6.1. Total Plasma Protein Concentration

Total plasma protein was determined by the Biuret method as described by the Sigma procedure No 541. Thus, 20 ml of bovine serum albumin (100 mg cm^{-3}), 20 ml of distilled water and 20 ml of plasma was added to 1.0 cm^3 of total protein reagent (Sigma Chemical Company) to produce the standard, blank and sample respectively. The reaction was allowed to proceed to completion (10 minutes at ambient temperature) and the absorbance of the sample was read against that of the blank at 540 nm using a Cecil Series 5000 UV/ VIS spectrophotometer. Having demonstrated that

the response of the assay was linear up to a protein concentration of 100 mg cm^{-3} the concentration of total protein in the sample was determined thus:

[Total Protein] (mg cm^{-3}) = Absorbance of test solution / Absorbance of standard x 100[a]

[a] where [Standard] = 100 mg cm^{-3}

2.6.2. Plasma Glucose Concentration

The concentration of glucose in the plasma was determined by the glucose oxidase method as outlined by the Sigma Procedure No 510. For the test, standard and blank 25 ml of sample, glucose standard (100 mg dl^{-1}) and water respectively were added to 0.5 cm^3 of distilled water. 5.0 cm^3 of combined enzyme colour reagent solution was added to all the tubes which were then incubated at 37°C for 30 minutes. The absorbance of the sample was then read against that of the blank at 450 nm using a Cecil Series 5000 U.V. Vis. spectrophotometer. Having shown that the response of the assay was linear up to a glucose concentration of 300 mg dl^{-1}, the concentration of glucose in the sample was calculated thus:

[Plasma glucose] (mg dl^{-1}) = Absorbance of test solution / Absorbance of standard x 100

[a] where [Standard] = 100 mg dl^{-1}

2.6.3. Plasma Triglyceride Concentration

The concentration of triglyceride in the plasma was determined by the enzymatic method as described by the Sigma Procedure No 334-UV. Initially, 1.0 cm^3 of triglyceride reagent was added in all cuvets and warmed to assay temperature (30 °C). Then, 0.02 ml of sample and 0.02 ml water were added into test and blank cuvets, respectively. The reaction was allowed to proceed to completion at assay temperature for 10 minutes and the absorbance of the blanks and samples recorded against water as reference at 340 nm using a Cecil Series 5000 U.V. Vis. spectrophotometer. The concentration of the triglycerides in the plasma was then determined thus:

Plasma Triglycerides] (mmol l^{-1}) =

(Absorbance of blank solution- Absorbance of test solution) x 726^a x 0.0113^b

726^a =(885 x 1.02 x 100) / 6.22×10^3 x 0.02 x 1

where, 885 = Molecular weight of triglycerides expressed as triolein

1.02 = Total volume (ml), 0.02 = Sample volume (ml)

6.22×10^3 = Molar absorptivity of NADH at 340 nm

1 = 1-cm lightpath, 100 = conversion of mg/ml to mg/dl, 0.0113^b = SI units

2.7. Definitions, Terms and Related Equations

Several nutritional parameters relevant to growth and feed utilisation efficiency were employed throughout the current programme of work and these are defined accordingly.

Weight Gain (%)

This parameter simply indicates the percent weight increment of the biomass. Thus:

Weight Gain (%) = (Final weight - Initial weight) / Initial weight x 100

Specific Growth Rate (% day^{-1})

Specific growth rate (SGR) is used to compare growth of fish on a relative daily basis expressed as percent increase in initial live weight over a defined period of time and hence reflecting the instantaneous rate of growth.

Specific Growth Rate (% day^{-1}) =

[Ln (final mean weight) - Ln (Initial mean weight)] / Experiment period (days) x 100

Feed Efficiency (%)

Feed efficiency relates the ability of the feed to support weight gain with respect to the amount of feed consumed or put simply, the extent to which feed is utilised for growth. Feed efficiency may be expressed as the feed conversion efficiency (FCE) or as the feed conversion ratio (FCR). The latter term is widely used in practical fish and animal nutrition field trials, however, scientifically FE is more acceptable since the efficiency is explained as a percentage term (Cowey, 1992);

Feed Efficiency (%) = Weight gain (g) / Feed intake (g) x 100

Food Conversion Ratio = Amount fed (g) / Weight gain (g)

Protein Efficiency Ratio and Apparent Net Protein Utilisation

The utilisation of protein for growth may be expressed as either the protein efficiency ratio (PER) or the net protein utilisation (NPU). The protein efficiency ratio simply quantifies the weight gained by the animal with respect to the amount of protein consumed and hence may be calculated according to the following expression:

Protein Efficiency Ratio = Weight gain (g) / Protein intake (g)

Protein Utilized kg^{-1} Growth (g) = Protein intake (g) / Weight gain (g) x 1000

Apparent net protein utilisation relates the utilisation of protein to its deposition in the carcass or muscle of the fish and hence indicates the efficiency of protein retention. Apparent net protein utilisation may be determined thus:

Apparent Net Protein Utilization (%) =

Final retained protein (g) - Initial retained protein (g) / Protein intake (g) x 100

Apparent Net Energy Utilization

Apparent net energy utilization indicates the efficiency of energy deposition in the carcass or muscle of fish and is calculated as follows:

Apparent Net Energy Utilization (%) =

Final retained energy (MJ)-Initial retained energy (MJ) / Energy intake (MJ) x 100

Digestible Energy (DE) Utilized kg^{-1} Growth (MJ) =

Digestible energy intake (g) / weight gain (g) x 1000

Condition Factor

Condition of body is an indicator of nutritional adequacy since well-fed fish often show high condition values. Thus:

CF = Fish weight (g) / (Fish length)^3 (cm)

Dress Out (%)

The quality of diet might change the fat accumulation in the viscera of the fish. For example, the fish fed high lipid or energy dense diets give lower DO (%) than the one fed low energy diets. It is calculated as follows:

DO (%) = (Fish weight (g) - Gut weight (g)) / Fish weight (g) x 100

Hepatosomatic Index (%)

It is also called liver index and high values may indicate that fat or glycogen is deposited in the liver. Thus:

HSI (%) = Liver weight (g) / Fish weight (g) x 100

2.8. General X-Radiography method

The X-radiographic technique applied in this study was that adapted by Sims *et al.* (1996) for gastric evacuation studies in dogfish (*Scyliorhinus canicula* L.). In contrast to Sims *et al.*

(1996), X- radiography was used in this research program for the purpose of return of appetite determinations rather than gastric evacuation studies.

A movable Philips "Practix" variable power output (kV) X-ray unit with light beam diaphragm attachment was used for taking all X-radiographic pictures. This system is located in the Biological Unit (BU) of University of Plymouth near to the aquarium where the experimental fish are maintained. All persons directly involved in the X-radiography were given and required to wear a thermo-luminescent detector (TLD) badge. The TLD monitored the cumulative X-ray dosage acquired over the duration of each three months, with the NRPB (National Radiological Protection Board, Didcot, Oxon.) providing periodic cumulative dose readings which were equivalent to 0 milliSieverts (mSv) throughout the return of appetite experiments. Blue sensitive film sheets (RP1, 24 x 30 cm, AGFA-Gevaert NV, Belgium), placed in hard cassettes (AGFA Blue R4, Curix rare earth screens, 24 x 30 cm) were used for all X-radiographs. Plastic cassettes protected the films from light and allowed an accurate image to be recorded on the films.

In summary, the anaesthetized fish to be X-rayed were placed directly on a plastic sheet covering a loaded cassette. The X-ray cassette was placed on a 3mm thick lead sheet (80 x 80 cm) situated on a stand just above floor level, with the X-ray generator head exactly 90 cm above the subjects. The light beam diaphragm unit enabled the subjects to be framed so that the area in which the X-rays would hit the subjects was exactly known. An exposure time of 0.16 or 0.2 seconds at an X-ray penetrating power of 40-45 kV potential difference (fixed 0.2mA) was used throughout the trials. The exposed film sheets were manually developed in an ventilated darkroom, situated within the Biological Unit by the author. The sheets of film were removed from the cassettes and individually immersed in developer

(G150, AGFA- Gevaert N. V., Belgium) for three minutes. After this period the film was removed from the developer with the use of plastic tongs, and rinsed thoroughly with water before being immersed in a tank of fixative agent (G350, AGFA- Gevaert N. V. Belgium) for between three to five minutes. Finally, the films were rinsed under running water to ensure the removal of all chemical agents from the newly developed film and air dried in a dryer cabinet at a temperature of 25^0C ± 0.5. Labels were attached to dry films to indicate the diet, time interval, and marker used to separate each film.

2.9. Return of Appetite Determinations

Apart from Experiment 1 (Chapter 3), return of appetite studies were undertaken after commencing a standard feeding trial for every single diet. Following digestibility trials and withdrawn of fish samples for proximate composition analysis, remaining fish were utilized for measurement of return of appetite using X-radiography.

Adult trout, *O. mykiss* (approximately mean weight 250 ± 15g SEM), were held as groups of approximately 20 fish in the previously mentioned facilities (2.1.2) at 15 °C ± 0.2 °C. During the acclimation period, the trout did not exhibit any unusual behavior and continued to feed normally on each respective test diet. A protocol was designed to allow each diet to be assayed at set time intervals (i.e: time = 0, 4, 8, 12, 24, 30 and 36 h) so that no fish was X-rayed more than once in a 96 hour period. This was to minimize stress in fish due to the handling and the X-radiographic procedure, and to ensure that all the previous meal contents (and therefore radio opaque glass beads) had been evacuated from the digestive tract prior to the next feeding. This procedure was employed for all experimental diets, and was repeated according to the protocol.

The first group of fish was fed to satiation with an unmarked diet for approximately 45 minutes and the total amount of feed delivered was recorded. Any surplus feed remaining on the bottom of the tank was removed and the total feed fed (g) was so corrected. The end of the feeding period was designated as time zero. The fish were then left to settle and noise was kept to a minimum within the aquaria so as to reduce conceivable stress and maximize second feeding at the next time period. After the required time interval, *eg.* t= 4h, the same group of fish were once again fed to satiation, this time with the test diet which contained X-ray dense indigestible dietary markers.

The total feed delivered for the second test diet was recorded and the fish allowed to settle for a period of 10 minutes. This was to reduce the risk of any of the trout vomiting during the resultant procedure, thus precluding them from the study. After the 'rest period' anaesthetized trout were X-rayed, 24 per time interval. The anaesthesia used was ethyl p-amino benzoate (Benzocaine, Sigma Chemical Co. Ltd, Poole, UK; 1g dissolved in 100ml of ethanol, added to freshwater at a concentration of 5ml l^{-1}). Immediately after the 'rest period' 12 trout were removed from the experimental tank via nets and placed in a temporary, aerated holding tank. Each trout was then weighed (to the nearest 0.01g) on an electronic balance, and placed temporarily into one of two plastic containers (containing aerated freshwater). This was repeated until all 12 trout were weighed and each container held six *O. mykiss*. A printout was obtained from the balance detailing the relevant statistics for the *O. mykiss* within each of the two containers. Benzocaine was then administered to one of the containers and when the fish were immobile they were X-rayed. All six fish were placed horizontally on the X-ray plate and a maker placed on the right hand corner of the plate. These procedures, from the time the fish were anaesthetized until they were returned to the holding tank and fully recovered, took 5-6 minutes. This procedure was then repeated for the

remaining six trout, with a different marker being used to distinguish the X-ray plate. Once all 12 of the trout were fully recovered in the holding tank the whole procedure was repeated on the remaining 12 fish in the experimental tank. The markers used to distinguish each x-ray plate within the group of four taken was also recorded on the corresponding printouts to facilitate the determination of individual trout weights from the developed X-ray plates. During the X-radiographic study no mortality was recorded due to handling or anaesthetic. Trout that were observed to vomit during the taking of the X-radiographs were removed from the studies. Low level of vomiting observed from the total amount of X-rays taken was as a direct result of ensuring that the anal side of the fish was touched as little as possible, as after anesthesia this was shown to trigger involuntary regurgitation of the recently ingested meal.

The X-radiographs of rainbow trout at specific time intervals were viewed on a light table (PLH Scientific Ltd., UK). The radio-opaque glass beads were clearly visible on the X-radiographs in the stomach. The smaller ballotini were aggregated toward the anterior of the stomach, the larger ballotini were well separated at the front of the stomach. Counts were made through a magnifying glass and could be made without variation between counts. The number of glass beads, of both sizes, within each stomach were recorded together with the weight of the fish (g). The amount of food consumed (expressed as % body weight) by individual fish was then calculated using the particular standard curve for the relationship between the weight of feed and number of and size of ballotini for each respective diet.

2.10. Gastric Evacuation Studies and Fish Sampling

Following the growth trial of Experiment 2 (Chapter 4.1), Experiment 4 (Chapter 5.1) and Experiment 6 (Chapter 6.1), gastric evacuation determinations were also performed for each treatment by serial slaughter in order to validate the X-Radiographic technique.

Experimental fish were deprived of food for 72 h and to ensure that the last meal had been completely evacuated before the start of return of appetite or gastric evacuation measurements. Each group of fish was then fed with associated diet. The feed was weighed (net weight to nearest mg) just before it was offered. After a multiple of 4 h or 6 h (i.e: sampling periods of 4, 8, 12, 24 and 48 h), fish were killed following prolonged immersion in ethyl p-amino benzoate (Benzocaine, Sigma Chemical Co. Ltd, Poole, UK; 1g dissolved in 100ml of ethanol, added to freshwater at a concentration of 5ml l^{-1}). From each trout a 2.0ml blood sample was withdrawn from the caudal vein with a medical syringe and centrifuged at 6500rpm for five minutes. The resultant supernatant was decanted off and placed within a capped 1.5ml microcentrifuge tube, labeled and frozen at – 70 °C for subsequent analysis.

From each sub-sample, fish weight (g), and fish length (fork length - cm) were measured and recorded. Then fish were dissected, gut weight (g) and liver weight (g) documented. The stomach was also removed to allow stomach fullness and gastric emptying data to be generated. Great care was taken to ensure minimal loss of digested material. Paper plugs were placed into the buccal cavity of the trout to prevent regurgitation. Fish sampled eight or more hours after initial feeding were placed in a freezer (-45 °C) for a period of up to 6 hours so as to solidify stomach contents and facilitate the removal of the stomach without loss of any stomach contents. The excised stomachs were placed into labeled, sealed plastic bags and frozen. The stomach contents were then removed, accurately weighed and dried at 105 °C

until a constant dry weight was obtained. All stomach contents were expressed as a percentage of the initial dry weight of the feed.

2.11. Statistical Analysis and Modeling

2.11.1. Allometric Analysis of Carcass and Muscle Components

Following growth trials and determinations of moisture, protein, lipid and ash content of carcass and muscle, all parameters were compared using one way ANOVA (Zar, 1996). Then the absolute weight of each parameter and the weight of whole carcass or muscle were log transformed and plotted. Finally, all slopes and intercepts were compared using Multiple Regression Analysis in Statgraphics (3.1). This analysis was performed to notice the possible misleading outcomes from the comparison of components using ANOVA only.

2.11.2. Modeling of Return of Appetite

The appetite return measurements were measured using a linear, exponential (first order) and sigmoidal (logistic) relationship as used by Sims (1994).

$$FI = a\,(1 - e^{-k*t}) \quad \ldots\ldots (1)$$

$$FI = 1/\,(a + b*e^{-k*t}) \quad \ldots\ldots (2)$$

Where, 'FI' represents the return of appetite or feed intake at time 't'. 'a', 'b' and 'k' are fitted parameters, with 'a' being the Y-intercept (asymptote to appetite return at t = 0) and 'k' the rate constant of appetite return. Each model was fitted to every appetite return data and the appetite return profiles of different dietary regimes were compared by ANCOVA. The parameters of the linear model were estimated using standard least square regression. The goodness of fit of the various models was compared by noting the magnitude of the

residual mean square (RMS) produced by each model (a measure of the variation in the data not explained by the model) and by comparison of the resultant r^2 values.

2.11.3. Modeling of Gastric Evacuation Rate

Regression analysis was applied to describe the relationship between untransformed, log transformed and square root transformed measurements of percent feed remaining and time after ingestion for each diet. The evacuation models were assayed by comparing the coefficients of determination (r^2), standard errors of the regression (S.E.R.), y- intercepts and residual plots. The linear regression models were further tested for deviation from linearity by using an F-test for linearity. Multiple regression and partial residual analysis were used to describe the relationship between each treatment. The decrease in stomach contents of *O. mykiss* was modeled using four relationships: linear, exponential, double exponential and square root (for discussion on the efficacy of each model see Jobling, 1981c; Medved, 1985; Ruggerone, 1989b and Bromley, 1994). The model which gave the best fit to the stomach emptying data varied for different dietary treatments. Therefore each data set was explained by each model and multiple regression analysis applied to determine any significant difference between the dietary treatments.

$$S_t = S_o - kt \quad \ldots\ldots (3)$$

$$S_t = S_0 e^{-k*t} \quad \ldots\ldots (4)$$

$$S_t = (S_0 - k*t)^{\wedge 2} \quad \ldots\ldots (5)$$

Where, 'S_0' represents the meal size consumed at time= 0 and 'S_t' the stomach contents at the given time 't' in hours and 'k' denoting the instantaneous rate of gastric evacuation. The equations were fitted by linear and non-linear regressions to obtain least square estimates of

the rate parameter in the model. The intercept on the y - axis was fixed at 100% stomach contents. The Marquardt search algorithm (Marquardt, 1963) of Statgraphics Version 3.1 determined the estimates to minimize the residual sum of squares of the function k. Slopes of each experiment' gastric evacuation models were compared statistically by Multiple Regression Analysis.

CHAPTER 3

EXPERIMENT 1

EFFECTS OF DIETARY LIPID LEVEL ON FEED INTAKE, NUTRIENT UTILISATION AND POSTPRANDIAL PLASMA NUTRIENT CONCENTRATION IN THE RAINBOW TROUT, *Oncorhynchus mykiss.*

3.1 INTRODUCTION

It is believed that fish, like all animals, eat to satisfy their energy requirements (Rozin & Mayer, 1961; Brett & Groves, 1979; Kaushik & Luquet, 1983). However, the significance of dietary energy level on feed intake has been indirectly studied by fish nutritionists whose principal aim has been to examine different feed ingredients in order to achieve superior growth performances from balanced rations.

Scientific studies on the factors modulating voluntary feed intake in fish is sparse and the information on evaluation of regulatory factors has been extrapolated from research conducted on higher vertebrates (Fletcher, 1982; Jobling, 1986a).

Dietary interactions play an important role in the regulation of feed intake as well as a variety of abiotic and biotic factors (Elliott, 1975b, 1982).

As far as the capacity of fish to regulate feed intake according to the energy content of the diet is concerned, there are conflicting claims regarding whether salmonid fish such as the rainbow trout should or should not be fed to satiation under practical conditions. For

instance, Lee & Putnam (1973), Majid (1986) and Boujard & Medale (1994) pointed out that rainbow trout, *Oncorhynchus mykiss* can modulate feeding behaviour according to the energy content of the diet by adjusting the daily ration level on a self-regulatory basis. On the contrary, Cho (1992) suggested that satiation feeding in fish is not appropriate and that the only approach to fulfil daily requirements of energy and nutrients with reduced waste is to estimate daily ration using the nutritional energetics strategy. However, Talbot (1993) claimed that an excess feeding regime is most applicable and that low protein/energy ratios seem to place the rainbow trout on a higher anabolic level. Similarly, Vahl (1979) hypothesized that maximum voluntary feed intake is one of the most important parameters in order to obtain maximum growth in fish.

The protein sparing effect of dietary lipid has been comprehensively established over 20 years in rainbow trout (De La Higuera *et al.*, 1976; Reinitz *et al.*, 1978; Watanabe *et al.*, 1986; Beamish & Medland, 1986; Davies, 1989; Corraze *et al.* 1993; Garcia-Riera *et al.*, 1993; Lanari *et al.*, 1995), Atlantic salmon (*Salmo salar*) (Hillestad & Johnsen, 1994; Helland & Grisdale-Helland, 1998), Arctic charr (*Salvelinus alpinus*) (Arzel *et al.*, 1994), channel catfish (*Ictalurus punctatus*) (Garling & Wilson, 1976) and tilapia (*Sarotherodon mossambicus*) (De Silva *et al.*, 1991). However, the influence of high lipid rations or low protein/energy ratios on voluntary feed intake has received little attention (Jobling & Wandsvik, 1983; Cho, 1992).

It has been mentioned by a number of workers (Cowey & Sargent, 1979; Cho *et al.*, 1982; Alsted & Jokumsen, 1989; Cowey, 1993) that high energy diets with an unbalanced protein / energy ratio cause excessive fat accumulation in adipose tissue. Indeed, excessive lipid deposition in hepatic tissue results in fatty liver disease.

There is also a great consumer concern for increased fat deposition in muscle causing poor flesh quality and processing problems (i.e. salmon smoke industry). Consequently, it follows that if rainbow trout are able to regulate energy intake according to their energy requirement, then there should not be any surplus fat associated with the viscera, muscle and liver tissue. It is important to establish modern commercial diets for rainbow trout to avoid undesirable meat quality.

This preliminary experiment was designed to elucidate whether rainbow trout can regulate feed intake finely according to their energy demand. Hence commercial diets of different energy densities (protein / energy ratio) (Low Fat; LF, Medium Fat; MF and High Fat, HF) were fed to rainbow trout either at restricted (Low Fat Restricted; LFR, Medium Fat Restricted; MFR and High Fat Restricted; HFR) or satiation (Low Fat Satiation; LFS, Medium Fat Satiation; MFS and High Fat Satiation; HFS) levels to examine their effects on feed consumption, growth performance, feed and nutrient utilization and proximate composition.

3.2 MATERIALS AND METHODS

3.2.1 Experimental Fish and Holding Facilities

All female (♀) rainbow trout, *Onchorynchus mykiss,* were supplied from a local trout hatchery (Mill Leat, Ermington, Devon) and acclimatized to aquarium conditions for 3 weeks prior to the feeding trial. Graded batches of 30 trout (IBW: 65.2 ± 1.52 g SEM) were placed into duplicate 400 l, fiberglass tanks within a closed, fresh water recirculation system with a parallel flow of 6.8 l through the tanks per minute at a temperature of 15 ± 0.2 °C. Photoperiod was set as 12 hours light / 12 hours dark using fluorescent discharge lamps with daylight simulation.

3.2.2 Feeding and Performance Indicators

Three commercial diets were employed for the study. These were supplied by Trouw Aquaculture Ltd (Wincham, Cheshire, UK) and varied in declared oil content ie: Trout Standard 40, Royal Crystal Supreme and High Performance which were stated to contain 15, 21 and 30 % oil, respectively. Each diet was based on a similar pellet size (4 mm) for use within the study. Measured chemical composition of experimental diets (closed formulations) is presented in Table 3.1.

Fish were fed by hand three times daily (0900, 1300 and 1700 h) and feed intake was recorded daily throughout the 56-day-feeding trial. Trout were weighed individually every two weeks to adjust the feed intake for restricted regimes and in order to observe the growth performance and monitor nutrient utilization These indicators were calculated as outlined in Chapter 2 (2.7).

Table 3.1 Proximate composition of analyzed[1] experimental diets (closed formulations)[2]

	Low Fat	Medium Fat	High Fat
Moisture (%)	9.7	7.6	4.6
Protein (% DM[3])	47.7	47.4	47.7
Lipid (% DM)	20.0	23.0	32.8
Ash (% DM)	7.9	7.2	6.3
N.F.E.[4] (% DM)	24.4	22.5	13.3
Digestible Protein (DP) (%)	39.7	40.0	40.5
Digestible Energy (DE) (MJ kg^{-1})	17.0	18.6	21.3
DP/DE Ratio (g DP/ MJ DE)	23.3	21.4	19.0

1. Analysis were performed as explained in Chapter 2.3, 2.4 and 2.5.
2. Commercial diets produced by Trouw Aquaculture (UK). These are closed formulations but typically contain over 50 % Low Temperature fish meal (LT 94), soybean meal, maize gluten meal and vitamin/mineral premix
3. Dry matter
4. Nitrogen Free Extract

3.2.3 Sampling and Analytical Procedures

After completing the feeding trial, fish were starved for one day and following re-alimentation, faecal material was stripped manually according to the method of Austreng (1978) and stored at - 25 °C for further analysis. Then ten fish were typically removed and stored for subsequent carcass and muscle analysis. Blood samples (approximately 2 ml) were obtained from 72 hour starved and fed fish following anesthetization. Then fish were quickly killed and length, weight, gut weight and stomach contents were recorded.

Blood samples were immediately centrifuged at 6000 rpm to obtain clear plasma and each sample kept frozen at -70 °C for subsequent analysis. Plasma glucose, protein and

triglyceride analytical reagents were obtained from Sigma Diagnostics (Sigma Chemical, Poole, Dorset, UK) and spectrophotometric assays were performed according to the manufacturer protocols and as described in Chapter 2 (2.6).

Random samples of 10 initial and experimental fish carcasses and muscle were dried at 105 °C to determine the moisture content. Crude protein was determined using the Kjeldahl method after acid digestion. Lipid analysis was performed according to Folch *et al.* (1959). Ash was determined by the ignition of samples in a muffle furnace at 550 °C overnight (12 hours). These analysis were as explained in Chapter 2.3 and in accordance with the official AOAC (1990) methods. Digestibility was determined by the AIA (Acid Insoluble Ash) technique for diets and faeces (Van Keulen & Young, 1977) as follows:

5 g of triplicated diets and faeces were ashed as described above. Each ashed sample was then washed into a centrifuged tube with 1.2 M HCl, and made up to 10 ml with the same acid. Following heating in a water bath (80 °C) for 5 minutes, samples were centrifuged at 6000 rpm for 5 minutes. The supernatant was discarded and 10 ml deionized water added to each sample. They were then shaken, centrifuged again and supernatant discarded. The same procedure was repeated one further time. Samples were then freeze-dried following supernatant removal. After drying, they were weighed and acid insoluble ash was calculated:

% AIA = $(W_{AIA} \times 100) / W_S$, where, W_{AIA} is the weight of the AIA recovered and W_S is the weight of the feed or faeces sample.

Energy contents of freeze-dried faeces and diets were determined in an adiabatic bomb calorimeter (Gallenkamp) as given in Chapter 2 (2.4).

3.2.4 Statistical Analysis

The statistical analysis to compare means between the six feeding regimes was made by One-way-ANOVA with the statistical software package, Statgraphics (Manugistics Incorporated, Rockville, MD, USA). Percentage data were arcsin transformed prior to ANOVA analysis. When statistically significant differences were detected by ANOVA, the multiple range test ($P< 0.05$) of Duncan (Steel & Torrie, 1960) was applied to test differences in mean values. Allometric analysis of carcass and muscle of the experimental fish were performed using multiple regression analysis to compare the slopes as outlined by Shearer (1994) and explained in Chapter 2.1.1. In all tests, the significance level was set at $P<0.05$ (95 % confidence level).

3.3 RESULTS

In this investigation, rainbow trout were fed three diets with varying protein/energy ratios for 6 weeks on either a restricted or satiation basis. Following the feeding trial, apparent digestibility coefficients of dry matter, protein, energy, lipid and carbohydrate for each treatment were determined (Table 3.2).

Table 3.2 Digestibility coefficients (%) of dietary nutrient components[1]

	Restricted			***Satiation***		
Treatments[2]	**LFR**	**MFR**	**HFR**	**LFS**	**MFS**	**HFS**
Dry Matter	64.1	78.6	81.9	67.9	63.9	86.0
Protein	83.0	88.6	89.9	83.2	79.9	92.3
Energy	77.0	82.6	85.0	75.0	76.0	86.0
Lipid	92.2	93.8	93.6	94.2	93.5	93.2
Carbohydrate	87.5	94.0	95.6	86.2	84.2	95.9

1.Coefficients based on pooled sample material from each dietary treatment (n=3).
2. LFR (Low Fat Restricted), MFR (Medium Fat Restricted), HFR (High Fat Restricted), LFS (Low Fat Satiation), MFS (Medium Fat Satiation) and HFS (High Fat Satiation)

Dry matter and energy digestibility of groups were elevated with the increase of the lipid level of diets. Apparent protein digestibility seemed to increase with the increase of dietary digestible energy concentration. Lipid digestibility of all treatments displayed a similar pattern between 92.2 and 94.2 %. Carbohydrate digestibility was generally higher in high fat treatments.

Fish fed to apparent satiation (LFS, MFS and HFS) consumed considerably more feed compared to restricted regimes (LFR, MFR and HFR) during the first four weeks of the

trial (Table 3.3). However, feed intake was generally decreased in satiation groups following the fourth week of feeding.

Table 3.3 Relative feed consumption of rainbow trout (g 100 g^{-1} biomass) (Biweekly basis)

	Restricted			*Satiation*		
Weeks	**LFR***	**MFR***	**HFR***	**LFS***	**MFS***	**HFS***
0-2	1.4	1.4	1.4	1.9	1.9	2.1
2-4	1.5	1.5	1.5	2.0	2.0	1.8
4-6	1.4	1.4	1.5	1.6	1.5	1.2
6-8	1.4	1.4	1.4	1.3	1.4	1.2
Mean Feed Intake	1.4	1.4	1.5	1.7	1.7	1.6

* LFR (Low Fat Restricted), MFR (Medium Fat Restricted), HFR (High Fat Restricted), LFS (Low Fat Satiation), MFS (Medium Fat Satiation) and HFS (High Fat Satiation)

LFS and MFS fish responded to diets very similar manner and utilized similar amount of feed whereas feed intake of HFS treatment was lower than that of LFS and MFS. When the overall feed intake is taken into account, the High Fat Satiation (HFS) group utilized 20 % more feed than High Fat Restricted (HFR) fish even though the final body weights of HFR and HFS were almost identical (Table 3.4). Final mean weight of LFS and MFS fish also very close to that of HFR and HFS fish. On the contrary the growth of LFR and MFR was significantly inferior compared to HFR and other satiation treatments at the end of the study (Table 3.4).

Table 3.4 Growth performance of rainbow trout fed three different oil levels practical feed for 56 days.

	Restricted			*Satiation*			
Treatments[1]	**LFR**	**MFR**	**HFR**	**LFS**	**MFS**	**HFS**	**±SEM[2]**
Initial mean weight (g)	65.8	64.2	65.9	64.5	65.9	64.7	1.52
Final mean weight (g)	192.2^{a}	194.9^{a}	226.1^{b}	225.4^{b}	222.7^{b}	226.1^{b}	8.34
Weight increment (%)	192	204	243	250	238	249	3.82
Feed efficiency (%)	127	131	139	122	117	129	11.73
Specific growth rate (% day^{-1})	1.8	1.8	2.0	2.1	2.0	2.0	0.29
Apparent net protein utilization (%)	50.6	56.0	53.6	50.3	48.1	52.0	5.7
Feed intake (% bw)	1.4	1.4	1.5	1.7	1.7	1.6	0.13
DP utilized kg^{-1} growth (g)	382	370	338	408	419	381	35.40
DE utilized kg^{-1} growth (MJ)	13.7	14.5	15.3	14.6	16.5	17.2	1.39
Condition factor	1.33^{a}	1.31^{a}	1.4^{c}	1.36^{ab}	1.38^{ab}	1.4^{c}	0.02
Dress out (%)	89.78^{c}	89.42^{c}	87.25^{a}	89.59^{c}	88.8^{bc}	87.36^{c}	0.36
Hepatosomatic index (%)	1.12	1.13	1.26	1.11	1.16	1.1	0.06

1. LFR (Low Fat Restricted), MFR (Medium Fat Restricted), HFR (High Fat Restricted), LFS (Low Fat Satiation), MFS (Medium Fat Satiation) and HFS (High Fat Satiation)

2. ± SEM, ± standard error of the pooled means, Values in each row allocated common superscripts or without superscripts are not significantly different from each other ($P > 0.05$).

An alternative growth response indicator (Specific growth rate) also showed the same phenomenon that LFR and MFR displayed inferior SGR compared to other treatments. Feed efficiency of all groups was excellent and lay between 117 % (MFS) and 139 % (HFR). Digestible protein (DP) utilized per kg^{-1} growth (the inverse formula of protein efficiency ratio) was calculated between 338 g (HFR) and 419 g (MFS). It was also detected that protein utilized per kg^{-1} growth increased in satiation trout compare to restricted ones fed the same feed. Digestible energy (DE) utilized per kg^{-1} growth was between 13.7 MJ (LFR) and 17.2 MJ (HFS). As was noticed in DP utilized per kg^{-1} growth, DE utilized per kg^{-1} growth elevated in satiation treatments compared to restricted fish fed the same feed.

Apparent net protein utilization of MFR was highest (56 %) whilst MFS demonstrated the lowest ANPU (48.1 %). Same parameter for other groups was between these medium fat treatments.

Hepatosomatic index did not show any significant variation among the treatments. However condition factor (CF) of high fat restricted and high fat satiation groups was significantly higher ($P<0.05$) than fish fed low fat or medium fat diets. In a similar manner, DO (dress out) of HFR and HFS was significantly lower ($P<0.05$) than other treatments.

The estimation of dietary energy partitioning (Table 3.5) showed that non-faecal energy loss was decreased from LFR to HFR groups proportionally, then it increased from LFS to HFS fish again. However this decrease or increase between treatments cannot be tested statistically.

Calculated retained energy in the carcass showed an increase with an increase in dietary lipid level which was more pronounced in the groups fed restricted rations. Estimated maintenance energy however displayed a similar pattern for all groups of trout.

Table 3.5 Estimation of dietary energy utilization by rainbow trout fed varying lipid diets either on a restricted or satiation regime calculated according to Cho & Kaushik (1985).

	Restricted			***Satiation***		
(%) Gross Energy	**LFR**	**MFR**	**HFR**	**LFS**	**MFS**	**HFS**
Gross Energy (GE)	100	100	100	100	100	100
Faecal Energy (FE)	23.0	17.4	15.0	25.0	24.0	14.0
Digestible Energy (DE)	77.0	82.6	85.0	75.0	76.0	86.0
Non-faecal Energy (ZE+UE+HiE)	14.0	10.3	9.2	12.6	13.4	13.8
Net Energy (NE)	63.0	72.3	75.8	62.4	62.6	72.2
Maintenance Energy	11.0	11.2	10.0	10.4	10.0	10.1
Retained Energy (RE)	52.0	61.1	65.8	52.0	52.6	62.1

The carcass and muscle proximate compositions of fish were presented in Table 3.6 and Table 3.7, respectively. It was observed that whole body moisture was inversely related to whole body lipid concentration and this component was positively related to the dietary lipid level. Whole body lipid was not affected by feeding rainbow trout on a restricted or satiation basis (e.g. % body lipid of LFR, 11.4; LFS, 12.1 or HFR, 16.8; HFS, 16.9).

Protein, lipid and ash components of whole carcass and whole fillet were calculated to be different significantly between groups following analysis of variance (ANOVA) as presented in Table 3.6 and 3.7. However, when the weight of fish was taken into consideration, no significant difference was determined in body protein and ash concentration (Table 3.8). Similarly muscle protein, lipid and ash were not significantly different ($P>0.05$) following multiple regression analysis. Only difference was observed in carcass lipid of HFR and HFS fish which were significantly higher ($P<0.05$) than other treatments. In a like manner, body moisture of HFR and HFS groups was significantly lower than fish fed low or medium fat diets.

Table 3.6 Proximate composition of the pooled carcasses of rainbow trout presented as a percentage of the whole fish.

	Initial	**LFR**	**MFR**	**HFR**	**LFS**	**MFS**	**HFS**	**± SEM**
Moisture	73.2^{d}	67.7^{bc}	67.3^{bc}	64.5^{a}	68.7^{c}	67.7^{bc}	65.4^{ab}	0.92
Protein	16.9^{ab}	17.4^{c}	17.5^{c}	16.1^{a}	17.2^{c}	16.7^{ab}	16.7^{ab}	0.29
Lipid	7.4^{a}	11.4^{b}	13.8^{b}	16.8^{c}	12.1^{b}	13.6^{b}	16.9^{c}	0.89
Ash	2.3^{b}	2.3^{b}	2.02^{a}	2.01^{a}	2.2^{ab}	2.2^{ab}	2.0^{a}	0.08

± SEM, ± standard error of the pooled means. Values in each row allocated common superscripts are not significantly different from each other ($P > 0.05$).

Table 3.7 Proximate composition of the pooled muscles as a percentage of the whole fish.

	Initial	LFR	MFR	HFR	LFS	MFS	HFS	± SEM
Moisture	76.6^{d}	66.1^{c}	64.9^{bc}	63.4^{ab}	64.1^{bc}	63.4^{ab}	61.8^{a}	0.77
Protein	16.2^{a}	19.7^{c}	19.2^{c}	19.9^{c}	19.7^{c}	18.9^{c}	17.8^{b}	0.41
Lipid	3.1^{a}	10.3^{b}	10.9^{bcd}	11.7^{cd}	11.5^{bcd}	10.7^{bc}	12.1^{d}	0.49
Ash	2.1^{b}	2.0^{b}	1.9^{ab}	1.9^{ab}	1.9^{ab}	1.9^{ab}	1.7^{a}	0.08

± SEM, ± standard error of the pooled means. Values in each row allocated common superscripts are not significantly different from each other ($P > 0.05$).

Table 3.8 Allometric analysis of carcass and muscle components of rainbow trout. (Data transformed and evaluated according to Shearer, 1994).

	Log (body protein) = a + b* Log (wt) R^2= 0.96		Log (body lipid)= a + b* Log (wt) R^2= 0.93		Log (body ash)= a + b* Log (wt) R^2= 0.78		Log (muscle pro.)= a +b* Log (wt) R^2= 0.93		Log (muscle lipid)= a + b* Log (wt) R^2= 0.83		Log (muscle ash)= a + b* Log (wt) R^2= 0.70	
	a	b	a	b	a	b	a	b	a	b	a	b
LFR	-0.98	1.1	-3.27	2.01	-1.59	0.98	-0.60	0.95	-1.34	1.18	-1.08	0.7
MFR	-0.98	1.1	-1.36	1.21	-1.65	0.98	-0.60	0.95	-1.34	1.18	-1.08	0.7
HFR	-0.99	1.1	-4.07	2.36	-1.65	0.98	-0.59	0.95	-1.3	1.18	-1.08	0.7
LFS	-0.99	1.1	-2.81	1.81	-1.61	0.98	-0.59	0.95	-1.34	1.18	-1.08	0.7
MFS	-1.00	1.1	-1.93	1.45	-1.60	0.98	-0.61	0.95	-1.34	1.18	-1.08	0.7
HFS	-1.01	1.1	-1.43	1.27	-1.65	0.98	-0.64	0.95	-1.34	1.18	-1.08	0.7
	S f=6.55	NS f= 0.4	S f= 3.90	S f= 4.45	S f= 3.16	NS f= 1.19	S f= 3.95	NS f= 0.44	NS f= 2.12	NS f= 1.62	NS f= 1.9	NS f= 1.61

S; significant, NS; nonsignificant

Plasma protein, glucose and triglyceride concentrations of starved and fed rainbow trout are presented in Table 3.9. Since these same parameters were not significantly different (P> 0.05) in either restricted and satiation regimes for each diet, data was therefore pooled and presented as low fat, medium fat and high fat groups, respectively. Plasma protein and glucose level of trout were significantly (P<0.05) elevated 4 hours following feeding. However, plasma triglyceride concentration appeared to decrease but no significant difference (P>0.05) was evident.

Table 3.9 Plasma nutrient concentrations in rainbow trout.

	Plasma Protein ($mg\ dl^{-1}$)			Plasma Glucose ($mmol\ l^{-1}$)			Plasma Triglyceride ($mmol\ l^{-1}$)		
	Starved	Fed	±SEM[1]	Starved	Fed	±SEM	Starved	Fed	± SEM
LF	5.49^{a}	6.14^{b}	0.24	3.41^{a}	4.68^{b}	0.18	4.29	3.75	0.42
MF	5.45^{a}	6.24^{b}	0.30	3.62^{a}	4.92^{b}	0.25	4.28	3.69	0.34
HF	5.69^{a}	6.24^{b}	0.33	3.88^{a}	5.13^{b}	0.21	4.34	3.66	0.48

± SEM, ± standard error of the pooled means Data in each row for each nutrient awarded different superscripts are significantly different from each other (P<0.05).

3.4 DISCUSSION

This investigation showed that growth and body composition in relation to carcass lipid levels could be modulated by the application of different feeding regimes, as previously mentioned by Jobling (1983) and demonstrated by Kiessling *et al.* (1989) in rainbow trout and Shearer *et al.* (1997) in chinook salmon, *Oncorhynchus tshawytscha.*

Regulation of feed intake in HFS seemed to be evident following the fourth week of the feeding trial. However, LFS and MFS fed fish seemed to adjust their feed intake after the sixth week of the experiment, but the difference between treatments cannot be tested statistically.

A considerable growth response was observed in all groups, indicating that fish from "satiation regimes" were placed in a higher anabolic plane to obtain maximum growth. HFR fed fish however, showed similar growth response to satiation groups which could imply that this group of fish were already consuming feed for maximum growth (Table 3.4).

Feed efficiency for all groups was more than 100 % which indicates that these extruded practical diets are adequately balanced as demonstrated by many studies (Johnsen & Wandsvik, 1991; Robert *et al.*, 1993). Increasing dietary lipid concentration however did not elevate Apparent Net Protein Utilization (ANPU), suggesting that diets including higher than 200 g kg^{-1} DM dietary lipid are unlikely to spare more protein than diets with approximately 200 g kg^{-1} DM dietary lipid.

Dress Out (%) was significantly lower in HFR and HFS compared to other treatments which may be the first indication of fat accumulation in fish. Hepatosomatic index was not

significantly different which might suggest that high dietary lipid level and feeding regime did not appreciably influence liver size.

Dietary energy level did not play a major role in short-term regulation of feed intake as Boujard & Medale (1994) also observed in trout. For instance, fish were starved for one week and then consumed 5 % body weight irrespective of dietary energy level in the study of the latter authors. They explained that this hyperphagia could be because of the reduction of body lipid reserves. However, these latter authors did not present carcass or muscle lipid content of the experimental fish on termination of their study. It is unlikely that carcass lipid levels were seriously depleted in a week. Therefore this reported hyperphagia could be explained by compensatory growth which has been extensively studied in fish (Jobling & Koskela, 1996).

Knowledge about the influence of body fat on voluntary feed intake is still very hypothetical even in higher animals. Scharrer & Langhans (1990) stated that loss of body lipid by starvation causes a transient hyperphagia, and that increased adiposity causes a transient hypophagia. This is in agreement with findings by Miglavs & Jobling (1989) with Arctic charr, *Salvelinus alpinus*.

By utilization of self-feeders, Boujard & Medale (1994) demonstrated that rainbow trout can regulate feed according to the energy density of the diet. However, Alanara (1994) observed a paucity of self feeding-response to the dietary energy content in rainbow trout fed at a higher stocking density than the former study.

It has been reported that dietary lipids increase the palatability of feeds to some extent in fish nutrition (De Silva & Anderson, 1995). Similarly, incorporation of fat in a diet for rats reduced feed intake but not significantly to maintain a constant level of digestible energy intake (De Castro, 1981), probably because this was offset by the improvement of palatability of the fat (Jacobs, 1967). Besides, Jen *et al.* (1985) stated that palatability of high fat diets in monkeys is more important than the metabolic effects in stimulating intake.

Allometric analysis (Table 3.8) of carcass proximate composition using logarithmic transformation of body component and fish weight indicated clearly that body protein and ash were endogenously controlled while carcass lipid was influenced by dietary lipid. However, muscle lipid showed no significance although comparison of muscle lipid was revealed to be significantly different before allometric analysis. Dietary interactions unarguably influence growth performance and feed utilization but body protein and ash content can be observed to be controlled endogenously when the weight of fish and actual amount of these constituents are taken into consideration.

The deposition of lipid in fish tissues is likely to be a continuous process, in which differential rates of deposition between varying tissues exist. Accordingly, lipid accumulation is most evident in body lipid stores (visceral fat) and least evident in skeletal muscle. However, at high dietary oil levels, accretion of lipids within muscle might become significant (Sheridan, 1994).

Energy digestibility values for high energy diets are higher than others and this might contradict the result of feed consumption between HFR and HFS. There is a possibility that the higher digestibility coefficient for energy (mainly as lipid) in the high fat satiation regime

was an artefact of the technique employed for measurement (ie: The faecal stripping or AIA method). The overall digestibility of the feeding trial may have varied due to natural rhythms and daily differences in meal consumption.

Elliott (1982) and Persson (1984) stated that the efficiency of digestion and absorption decreases with increasing ration size. The digestible energy intake difference between HFR and HFS groups could explain the reduction of digestion efficiency in HFS, although the digestibilities of energy and nutrients were very high. However, fish were stripped after a satiation meal at the end of the trial, not after feeding them continuously. Multiple-meal experiments demonstrated that the administration of a second meal speeds up the evacuation of the initial meal while the evacuation of the second meal is slowed (Ruggerone, 1989a; Bromley, 1994). Therefore, digestibility results of this study may not indicate the overall digestion efficiency under normal feeding conditions.

It has also been observed that rainbow trout may not have the capability to regulate their feed intake according to the energy requirement in the short term. Considerable alteration apparently occurred after the fourth week in satiation regimes, but at this stage, this can be attributed to the decrease of stomach volume indirectly because of increases of adipose tissue, or the regulation of body fat reserves via pancreatic hormones. In this context, Jobling & Miglavs (1993) and Shearer *et al.* (1998) (cited in; Shearer *et al.*, 1997) suggested that high adiposity suppresses feed intake.

In this respect, Brett & Groves (1979) claimed that fish receiving energy dense diets can utilise more nutrients at maximum physical intake, and are thus able to grow at a higher rate. Our results do not support this claim, because High Fat Restricted regimes reached the

maximum growth (2.0 % SGR) whilst consuming 20 % less than High Fat Satiation. On the other hand, Jobling (1986b) suggested that high rates of energy absorption could result in metabolic disturbances and may be the direct cause of abnormally high levels of fat deposition in farmed fish. However, the fish used in this study did not show any metabolic disturbance (e.g. normal liver size). Eight weeks were probably not enough to monitor metabolic disturbances such as fatty liver, carcass, muscle and impaired locomotion.

In this experiment, relative feed consumption was reduced after four weeks in HFS fish probably because of visceral fat accumulation. In this regard, the lipostatic theory of long term appetite control has been proposed for farm animals, and states that the hypothalamus is sensitive to blood metabolites which in turn are influenced by fat mobilization (Kennedy, 1953; Deutsch & Gonzales, 1981; Cook *et al.*, 1997). Forbes (1995) stated that " this 'long term' signal must be integrated with the various 'short term' signals in order that the sum total of the food eaten at a series of meals is appropriate to the animal's long term requirements". Furthermore, since the mechanisms between satiety and adipose tissue have not yet been quantified in farm animals, lipostatic theory is still unproven (Baile, 1971).

Plasma protein and glucose concentration tended to increase following feeding, whilst postprandial blood triglyceride level was depressed even though no significance was detected. Actually some pancreatic hormones could be responsible for such a decrease in triglyceride concentration. However more sampling intervals are necessary to draw a more defined picture. The effect of pancreatic hormones on protein, lipid and carbohydrate metabolism has been demonstrated although the regulation of lipid metabolism is not fully understood. For instance, insulin, glucagon and glucagon-like peptide play modulatory roles

in the regulation of lipid and carbohydrate metabolism in salmonids (Sheridan & Mommsen, 1991; Harmon & Sheridan, 1992a, 1992b).

In summary, dietary lipid level and protein / energy ratio appeared to affect carcass lipid level directly irrespective of the feeding regime which is in agreement with Wathne (1995) and Shearer *et al.* (1997). Hence, feeding fish on a satiation basis would not be applicable as long as maximum growth is obtained on restricted feeding regime. Consequently, it could be reasonable to suggest that claims by Vahl (1979), Talbot (1993) and Brett & Groves (1979) that fish should be fed as much as they can eat, since they can regulate feed intake, are not relevant. Maximum growth could be obtained by feeding fish with high energy diets on a restricted basis. In this case, undesired fat deposition could be hindered and waste output is minimized. Above mentioned studies could be supported by Cacho *et al.* (1990) who studied the relationship between dietary protein and feeding rate in channel catfish, *Ictalurus punctatus* using bioeconomic analysis. These workers reported that similar growth performance could be obtained by feeding fish low protein diets at high rations or high protein diets at low rations.

In can be concluded that Low Fat diet (LF) with 17.0 MJ kg^{-1} DE and 23 g DP MJ DE protein/energy ratio would provide similar growth performance and nutrient utilization with significantly lower carcass lipid concentration compared to the HF diet. If high energy diets (i.e HF) is used, a restricted feeding regime should be employed in order to utilize less energy and protein per kg growth compared to a satiation feeding strategy.

The results of this preliminary experiment highlight the need to demonstrate the significance of dietary lipid or energy concentration in the regulation of feed intake in fish. In order to

improve our understanding of this matter, the following section reports a series of investigations in which gastric evacuation rate and postprandial plasma nutrients are also measured at set time intervals in rainbow trout. These parameters are supported by quantification of return of appetite for each experimental diet. For this purpose, experimental diets having different protein and energy concentration were fed to rainbow trout on a satiation basis. Feeding and growth response (Chapter 4.1), the gastric evacuation, return of appetite rates and postprandial plasma protein, glucose and triglyceride levels were examined (Chapter 4.2).

CHAPTER 4

THE INFLUENCE OF DIETARY ENERGY AND NUTRIENT DENSITY ON FEED INTAKE, NUTRIENT UTILIZATION, GASTRIC EVACUATION AND RETURN OF APPETITE IN RAINBOW TROUT, *Oncorhynchus mykiss*.

EXPERIMENT 2

4.1 EFFECT OF DIETARY ENERGY DENSITY ON FEED INTAKE AND NUTRIENT & ENERGY UTILIZATION IN RAINBOW TROUT, *Oncorhynchus mykiss.*

4.1.1 INTRODUCTION

The response of rainbow trout to diets of varying energy density has been studied (Lee & Putnam, 1973; Takeuchi *et al.*, 1978; From & Rasmussen, 1984; Davies, 1989; Kim & Kaushik, 1990; Alsted, 1991; Weatherup *et al.*, 1997) and reviewed (Cho *et al.*, 1982; Cho & Kaushik, 1985) extensively. However, many of the results are contradictory and implications to the aquafeed industry with respect to the use of high oil feeds are questionable and need to be re-evaluated (Cho, 1992; Mäkinen, 1994; Wathne, 1995).

The first experiment of this research programme demonstrated three important issues:

- Rainbow trout were unable to regulate their feed intake in the short term since fish fed a high energy commercial diet (DE: 21.3 MJ kg^{-1}) managed to adjust their feed intake after the fourth week of the feeding trial. The paucity of an initial regulatory response in feeding behaviour of trout was possibly connected to the palatability of dietary lipid.
- Diets with different digestible energy (DE) levels (17.0, 18.6 and 21.3 MJ kg^{-1}) resulted in similar growth performance and nutrient utilization in rainbow trout.
- Carcass lipid concentration increased significantly ($P<0.05$) in fish fed diets with 32.8 % dietary lipid level with high DE concentration (21.3 MJ kg^{-1}) whilst body protein and ash were independent of the respective dietary treatments.

In order to test whether rainbow trout may have the capacity to control their energy intake, diets with a wide range of nutrient and energy levels should be fed to rainbow trout. This also may provide information about what range of DE concentrations achieve similar growth and carcass profile. Furthermore, it is assumed that a similar (maximum) growth response will be observed in fish fed different nutrient and energy dense diets as a result of similar digestible nutrient and energy intake if there is a precise regulation of feed intake. To what extent do rainbow trout increase their feed consumption for maximum growth potential? Once maximum growth is achieved, excessive feed intake and lipid deposition should not appear. Also, a similar feed intake will be achieved if fish eat for gastric fullness irrespective of the energy density of the diet. In this case, it will be suggested that high nutrient dense diets do not influence feed intake in the short term. If so, when will diet quality factors begin to influence feed consumption? How will apparent net protein and net energy utilization be influenced?

In order to address these points, six diets were formulated in which the protein/energy ratio remained similar, but an overall dilution of the protein and energy level was achieved across the diet range (ie: 52.1 % digestible protein (DP), 21.3 MJ kg^{-1} digestible energy (DE) (Diet 1; D.1); 47.2 % DP, 20.3 MJ kg^{-1} DE (Diet 2; D.2); 41.7 % DP, 18.8 MJ kg^{-1} DE (Diet 3; D.3); 35.2 % DP, 15.5 MJ kg^{-1} DE (Diet 4; D.4); 29.0 % DP, 12.5 MJ kg^{-1} DE (Diet 5; D.5) and 23.6 % DP, 9.0 MJ kg^{-1} DE (Diet 6; D.6) were fed to juvenile rainbow trout). Diets (1-3) were effectively altered by incorporating extruded wheat meal as an available energy source. The three remaining diets (4-6) were diluted by α-cellulose to obtain the required protein and energy concentrations. Feed intake, growth performance, nutrient and energy assimilation and proximate carcass and muscle composition were all investigated.

4.1.2 MATERIALS AND METHODS

4.1.2.1. Experimental Fish and Holding Facilities

500 juvenile female rainbow trout, *Onchorynchus mykiss,* were obtained from a private fish farm (Mill Leat Trout Farm, Ermington, Devon, UK), were acclimatized to laboratory conditions for 3 weeks prior to the commencement of the feeding trial.

Batches of 40 trout (mean weight 27.1 ± 0.26 g SEM) were placed into duplicate 400 l, fiberglass tanks within a closed fresh water recirculation system as explained in 3.2.1.

4.1.2.2 Feeding and Performance Indicators

Six experimental diets were formulated (Table 4.1.1) and manufactured as described in Chapter 2.2.2. Trout were fed to apparent satiation by hand three times daily (09.00, 13.00 and 17.00 h). Feed provision was recorded daily throughout the 84-day-trial. Trout deprived of feed for one day (without being anaesthetized) were weighed individually every four weeks to observe growth performance and nutrient utilization. Parameters relevant to growth and feed utilisation efficiency were calculated as outlined in Chapter 2.7.

Table 4.1.1 Dietary formulation and chemical composition of experimental diets.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
LT Fish Meal[1]	66.5	55.5	45.6	40.0	35.0	28.5
Blood Meal[2]	3.0	3.0	3.0	3.0	3.0	3.0
Poultry Meat Meal[3]	8.3	8.3	8.3	8.3	8.3	8.3
Extruded Wheat Meal[4]	-	14.0	24.0	15.7	4.7	-
Fish Oil[5]	18.5	15.0	12.1	11.2	10.4	9.0
Vitamin/Mineral Premix[6]	2.5	2.5	2.5	2.5	2.5	2.5
α- Cellulose[7]	-	0.5	3.3	18.1	35.0	47.5
Cr_2O_3[7] (Dietary marker)	0.25	0.25	0.25	0.25	0.25	0.25
Binder[7] (CMC[8])	1.0	1.0	1.0	1.0	1.0	1.0
Nutrient Analysis						
Protein (% DM[9])	56.3	51.7	45.8	40.3	34.6	29.2
Lipid (% DM)	25.5	22.2	19.0	16.7	15.4	13.0
Ash (% DM)	11.3	10.4	9.2	8.5	7.2	6.5
NFE[10] (% DM)	7.0	15.7	26.1	34.5	42.8	51.3
Digestible Protein* (DP) (%)	52.1	47.2	41.7	35.2	29.0	23.6
Digestible Energy* (DE)(MJ kg^{-1})	21.3	20.3	18.8	15.5	12.5	9.0
DP/DE Ratio (g DP MJ^{-1} DE)	24.4	23.2	22.2	22.8	23.3	26.3

1. Low Temperature fish meal, Norsea Mink, LT 94. Donated by Trouw Aquaculture, Wincham, Cheshire, UK.

2. Int. Feed Number, 5-00-381, Trouw Aquaculture, Wincham, Cheshire, UK.

3. Int. Feed Number, 5-03-798, " " " " "

4. Int. Feed Number, 4-05-205, " " " " "

5. Int. Feed Number, 7-01-994, Boost Oil, Cod liver oil, Seven Seas, Hull, UK.

6. (Closed Formulation). Trouw Aquaculture, Wincham, Cheshire.

7. Sigma Chemical Company, Poole, Dorset, UK.

8. Carboxy methyl cellulose

9. Dry matter

10. Nitrogen Free Extract

* see 4.1.2.3

4.1.2.3 Sampling and Analytical Procedures

At the end of the feeding trial, faeces were obtained for apparent digestibility determination by the stripping technique as outlined by Austreng (1978) and described in Chapter 2.2.

Ten fish from each treatment were also removed and stored for subsequent carcass and muscle analysis following measuring their length, body, gut and liver weights. Random samples ie: 10 from the initial stock and from each of the respective treatments were stored for subsequent proximate analysis on whole carcass and complete fillets. Crude protein, lipid and ash analysis were determined as outlined in Chapter 2..3.

Digestibility was determined by the indirect method (see 2.5.2 for details) using chromium oxide as the marker (Furukawa & Tsukahara, 1966; Singh & Nose, 1967). Energy content of freeze-dried samples of diets and faeces was determined by calorimetry (adiabatic bomb calorimeter, Gallenkamp) (Chapter 2.4.)

4.1.2.4 Statistical Analysis

Statistical analysis employed for the interpretation of experimental data was as explained in Chapter 2.11.1 and applied in Chapter 3.

4.1.3 RESULTS

In this experiment, rainbow trout were fed six diets of varying digestible protein and energy concentration for 12 weeks. Following the feeding trial, apparent digestibility coefficients of dry matter (DM), protein, energy and lipid for each test diet were determined (Table 4.1.2).

Table 4.1.2 Digestibility coefficients (%) of dietary nutrient components*

Diet No.	D.1	D.2	D.3	D.4	D.5	D.6
Dry Matter	86.6	83.9	81.5	79.3	58.1	30.2
Protein	92.5	91.4	91.1	87.4	83.7	80.8
Energy	93.9	90.9	87.6	75.0	63.0	47.0
Lipid	91.8	91.7	90.6	90.8	91.4	91.0

* Coefficients based on pooled sample material from each dietary treatment.

Dry matter and energy digestibility of diets was reduced substantially in accordance with decreased nutrient and energy density. There was also a proportional decrease in apparent protein digestibility, but not as high a variation compared to dry matter and energy digestibility coefficients. For instance, protein digestibility varied between 92.5 % (D.1) and 80.8 % (D.6) and energy digestibility was between 93.9 % (D.1) and 47.0 % (D.6). On the other hand, values around 90 % were observed for lipid digestibility in all groups irrespective of the dietary energy dilution from 21.3 MJ kg^{-1} (D.1) to 9.0 MJ kg^{-1} (D.6).

In this experiment, all groups of fish were fed to apparent satiation. Fish fed D.1 decreased their feed intake following the fourth week of the trial (Table 4.1.3). On the other hand, suppression of feed intake was apparent in other treatments after eight weeks. Overall mean feed intake was similar in fish fed D.1 and D.2. A marginal proportional increase was

observed in mean feed intake for trout receiving diets 3, 4 and 5, respectively. This increase was more pronounced in D.6 groups of trout. However it could not be tested statistically whether any significant difference existed between trout fed different nutrient-energy dense diets.

Table 4.1.3 Relative feed consumption of rainbow trout (g 100 g^{-1} biomass)

Week	D.1	D.2	D.3	D.4	D.5	D.6
0-4	2.1	1.8	2.2	2.1	2.1	1.8
4-8	1.6	1.9	1.9	2.3	2.3	3.1
8-12	1.7	1.7	1.7	1.7	2.0	2.3
Mean Feed Intake	1.8	1.8	1.9	2.0	2.1	2.4

Although different nutrient and energy intakes were observed in fish fed D.1, D.2 and D.3 test diets, similar growth performance was noted (Table 4.1.4). The first 3 groups of trout grew significantly better compared to D.4, D.5 and D.6 fish and a significant difference was evident between D.4, D.5 and D.6 treatments. The calculated specific growth rate (SGR) also followed the same trend in that D.6 (9.0 MJ kg^{-1} DE) fed trout displayed the poorest growth over the trial period.

The feed efficiency of fish receiving diets 1, 2 and 3 exceeded 100 % and this same parameter for the last three groups displayed a decreasing order with the D.6 trout, exhibiting the lowest value ie: 77 %.

Table 4.1.4 Growth performance of rainbow trout fed different energy-dense diets for 84 days.

	D.1	D.2	D.3	D.4	D.5	D.6	±SEM
Initial mean weight (g)	27.0	27.1	27.1	27.1	27.1	27.1	0.32
Final mean weight (g)	143.6^{d}	145.2^{d}	149.7^{d}	128.5^{c}	98.1^{b}	74.9^{a}	4.71
Weight increment (%)	431	435	453	374	262	176	4.60
Feed efficiency (%)	114	117	114	97	74	52	4.22
Specific growth rate (% day^{-1})	2.0	2.0	2.1	1.9	1.5	1.2	0.21
Apparent net protein utilization (%)	36.5	40.8	47.0	43.4	39.6	27.1	1.12
Apparent net energy utilization (%)	54.1	54.8	55.5	49.9	48.8	46.2	1.24
Feed intake (% bw)	1.8	1.8	1.9	2.0	2.1	2.4	0.17
DE utilized kg^{-1} growth (MJ)	19.6	17.4	17.1	16.1	16.9	17.6	1.76
DP utilized kg^{-1} growth (g)	478	404	379	367	394	461	4.22
Condition factor	1.43^{c}	1.43^{c}	1.42^{c}	1.35^{b}	1.31^{ab}	1.27^{a}	0.03
Dress out (%)	86.5	87.1	87.0	87.5	86.4	86.3	0.32
Hepatosomatic index (%)	1.28^{b}	1.17^{a}	1.17^{a}	1.15^{a}	1.19^{ab}	1.13^{a}	0.04

± SEM, ± standard error of the pooled means. Values in each row allocated common superscripts or without superscripts are not significantly different from each other ($P > 0.05$)

Digestible protein (DP) utilized kg^{-1} growth declined from 478 g (D.1) to 367 g (D.4) and increased again to 461 g (D.6). In a similar manner, digestible energy (DE) utilized kg^{-1} growth decreased from 19.6 MJ (D.1) to 16.1 MJ (D.4) and elevated again to 17.6 MJ (D.6). Most efficient protein utilization (apparent net protein utilization) was observed in trout fed D.3. ANPU increased from D.1 (36.5 %) to D.3 (47.0 %) groups, and declined to 27.1 % in the D.6 groups of fish. Moreover, the best apparent energy utilization was determined in D.3 with 55.5 %, but the difference was marginal with respect to the same

parameter for D.1 (54.1 %) and D.2 (54.8 %) fish. Again ANEU was reduced to 49.9, 48.8 and 46.2 % in D.4, D.5 and D.6, respectively (Table 4.1.4).

Condition factor (CF) of D.1, D.2 and D.3 trout was significantly higher ($P<0.05$) than that of D.4, D.5 and D.6 fish. Dress out (%) of all treatments did not show any significant difference ($P>0.05$). Hepatosomatic index (HSI) of D.1 group was however significantly higher ($P<0.05$) than the other five groups of trout.

The estimation of dietary energy apportion (Table 4.1.5) showed that non-faecal energy loss declined proportionally from D.1 (24.2 % of Gross Energy) to D.6 (11.0 % of Gross Energy). Retained carcass energy (determined by calculation) displayed the same order; the D.1 group being the highest (50.5 % of Gross Energy) and D.6 lowest (21.7 % of Gross Energy). This reduction in carcass retained energy was also in accordance with dietary lipid levels.

Analysis of proximate composition of rainbow trout (Table 4.1.6) showed that whole carcass protein and ash were not significantly different ($P>0.05$) in all treatments (Table 4.1.8). Carcass lipid component however was significantly higher ($P<0.05$) in D.1, D.2 and D.3 fish compared to D.4, D.5 and D.6 fish. On the other hand, muscle (whole fillet) protein, lipid and ash concentration displayed no significance between the treatments (Table 4.1.7) after weight of the fish was taken into account (Table 4.1.8). It was also observed that body or muscle moisture was inversely related to body or muscle lipid content of rainbow trout used in this investigation.

Table 4.1.5 Estimation of dietary energy utilization by rainbow trout fed varying energy diets.

(%) Gross Energy	D1	D2	D3	D4	D5	D6
Gross Energy (GE)	100	100	100	100	100	100
Faecal Energy (FE)	6.6	9.0	12.4	25.0	37.0	53.0
Digestible Energy (DE)	93.4	91.0	87.6	75.0	63.0	47.0
Non-Faecal Energy (ZE+UE+HiE)	24.2	22.0	19.5	19.2	15.6	11.0
Net Energy (NE)	69.2	69.0	68.1	55.8	47.4	36.0
Maintenance Energy	18.7	19.1	19.5	18.3	16.7	14.3
Retained Energy (RE)	50.5	49.9	48.6	37.5	30.7	21.7

Table 4.1.6 Proximate composition of the pooled carcasses of rainbow trout presented as a percentage of the whole fish.

	Initial	D.1	D.2	D.3	D.4	D.5	D.6	± SEM*
Moisture	71.7	66.4	67.9	69.0	71.2	71.2	71.1	0.59
Protein	14.9	16.3	16.2	16.4	15.5	15.1	15.0	0.25
Lipid	10.4	14.6^{c}	13.5^{bc}	12.2^{b}	10.6^{a}	11.0^{a}	10.8^{a}	0.53
Ash	2.3	2.2	2.2	2.3	2.3	2.3	2.4	0.07

* ± standard error of the pooled means (n=10). Values in each row sharing common superscript are not significantly different from each other (P > 0.05) (see Table 4.1.8)

Table 4.1.7 Proximate composition of pooled muscle of rainbow trout as a percentage of the muscle.

	Initial	D.1	D.2	D.3	D.4	D.5	D.6	± SEM*
Moisture	77.6	70.1	70.0	71.1	71.3	72.8	73.3	0.33
Protein	16.5	17.2	17.0	18.5	18.3	18.1	18.0	0.28
Lipid	3.0	10.3	8.8	8.7	8.6	7.6	6.9	0.51
Ash	2.2	1.7	1.7	1.7	1.8	1.9	1.9	0.03

* ± standard error of the pooled means (n=10). Values in each row are not significantly different from each other (P > 0.05) (see Table 4.1.8).

Table 4.1.8 Allometric analysis of carcass and muscle components of rainbow trout

	Log (body protein)= a + b* Log (wt) R^2= 0.99		Log (body lipid)= a + b* Log (wt) R^2= 0.97		Log (body ash)= a + b* Log (wt) R^2= 0.96		Log (muscle pro)= a + b* Log (wt) R^2= 0.99		Log (muscle lipid)= a + b* Log (wt) R^2= 0.89		Log (muscle ash)= a + b* Log (wt) R^2= 0.98	
	a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
D.1	-1.89	1.02	-2.19	2.14	-2.97	0.87	-2.06	1.07	-3.10	1.18	-3.67	0.92
D.2	-1.90	1.02	-2.27	1.95	-3.19	0.87	-2.09	1.07	-3.34	1.18	-3.64	0.92
D.3	-1.88	1.02	-2.38	1.65	-3.15	0.87	-1.99	1.07	-3.28	1.18	-3.68	0.92
D.4	-1.89	1.02	-2.50	1.18	-3.19	0.87	-2.00	1.07	-3.29	1.18	-3.64	0.92
D.5	-1.96	1.02	-2.44	1.09	-3.21	0.87	-2.01	1.07	-3.42	1.18	-3.60	0.92
D.6	-1.96	1.02	-2.46	1.05	-3.20	0.87	-1.99	1.07	-3.42	1.18	-3.61	0.92
	S f=2.80	NS f=1.35	S f= 7.94	S f= 2.95	S f= 6.48	NS f= 0.29	S f= 4.57	NS f= 1.10	S f= 2.78	NS f= 0.55	S f= 4.36	NS f= 0.23

(S; significant, NS; nonsignificant)

4.1.4 DISCUSSION

The protein digestibility values of D.1, D.2 and D.3 diets obtained from the present experiment were higher compared to those reported by Lanari *et al.* (1993) and Cho *et al.* (1976) and closer to those reported by Kim & Kaushik (1992) and Kaushik *et al.* (1989) for this species. However, protein digestibility was reduced in D.4, D.5 and D.6 proportionally due to the high level of indigestible material used for bulk dilution. Dry matter digestibility was also reduced with a reduction of energy and nutrient density in these diets. For example, dry matter digestibility of D2, D.3. D.4. D.5 and D.6 were 3, 6, 9, 49 and 186 % inferior respectively compared to that of D.1. The same parameter was 7 and 20 % inferior in trout fed 10 % and 20 % diluted diets, respectively in a study conducted by Hilton *et al.* (1983). It can be suggested that an appreciable effect on dry matter digestibility may be observed in rainbow trout fed more than 15 % diluted diets.

This nutrition trial supported findings from the previous study (Chapter 3) that the growth of rainbow trout fed three different energy diets (D1, D2 and D3) was similar with almost identical feed intake as also previously observed by Alsted (1991). The results of mean feed consumption (Table 4.1.3) indicate that the capacity of the cardiac stomach may be a more important determinant than either protein or energy concentration of the diets in the regulation of feed intake. The present study also supported the views of Jobling (1983), Wathne (1995), Shearer *et al.* (1997) and the author (Chapter 3) that carcass lipid level is positively related to dietary lipid concentration.

A good growth performance (Table 4.1.4) was observed in all groups, however D1, D2 and D3 groups showed superior growth responses as would be expected because of higher

digestible protein and energy intake. The close agreement between the final weights ($P>0.05$) of D1, D2 and D3 fish indicated that these groups were at a similar anabolic plane to obtain maximum growth throughout the feeding trial. These results also confirmed the growth performance data of the previous experiment (Chapter 3), From & Rasmussen (1984) and Jobling (pers. comm., 1998) that rainbow trout grow quite similarly when they are fed diets with a certain DE or DP concentrations on a satiation basis with a scope for maintaining an adequate nutrient intake.

Regulation of feed intake in D1 seemed to be evident from the fourth week of the feeding trial whilst D2, D3 and D4 fed fish regulated their feed intake from the sixth week of the experiment to the level reported for the D1 group. In this context, the present study confirms the view that finite control of protein and energy intake does not occur in trout in the short term, since D1, D2 and D3 fish displayed quite different nutrient and energy intakes. For instance, D1 group utilized 19.6 MJ digestible energy (DE) per kg^{-1} growth which is 12.7 % and 14.6 % higher than those of D2 and D3 fish respectively. In a like manner, D1 group trout utilized 18.4 and 26.2 % more digestible protein (DP) per kg^{-1} growth than D2 and D3 fish, respectively.

In this study, high rates of energy and nutrient absorption did not cause any visual metabolic disturbances (i.e. abnormal liver size; personal observation). This is in connection with the aforementioned study (Chapter 3) in which no metabolic disturbance was observed. Thus it might be implied that more time is required to notice such effects in salmonids.

Feed efficiency for first three groups (D1, D2 and D3) exceeded 100 % whereas D4, D5 and D6 accomplished 97.0, 74.1 and 51.6 % respectively. Protein efficiency ratio and net

protein utilization parameters also confirmed that high protein fed fish (D1 and D2) excreted excessive nitrogen without utilizing all protein for growth, whilst D3 (47.0 %) demonstrated the best overall performance. The satiation feeding regime employed in this study might have also resulted in a reduction of digestion efficiency in trout fed high energy diets as previously been reported (Ursin, 1967; Windell *et al.*, 1969 and 1978).

Dress Out (%) of all treatments was not significantly different probably because of fat accumulation in D1, D2 and D3 groups and heavier gastrointestinal weights (personal observation) in D4, D5 and D6 fed fish. Hepatosomatic index of D1was significantly different from others which might indicate that high dietary energy and protein influenced liver size probably in relation to lipid accumulation.

The estimation of partitioning of dietary energy (Table 4.1.5) suggested that non-faecal energy losses in D1, D2 and D3 are higher than D4, D5 and D6. Similarly, calculated retained energy level in the carcass followed the same trend. Feed intake of trout in this trial was not affected by protein content. Thus the SDA (specific dynamic action) effect associated with the deamination of the excess amino acids (Beamish & Thomas, 1984; Kaushik & Cowey, 1991) probably did not play a major role in the regulation of feed intake as mentioned by Fletcher (1984). However, the significance of SDA has been proposed by Beukema (1968), Muir & Niimi (1972), Vahl & Davenport (1979) and Medland & Beamish (1985) and implied by Jobling (1981a), Lucas & Priede (1992) and (Sims, 1994).

As far as the dietary energy dilution is concerned, Grove *et al.* (1978) reported that rainbow trout compensated their energy intake by consuming more feed which was diluted from 20 MJ kg^{-1} to 9.12 MJ kg^{-1} with kaolin. However, these authors did not present any data as to

whether rainbow trout were able to consume enough nutrient and energy for maximum growth. The feed intake and growth performance results of the present study therefore do not support the findings reported by Grove *et al.* (1978). This study revealed that rainbow trout may not be able to compensate their DE intake and consequently they do not grow at a high anabolic plane when they are fed diets whose dietary digestible energy concentration are diluted with an inert material up to 15 % (in this case) or 10 % (according to Hilton *et al.*, 1983).

Dilution of dietary nutrient and energy level with water has also been studied in trout (Bromley & Smart, 1981; Ruohonen *et al.*, 1997; Ruohonen *et al.*, 1998). From these studies, rainbow trout seem to compensate their feed intake and growth when water is included in their diets up to 50 %. This latter point is of interest to the practice of feeding moist diets (eg: silage and processed fish offal) to salmonids.

In the present investigation, it was observed that 21.7 % dilution of dietary digestible energy concentration (15 % dilution in term of α-cellulose) impaired the growth of D4 trout compared to D3. This is in support of Hilton *et al.* (1983) who recommended not more than 10 % cellulose in the rainbow trout diets. However, Bromley & Adkins (1984) reported no significant differences in growth performance in trout fed up to 30 % cellulose diluted diets. These latter authors utilized a high nutrient and energy dense diet (66 % crude protein, 20.5 MJ kg^{-1} gross energy with 95 % fish meal) as a control and diluted with α-cellulose. 30 % diluted diet contains 46 % crude protein and 14.35 MJ kg^{-1} gross nutrient energy. In this case, it may be suggested that trout compensated their energy requirements for maximum growth by utilizing dietary protein energy instead of non-protein energy components. It appears that the compensation of feed intake and growth in trout is possible up to a certain

dietary protein level (From & Rasmussen, 1984). Nevertheless, we need to establish more defined standards such as those for compensation to dietary dilution and less carcass lipid accumulation.

In a study directed by Shiau *et al.* (1988), tilapia (*Oreochromis niloticus* x *O. aureus*) (initial mean weight 5.14 g) were fed diets containing 2, 4, 10 and 14 % carboxymethylcellulose (CMC) on a restricted basis (3 % body weight per day). They found that the fish fed the diet with 2 % CMC showed superior growth performance. Moreover, Shiau *et al.* (1989) investigated the growth performance in the same species fed five different dietary fibres and as controls, glucose and dextrin was provided at an inclusion level of 10 %. These authors demonstrated that glucose or dextrin enriched diets produced superior growth and nutrient utilization because fish were fed similar level (3 % bw day^{-1}) of feed and consequently obtained more nutrient and energy from dextrin and glucose enriched diets. Since the fish did not feed on a satiation basis, it may not be possible to demonstrate whether other groups of fish were proceeding towards increasing their feed intake for optimum digestible nutrient and energy intake.

Results of carcass (Table 4.1.6) and muscle (Table 4.1.7) composition of fish support our aforementioned experiment (Chapter 3) that body protein and ash content are both independent of the dietary treatment. On the contrary, carcass lipid level of high energy-high protein treatment (D.1) was significantly higher ($P<0.05$) compared to D4, D5 and D6 treatments respectively. Comparison of slopes and intercepts derived from the allometric deposition of each nutrient was presented in Table 4.1.8. Body protein energy decreases with increase of dietary lipid level and vice versa. Inversely, body lipid energy level was deemed to increase with increasing dietary lipid as also reported by Einen & Roem (1997).

The same phenomenon is positive for muscle, however not as significant compared to the whole carcass.

In conclusion, D.3 (41.7 % DP & 18.8 MJ kg^{-1} DE) with 22.2 g DP per MJ DE can be recommended for maximum growth and nutrient utilization in rainbow trout from a practical standpoint.

Rainbow trout do not seem to be able to finely adjust their feed intake as also observed by Alanärä (1994) and Mäkinen (1994). This is in contrast to the views of Lee & Putnam (1973), Vahl (1979), Brett & Groves (1979) and Talbot (1993) who collectively expressed that trout are able to regulate meal consumption according to the energy concentration of the diet which may meet a set target energy intake.

Similar growth performance was observed in trout fed diets containing between 18.8 MJ and 21.3 MJ kg^{-1} DE which was in agreement with the results of Experiment 1 (Chapter 3). In that study, similar growth response had detected in fish fed diets with between 17 MJ and 21.3 MJ kg^{-1} DE. However, fish fed D.4 (15.5 MJ kg^{-1} DE) showed inferior growth which might be explained by 15 % α- cellulose inclusion in the diet.

Moreover, together with the results of Chapter 3, it can be suggested that 255 g kg^{-1} DM or higher dietary lipid level is likely to elevate the carcass lipid concentration significantly ($P<0.05$).

Regulation of feed intake was observed in D.1, D.2 and D.3. groups, however a relative reduction of feed intake was visualised in these groups following the tenth week of the trial.

Similar feed intake results may support the idea that rainbow trout may adjust their feed intake according to the degree of stomach fullness. It is possible that postprandial plasma nutrients may play regulatory role as well as gastric fullness. In this respect, there is a necessity towards investigating some physiological parameters for comprehending the overall response of rainbow trout to the varying level of energy and nutrient dense diets. Therefore the next experiment was planned using the same diet formulations in order to examine the gastric evacuation, return of appetite and postprandial plasma nutrient concentrations in rainbow trout. In this manner, the relative importance of these factors in the modulation of feed consumption in trout could be investigated.

EXPERIMENT 3

4.2. EFFECTS OF NUTRIENT & ENERGY DENSITY ON GASTRIC EVACUATION, RETURN OF APPETITE AND PLASMA NUTRIENTS IN RAINBOW TROUT, *Oncorhynchus mykiss.*

4.2.1 INTRODUCTION

The prediction of the return of appetite in cultured fish is important since one of the most significant considerations in aquaculture is to determine the appropriate feeding frequency and optimum ration size. If fish are fed continuously, then not only will uneaten feed be lost but also the environment may become polluted (Grove *et al.*, 1978). Furthermore, considerable amounts of dry matter may escape from gastric and intestinal digestion and assimilation following satiation feeding (Windell & Norris, 1969; Windell *et al.*, 1969). This claim has not been examined in rainbow trout adequately although the feed consumption results of Chapter 3 and Chapter 4.1 provided rational information that feeding trout with high energy and nutrient dense diets on a satiation basis may result in a reduction of assimilation efficiency.

It is obvious that the quantification of the rate of evacuation of a meal from the cardiac stomach and comparison of this pattern with the time at which appetite returns can provide important information towards understanding the processes of digestion and optimizing feeding regimes for farmed fish (Fletcher, 1982). Besides, information on evacuation rates with knowledge of the type and quantity of prey obtained from the stomach of wild fish has been widely used to estimate the feeding rates of fish populations (Jobling *et al.*, 1977; Talbot, 1985; Bromley, 1987).

It is interesting to note that almost identical feed consumption and growth performances were observed in trout fed diets containing 21.3 MJ kg^{-1} (Diet 1), 20.3 MJ kg^{-1} (Diet 2) and 18.8 MJ kg^{-1} (Diet 3) in Chapter 4.1. It may well be as a consequence of similar gastric evacuation and appetite revival rates associated with a different digestible energy intake.

It has been well established that rainbow trout increase their feed intake in order to obtain sufficient nutrient, energy and consequently maximum growth potential when the energy concentration of the diet is diluted (Bromley & Smart, 1981; Ruohonen *et al.*, 1998). This is achieved by enhancing the gastric evacuation rate. Besides, postprandial plasma nutrients may have significance in the compensation of feed intake and growth as well as the gut capacity as proposed by Vahl (1979).

The objectives of the present investigation are the quantification of gastric evacuation and return of appetite rates with postprandial plasma nutrients in rainbow trout fed different energy and nutrient dense diets utilized in Chapter 4.1. It is now hypothesized that if fish are allowed to eat as much as their energy requirement then there will be significantly different gastric evacuation rates in fish fed diets of varying energy density. Similarly, appetite revival time in fish fed energy dense diets may be significantly longer compared to those fed lower energy diets. The influence of both digestible energy and digestible protein density on gastric evacuation rates and return of appetite in trout remains to be explored.

In this investigation, return of appetite measurements were conducted by re-feeding groups of rainbow trout under defined experimental conditions. Gastric evacuation determinations were achieved by X-radiography in a preliminary trial. The serial slaughter technique was

also employed to validate the efficacy of the X-radiography method, since this is subject to criticism for use in fish (Jorgensen & Jobling, 1988).

Gastric evacuation modeling has also been the center of discussion over two decades as to whether linear, square root or exponential equations best describe the evacuation pattern in salmonids (Persson, 1979, 1981; Jobling 1981c; Grove, 1986). Therefore the present study was also directed to provide further information as a result of comparison of certain models.

4.2.2 MATERIALS AND METHODS

4.2.2.1 Experimental Fish and Holding Facilities

Rainbow trout, *Oncorhynchus mykiss* from the previous feeding experiment (Chapter 4.1) were used for the subsequent return of appetite and gastric evacuation measurements (serial slaughter). Following the feeding trial (Chapter 4.1), fish were ranked into two groups and subordinate groups (average body weight 80-120 g) were fed to apparent satiation (until no feed is eaten). Dominant groups (average body weight 130-180 g) were also fed on restricted (0.8 % of total biomass day^{-1}) with each respective diet. Eight weeks later, experimental fish (mean weight 185.0 ± 12.0 g SEM) (24 fish per group) were assigned to the return of appetite experiment. 140 fish (186.2 ± 15.1 g SEM) were also supplied for the gastric evacuation determination of trout by X-Radiography. Experimental conditions were as outlined in Chapter 2.1.

4.2.2.2 Test Diets

Formulation and chemical composition of experimental diets (Table 4.2.1) are the same as those used in Chapter 4.1 (Table 4.1.1). For the preliminary assessment of efficacy of X-Radiography in gastric evacuation study, 3.8 % of radio opaque ballotini powders (0.65-0.90 mm) by weight was added in the first batch of Diets 1 and 6, respectively (Table 4.2.2). The numbers of marker "ballotini" in known weights of diet were determined by X-radiography to ensure even distribution as outlined by McCarthy *et al.* (1993b) and Carter *et al.* (1995). The relationship between the weight of feed (FW) and the number of beads (N) was linear: $FW_{D.1} = 0.0233 \times N_{D.1}$, $r^2 = 0.98$ and $FW_{D.6} = 0.023 \times N_{D.6}$, $r^2 = 0.93$.

Table 4.2.1 Dietary formulation and chemical composition of the experimental diets.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
LT Fish Meal[1]	66.5	55.5	45.6	40.0	35.0	28.5
Blood Meal[1]	3.0	3.0	3.0	3.0	3.0	3.0
Poultry Meat Meal[1]	8.3	8.3	8.3	8.3	8.3	8.3
Extruded Wheat Meal[1]	-	14.0	24.0	15.7	4.7	-
Fish Oil[1]	18.5	15.0	12.1	11.2	10.4	9.0
Vitamin/Mineral Premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
α- Cellulose[1]	-	0.5	3.3	18.1	35.0	47.5
Cr_2O_3[1] (Dietary marker)	0.25	0.25	0.25	0.25	0.25	0.25
Binder[1] (CMC*)	1.0	1.0	1.0	1.0	1.0	1.0
Nutrient Analysis						
Protein (% DM)	56.3	51.7	45.8	40.3	34.6	29.2
Lipid (% DM)	25.5	22.2	19.0	16.7	15.4	13.0
Ash (% DM)	11.3	10.4	9.2	8.5	7.2	6.5
NFE (% DM)	7.0	15.7	26.1	34.5	42.8	51.3
Digestible Protein (DP) (%)	52.1	47.2	41.7	35.2	29.0	23.6
Digestible Energy (DE)(MJ kg^{-1})	21.3	20.3	18.8	15.5	12.5	9.0
DP/DE Ratio (g DP MJ^{-1} DE)	24.4	23.2	22.2	22.8	23.3	26.3

1. Same ingredients as given in Table 4.1.1.

Table 4.2.2 Dietary formulation and chemical composition of Diets 1 and 6 with the incorporation of ballotini particles

Ingredients	**Diet 1**	**Diet 6**
LT Fish Meal[1]	64.1	27.5
Blood Meal[1]	2.9	2.9
Poultry Meat Meal[1]	8.0	8.0
Extruded Wheat Meal[1]	-	-
Fish Oil[1]	17.8	8.6
Vitamin/Mineral Premix[1]	2.4	2.4
α- Cellulose[1]	-	45.8
Ballotini[2]	3.7	3.7
Binder[1]	1.0	1.0
Nutrient Analysis		
Protein (% DM)	54.3	28.1
Lipid (% DM)	24.6	12.6
Ash (% DM)	10.9	6.3
NFE (% DM)	6.7	49.5
Digestible Protein (DP) (%)	50.2	22.7
Digestible Energy (DE)(MJ kg^{-1})	20.6	8.7
DP/DE Ratio (g DP MJ^{-1} DE)	24.4	26.3

1. Same ingredients as given in Table 4.1.1.
2. Size: 0.6-0.9 mm (Jensons Ltd UK)

4.2.2.3 Return of Appetite Determinations

Return of appetite determinations were performed by re-feeding fish as separate groups. Following a–72-hour starvation period, fish were fed each respective diets for about 45 minutes until all fish reached apparent satiation (Ishiwata, 1968; Windell *et al.*, 1978). This was determined by monitoring the bottom of the tanks where 1-2 feed pellets remained. After removing and weighing residual feed, the amount of feed consumed was recorded. Fish were fed each respective diet again to apparent satiation 4 hours after first feeding. The level of re-feeding at the specified time interval was equal to the extent of appetite return. The uneaten feeds were collected and weighed and subtracted from the amount of the subsequent feed consumed. Then all groups were starved for 72-hours and the same procedure was repeated for subsequent time periods of 8h, 12h, 24h, 30h and 36h. Appetite return determinations were performed four times for each time interval. During the course of the experiment, the total biomass of fish was weighed during the second day of starvation without anaesthetic in order to perform weight specific calculations.

4.2.2.4 Gastric Evacuation Study by X-Radiography

Methods used for X-Radiography for the determination of gastric emptying rate were as outlined in Chapter 2.8. In order to test the power of the X-Radiography for the determination of gastric evacuation rates, two groups of 60 fish were fed Diet 1 or Diet 6 with ballotini for two weeks prior to the experiment. Fish were starved for 72 h to ensure that the last meal had been completely evacuated as observed in the preliminary assessment and reported by Windell *et al.* (1969) and Grove *et al.* (1978). Subsequently each group of fish was fed with marked diets (Table 4.2.2) until all fish reached apparent satiation. Fish from each of the two treatments were removed at

selected time intervals: time= 0 (as soon as feeding is completed), 6h, 12h, 18h, 24h, 30h and 36h. On each occasion, eight fish were sacrificed following prolonged immersion in ethyl p-amino benzoate (Benzocaine, Sigma Chemical Co. Ltd., Poole, UK; 4 g dissolved in 100 ml of ethanol, this added to fresh water at a concentration of 5 ml l^{-1}). Fish were then X-rayed using a portable Phillips Practix X-ray unit with light beam diaphragm attachment. The X-radiographic pictures of rainbow trout were viewed on a light table (PLH Scientific Ltd, UK) and glass beads were counted. Weight of feed recovered from each fish was calculated according to the calibration formula and expressed in weight specific terms. The X- radiography technique employed was as used by Sims *et al.* (1996) and described fully Chapter 2.8. X-rayed fish were then placed in a freezer (-20 ° C) for a period of up to 12 hours so as to solidify stomach contents and facilitate removal without loss.

Finally, stomach contents were removed into separate aluminium dishes and were accurately weighed and dried at 105 °C until a constant dry weight was obtained. All stomach contents were expressed as a percentage of the initial dry weight of the feed. Rate of digestion was considered a function of gastric evacuation measured by using a dry weight method.

4.2.2.5 Gastric Evacuation Study by Serial Slaughter and Fish Sampling

After completing return of appetite measurements, the fish used for return of appetite experiments together with those reserved for the gastric evacuation investigation were pooled. 60 fish were placed in each of the six tanks and returned for one week on the respective diets prior to post-mortem analysis of the stomach contents.

The sampling procedure was that same as detailed in 4.2.2.4. In summary, 8 fish from each six treatments were sacrificed following feeding all groups of fish with respective diets. After weighing sampled fish, paper plugs were placed in the buccal cavity of the trout following weighing and measuring individually to prevent regurgitation of digesta. Then 2.0 ml blood was withdrawn from the caudal vein of each trout. Digesta from each fish were carefully recovered and analysed as explained in 4.2.2.4. Stomach evacuation data derived from both X-radiography and serial slaughter were compared in order to assess whether the X-radiography technique could be used for gastric evacuation determinations.

Sampled blood was centrifuged (6500rpm) for 5 minutes to obtain clear plasma. The supernatant of each sample of blood was pipetted into a clean, labelled tube and kept frozen at -70 $^{\circ}$ C until plasma was analysed. Plasma glucose, protein and triglyceride reagents were supplied from Sigma Diagnostics (Sigma Chemical Co. Ltd., Poole, Dorset, UK) and spectrophotometric assays performed according to the protocol outlined in Chapter 2.6.

4.2.2.6 Statistical Analysis

The plasma nutrient data were subjected to analysis of variance (ANOVA) and the multiple range test ($P<0.05$) of Duncan (Steel & Torrie, 1960) using the statistical software package, Statgraphics (Manugistics Incorporated, Rockville, MD, USA). Return of appetite and gastric evacuation data were also for different time intervals compared using ANOVA following the arcsin transformation.

As explained in Chapter 2.10., regression analyses were applied to the gastric evacuation and return of appetite data and following equations were fitted where necessary:

$S_t = S_0 - k*t$ (Linear) ..... (1)

$S_t = (S_0 - k*t)^2$ (Square root) ..... (2)

$S_t = S_0 - e^{k*t}$ (Exponential) ..... (3)

$RA = 1/ a+b*e^{-k*t}$ (Sigmoid) ..... (4)

$RA = a(1-e^{-kt})$ (First Order) ..... (5)

Where, 'S_0' is the meal amount consumed at time = 0, 'S_t' represents the gastric content at the given time 't' and 'k' is the instantaneous rate of stomach evacuation for the first three regressions. 'a' and 'b' are the asymptotes to appetite return and 'k' is the rate constant of appetite revival at the given time 't' for the last two regressions. The fitted curves for return of appetite in trout fed different sources of carbohydrate were compared statistically by multiple regression analysis in order to test whether there was any significant difference.

4.2.3 RESULTS

4.2.3.1 Validation of X-Radiography for gastric evacuation determinations.

In the preliminary gastric evacuation trial in order to assess the efficacy of X-Radiography, significant results were observed in D.1 (Figure 4.2.1) and D.6 (Figure 4.2.2) fed fish. For instance, the evacuation slopes of D.1 derived from X-Radiography and serial slaughter differed significantly ($P<0.05$). Similarly, gastric evacuation rate of D.6 derived from X-Radiography and serial slaughter was found to be significantly different ($P<0.05$). It was apparent from the X-ray pictures that glass beads did not demonstrate a typical evacuation pattern, on the contrary they were selectively retained in the cardiac stomach region.

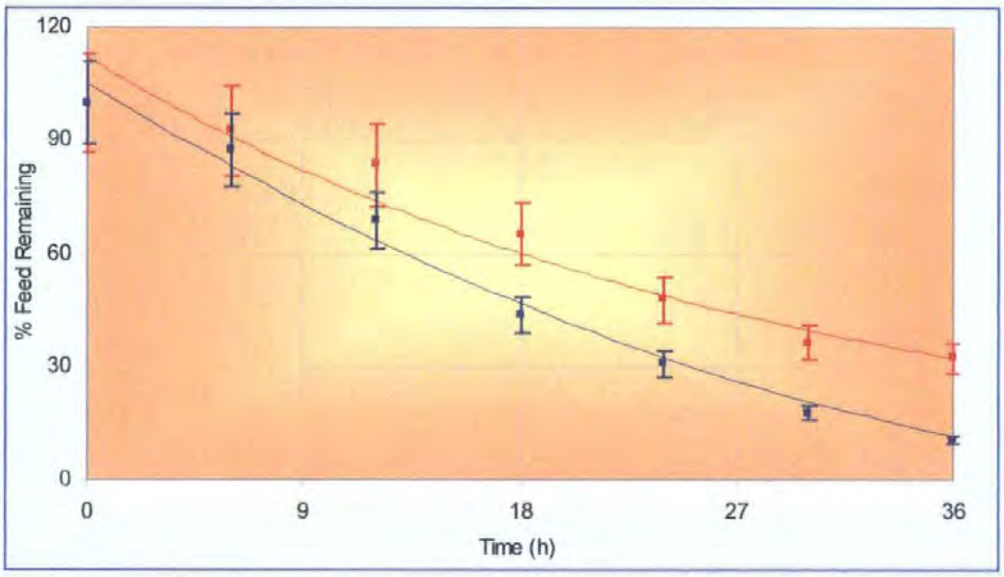


Figure 4.2.1 Percentages of gastric evacuation in rainbow trout fed D.1 following X-Radiography (■) and serial slaughter (■).

Fitted equations were $\mathbf{S_t = (10.6 - 0.14*t)^2}$, $\mathbf{R^2 = 0.81}$, and $\mathbf{S_t = (10.26 - 0.19*t)^2}$, $\mathbf{R^2 = 0.90}$, respectively. 'S_t' denotes percentage stomach content at time 't', n = 56. Slopes were significantly different ($P<0.05$) (d.f. 3:108, f= 10.9)

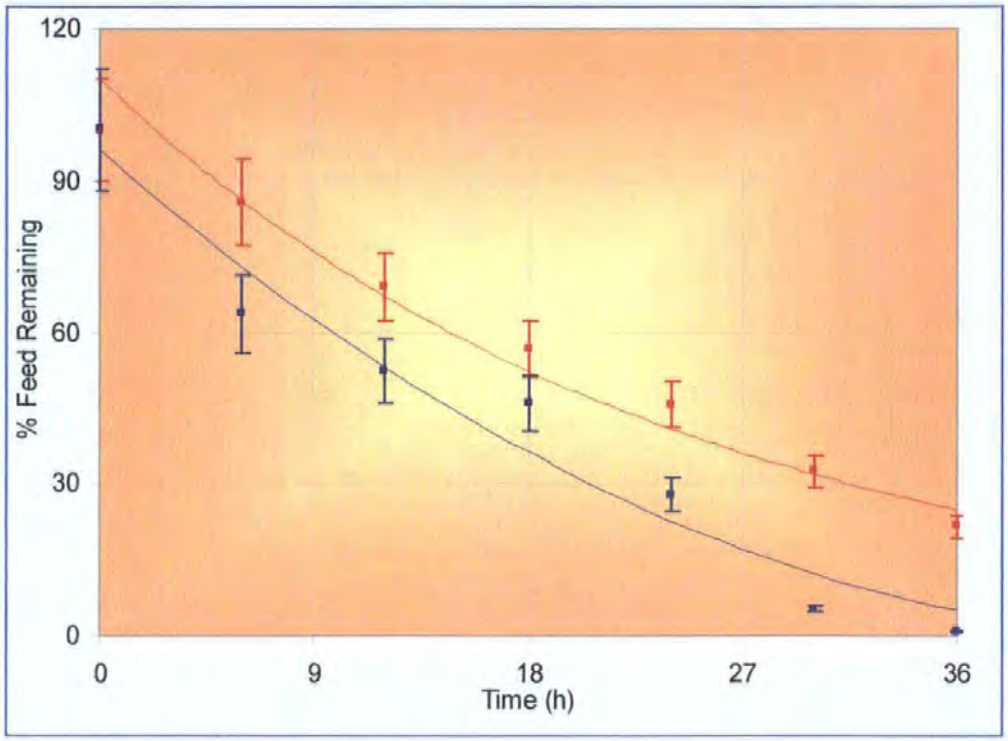


Figure 4.2.2 Percentages of gastric evacuation in rainbow trout fed D.6 following X-Radiography (■) and serial slaughter (■).

Fitted equations were $S_t = (10.3 - 0.16*t)^2$, $R^2 = 0.76$, and $S_t = (9.8 - 0.21*t)^2$, $R^2 = 0.90$, respectively. 'S_t' denotes percentage stomach content at time 't', n = 56. Slopes were significantly different ($P<0.05$) (d.f. 3:108, f= 12.2).

4.2.3.2 Gastric Evacuation and Return of Appetite Determinations

Following comparative stomach content analysis for the gastric evacuation modelling, square root models gave better fits compared to linear and exponential equations for the data set under examination. Fitted models were compared by multiple regression analysis. In order to choose the best fit, minimum residual mean sum of squares (RMS), intercepts nearest to 100 and consequently highest r^2 were taken into account. Minimum RMS and the highest r^2 for the evacuation of all treatments were obtained

in the square root model (Table 4.2.3). The comparisons of slopes for square root models are presented Table 4.2.5.

According to the square root fit for evacuation of Diet 1, 2, 3 and 4, no significant difference ($P>0.05$) at 95 % confidence level was evident (Table 6.2.5). However, the instantaneous rates of Diets 1, 2, 3 and 4 were significantly different than the rates of Diet 5 and Diet 6 treatments (Table 4.2.5). The evacuation slopes of Diet 5 and Diet 6 did not demonstrate any considerable difference ($P>0.05$).

First order and sigmoid equations were used for the description of return of appetite data (Table 4.2.4). Although both models fitted well, first order equations resulted in a better fit due to the lower residuals mean sum of squares in Diets 3, 4, 5 and 6. On the other hand, the return of appetite data of Diets 1 and 2 were marginally better explained by sigmoid models.

Table 4.2.3 Fitted equations for the gastric evacuation rates in rainbow trout fed different nutrient & energy dense diets.

Diets	Model[1]	S_0	k	r^2	RSM[2]
D.1	*Square Root*	10.26	-0.19	0.90	118
	Exponential	107.7	-0.05	0.87	149
	Linear	99.02	-2.65	0.89	127
D.2	*Square Root*	10.17	-0.20	0.87	163
	Exponential	106.23	-0.05	0.85	191
	Linear	96.02	-2.65	0.85	188
D.3	*Square Root*	9.89	-0.20	0.86	159
	Exponential	101.59	-0.055	0.85	166
	Linear	90.53	-2.47	0.83	190
D.4	*Square Root*	10.03	-0.20	0.91	100
	Exponential	103.65	-0.054	0.89	128
	Linear	94.26	-2.63	0.91	104
D.5	*Square Root*	9.85	-0.22	0.94	68
	Exponential	101.17	-0.065	0.92	87
	Linear	88.55	-2.65	0.92	96
D.6	*Square Root*	9.8	-0.21	0.88	143
	Exponential	99.93	-0.06	0.86	167
	Linear	89.25	-2.61	0.87	149

[1] Coefficients derived from the square root, $S_t = (S_0 - k*t)^2$, linear, $S_t = (S_0 - k*t)$ and exponential, $S_t = (S_0 * e^{-k*t})$, where 'S_t' is the percentage digesta remaining in the cardiac stomach and time 't'. 'S_0' is the percentage meal at time=0, k" is the rate of evacuation

[2] Residual Mean sum of Squares

Return of appetite slopes of Diet 1, 2 and 3 were not observed to be significantly different ($P>0.05$). However the slopes of these latter dietary treatments were significantly different ($P<0.05$) compared to Diet 5 and 6, respectively. On the other hand Diet 3 and Diet 4 did not demonstrate any considerable difference

(P<0.05) in appetite return patterns. Again, the instantaneous appetite revival rate of Diet 5 and 6 did not show any noticeable significance (P<0.05).

Table 4.2.4 Fitted equations for the return of appetite rates in rainbow trout fed different nutrient and energy dense diets.

Diets	Model[1]	a	b	k	r^2	RSM[2]
	First Order	237.7	-	-0.017	0.90	185
D.1	*Sigmoid*	0.0096	0.28	-0.21	0.95	104
	First Order	343.7	-	-0.01	0.93	106
D.2	*Sigmoid*	0.0098	0.18	-0.17	0.96	74
	First Order	167.73	-	-0.03	0.97	54
D.3	*Sigmoid*	0.0091	0.1	-0.19	0.96	83
	First Order	144.2	-	-0.041	0.98	45
D.4	*Sigmoid*	0.0093	0.085	-0.18	0.96	74
	First Order	116.5	-	-0.05	0.97	45
D.5	*Sigmoid*	0.01	0.063	-0.15	0.93	98
	First Order	107.16	-	-0.058	0.98	31
D.6	*Sigmoid*	0.01	0.083	-0.19	0.95	69

[1] Coefficients derived from the fitted first order, $FI= a(1-e^{-k*t})$ and sigmoid relationships, $FI= 1/a + b* e^{-k*t}$, where 'FI' is the percentage feed intake or appetite return, 'a', 'b' and 'k' are the constants and 't' is time (h)
[2] Residual Mean sum of Squares

Table 4.2.5. Statistical summary of comparison of the fitted gastric evacuation & return of appetite slopes in square root & first order form, respectively.

Treatments	Gastric Evacuation[1] ANCOVA[3] d.f. (3:108)		Return of Appetite[2] ANCOVA d.f. (3:46)	
	f	P	f	P
D.1 & D.2	0.6	>0.05	0.0	>0.05
D.1 & D.3	0.0	>0.05	2.0	>0.05
D.1 & D.4	1.8	>0.05	5.7	<0.05
D.1 & D.5	12.8	<0.05	14.5	<0.05
D.1 & D.6	8.8	<0.05	13.5	<0.05
D.2 & D.3	0.4	>0.05	1.6	>0.05
D.2 & D.4	0.1	>0.05	5.4	<0.05
D.2 & D.5	4.5	<0.05	15.0	<0.05
D.2 & D.6	4.2	<0.05	13.9	<0.05
D.3 & D.4	1.1	>0.05	1.3	>0.05
D.3 & D.5	8.2	<0.05	8.9	<0.05
D.3 & D.6	6.3	<0.05	7.9	<0.05
D.4 & D.5	5.4	<0.05	4.77	<0.05
D.4 & D.6	4.3	<0.05	4.24	<0.05
D.5 & D.6	0.0	>0.05	0.0	>0.05

[1] The fitted square root model $S_t = (S_0 - b*t)^2$, where 'S_t' is the percentage meal remain in the cardiac stomach at time 't'.
[2] The fitted first order model $Fl = a(1 - e^{-b*t})$, where 'Fl' is the percentage of feed consumed. [3]Significant differences at the 95 % confidence level ($P<0.05$) in shape of slopes determined by multiple regression analysis (ANCOVA).

Gastric evacuation and return of appetite models for D.1, D.2, D.3, D.4, D.5 and D.6 treatments are presented in Figures 4.2.3, 4.2.4, 4.2.5, 4.2.6, 4.2.7 and 4.2.8, respectively. The amount of meal ingested is presented in each figure as a percentage of the average satiation amount. The gastric evacuation curve of the population of fish following a satiation meal at the same temperature (15 °C) is presented on the same graph.

Two sigmoid and four first order equations described the appetite revival data of experimental groups (Figure 4.2.3, 4.2.4, 4.2.5, 4.2.6, 4.2.7 and 4.2.8, respectively). There was always a significant increase in feed intake ($P<0.05$) at time 4h in all groups of trout. Feed intake of all groups at time 30h and 36h was not significantly different ($P>0.05$). However, appetite return patterns of groups displayed some variances. For instance, fish fed Diet 1 and Diet 2 did not increase their feed intake significantly ($P>0.05$) between time 6h and 12h.

The time required for 95 % of appetite return was predicted as 31.0, 32.4 and 27.9 hours for D.1, D.2 and D.3 treatments, respectively (Table 4.2.7) according to the fitted first order equations. It was 26.2, 28.8 and 27.5 hours for D.4, D.5 and D.6 groups, respectively.

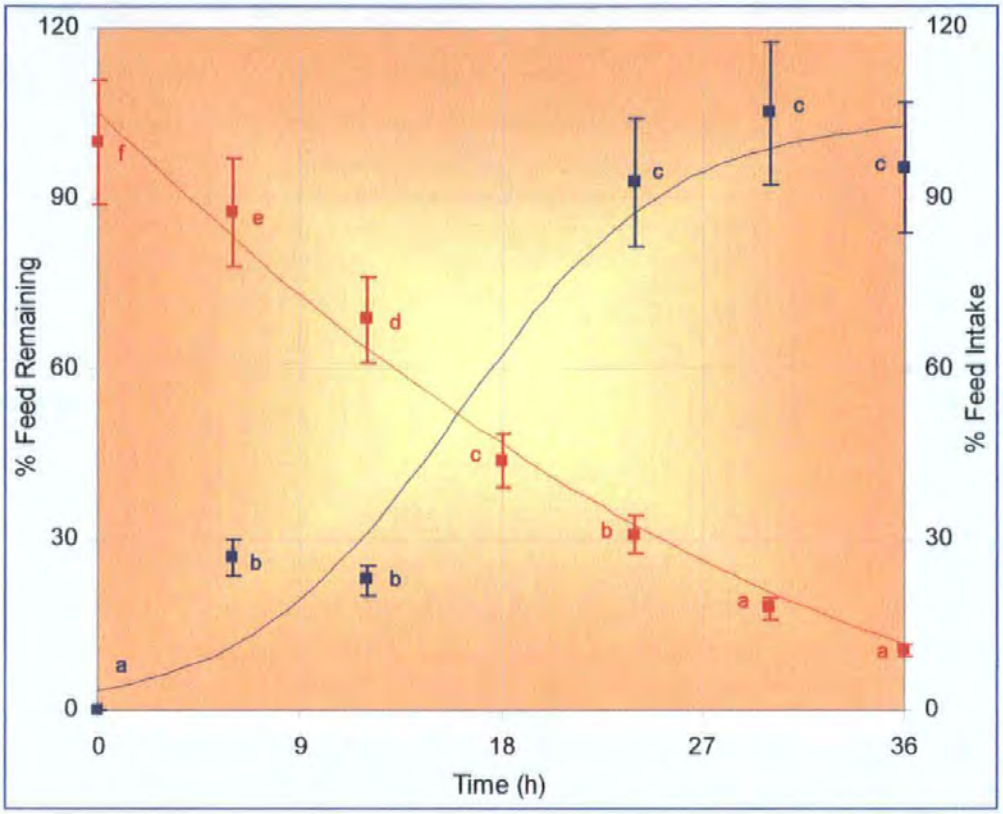


Figure 4.2.3 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed D1.

Stomach evacuation rate was described by a square root model;

$S_t = (10.26 - 0.19*t)^2$, $R^2 = 0.90$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (Sigmoid);

$FI = 1/ (0.0096 + 0.28* e^{-0.21* t})$, $R^2 = 0.95$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 4.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

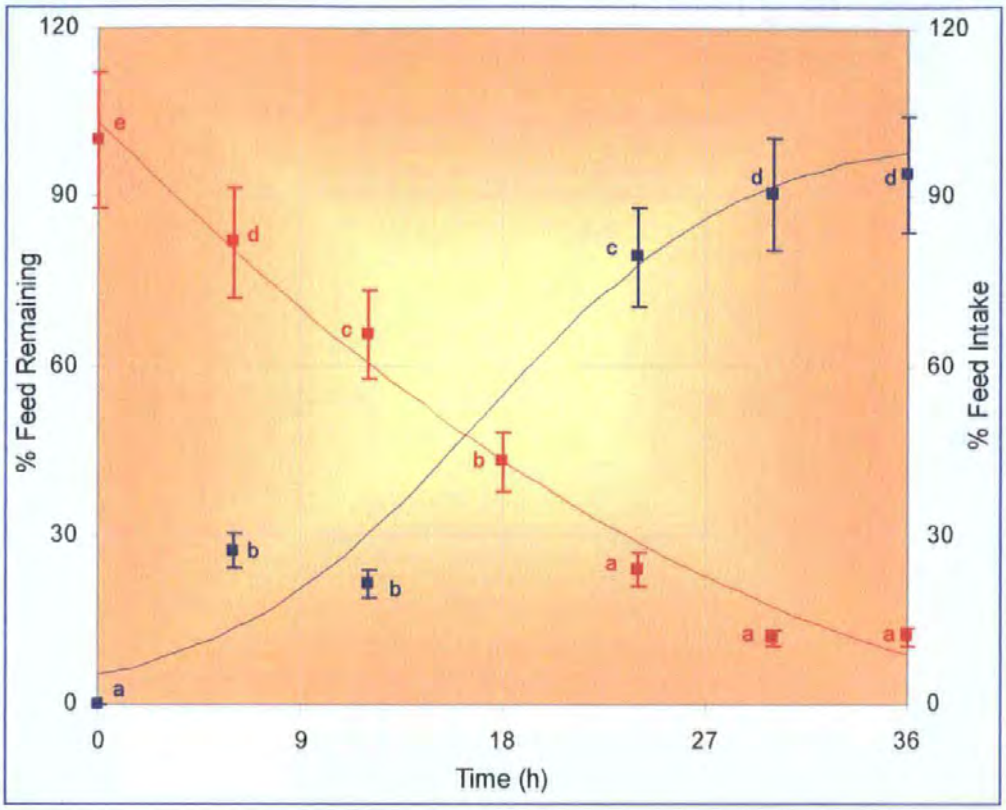


Figure 4.2.4 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed D.2.

Stomach evacuation rate was described by a square root model;

$\mathbf{S_t = (10.17-0.2*t)^2}$, $\mathbf{R^2 = 0.87}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (Sigmoid);

$\mathbf{FI = 1/ (0.0098 + 0.18* e^{-0.17* t})}$, $\mathbf{R^2 = 0.96}$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 4.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

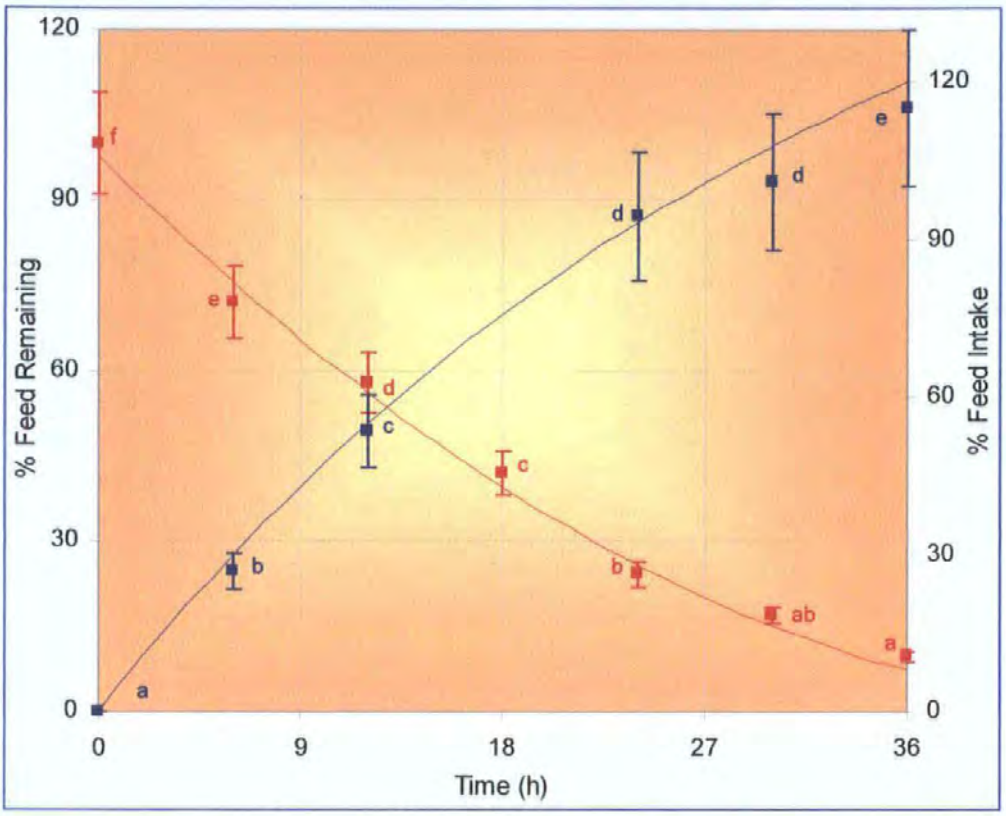


Figure 4.2.5 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed D.3.

Stomach evacuation rate was described by a square root model;

$\mathbf{S_t = (9.89-0.2*t)^2}$, $\mathbf{R^2 = 0.86}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$\mathbf{FI = 167.73* (1-e^{-0.03* t})}$, $\mathbf{R^2 = 0.97}$ Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 4.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

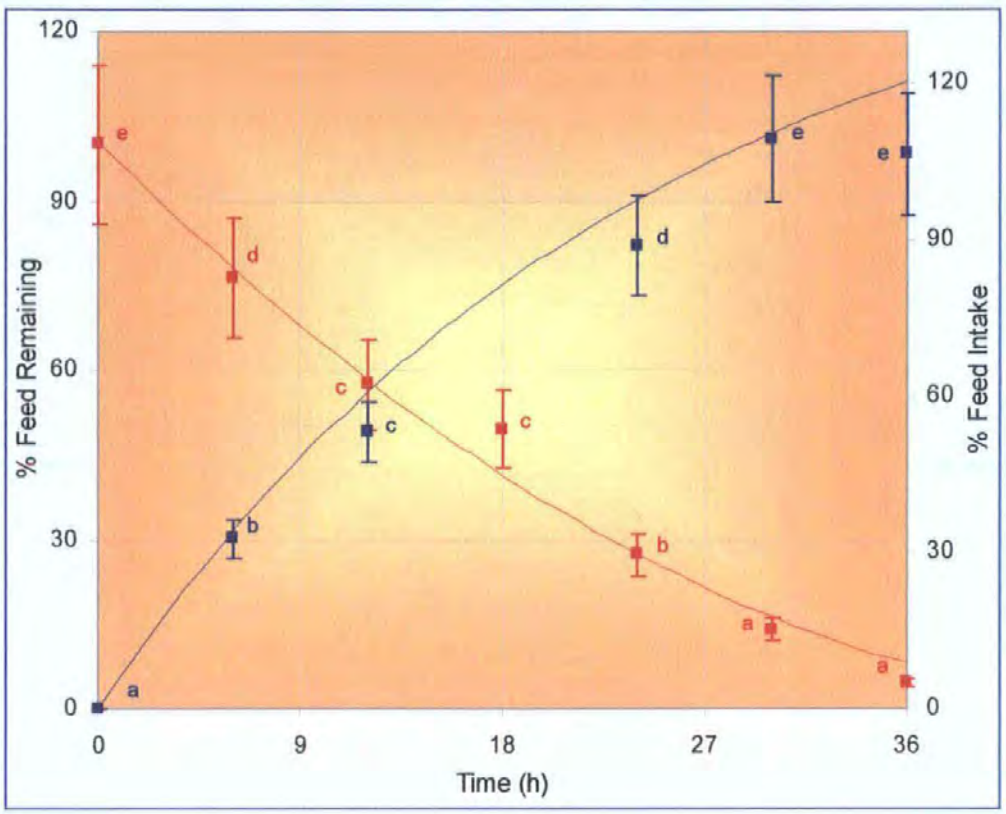


Figure 4.2.6 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed D.4.

Stomach evacuation rate was described by a square root model;

$S_t = (10.03-0.2*t)^2$, $R^2 = 0.91$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order),

$FI = 144.2*(1-e^{-0.041*t})$, $R^2 = 0.98$ Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 4.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

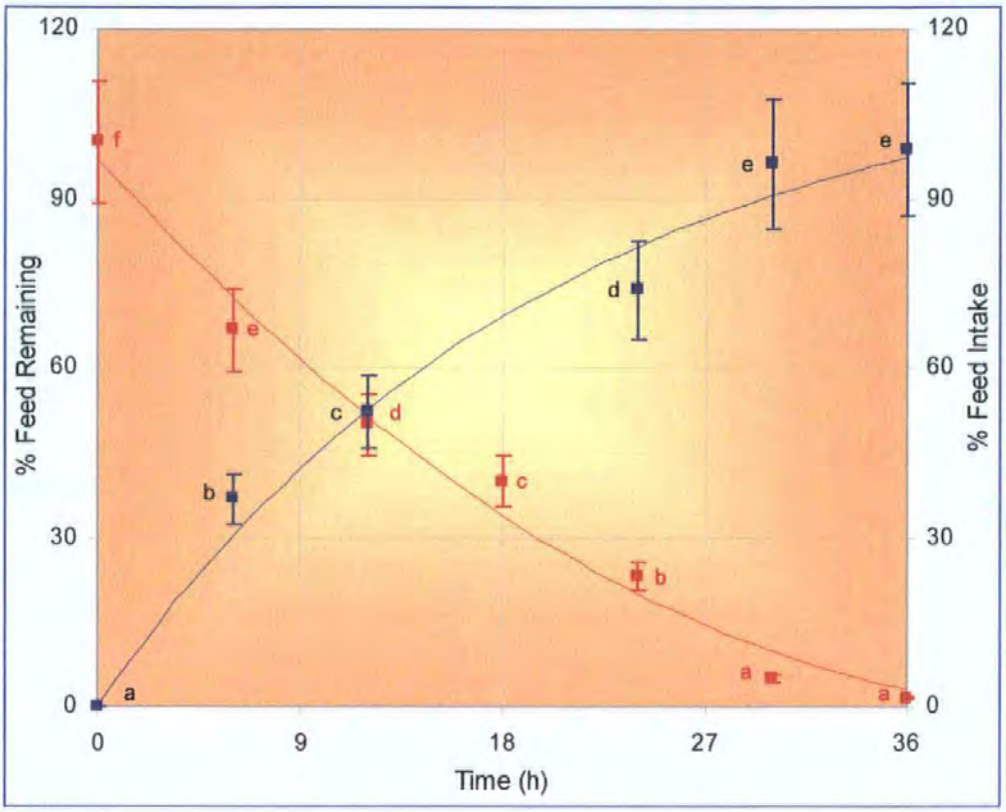


Figure 4.2.7 Percentages of stomach evacuation (■) and return of appetite (■) rates in trout fed D.5.

Stomach evacuation rate was described by a square root model;

$S_t = (9.85-0.22*t)^2$, $R^2 = 0.94$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$FI = 116.5* (1-e^{-0.05* t})$, $R^2 = 0.97$ Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 4.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

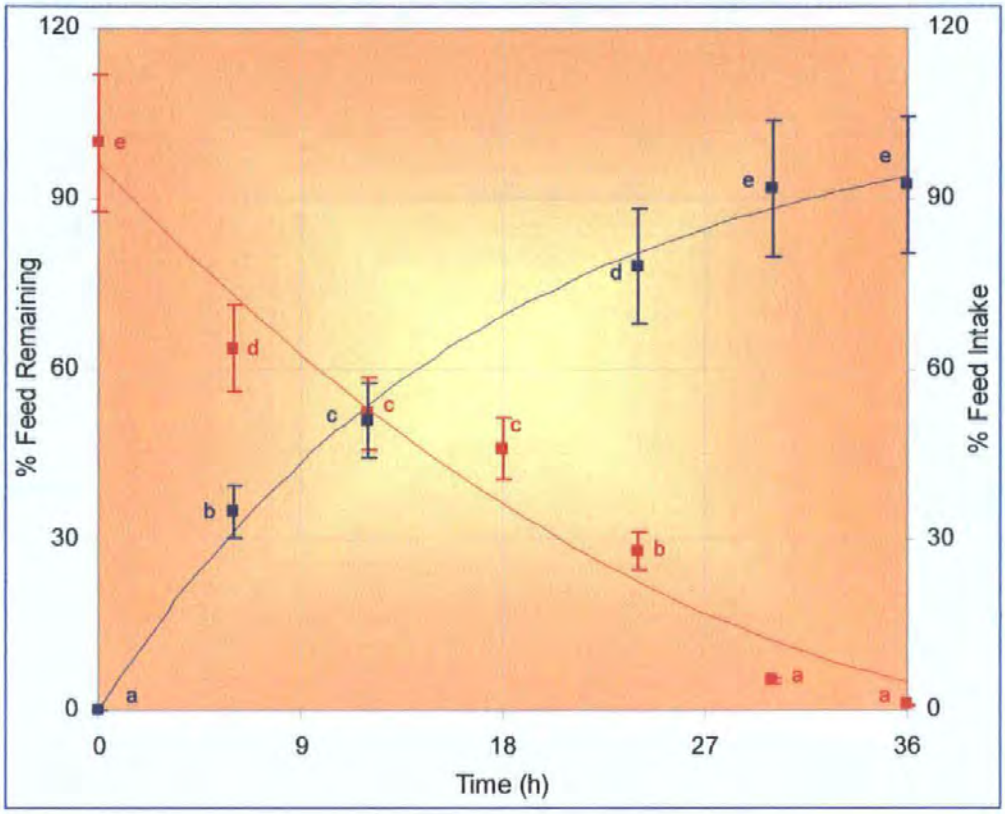


Figure 4.2.8 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed D.6.

Stomach evacuation rate was described by a square root model;

$\mathbf{S_t = (9.8-0.21*t)^2}$, $\mathbf{R^2 = 0.88}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$\mathbf{FI = 107.16* (1-e^{-0.058* t})}$, $\mathbf{R^2 = 0.98}$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 4.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

Primarily, a significant evacuation ($P<0.05$) was observed every 6 hours until the sampling time of 30h in all groups and no considerable difference ($P>0.05$) was detected in evacuation pattern between time 30h and time 36h for all treatments. However, trout fed Diet 4 (Figure 4.2.6) and Diet 6 (Figure 4.2.8) displayed a similar delay between 12h and 18h. postprandially.

The evacuation time of 95 % of the digesta from the cardiac stomach was calculated as 42.2 hours for D.1, 39.7 hours for D.2 and 38.3 hours for D.3 fed trout (Table 4.2.6). 95 % clearance time of the D.4, D.5 and D.6 groups was 39.0, 34.6 and 36.0 hours, respectively.

Table 4.2.6 Predicted gastric evacuation times for rainbow trout[1].

		Calculated times (h) for gastric evacuation (%)			
Model	Treatments	25	50	75	95
Square Root	D.1	**8.4**	**16.8**	**27.7**	**42.2**
	D.2	**7.6**	**15.5**	**25.9**	**39.7**
	D.3	**6.2**	**14.1**	**24.5**	**38.3**
	D.4	**6.9**	**14.8**	**25.2**	**39.0**
	D.5	**5.4**	**12.7**	**22.1**	**34.6**
	D.6	**5.4**	**13.0**	**22.9**	**36.0**

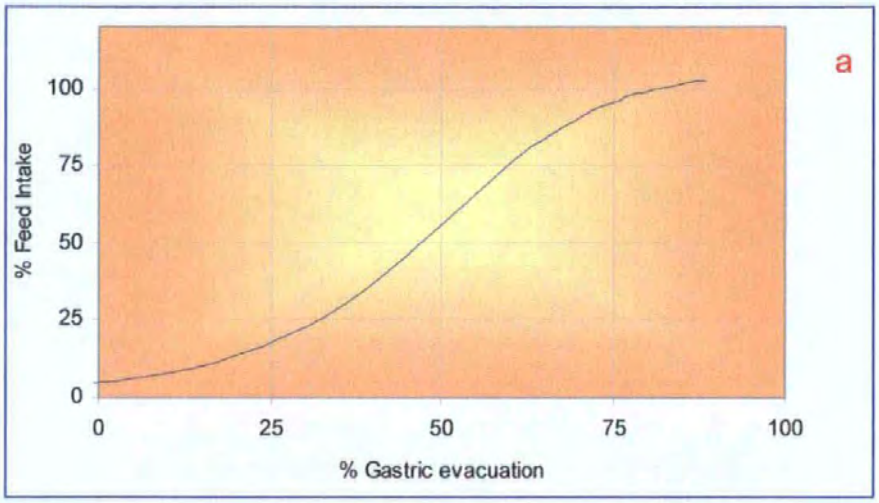
1. Calculations are based on the fitted square root models.

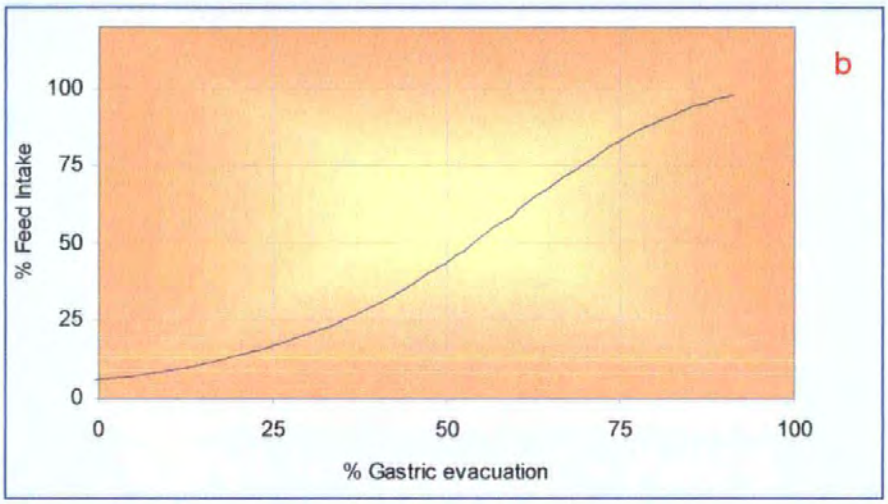
Table 4.2.7 Comparison of predicted return of appetite times for rainbow trout fed different nutrient and energy dense diets[1].

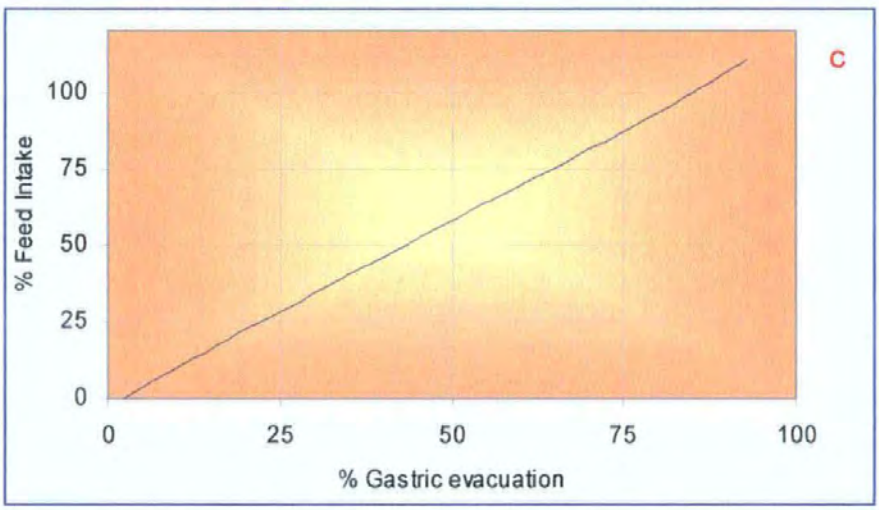
Model	Treatments	Calculated times (h) for appetite revival (%) 25	50	75	95
Sigmoid	D.1	**10.6**	**15.7**	**20.6**	**27.2**
	D.2	**10.5**	**16.9**	**23.1**	**32.4**
	D.3	**6.2**	**11.7**	**16.7**	**22.3**
	D.4	**5.7**	**11.5**	**16.9**	**23.6**
	D.5	**5.0**	**12.3**	**19.6**	**31.9**
	D.6	**5.4**	**11.2**	**17.0**	**26.6**
First Order	D.1	**6.5**	**13.9**	**22.3**	**31.0**
	D.2	**7.6**	**15.8**	**24.6**	**32.4**
	D.3	**5.4**	**11.8**	**19.8**	**27.9**
	D.4	**4.7**	**10.4**	**17.9**	**26.2**
	D.5	**4.8**	**11.2**	**20.7**	**28.8**
	D.6	**4.6**	**10.9**	**20.8**	**27.5**

1. Calculations are based on the fitted first order and sigmoid models given in Table 4.2.5.

Regardless of the models employed, an almost 100 % relationship was apparent between appetite revival and gastric evacuation rates in rainbow trout fed D.1, D.2, D.3, D.4, D.5 and D.6, respectively. These relationships are presented in Figure 4.2.9 for D.1 (Fig. 4.2.9a), D.2 (Fig. 4.2.9b), D.3 (Fig. 4.2.9c), D.4 (Fig. 4.2.9d), D.5 (Fig. 4.2.9e) and D.6 (Fig. 4.2.9f) groups, respectively. Also estimated equations are tabulated in Table 4.2.8.







See legend in Figure 4.2.9 (Page 115)

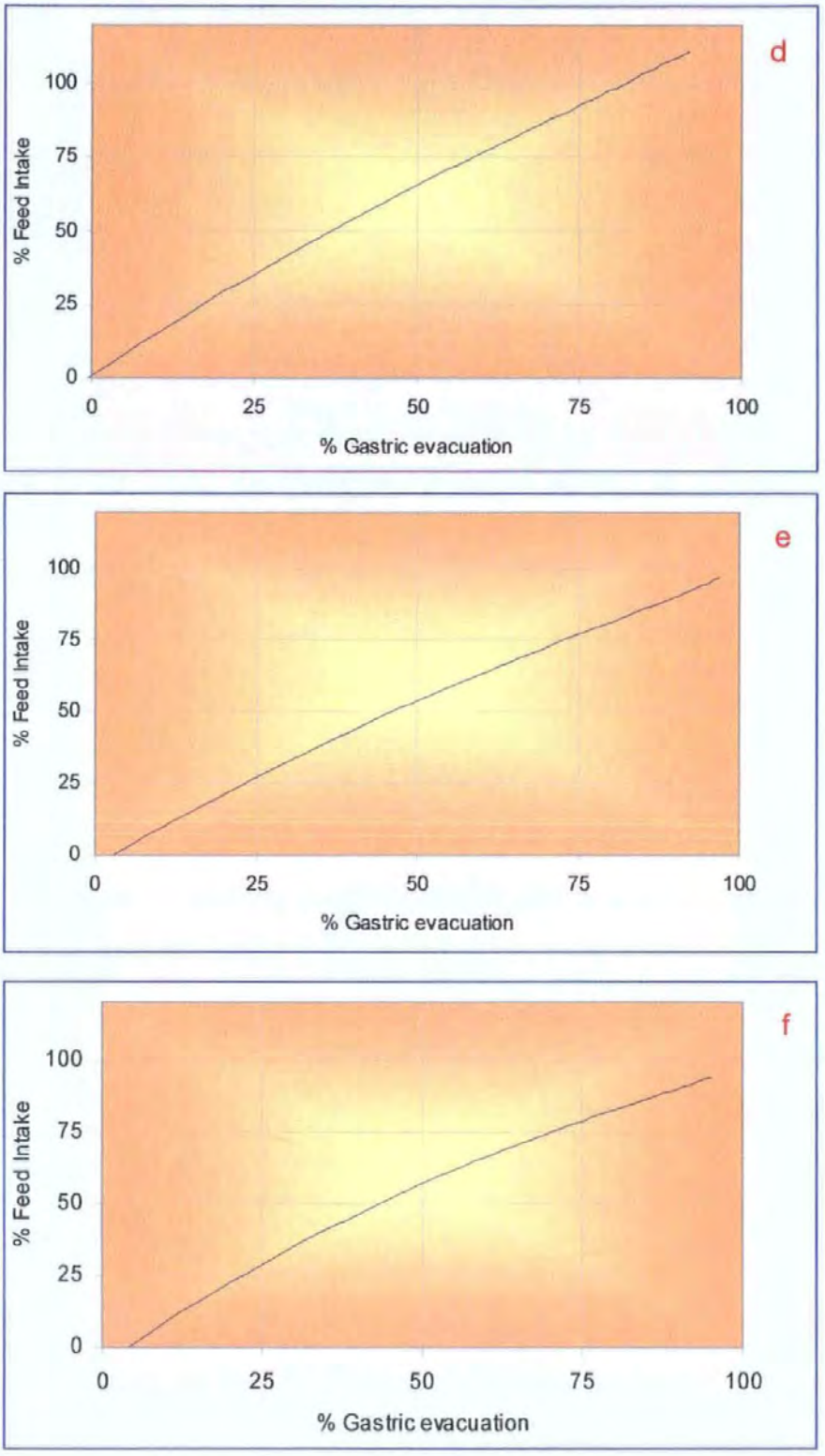


Figure 4.2.9 Relationship between return of appetite (% Feed Intake) and gastric evacuation (%) in rainbow trout fed D.1 (a), D.2 (b), D.3 (c), D.4 (d), D.5 (e) and D.6 (f) (see Table 4.2.8 for the fitted equations).

Table 4.2.8 Fitted equations for the relationship between return of appetite and gastric evacuation in rainbow trout fed different nutrient and energy dense diets.

Diet	Model[1]	a	b	k	R^2	RMS
D.1	*Sigmoid*	0.009	0.29	-0.07	1.0	0.7
	Square Root	2.04	-	0.1	0.98	0.2
	Linear	-6.1	-	1.29	0.97	44.8
D.2	*Sigmoid*	0.009	0.2	-0.05	1.0	0.6
	Square Root	2.14	-	0.09	0.99	0.1
	Linear	-6.42	-	1.14	0.97	1.0
D.3	*Sigmoid*	0.008	0.009	-0.045	0.99	11.0
	Linear	-2.16	-	1.2	1.0	0.3
	First Order	211.2	-	-0.007	0.98	25.3
D.4	*Sigmoid*	0.008	0.07	-0.05	0.99	12.1
	Linear	5.13	-	1.17	1.0	2.7
	First Order	184.8	-	-0.009	0.99	7.0
D.5	*Sigmoid*	0.01	0.1	-0.05	0.99	9.6
	Linear	1.83	-	1.0	1.0	2.9
	First Order	172.9	-	-0.008	1.0	8.6
D.6	*Sigmoid*	0.01	0.1	0.05	0.99	9.3
	Linear	3.93	-	0.99	0.99	8.9
	First Order	191.1	-	-0.007	1.0	2.7

[1] Coefficients derived from the fitted sigmoid $Y= 1/ (a+b* e^{-k*x})$, linear $Y= a + k*x$, first order $Y= a*(1-e^{-k*x})$ and square root function $Y= (a + k*x)^2$, where 'Y' is the return of appetite (% Feed Intake) and 'x' is gastric evacuation (%).

4.2.3.3 Postprandial Plasma Nutrients

Postprandial plasma nutrient profiles are presented in Figure 4.2.10, 4.2.11, 4.2.12, 4.2.13, 4.2.14 and 4.2.15, respectively. Apart from D3 group (Figure 4.2.12), no significant change ($P>0.05$) was observed in plasma protein (mg dl^{-1}) concentration of rainbow trout fed D1, D2, D4, D5 and D6, respectively. The plasma protein level in the D3 group however rose significantly ($P<0.05$) between 18 and 24 hours following feeding (Figure 4.2.12).

Plasma glucose (mmol l^{-1}) levels for D1, D2 and D3 increased and were significantly ($P<0.05$) higher at 24h. On the other hand, the plasma glucose levels for D4, D5 and D6 groups of fish were significantly suppressed ($P<0.05$) until 18 hours, but returned to the initial concentration 24 hours after feeding.

Postprandial triglyceride levels (mmol l^{-1}) for D1, D2, D3 and D4 were elevated significantly ($P<0.05$) and reached a maximum 12 hours after feeding returning to initial concentrations after 24 hours. There was also a significant increase ($P<0.05$) in triglyceride level of D6 (Figure 4.2.15), but this was evident at time 6h. In contrast to D1, D2, D3, D4 and D6 groups, no significant change ($P>0.05$) was observed in triglyceride concentration for D5 (Figure 4.2.14).

Rainbow trout utilized in this study displayed the same phenomenon that all three circulating nutrients for each treatment returned towards their initial (three day starved) concentration 48 hours following re-feeding (two days starved).

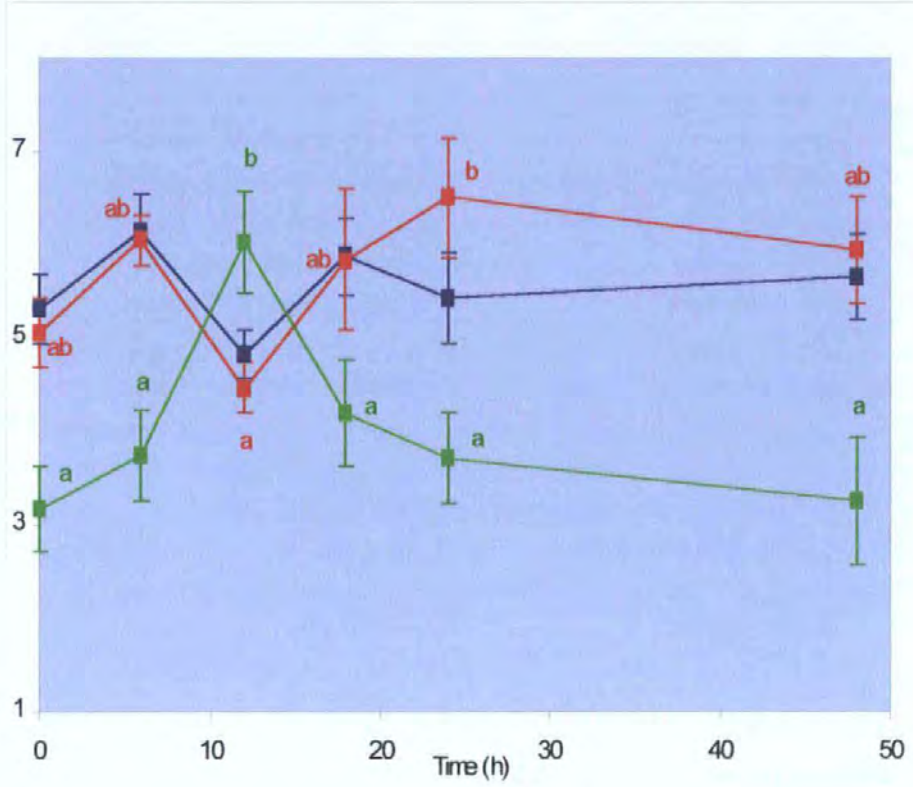


Figure 4.2.10 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed Diet 1 (D1).

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

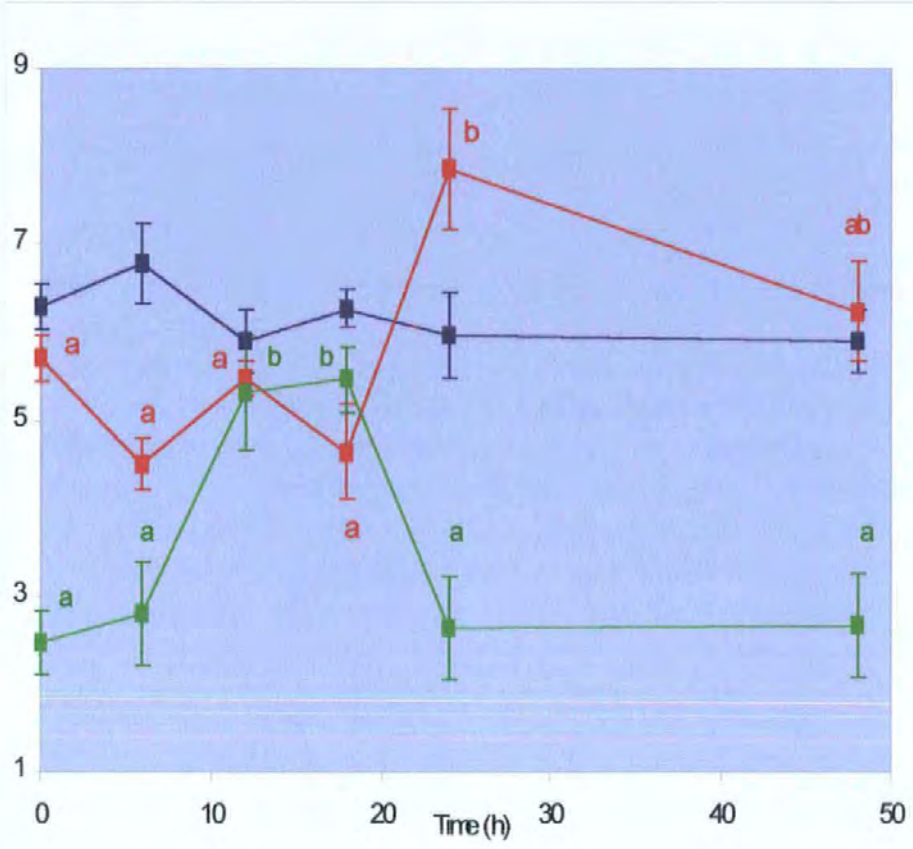


Figure 4.2.11 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed Diet 2 (D2).

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

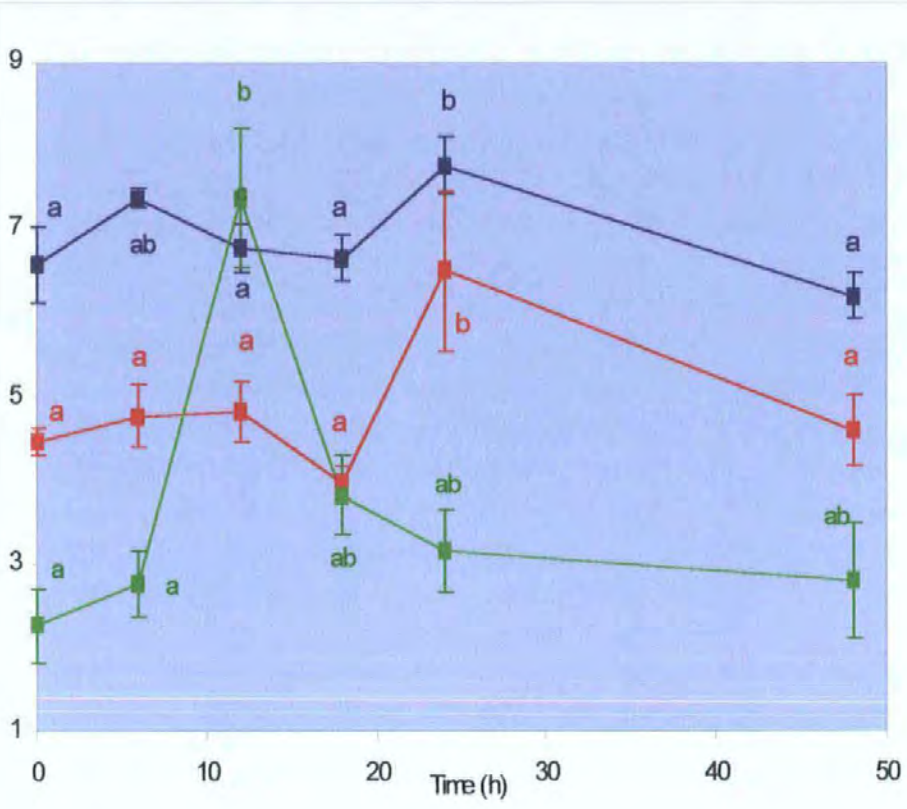


Figure 4.2.12 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed Diet 3 (D3).

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

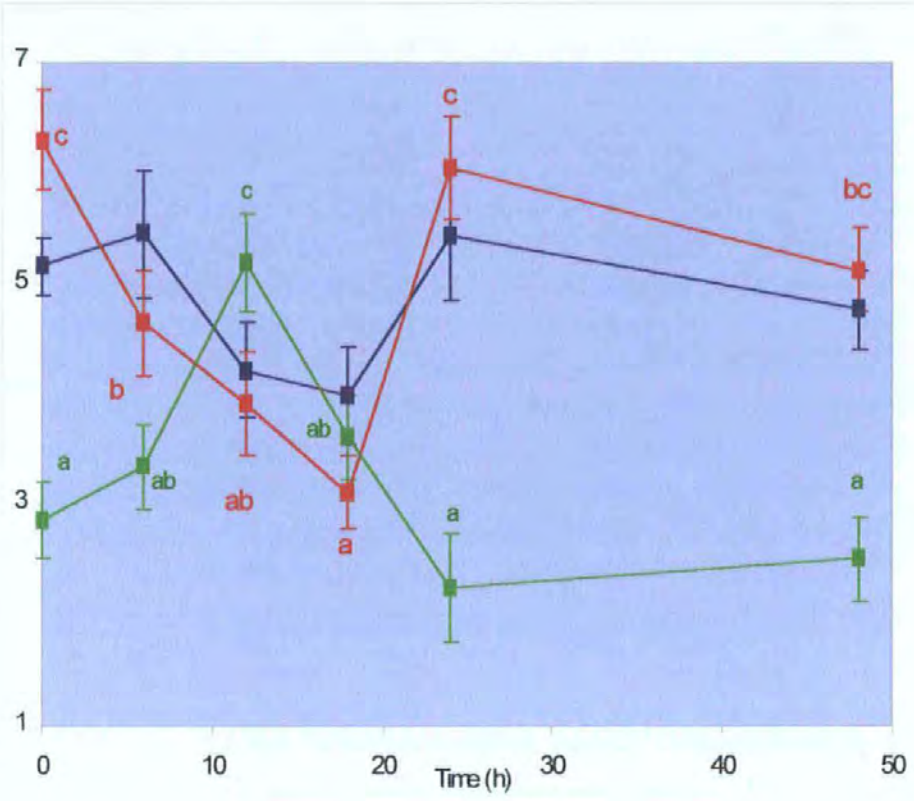


Figure 4.2.13 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed Diet 4 (D4).

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

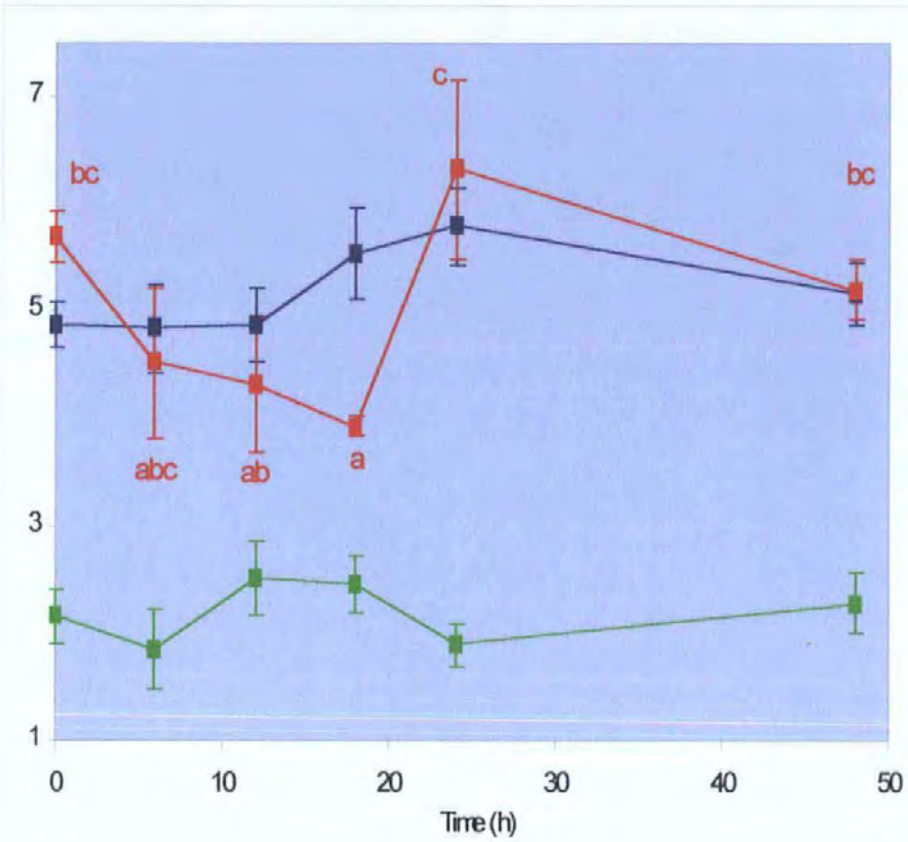


Figure 4.2.14 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed Diet 5 (D5).

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

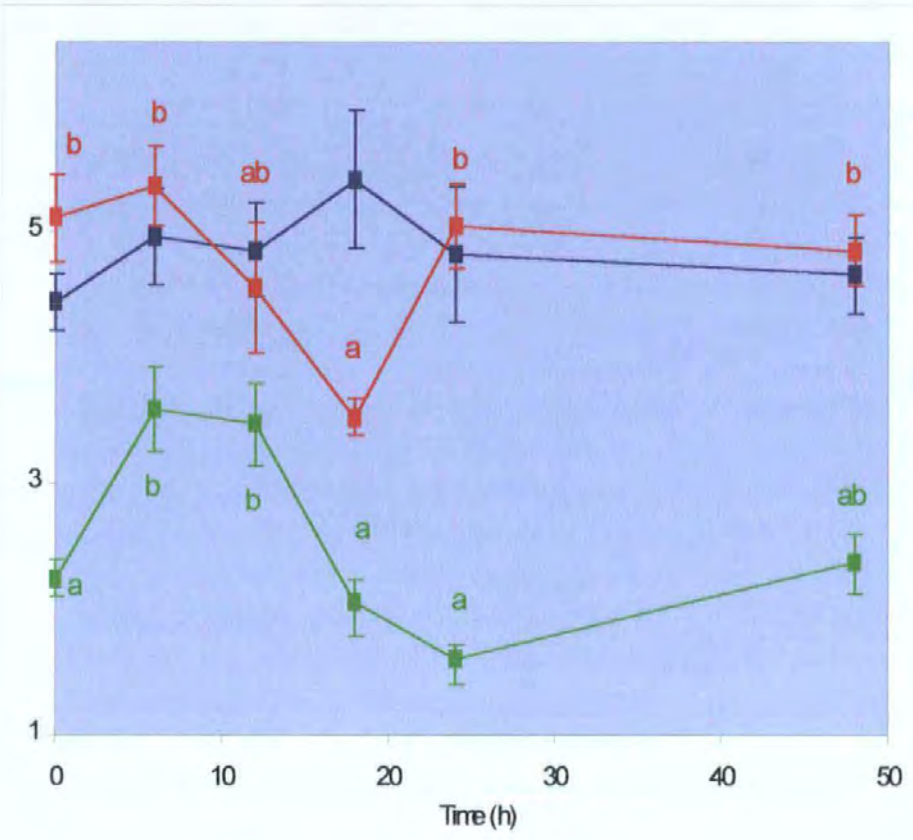


Figure 4.2.15 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed Diet 6 (D6).

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

4.2.4 DISCUSSION

The preliminary results of the present study suggested that X-Radiography was not a valid technique for stomach evacuation determinations since the X-ray dense "ballotini" (0.65-0.90 mm) did not display the same transit flow as the digesta and consequently were retained in the stomach compartment of the trout. Supportive information was reported by Jobling & Jorgensen (1988) that gastric evacuation data derived by the X-Radiography technique was not appropriate for use in Arctic charr (*Salvelinus alpinus*). Furthermore, Grove (1986) observed that radio-opaque particles were retained selectively in the gut of *Scopthalmus maximus* suggesting that X-Radiography was not a satisfactory method for this species when particulate radio opaque markers are employed. However, the efficacy of X-Radiography in order to quantify the digestion rate has previously been demonstrated (Talbot & Higgins, 1983; Sims *et al.*, 1996) for both salmonids and elasmobranchs.

Gastric evacuation pattern appeared to exert the major influence on the return of appetite in rainbow trout as compared to systemic factors such as circulating plasma nutrients and metabolites.

The gastric evacuation rates (GER) of all treatments were described by six square root equations. Exponential, rectilinear and surface area models of stomach emptying stated in the literature and square root equations of evacuation pattern described in this study share the common style that evacuation rate is fast initially and slows down with time as the amount of the digesta in the cardiac stomach declines (Cooke, 1975; Medved, 1985; Grove, 1986; Mayer, 1994). It is a general phenomenon for pelleted feeds which

are quickly broken down to a chyme-like consistency as was also observed by Bromley (1987) in *Scopthalmus maximus*.

Calculated times for the evacuation of 95 % digesta from the cardiac stomach varied between 34.6 hours (D.5) and 42.2 hours (D.1) which were lower compared to the values (40-50 hours for 200 gram trout at 15 °C) tabulated by Grove *et al.* (1978). Although a wide range of diets (21.3 MJ kg^{-1} - 9.0 MJ kg^{-1} DM) were used in the present investigation, it was unlikely that a 200 gram fish evacuated its stomach in 50 hours, thus the diagram represented by Grove *et al.* (1978) is not always applicable. In contrast to the present findings, Windell *et al.* (1969) reported 36 hours as the total clearance of 1 % bw capsules from the stomach of a 90 gram rainbow trout at 15 °C. This value may not be comparable since the fish sizes and meal intakes used in the latter authors' study were smaller than the ones employed in the present study.

Square root models applied for the gastric evacuation data provided a rational approach in that distension of the cardiac stomach wall is more important than the surface area of the digesta in the regulation of the stomach emptying as previously described by Jobling (1981b) and demonstrated by Gwyther & Grove (1981) in *Limanda limanda* and Grove *et al.* (1985) in *Scopthalmus maximus*. However, it cannot be stated that the fitted square root equations are exclusive since the differences between the RSM (residuals of mean square) and r^2 of the linear, exponential and square root models were marginal. Actually, the choice of a model for stomach emptying cannot be made on a biological basis alone. Even if all the factors were known, a biologically based model would be very complex (Elashoff *et al.*, 1982). Therefore comparing different evacuation rates of fish fed different quality diets would

provide more information towards the understanding digestive physiology rather than trying to standardise certain models (for discussion see Bromley, 1994).

The multiple regression (ANCOVA) analysis provided substantial information if there were any statistically significant differences among the slopes for the experimental treatments at the 95 % confidence level. From the results, it is clear that a change in dietary digestible energy content (21.3 – 15.5 MJ kg^{-1}) does not affect the rates of gastric evacuation in rainbow trout. The gastric evacuation slopes of D.1, D.2, D.3 and D.4 were not significantly different ($P > 0.05$). However, the slopes of these treatments were found to be significantly different from those of D.5 and D.6, respectively. No significant difference ($P>0.05$) was revealed in the slopes of D.5 and D.6 indicating that 30 % and 45 % inclusion of an inert material in the diets of trout did not alter instantaneous rate of stomach evacuation significantly.

Similar gastric evacuation results support the voluntary feed intake and growth responses of rainbow trout fed D1 (21.3 MJ kg^{-1} DE), D2 (20.3 MJ kg^{-1} DE) and D3 (18.8 MJ kg^{-1} DE). On the other hand, the same measured parameter was significantly different (faster gastric evacuation rate) in D5 and D6 fed fish. In this respect, it may be suggested that gastric evacuation (GE) and feed intake (FI) rates could be controlled within a certain limit of dietary DE concentrations.

It may also suggest that rainbow trout maintain a uniform rate of dry matter consumption. In this connection, Grove (1986) and Jobling (1986a) hypothesised that the stomach may release (via neurons or hormonal feedback mechanisms) varying volumes of digesta such that the intestine receives a constant amount of dry matter or

energy. Besides Jobling & Wandsvik (1983) and Sims (1994) suggested that certain receptors situated in the upper intestine may monitor the total, digested or metabolizable energy level and consequently this information can modulate feed consumption according to the diet quality. On the contrary, the similar feed intake results of D.1, D.2 and D.3 (Chapter 4.1) do not support these claims, at least in the short term.

Satiation times (40-50 minutes) for all groups of trout were quite similar in this experiment irrespective of the diet quality. This is in accordance with Ishiwata (1968), Windell *et al.* (1969), Grove *et al.* (1978) for rainbow trout and Brett (1971), Elliott (1975a) and Nagata (1989) for other salmonids. Observed similar satiation times for trout offered different nutrient and energy dense diets could be a supportive point for the claim that rainbow trout eat to maintain a constant dry matter intake. In this manner, Grove & Holmgren (1992) suggested that the pyloric part of the stomach in rainbow trout is not affected whilst the cardiac part of the stomach distends following feeding a satiation meal. This may indicate that the amount of delivered digesta from cardiac stomach to upper intestine is approximately constant since the pyloric part of the stomach is unaffected by the mass of the digesta in the cardiac stomach. However, little is known of the mechanisms and which neurons and endocrine cells are playing a modulatory role in this process.

Faster evacuation rates derived especially from D.5 and D.6 fed trout supports the common view that rainbow trout increase their feed intake when the energy content of the diet is diluted (Lee & Putnam, 1973; Grove *et al.*, 1978; Hilton *et al.*, 1983). A similar finding was documented in goldfish, *Carassius carassius* (Rozin & Mayer,

1961), turbot, *Scapthalmus maximus* (Flowerdew & Grove, 1979) and *Pleuronectes platessa* (Jobling, 1981c).

Energy concentration has been suggested to be more important than specific nutrients in the control of feed intake (Jobling, 1981b; Jobling & Wandsvik, 1983). On the other hand, similar gastro-intestinal evacuation rates in other fish fed different dietary energy concentration have also been reported. For instance, the sand dab, *Limanda limanda* (Gwyther & Grove, 1981), tilapia, *Sarotherodon mossambicus* (De Silva & Owoyami, 1983), cod, *Gadus morhua* (Dos Santos & Jobling, 1988) and more recently dogfish, *Scyliorhinus canicula* L. (Sims, 1994) did not demonstrate a significant response when offered different energy and nutrient dense diets. Therefore, it appears that macro- and micro nutrients are interrelated and required to be investigated mutually.

A very high relationship (r^2 approximately 1.0) between gastric evacuation and return of appetite was found following plotting the data according to first order, linear and square root equations (Table 4.2.8 and Figure 4.2.9). This high correlation indicated that rainbow trout adjusted feed intake so that stomach capacity was at near maximum fullness. Therefore it can be suggested that gastric tension receptors were the main regulatory factors in relation to the amount of digesta in the cardiac stomach in the short term. Consequently the appetite of trout was controlled by the gastric emptying of meal in a weight dependent manner in the short term. In this context, the nutritional status and history of the experimental fish is also a very important point to be considered. For instance, results of the present study were derived from fish starved 72 hours and fed a single satiation meal. Therefore I have limited my discussion according to the constraints of the study as undertaken.

With reference to the plasma nutrient profiles displayed in Figures 4.2.10– 4.2.15, respectively there were no obvious differences in measured postprandial metabolic factors. It was, however, noteworthy that the trend for each nutrient with time were in accordance to their relative levels in the diets. For instance, the higher nutrient densities of the undiluted feeds generally resulted in elevated plasma nutrient levels compared to the α-cellulose treatments which were all suppressed. It can be stated that this response in relation to their effect on appetite regulation is likely to be negligible compared to the direct influence of gastric evacuation rate.

In conclusion, this investigation has confirmed the general view of Windell & Norris (1969), Grove *et al.* (1978) that stomach evacuation rate is an important feature in the modification of feeding behaviour of rainbow trout. Gastric distension is probably to be a main factor in the short-term satiety of trout whilst energy density of the meal may be less significant component.

From the present investigation, it has been understood that dietary digestible energy concentration is apparently the most important factor in the regulation of feed intake in the longer term. If the basis of appetite regulation is dependent on the bulk of food, then the bulk density effect of dietary carbohydrates may influence appetite in trout. What is not known is how the bulk dependent evacuation process of trout may be modified by available digestible energy from dietary carbohydrate? In these experiments (Chapter 4.1 & 4.2), a non nutritive material (α-cellulose) has been used for dilution of the dietary energy and protein concentration in experimental diets. What would be the effects when an actual feed ingredient such as extruded wheat meal was used for dilution of dietary nutrient and energy? Would the evacuation rate be similar to those

derived from this study? Or will the postprandial glucose concentration influence the gastric emptying pattern? Will there be an interaction between postprandial protein, glucose and triglyceride concentrations? These are pertinent questions in relation to practical diet formulations where such carbohydrate rich cereals are often used.

Therefore, the next series of experiments described will address the influence of dietary carbohydrate levels on feed consumption, nutrient utilization and gastric evacuation rates in trout. The protein sparing effect of dietary carbohydrates will also be assessed since this is a key issue in the design of commercial feeds for salmonids.

CHAPTER 5

THE EFFECT OF DIETARY CARBOHYDRATE LEVEL ON FEED INTAKE, NUTRIENT UTILIZATION, GASTRIC EVACUATION AND RETURN OF APPETITE IN RAINBOW TROUT, *Oncorhynchus mykiss*.

EXPERIMENT. 4

5.1 EFFECTS OF CARBOHYDRATE LEVEL ON FEED INTAKE AND NUTRIENT & ENERGY UTILIZATION IN RAINBOW TROUT, *Oncorhynchus mykiss.*

5.1.1 INTRODUCTION

Rainbow trout, *Oncorhynchus mykiss* do not possess a defined carbohydrate requirement *per se* partly due to their carnivorous mode of nutrition with a low ability to metabolize high levels of carbohydrate (NRC, 1993; Wilson, 1994). Therefore carbohydrate nutrition has been of less significance compared to lipid nutrition in practical salmonid diets. However, it has been reported by a number of studies (Cowey & Sargent, 1979; Steffens, 1989; Smith, L., 1989; Cowey & Walton, 1989) that it is beneficial to include a reasonable carbohydrate level in formulated feeds for salmonids as a partial energy source and a filler component.

As previously demonstrated in Chapter 3 and Chapter 4.1, body lipid content of rainbow trout may be directly correlated to dietary lipid level even if the protein/energy ratio is ensured to be within an optimum range (22-24 g digestible protein per MJ^{-1} digestible energy). The consequences of employing energy-dense diets in relation to high levels of dietary lipid (i.e. surplus lipid retention) should be especially noted. In this manner, the value of carbohydrate as an energy source should be of interest since this nutrient component is the only viable alternative to the use of expensive oils and protein rich ingredients.

Over three decades, there has been a paradoxical interpretation in relation to the carbohydrate nutrition of rainbow trout. On one hand, some nutritionists (Phillips *et al.*, 1948; Luquet, 1971; Cowey *et al.*, 1977a) suggested that no more than 20 % carbohydrate should be included in diets for rainbow trout. However, others claimed that 30 % carbohydrate did not cause any inferior growth or negative health condition (Kaushik & Oliva-Teles, 1985; Kaushik *et al.*, 1989; Steffens, 1989; Kim & Kaushik, 1992).

Yet, the technological improvement (i.e. extrusion and expansion) of carbohydrate availability by increasing the digestibility of carbohydrate ingredients plays a significant role in this context. However, there has not been significant attempts so far to show to what extent energy from carbohydrate can spare protein for growth or how much lipid can be substituted to decrease the visceral fat accumulation under both restricted and satiation feeding regimes. There is also a need to evaluate the influence of highly digestible carbohydrates on dietary energy partitioning as well as apparent net protein and energy utilization efficiency.

Furthermore, there are also a number of investigations which examined the effect of carbohydrate level on proximate body composition of trout. On the contrary, inadequate analysis of previous data has resulted in some misinterpretations. These include statements such as that body protein was decreased in fish fed high levels of carbohydrates (Austreng *et al.*, 1977; Beamish & Medland, 1986) without fish size being taken into consideration.

Therefore, the contrasting information and different interpretations regarding carbohydrate nutrition in fish have prompted this investigation. The aim was to elucidate the influence of different dietary carbohydrate levels (as approximately 15, 30 and 45 % of extruded wheat meal) on relative feed consumption rate, growth performance, nutrient and energy utilization and carcass and muscle proximate composition in rainbow trout fed semi-practical diets.

5.1.2 MATERIALS AND METHODS

5.1.2.1 Experimental Fish and Maintenance Facilities

500 rainbow trout, *Onchorynchus mykiss* (all female) were acclimatized to laboratory conditions for 3 weeks prior to the 12-week-feeding trial. Batches of 40 trout (mean weight 33.0 ± 0.46 g SEM) were placed into duplicate 400 l, fiberglass tanks within a closed fresh water recirculation system as described in Chapter 2.1.

5.1.2.2 Feeding and Performance Indicators

Three diets containing 15.2 (LC, low carbohydrate), 32.2 (MC, medium carbohydrate) and 43.5 (HC, high carbohydrate) % extruded wheat meal were formulated (Table 5.1.1) and manufactured as described previously in Chapter 2.2. Fish were fed either restricted (LCR, low carbohydrate restricted; MCR, medium carbohydrate restricted and HCR, high carbohydrate restricted) or satiation (LCS, low carbohydrate satiation; MCS, medium carbohydrate satiation and HCS, high carbohydrate satiation) by hand three times (09.00, 13.00 and 17.00 h) per day. The restricted regimes were designed to provide a set protein intake relative to the live weight of the fish. Therefore allowance was made for the dilution effect of increasing carbohydrate level in these diets. Feed provision was recorded daily throughout the 84-day-trial. Trout (without being anaesthetized) were weighed individually every two weeks following a 24-hour feed deprivation period. Parameters relevant to growth and feed utilisation efficiency were calculated as outlined in Chapter 2.7.

Table 5.1.1 Diet Formulation and chemical composition of experimental diets.

Ingredients	*Diets*[1]		
	LC	**MC**	**HC**
LT Fish Meal[a]	52.6	42.8	35.0
Poultry Meat Meal[b]	12.0	9.6	8.0
Blood Meal[c]	3.0	2.4	2.0
Extruded Wheat Meal[d]	15.3	32.2	43.5
Fish Oil[e]	10.81	8.65	7.2
Vitamin/Mineral Premix[f]	2.0	2.0	2.0
∝-cellulose[g]	1.89	-	-
Cr_2O_3[g]	0.4	0.4	0.4
Binder[g] (CMC)*	2.0	2.0	2.0
Nutrient Analysis			
Protein (% DM)	48.7	41.7	37.3
Lipid (% DM)	20.5	17.5	15.2
Ash (% DM)	10.4	8.9	7.7
Carbohydrate (% DM)	13.2	22.0	30.5
Digestible Protein (DP) (%)	43.6	34.0	30.7
Digestible Energy (DE) (MJ kg^{-1})	20.2	17.3	16.4
DP/DE Ratio (g DP MJ^{-1} DE)	21.6	19.7	18.7

a. Low Temperature Fish Meal, Norsea Mink, LT 94. Donated by Trouw Aquaculture, Wincham, Cheshire, UK

b. Int. Feed Number, 5-03-798, Trouw Aquaculture, Wincham, Cheshire, UK

c. Int. Feed Number, 5-00-381, " " " " "

d. Int. Feed Number, 4-05-205, " " " " "

e. Atlantic Herring Oil (7-08-048), Seven Seas, Marfleet, Hull, UK

f. (Closed Formulation), Trouw Aquaculture, Wincham, Cheshire, UK

g. Sigma Chemical Company, Poole, Dorset, UK

1. LC (low carbohydrate), MC (medium carbohydrate) and HC (high carbohydrate)
*, Carboxy methyl cellulose

5.1.2.3 Sampling and Analytical Procedures

Digestibility, fish sampling and all analytical procedures were as detailed in Chapter 2.3, 2.4 and 2.5.

5.1.2.4 Statistical Analysis

Statistical analysis employed for the interpretation of experimental data was as explained in Chapter 3.1.2.4.

5.1.3 RESULTS

Apparent digestibility coefficients of dry matter, protein, energy, lipid and carbohydrate for each group were calculated (Table 5.1.2) after the digestibility trial. Low carbohydrate groups (LCR and LCS) displayed relatively higher dry matter digestibility coefficients. A decreasing trend between low carbohydrate and high carbohydrate treatments was also observed that protein, energy and carbohydrate digestibility reduced with increasing the carbohydrate level. On the contrary lipid digestibility show a similar pattern for all treatments. Some differences were detected in restricted and satiation groups fish fed the same diet, however no statistical evaluation can be made since the samples were pooled from each dietary treatment.

Table 5.1.2 Digestibility coefficients of dietary nutrient components*

	Restricted[1]			***Satiation***[2]		
	LCR	**MCR**	**HCR**	**LCS**	**MCS**	**HCS**
Dry Matter	83.8	76.9	77.1	86.0	71.0	69.1
Protein	88.2	84.5	85.7	90.8	78.6	79.0
Energy	89.8	84.8	78.0	91.5	82.4	76.6
Lipid	89.5	90.4	88.7	90.4	88.6	88.1
Carbohydrate	93.2	85.4	89.0	94.0	89.1	84.7

* Coefficients based on pooled sample material from each dietary treatment.

1. LCR (low carbohydrate restricted), MCR (medium carbohydrate restricted) and HCR (high carbohydrate restricted

2. LCS (low carbohydrate satiation), MCS (medium carbohydrate satiation) and HCS (high carbohydrate satiation)

Fish fed to apparent satiation (LCS, MCS and HCS) displayed a feed intake which was more uniform and closer to the 2 % bw fixed feeding level (Table 5.1.3). Low

carbohydrate satiation (LCS) group consumed more feed compared to LCR, but fish receiving this diet reduced their feed intake following the tenth week of the trial. Similarly MCS and HCS fish decreased feeding rate after the tenth week of the feeding trial.

Table 5.1.3 Feed consumption of rainbow trout (g 100 g^{-1} biomass)

Week	*Restricted*			*Satiation*		
	LCR	**MCR**	**HCR**	**LCS**	**MCS**	**HCS**
0-2	1.7	2.0	2.3	2.4	2.3	2.2
2-4	1.7	2.0	2.2	2.1	2.2	2.0
4-6	1.5	1.6	1.9	1.9	1.9	1.8
6-8	1.5	1.8	2.1	1.9	1.8	2.1
8-10	1.4	1.6	1.9	1.9	1.8	2.1
10-12	1.4	1.7	2.0	1.6	1.4	1.6
Mean F.I.	1.5	1.8	2.1	2.0	1.9	2.0

When overall mean feed intake is taken into account, MCR, MCS and HCR, HCS fish fed the same diet however showed similar feeding responses. Also it can be stated that apart from LCR and MCR groups, mean feed intake of other treatments (HCR, LCS, MCS and HCS) were observed to be similar (Table 5.1.3).

Although the feeding response of the above mentioned groups were very similar, they displayed significant differences in growth rates (Table 5.1.4). LCS trout showed the highest

performance ($P<0.05$). MCR and MCS groups followed LCS fish and they grew significantly superior ($P<0.05$) compared to LCR, HCR and HCS treatments. On the other hand any statistical significance between the growth of LCR, HCR and HCS was not evident ($P>0.05$). The Specific Growth Rate (SGR) also supported the same view that the growth performance of rainbow trout used in this experiment was LCS > MCR = MCS > HCR = HCS = LCR.

Feed efficiency of all groups except HCR and HCS was determined as more than 100 %, feed efficiency of LCR highest. This parameter was 91.6 and 92.2 % for HCR and HCS trout, respectively. Digestible protein (DP) utilized per kg^{-1} growth was observed between 312 (MCR) and 420 g (LCS). It was detected that apparently more protein was utilized per kg^{-1} growth in the groups fed high protein diet (LCR and LCS). Digestible energy (DE) utilized per kg^{-1} growth lay between 15.9 (MCR) and 19.5 MJ (LCS).

Apparent net protein utilization (ANPU) of MCR trout was highest (53.7 %) while LCS demonstrated the lowest ANPU (41.5 %). Apparent net energy utilization (ANEU) was observed to be in accordance with the ANPU parameter that ANEU of MCR fish displayed the highest value, whilst that of LCR was minimum (38.3 %) (Table 5.1.4).

The condition factor of LCS trout was significantly higher ($P<0.05$) than other groups, but there is a significant difference between the condition factor of LCS and MCR fish. Dress Out (%) of rainbow trout ranged between 86.7 and 88.1 %, however no significance ($P>0.05$) was evident. Hepatosomatic Index (HSI) increased significantly ($P<0.05$) with the

carbohydrate level. On the other hand, feeding strategy did not affect liver size (e.g. HSI of LCR and LCS was 1.1 % or HSI of MCR and MCS was 1.4 %) (Table 5.1.4).

Table 5.1.4 Growth performance of rainbow trout fed different levels of carbohydrate diets either fed restricted or satiation for 84 days.

	Restricted			***Satiation***			
Parameters	**LCR**	**MCR**	**HCR**	**LCS**	**MCS**	**HCS**	**±SEM***
Initial Mean Weight (g)	34.0	33.8	33.7	33.3	33.8	33.9	0.46
Final Mean Weight (g)	132.0^{a}	147.4^{b}	137.5^{a}	169.0^{c}	145.2^{b}	132.7^{a}	5.21
Weight Increment (%)	289	337	307	407	330	292	2.80
Feed Efficiency (%)	116	108	92	104	102	92	2.73
SGR (%)	1.6	1.8	1.7	1.9	1.7	1.6	0.18
ANPU (%)	44.8	53.7	50.7	41.2	49.5	50.3	2.56
ANEU (%)	38.3	53.3	48.9	42.0	50.5	48.2	1.85
Feed Intake (bw %)	1.5	1.8	2.1	2.0	1.9	2.0	0.08
DP utilized per kg^{-1} growth (g)	374	312	335	420	333	332	3.28
DE utilized per kg^{-1} growth (MJ)	17.4	15.9	17.9	19.5	16.9	17.8	4.62
Condition Factor (%)	1.26^{a}	1.27^{ab}	1.26^{a}	1.31^{b}	1.23^{a}	1.24^{a}	0.02
Dress Out (%)	88.1	87.4	86.7	88.1	86.7	87.3	0.32
Hepatosomatic Index (%)	1.1^{a}	1.4^{b}	1.7^{c}	1.1^{a}	1.4^{b}	1.6^{c}	0.05

*Values in each row allocated common superscripts or without superscripts are not significantly different from each other ($P > 0.05$).

Dietary energy partitioning of rainbow trout (Table 5.1.5) displayed that non-fecal energy loss was minimum in Low Carbohydrate Satiation (LCS) group, however this increase could not be tested statistically. Estimated maintenance energy was between 15.75 (LCS) and 20.22 (LCR) % of Gross Energy (GE) intake.

Table 5.1.5 Estimation of dietary energy utilization by rainbow trout fed different levels of carbohydrate diets either restricted or satiation regime. (Cho & Kaushik, 1985)

	Restricted			***Satiation***		
Gross Energy (%)	**LCR**	**MCR**	**HCR**	**LCS**	**MCS**	**HCS**
Gross Energy (GE)	100	100	100	100	100	100
Faecal Energy (FE)	9.4	18.5	20.6	9.4	18.5	20.6
Digestible Energy (DE)	90.6	81.5	79.4	90.6	81.5	79.4
Non-faecal Energy (ZE + UE + HiE)	29.1	19.8	24.3	36.9	23.0	25.3
Net Energy (NE)	61.5	61.7	55.1	53.8	58.4	54.1
Maintenance Energy	20.2	18.2	16.3	15.8	17.3	15.8
Retained Energy (RE)	41.3	43.4	38.8	38.0	41.1	38.3

The carcass and whole fillet proximate compositions of rainbow trout fed different levels of carbohydrate are presented in Table 5.1.6 and Table 5.1.7, respectively. Carcass and muscle components (protein, lipid and ash) were found not to be significantly different between treatments ($P>0.05$). Thus it was observed that body protein, lipid and ash content of trout was not affected by diets including different carbohydrate concentration

or feeding regime (Table 5.1.8). These results demonstrate that fish size is a necessary parameter to consider in order to avoid contradictory results.

Table 5.1.6 Proximate composition of the pooled carcasses of experimental animals presented as a percentage of the whole fish.

	Initial	LCR	MCR	HCR	LCS	MCS	HCS	±SEM*
Moisture	72.0	70.5	70.4	68.9	69.0	70.4	70.1	0.41
Protein	15.3	16.4	16.5	16.6	16.9	16.2	16.4	0.24
Lipid	10.4	10.1	11.2	11.6	11.8	11.4	11.4	0.25
Ash	2.4	2.5	2.4	2.4	2.3	2.5	2.4	0.05

*± standard error of the pooled means (n=10). Values in each row are not significantly different from each other (P > 0.05) (see Table 5.1.8)

Table 5.1.7 Proximate composition of pooled muscle of test animals presented as a percentage of the muscle.

	Initial	LCR	MCR	HCR	LCS	MCS	HCS	±SEM*
Moisture	77.9	72.6	72.7	72.5	72.1	72.4	72.1	0.28
Protein	16.7	18.7	18.2	17.9	18.6	17.4	18.5	0.17
Lipid	4.3	7.5	7.8	8.0	7.8	8.4	8.4	0.24
Ash	2.3	1.9	1.9	2.0	1.9	2.0	2.0	0.04

*± standard error of the pooled means (n=10). Values in each row are not significantly different from each other (P > 0.05) (see Table 5.1.8)

Table 5.1.8 Allometric analysis of carcass and muscle components of rainbow trout.

	Log (body protein)= a + b* Log (wt) R^2= 0.97		Log (body lipid)= a + b* Log (wt) R^2= 0.94		Log (body ash) = a + b* Log (wt) R^2=0.78		Log (muscle pro.)= a +b* Log (wt) R^2=0.98		Log (muscle lipid) = a + b* Log (wt) R^2=0.82		Log (muscle ash) = a + b* Log (wt) R^2=0.91	
	a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
LCR	-0.76	0.99	-1.07	1.03	-1.42	0.90	-0.71	0.99	-1.44	1.16	-1.58	0.94
MCR	-0.76	0.99	-1.04	1.03	-1.42	0.90	-0.72	0.99	-1.43	1.16	-1.58	0.94
HCR	-0.76	0.99	-1.03	1.03	-1.42	0.90	-0.73	0.99	-1.41	1.16	-1.58	0.94
LCS	-0.74	0.99	-1.01	1.03	-1.42	0.90	-0.71	0.99	-1.44	1.16	-1.58	0.94
MCS	-0.77	0.99	-1.03	1.03	-1.42	0.90	-0.74	0.99	-1.39	1.16	-1.58	0.94
HCS	-0.76	0.99	-1.03	1.03	-1.42	0.90	-0.72	0.99	-1.39	1.16	-1.58	0.94
	S F= 5.1	NS F= 1.4	S F= 2.7	NS F= 0.5	NS F= 0.9	NS F= 2.0	S F= 7.5	NS F= 0.7	S F= 2.5	NS F= 2.3	NS F= 0.8	NS F= 0.6

S, significant; NS, nonsignificant

5.1.4 DISCUSSION

The present investigation has clarified certain perspectives in the carbohydrate nutrition of rainbow trout in relation to the feeding behaviour and physiology. Carbohydrate digestibility was effectively reduced by the incorporation of increasing carbohydrate level in this experiment as previously reported by Inaba *et al.* (1963) and Rychly & Spannhof (1979). This finding was also in good agreement with Takeuchi *et al.* (1990) who determined 82.1 % carbohydrate, 88.5 % energy digestibility coefficients. However, carbohydrate digestibility was superior in medium (MCR and MCS) and high carbohydrate (HCR and HCS) groups compared to the results of Singh & Nose (1967) who determined 77.2 % and 74.8 % in the rainbow trout diets containing 20 and 30 % dextrin, respectively.

Dry matter and energy digestibility also declined with increasing the level of extruded wheat in the diet. The relatively low digestibility of extruded wheat with an approximately 30 % inclusion level by rainbow trout might be due to the absorption of amylase by starch and the inhibition of hydrolysis of the starch as suggested by Furuichi & Yone (1980) and Spannhof & Plantikow (1983). It could also be explained by acceleration of the chyme transport through the intestine in order to obtain more digestible energy, thus reducing scope for hydrolysis and digestion (Bergot & Breque, 1983; Bergot, 1993).

In a similar manner to carbohydrate digestibility, dietary carbohydrate level also influenced the rate of protein digestion inversely. Apparent protein digestibility coefficients are lower compared to the results reported by Kaushik *et al.*, (1989), Takeuchi *et al.* (1990),

Henrichfreise & Pfeffer (1992) and Pfeffer & Henrichfreise (1994) who reported that the protein digestibility of wheat grain or wheat starch was between 90- 98 %. The apparent lipid digestibility however was not affected by incorporation of different source of dietary carbohydrate as previously shown in trout (Bergot, 1993). However, lipid digestibility was lower compared to that of Takeuchi *et al.* (1990).

The relative feed consumption of rainbow trout (Table 5.1.3) fed up to 45 % extruded wheat meal did not show a dramatic difference between satiation treatments. It also did not result in any negative effects on the physical health status of fish as previously demonstrated by Kaushik *et al.* (1989). However, Baeverfjord (1992) reported that 250 g kg^{-1} extruded starch caused intracellular damage due to surplus deposition of glycogen in the liver of rainbow trout. Hepatosomatic index (Table 5.1.4) was proportionally increased with carbohydrate level probably because of hepatic glycogen deposition (Phillips *et al.*, 1948; Lee & Putnam, 1973; Reftsie & Austreng, 1981; Hilton & Atkinson, 1982; Hilton *et al.*, 1987) although these workers did not explain feeding response of fish. However, it appeared that feed intake of trout was not influenced by chemical alteration of the liver during the first ten weeks of the feeding trial. On the other hand, during the last two weeks of the experiment, the appetite of fish could have been affected by intracellular damage due to surplus deposition of glycogen in the liver of rainbow trout as demonstrated by Baeverfjord (1992). Moreover, glucostatic receptors might have been affected in the long-term (after tenth week of the experiment). However, these factors need to be elucidated more closely.

Mean feed intake was 2.0, 1.9 and 2.0 % body weight day^{-1} in the low carbohydrate satiation (LCS), medium carbohydrate satiation (MCS) and high carbohydrate satiation (HCS) regimes, respectively. Very close apparent feed consumption of these groups may indicate that plasma glucose concentration may not be significantly elevated by carbohydrate level or plasma glucose level did not play a major role in modulation of feed intake. These points will therefore be addressed in the next series of experiments (Chapter 5.2).

Superior growth performance was observed in the low carbohydrate satiation regime probably because protein and energy density of the diet was adequately balanced and consequently the scope of fish for growth was near optimum (SGR: 1.9 %). On the other hand, medium carbohydrate satiation (MCS) and high carbohydrate satiation (HCS) groups grew 16.4 and 27.3 % inferior compared to LCS fish, respectively. This is probably because all groups fed for gastric fullness, however carbohydrate diluted diets provided less digestible energy for maximum growth. This is also in agreement with the common view that high levels of carbohydrate inclusion in trout diets decreases the carbohydrate digestibility. For example medium (220g kg^{-1} DM) carbohydrate groups grew superior compared to high carbohydrate (305g kg^{-1} DM) groups despite similar digestibility coefficients. It may therefore be suggested that inclusion of approximately 30 % extruded wheat meal for rainbow trout diets provide a good growth performance (1.7-1.8 SGR), nutrient and energy utilization (50 % ANPU or ANEU) and digestibility under a near -to- satiation feeding regime.

Growth performance (SGR) of the HCR or HCS groups fed 43.5 % extruded wheat meal was superior compared to that of Kaushik *et al.* (1989) who fed rainbow trout diets one of which was

38 % extruded wheat at 18 °C for 18 weeks and obtained 1.3 % day^{-1} SGR. However the dietary lipid level in the study of these workers was 8.7 % whereas the fish fed the high carbohydrate diet (HC) in the present investigation was 15.2 %. Therefore dietary lipid level or lipid carbohydrate interaction may play a role on the growth performance of trout. Moreover protein and energy retention was 34.0 and 33.3 % respectively in the aforementioned study whilst HCS group of this investigation displayed 50.3 and 48.2 % protein and energy retention efficiency, respectively. In this context, it can be suggested that optimum growth and nutrient utlization achieved by adjusting dietary lipid and carbohydrate level according to digestible energy (DE) requirement of fish under the examination.

Specific growth rate (SGR) of MCR (1.8) or MCS (1.7) in this study was inferior compared to that (2.2 %) reported by Kim & Kaushik (1990) who fed trout diets one of which contained 33 % wheat middlings. One possible reason for this is the difference in dietary lipid levels as mentioned previously.

From a protein sparing standpoint, MCR (medium carbohydrate restricted) fish spared considerable protein for growth when compared to LCR fish, thus they grew 11.7 % above the LCR (low carbohydrate restricted) group despite having the same protein intake. Consequently, MCR utilised approximately 19.7 % less digestible protein and 10 % less digestible energy per kg^{-1} growth compared to LCR group. Similar growth performance of MCR & MCS, and of HCR and HCS treatments could be explained by the fact that restricted feeding regimes were near to satiation level and consequently these groups consumed similar amounts of feed. Final weights of high carbohydrate restricted or satiation regimes and low carbohydrate restricted group were not

significantly different, even though HCR or HCS consumed approximately 33 % more feed than LCR. It should be noted that low digestion efficiency of the high carbohydrate diet resulted in higher feed intake and more faecal output.

However, apparent net protein utilisation of LCR was 12.7 % inferior compared to HCR or HCS. This may thus suggest that high level dietary carbohydrate (305 g kg^{-1}) could spare protein within the limit of this study, however the protein sparing action of such high levels of carbohydrate is open to discussion because of their reduced digestibility coefficients and utilization efficiencies. The highest apparent net energy utilization (ANPU) in medium carbohydrate restricted groups supported this view that ANPU was lowest in LCS fish although low carbohydrate satiation group revealed the best growth performance.

The estimation of partitioning of dietary energy (Table 5.1.5) according to Cho & Kaushik (1985) suggested that non-faecal energy loss in LCS was the highest and contributed nearly one third of gross energy consumed. This calculation is an indication of lowest ANPU and ANEU of low carbohydrate satiation group.

The dress out (%) of fish in all groups was not significantly different ($p>0.05$) which was the first indication of similar carcass composition of experimental treatments. Allometric analysis of proximate composition of carcass and muscles (Table 5.1.6, 5.1.7 and 5.1.8) showed a very uniform picture in the level of lipid content in all treatments. Therefore, it could be suggested that inclusion of complex digestible dietary carbohydrates up to 45 % does not affect carcass and muscle proximate composition of trout under present experimental conditions.

Generally it can be suggested that diets enriched with digestible carbohydrate and having digestible energy concentration between 16.4 and 20.2 MJ kg^{-1} may not change body composition of trout significantly. This view is supported by Chapter 3 and Chapter 4.1 in which the influence of different energy diets on carcass lipid concentration was studied and revealed that high energy diets with more than 25 % dietary lipid concentration affects carcass lipid level significantly ($P<0.05$).

Regulation of feed intake was observed in all satiation treatments, however a relative reduction of feed intake was visualised in these groups following the tenth week of the trial. Similar feed intake results may support the idea derived from Chapter 4.2 that rainbow trout may adjust their feed intake according to the degree of stomach fullness. However this claim should be tested whether the stomach fullness is the consequence or the main cause modifying feeding behaviour of trout. Also postprandial plasma nutrients may play a regulatory function as well as gastric fullness. In this respect, there is a necessity towards investigations of some physiological parameters for comprehending the overall response of rainbow trout to the varying level of carbohydrate diets.

It was in this context that the next experiment was designed employing the same diets (LC, MC and HC) in order to examine their effects on gastric evacuation, return of appetite and postprandial plasma glucose, protein and triglyceride concentration in rainbow trout.

EXPERIMENT 5

5.2 EFFECTS OF DIETARY CARBOHYDRATE LEVEL ON GASTRIC EVACUATION, RETURN OF APPETITE AND POSTPRANDIAL PLASMA NUTRIENT CONCENTRATION IN RAINBOW TROUT, *Oncorhynchus mykiss.*

5.2.1 INTRODUCTION

It has been extensively reported that return of appetite is probably controlled by stomach evacuation rate in fish (Elliott & Persson, 1978; Gwyther & Grove, 1981; Grove *et al.*, 1985; Singh & Srivastava, 1985; Sims, 1994). The results of Chapter 4.2 have also supported this hypothesis irrespective of dietary energy concentration (9.0-21.3 MJ kg^{-1} DE) fed. However, this might contradict the idea forwarded by Grove (1986), Jobling (1986a) and Sims (1994) that a constant flow of digestible or metabolizable energy is delivered from the cardiac stomach into the intestine. In this case, energy intake will play a more important role compared to dry matter intake. On the contrary, total dry matter intake was likely to be more significant according to the findings of Chapter 4.2.

Supporting information was obtained from the previous experiment (Chapter 5.1) that rainbow trout fed three different carbohydrate levels (13.2, 22.0 and 30.5 %) on a satiation basis showed very close feeding behaviour with regards to mean feed consumption. It was speculated that these groups (LCS, low carbohydrate satiation; MCS, medium carbohydrate satiation and HCS, high carbohydrate satiation) might have

fed for dry matter since the absolute protein and energy intakes of fish were quite different.

Interactions between the meal volume and dietary composition are the most important considerations in gastric evacuation and appetite revival phenomenon in rainbow trout as well as in higher animals (Hunt, 1980; Deutsch & Gonzales, 1981; Kallogeris *et al.* 1983; Jobling, 1986a; Mayer 1994; Porrini *et al.*, 1997).

Since dietary carbohydrate is commonly used as a filler component in practical trout diets, it would be relevant to feed rainbow trout different levels of dietary carbohydrate to study whether feed intake is regulated by stomach fullness related to the dry matter or digestible energy content of the diet.

Plasma circulating nutrients and hormones have also been proposed as informing the brain about the animals' metabolic and physiological state, and may be involved in the control of feed intake in higher animals (Kissileff & Van Itallie, 1982; Forbes, 1995). Plasma glucose concentration may suppress appetite in trout fed a high-level carbohydrate diet (Hilton *et al.*, 1987). The implications are important with respect to our knowledge of fish feeding physiology in general, and the development of suitable aquafeeds for intensive fish production with carbohydrates as an energy source.

Therefore three diets (used in Chapter 5.1) with differing dietary carbohydrate levels (13.2, 22.0 and 30.5 %) were fed to rainbow trout in order to examine the gastric evacuation rate, appetite revival and associated postprandial plasma nutrient levels.

The use of X-Radiography could not be validated in the gastric evacuation study as reported in Chapter 4.2, since the "ballotini" glass beads were selectively retained in the cardiac stomach. However, the same technique is used for return of appetite determinations in the present investigation since feed intake is independent of bead retention.

Physiological investigations in fish concerning regulation of feed intake are comparatively scarce. Therefore the author aims to derive some information on physiological mechanisms controlling feed consumption in relation to the dietary carbohydrate concentration.

5.2.2 MATERIALS AND METHODS

5.2.2.1 Experimental Fish and Holding Facilities

Rainbow trout, *Oncorhynchus mykiss* were supplied from the previous feeding experiment and held in the aquarium. Prior to return of appetite and gastric evacuation studies, fish were ranked into two groups and subordinate groups fed to apparent satiation three times daily (until no feed is consumed) for four weeks. Dominant groups were also fed restricted (0.6 % total biomass day^{-1}) with the respective diets. Then, experimental fish (mean weight 205.0 ± 2.0 g SEM) (30 fish per group) were assigned to the return of appetite experiment. Second groups of 30 fish were maintained for the gastric evacuation study. Experimental conditions were as outlined in Chapter 2.1.

5.2.2.2 Test Dits

Formulation and chemical composition of experimental diets are presented in Table 5.2.1 and 5.2.2, respectively. The first test diets (Table 5.2.1) were identical to those used in Chapter 5.1. However X-ray dense marker (3.8 % of the diet in weight) was incorporated into the second test diets. The numbers of marker "ballotini" in known weights of diet were determined by X-radiography to ensure even distribution. The relationship between the weight of feed (FW) and the number of beads (N) was linear:

Weight of Low Carbohydrate diet (FW_{LC})= 0.0255*N, R^2 = 0.95, n= 20 different amounts of feed X-rayed.

Weight of Medium Carbohydrate diet (FW_{MC}) = 0.0240*N, R^2 = 0.97, n= 20

Weight of High Carbohydrate diet (FW_{HC})= 0.0224*N, R^2 = 0.97, n= 20

Table 5.2.1 Diet Formulation (% dry matter) and chemical composition of experimental diets* without ballotini.

Ingredient	**LC**	**MC**	**HC**
LT Fish Meal	52.6	42.8	35.0
Poultry Meat Meal	12.0	9.6	8.0
Blood Meal	3.0	2.4	2.0
Extruded Wheat Meal	15.3	32.1	43.4
Fish Oil	10.8	8.7	7.2
Vitamin/Mineral Premix	2.0	2.0	2.0
∝-cellulose	1.9	-	-
Cr_2O_3	0.4	0.4	0.4
Binder (CMC)	2.0	2.0	2.0
Protein (% DM)	48.7	41.7	37.3
Lipid (% DM)	20.5	17.5	15.2
Ash (% DM)	10.4	8.9	7.7
Carbohydrate (% DM)	13.2	22.0	30.5
Digestible Protein (DP) (%)	43.6	34.0	30.7
Digestible Energy (DE) (MJ kg^{-1})	20.2	17.3	16.4
DP/DE Ratio (g DP MJ^{-1} DE)	21.6	19.7	18.7

* Same diet specifications as given in Table 5.1.1.

Table 5.2.2 Diet Formulation (% dry matter) and chemical composition of experimental diets with ballotini

Ingredient	LC	MC	HC
LT Fish Meal[1]	51.3	41.8	34.2
Poultry Meat Meal[1]	11.7	9.4	7.8
Blood Meal[1]	2.9	2.3	2.0
Extruded Wheat Meal[1]	14.9	31.4	42.3
Fish Oil[1]	10.6	8.4	7.0
Vitamin/Mineral Premix[1]	2.0	2.0	2.0
∝-cellulose[1]	1.9	-	-
Marker[2] (Ballotini)	3.8	3.8	3.8
Binder[1] (CMC)	0.9	0.9	0.9
Protein (% DM)	47.6	40.7	36.4
Lipid (% DM)	20.0	17.0	14.8
Ash (% DM)	10.1	8.7	7.5
Carbohydrate (% DM)	12.9	21.5	29.8
Digestible Protein (DP) (%)	42.6	33.2	30.0
Digestible Energy (DE) (MJ kg^{-1})	19.7	16.9	16.0
DP/DE Ratio (g DP MJ^{-1} DE)	21.6	19.7	18.8

1. Same ingredients as presented in Table 5.1.1
2. Size: 0.65-0.90 mm (Jensons Ltd. UK)

5.2.2.3 Return of Appetite Determinations

A protocol (Table 5.2.3) was designed to allow each diet to be assayed at set time intervals (t = 0, 4, 8, 12, 24, 48 h) so that no individual fish was X-rayed more than once in a 144 h period.

Table 5.2.3 X-Radiography protocol for determination of appetite return.

	LC	MC	HC
Day 1	Time 4h	Time 8h	Time 12h
Day 7	Time 8h	Time 12h	Time 4h
Day 13	Time 12h	Time 4h	Time 8h
Day 20	Time 24h	Time 24h	Time 24h
Day 28	Time 48h	Time 48h	Time 48h

Following a –72- hour starvation period, fish were fed diets without an X-ray dense marker (Table 5.2.1) until all fish reached apparent satiation. This was determined by monitoring the bottom of the tanks where a small amount of uneaten feed (1-2 pellet) remained. The satiation time for rainbow trout was observed to be between 40-50 minutes (personal observation). After removing uneaten feed, fish were starved until the second meal was applied.

The second meal with the X-ray opaque beads (0.65-0.90 mm) was offered to respective groups according to the protocol (Table 5.2.3) until all fish reached satiation. The level of re-feeding at the specified time interval was equal to the extent of appetite return. Subsequently, 10 LC fish were anaesthetised (Benzocaine, Sigma Chemical Co. Ltd., Poole, UK; 1 g dissolved in 100 ml of ethanol, this added to fresh water at a concentration of 5 ml l^{-1}), weighed and X-rayed using a portable Phillips Practix X-ray unit with light beam diaphragm attachment. Then X-rayed fish were transferred to their original tank following recovery, after which a second group of 10 fish were removed and treated in a similar manner.

The recovered group of fish were then maintained on the same diet for 2 days and deprived of food for three days before beginning further appetite revival measurements. During the X-radiographic studies, no mortality or evidence of vomiting of feed was observed. The X-radiographic pictures of rainbow trout were viewed on a light table (PLH Scientific Ltd, UK) and glass beads were counted. Weight of feed consumed by each fish was calculated according to the calibration formula and expressed in weight specific terms. Return of appetite of fish for each set time interval was expressed as a percentage of the mean feed intake of fish at time = 0. The X- radiography technique employed was that employed by Sims *et al.* (1996) and described in Chapter 2.8.

5.2.2.4 Gastric Evacuation Study and Fish Sampling

After completing return of appetite measurements, the fish used for return of appetite experiment and reserved for gastric evacuation study were pooled. 60 fish were placed in each of the three tanks and allowed one week by feeding respective diets prior to sampling. The method used for the stomach evacuation study was as outlined in Chapter 4.2.2.5.

5.2.2.5 Statistical Analysis

Statistical analysis used in the present study is the one used in Chapter 4.2 and outlined in Chapter 2.11.

5.2.3 RESULTS

5.2.3.1 Gastric Evacuation and Return of Appetite Rates

As a result of a series of comparative analysis for gastric evacuation data, linear, square root and exponential models gave the appropriate fit for the data set under the examination. Therefore all three models were used for every single data set and slopes of equations were compared by multiple regression analysis. The comparison of slopes for linear, exponential and square root models are presented Table 5.2.4, Table 5.2.5 and Table 5.2.6, respectively.

Table 5.2.4 Statistical summary of comparison of the fitted slopes in linear form.

		Regression[1]			Multiple Regression Analysis[2]		
Linear	**RMS[3]**	**A**	**b**	**r^2**	**F**	**d.f.**	**P**
LC	93	101.4	-2.59	0.85	-31	1:108	>0.05
MC	67	92.5	-2.62	0.89			
LC		101.4	-2.59	0.85	0.36	1:108	>0.05
HC	64	92.7	-2.71	0.90			
MC		92.5	-2.62	0.89	0.21	1:108	>0.05
HC		92.7	-2.71	0.90			

[1] Coefficients derived from the fitted linear model $S_t = (S_0 - b*time)$

[2] Significant differences ($P<0.05$) in shape of slopes determined by multiple regression analysis. [3] Residual Mean sum of Squares

Comparison of the linear regression slopes for evacuation of three dietary treatments was not found to be significantly different ($P>0.05$). However, application of exponential (Table 5.2.5) and square root models (Table 5.2.6) displayed that the slope of the LC group was significantly different ($P<0.05$) than that of MC and HC, while there was no significant difference ($P>0.05$) between the slopes of MC and HC groups.

Table 5.2.5 Statistical summary of comparison of the fitted slopes in exponential form.

		Regression[1]			Multiple Regression Analysis[2]		
Exponential	**RMS[3]**	**a**	**b**	**r^2**	**F**	**d.f.**	**P**
LC	117	108.1	-0.045	0.82	8.02	1:108	<0.05
MC	66	103.4	-0.057	0.89			
LC					18.54	1:108	<0.05
HC	72	103.6	-0.06	0.89			
MC					2.96	1:108	>0.05
HC							

[1] Coefficients derived from the fitted exponential model $S_t = (S_0 * e^{-b * time})$

[2] Significant differences ($P<0.05$) in shape of slopes determined by multiple regression analysis

[3] Residual Mean sum of Squares

As far as the choice of the best model is concerned, minimum residual mean sum of squares (RMS), intercepts nearest to 100 and consequently highest r^2 were taken into consideration. Minimum RMS for MC and HC groups was obtained in the square root model with the highest r^2 (Table 5.2.6). The RMS of LC group was lower in linear and square root equations compared to exponential one. R^2 of LC in linear and square root models was same (0.85) and residual of linear was only 3.9 % lower than that of square root equation. However, since the slope of LC was not significantly different ($P>0.05$) than that of MC and HC treatments and vice versa for the square root model as was expected, square root equations were selected for the gastric evacuation of LC, MC and HC groups.

Table 5.2.6 Statistical summary of comparison of the slopes in square root model.

		Regression[1]			Multiple Regression Analysis[2]		
Square Root	**RMS[3]**	**a**	**b**	**r^2**	**F**	**d.f.**	**P**
LC	97	10.3	0.18	0.85	3.76	1:108	<0.05
MC	54	10.0	0.21	0.91			
LC					8.35	1:108	<0.05
HC	54	10.02	0.22	0.92			
MC					1.75	1:108	>0.05
HC							

[1] Coefficients derived from the fitted square root function $S_t = (S_0 - b*time)^2$

[2] Significant differences ($P<0.05$) in shape of slopes determined by multiple regression analysis.

[3] Residual Mean sum of Squares

First order and Sigmoid equations were used for return of appetite modelling (Table 5.2.7). Although both models fitted well, sigmoid equations gave a marginally better fit due to lower residuals mean sum of squares.

Table 5.2.7 Fitted equations for the return of appetite.

Diets	**Model[1]**	**a**	**b**	**k**	**r^2**	**RSM[2]**
LC	Sigmoid	0.0092	0.039	-0.08	0.76	27
	First Order	114.8	-	-0.04	0.74	30
MC	Sigmoid	0.0097	0.047	-0.106	0.80	27
	First Order	112.2	-	-0.046	0.79	28
HC	Sigmoid	0.0099	0.034	-0.105	0.80	21
	First Order	102.2	-	-0.064	0.84	21

[1] Coefficients derived from the fitted Sigmoid, $FI = 1/(a + b* e^{-k*t})$ and First order relationships $FI = a* (1-e^{-k*t})$ [2] Residual Mean sum of Squares

Gastric evacuation and return of appetite models for LC, MC and HC treatments are presented in Figure 5.2.1, Figure 5.2.2 and Figure 5.2.3, respectively. These two models were displayed in the same figure in order to be compared closely.

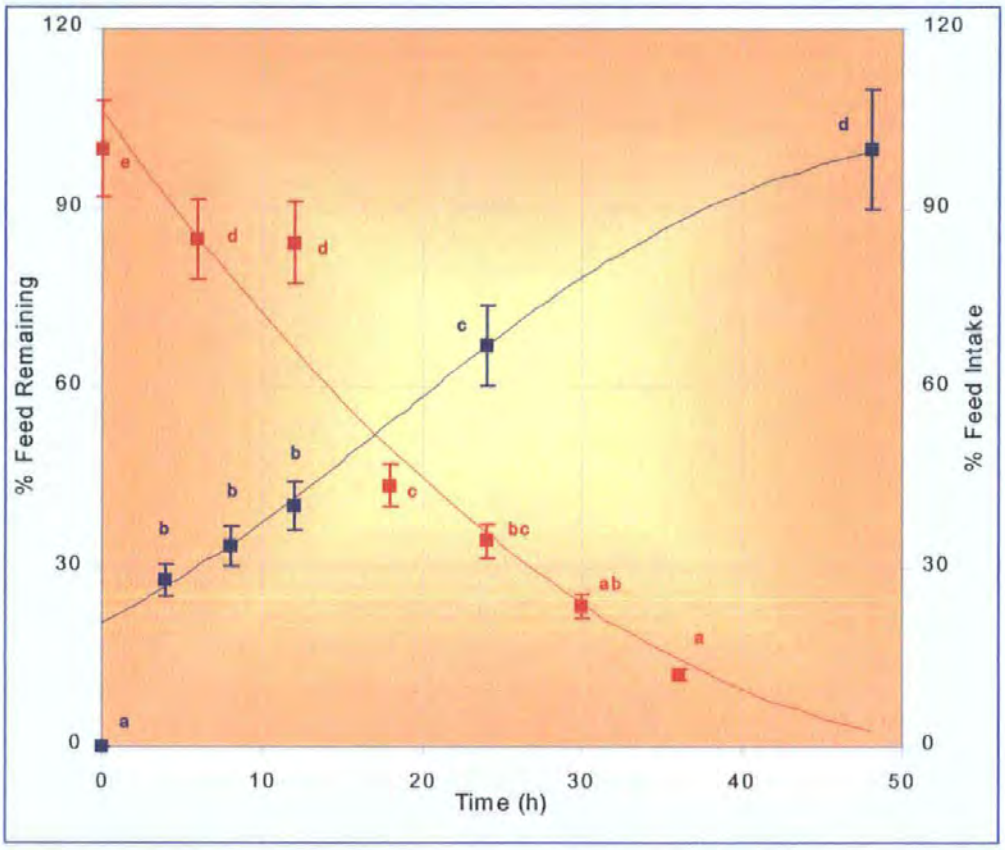


Figure 5.2.1 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed low carbohydrate diet (LC).

Stomach evacuation rate was described by a square root model; $\mathbf{S_t = (10.3 - 0.18*t)^2}$, $\mathbf{R^2 = 0.85}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (Sigmoid);

$\mathbf{FI = 1 / 0.0092 + 0.039* e^{-0.08* t}}$, $\mathbf{R^2 = 0.76}$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20. Data points in each graph allocated different letters are significantly different from each other ($P< 0.05$). Bars denote ± 5 standard error of the mean.

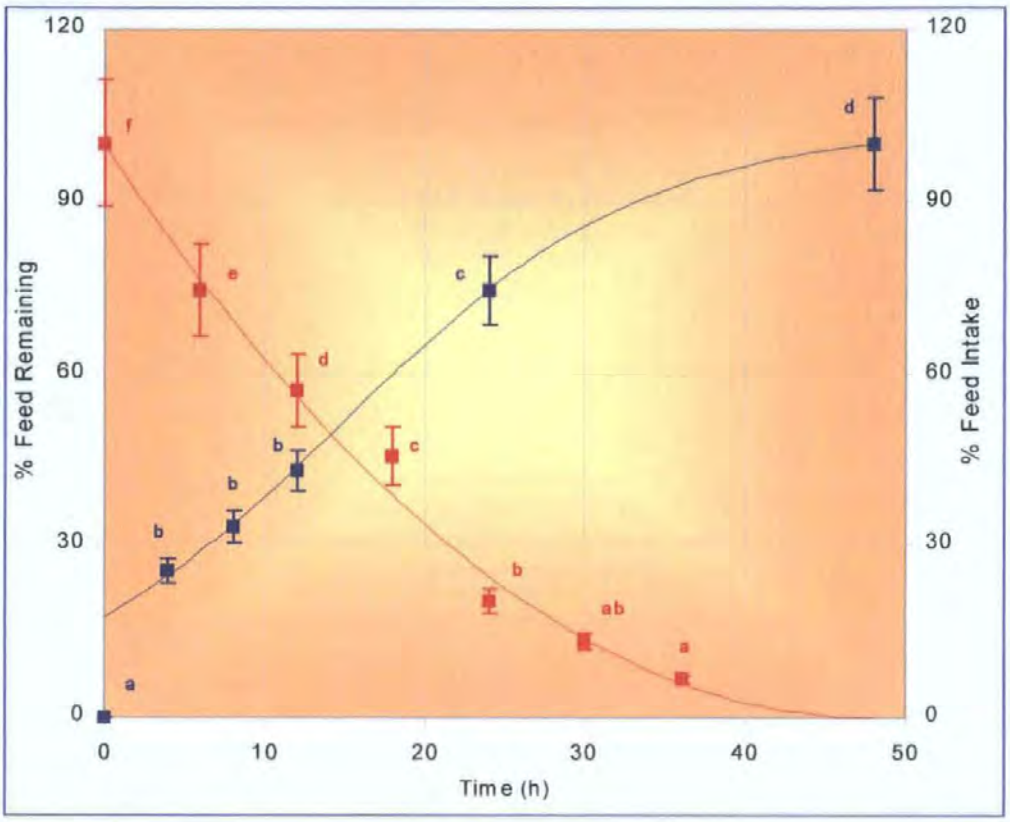


Figure 5.2.2 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed medium carbohydrate diet (MC).

Stomach evacuation rate is described by a square root model;

$\mathbf{S_t = (10.0 - 0.21*t)^2}$, $\mathbf{R^2 = 0.91}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (Sigmoid);

$\mathbf{FI = 1 / 0.0097 + 0.047* e^{-0.106* t}}$, $\mathbf{R^2 = 0.80}$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

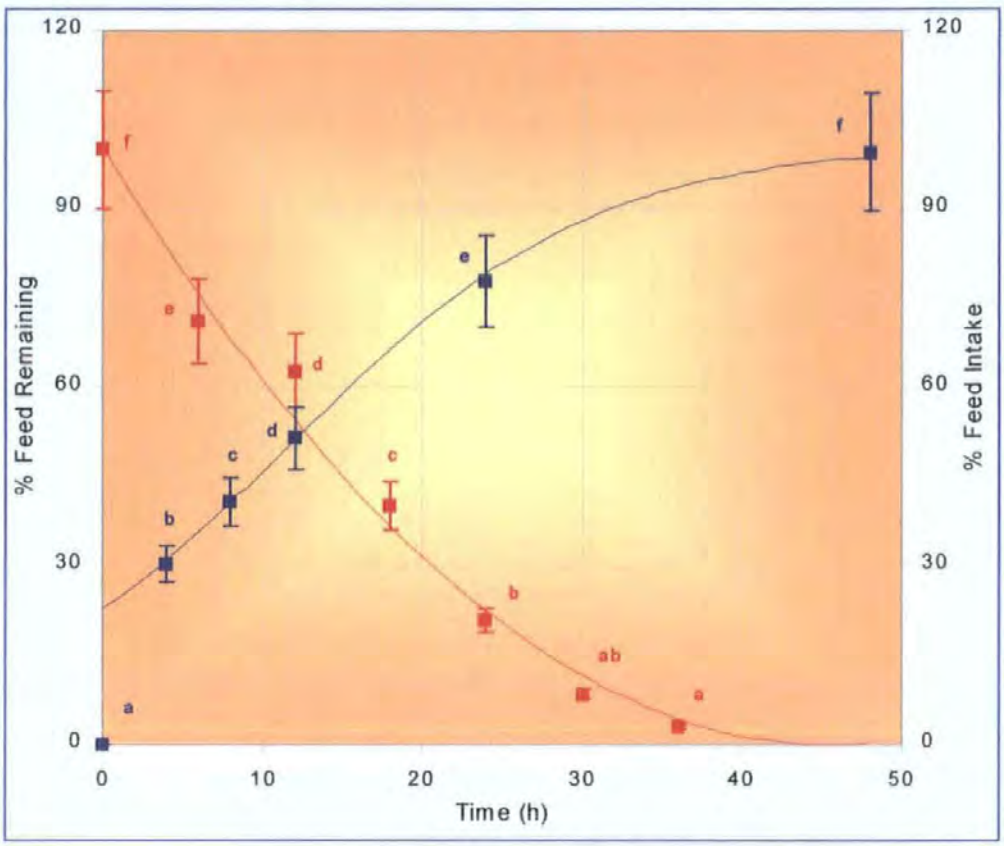


Figure 5.2.3 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed high carbohydrate diet (HC).

Stomach evacuation rate is described by a square root model;

$\mathbf{S_t = (10.02 - 0.22*t)^2}$, $\mathbf{R^2 = 0.92}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (Sigmoid);

$\mathbf{FI = 1 / 0.0099 + 0.034* e^{-105* t}}$, $\mathbf{R^2 = 0.80}$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

The gastric evacuation of LC (Figure 5.2.1), MC (Figure 5.2.2) and HC (Figure 5.2.3) diets was described by a square root relationship. The evacuation curve of LC group was significantly ($P<0.05$) different than other treatments (Table 5.2.6).

Basically, a significant evacuation ($P<0.05$) was observed first 6 hours, and a delay was detected between 6 and 12 hours following feeding in LC treatment. Then, emptying was sustained until 95 % of the digesta was cleared from the cardiac stomach 44.8 hours after feeding (Table 5.2.8). The clearance of MC and HC diets was similar and a significant amount of the digesta was emptied for each time interval until 30 hours. There was no important difference between the evacuation level at 30 and 36 hours. The transit time for 95 % of digesta was 37.0 and 35.4 h for MC and HC diets, respectively (Table 5.2.8).

Table 5.2.8 Predicted gastric evacuation times[1].

		Calculated times (h) for stomach evacuation (%)				
Model	Treatments	5	25	50	75	95
Square Root	LC	**3.1**	**9.1**	**18.0**	**29.4**	**44.8**
	MC	**1.2**	**6.4**	**14.0**	**23.8**	**37.0**
	HC	**1.3**	**6.2**	**13.4**	**22.8**	**35.4**
Exponential	LC	**2.9**	**8.1**	**17.2**	**32.5**	**68.3**
	MC	**1.5**	**5.6**	**12.75**	**24.9**	**53.1**
	HC	**1.45**	**5.4**	**12.15**	**23.7**	**50.5**

1. Calculations are based on the fitted square root and exponential models.

Three sigmoid equations described the appetite revival data of experimental groups (Figure 5.2.1, 5.2.2 and 5.2.3). There was no significant relationship in return of appetite of LC and MC fish between 4 and 12 hours whilst HC group elevated their feed intake significantly at each time interval.

The time required for 95 % of appetite return was predicted as 42.3, 38.2 and 38.1 hours for LC, MC and HC treatments, respectively (Table 5.2.9). According to the fitted first order equations, these times for 95 % appetite revival was 44, 40.8 and 41.5 hours for LC, MC and HC treatments, respectively (Table 5.2.9).

Table 5.2.9 Comparison of predicted return of appetite times[1].

		Calculated times (h) for appetite revival (%)			
Model	Treatment	25	50	75	95
Sigmoid	LC	**3.0**	**16.6**	**28.1**	**42.3**
	MC	**4.2**	**14.4**	**24.2**	**38.2**
	HC	**1.2**	**11.6**	**21.9**	**38.1**
First Order	LC	**6.2**	**14.3**	**26.5**	**44.0**
	MC	**5.5**	**12.9**	**24.0**	**40.8**
	HC	**4.4**	**10.5**	**20.7**	**41.5**

1. Calculations are based on the fitted sigmoid and first order models given in Figure 5.2.1, 5.2.2 and 5.2.3, respectively.

A very high correlation was determined between the gastric evacuation and return of appetite for each treatment by using fitted equations. These relationships are presented in Figure 5.2.4, 5.2.5 and 5.2.6 for LC, MC and HC groups,

respectively. Also estimated equations are tabulated in Table 5.2.10. Irrespective of the models applied, approximately 100 % relation was predicted between appetite revival and gastric evacuation rates in rainbow trout fed Low, Medium and High Carbohydrate diets.

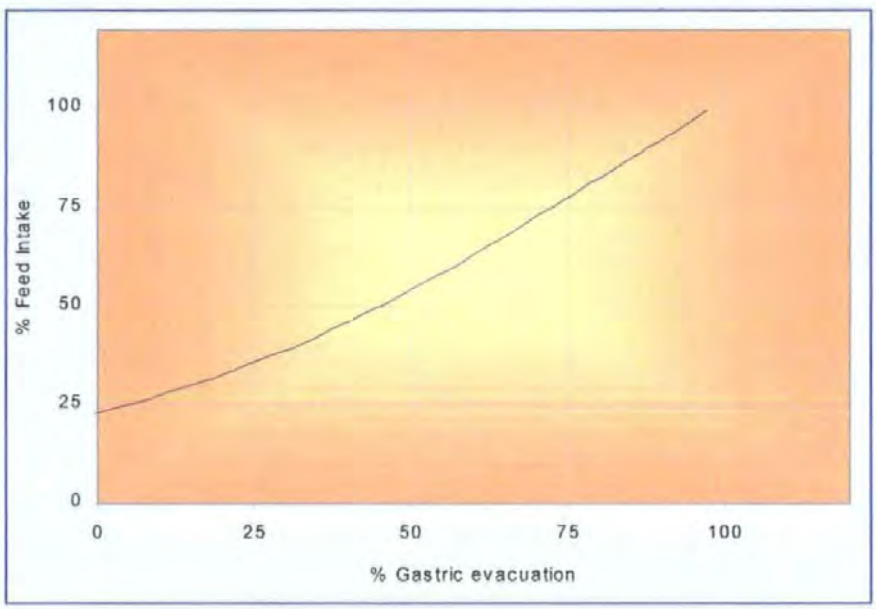


Figure 5.2.4 Relationship between return of appetite (% Feed Intake) and gastric evacuation (%) in rainbow trout fed LC diet (for the fitted equations see Table 5.2.10).

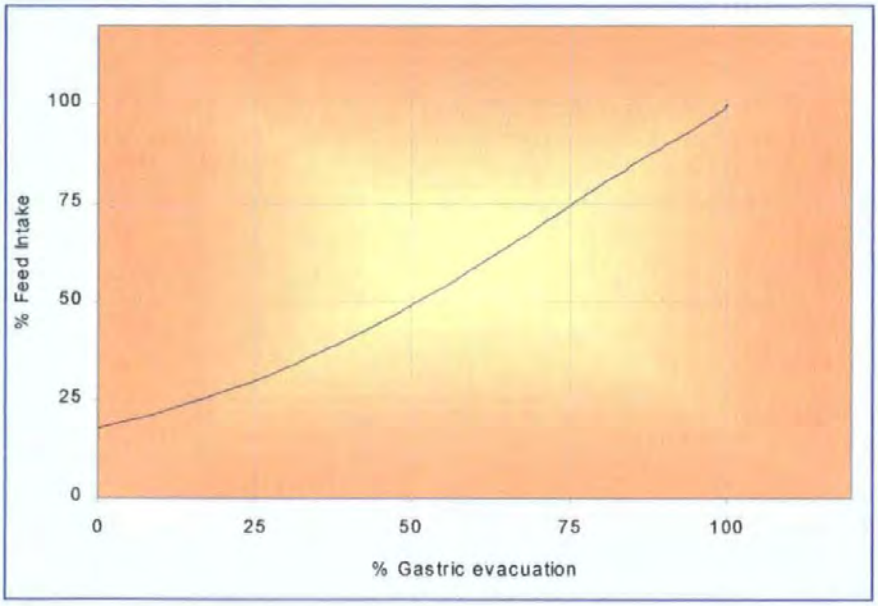


Figure 5.2.5 Relationship between return of appetite (% Feed Intake) and Gastric Evacuation (%) in rainbow trout fed MC diet (for the fitted equations see Table 5.2.10).

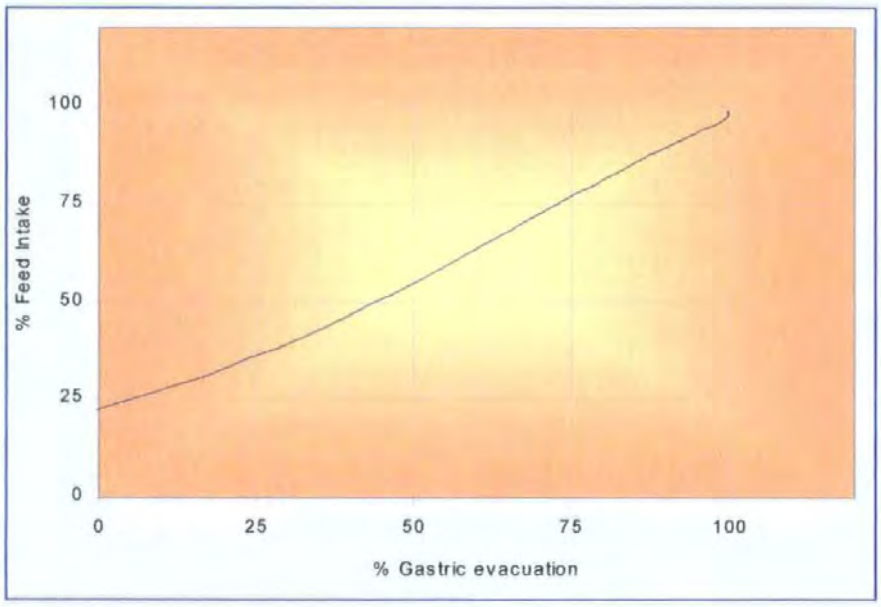


Figure 5.2.6 Relationship between return of appetite (% Feed Intake) and Gastric Evacuation (%) in rainbow trout fed HC diet (for the fitted equations see Table 5.2.10).

Table 5.2.10 Fitted equations for the relationship between return of appetite and gastric evacuation rates[1].

Diet	Model[1]	a	b	R^2	Residual
LC	*Linear*	17.3	0.81	0.99	432
	Exponential	3.9	0.015	0.99	0.064
	Square Root	4.69	0.055	1.0	0.16
MC	*Linear*	8.64	0.89	1.0	375
	Exponential	2.99	0.017	1.0	0.156
	Square Root	4.04	0.06	1.0	0.197
HC	*Linear*	17.3	0.80	1.0	127
	Exponential	3.25	0.014	0.99	0.156
	Square Root	4.79	0.052	1.0	0.207

[1] Coefficients derived from the fitted linear $Y = a + b^* X$, exponential $Y = e^{a + b^* X}$ and square root function $Y = (a + b^* X)^2$, where 'Y' is the return of appetite (% Feed Intake) and 'X' is gastric evacuation (%).

5.2.3.2 Plasma Nutrients

Postprandial plasma nutrients of rainbow trout fed low, medium and high carbohydrate diets are presented in Figure 7, 8 and 9, respectively. There was a significant increase ($P<0.05$) in circulating protein (mg dl^{-1}) concentration of all three treatments. This attained a maximum value four hours after feeding in LC and MC groups whilst the protein level of HC fish reached the maximum at 8 hours. Typically, postprandial plasma protein of these treatments returned to their initial concentrations 48 hours after initial meal consumption.

Plasma glucose (mmol l^{-1}) level of LC, MC and HC trout was also elevated and reached the maximum concentration ($P<0.05$) at time 4, 8 and 24h, respectively. On the other hand, a transient hyperglycemia was observed in rainbow trout fed High Carbohydrate, since plasma glucose level sustained significantly high ($P<0.05$) even 48 hours following alimentation.

No significant relationship ($P>0.05$) was detected in postprandial triglyceride level (mmol l^{-1}), although triglyceride concentration of all treatments displayed an initial elevation. This observed insignificance was despite the difference between dietary lipid concentration of LC (20.5 %), MC (17.5 %) and HC (15.2 %) diets.

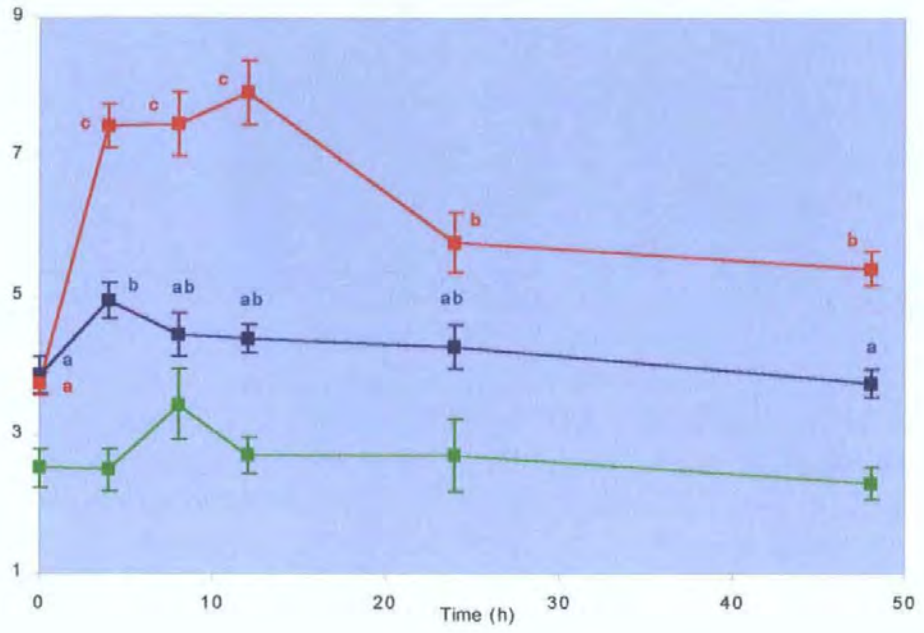


Figure 5.2.7 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed low carbohydrate (LC) diet.

Data points in each graph allocated different letters are significantly different from each other ($P< 0.05$). Bars denote ± 5 standard error of the mean.

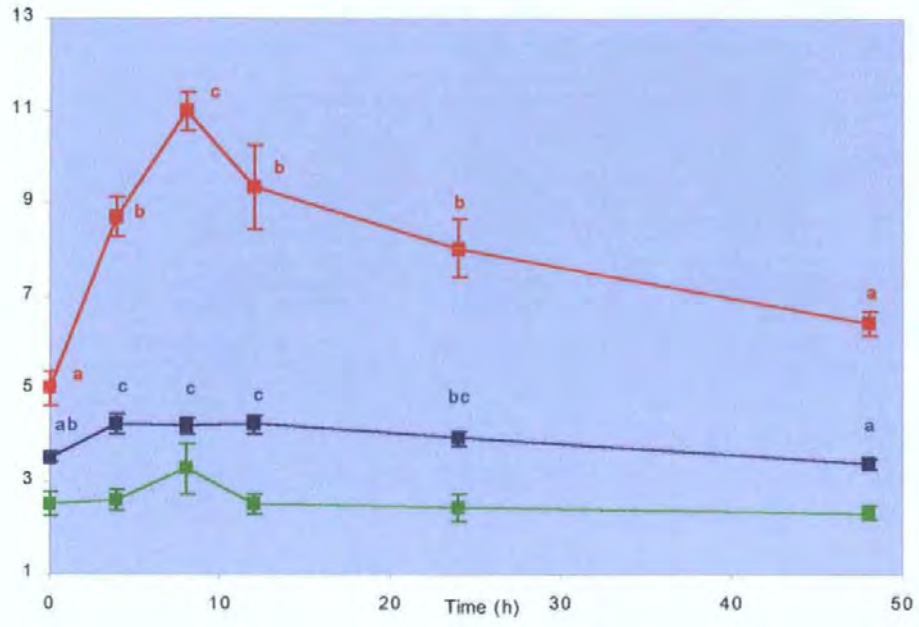


Figure 5.2.8 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed medium carbohydrate (MC) diet.

Data points in each graph allocated different letters are significantly different from each other ($P< 0.05$). Bars denote ± 5 standard error of the mean.

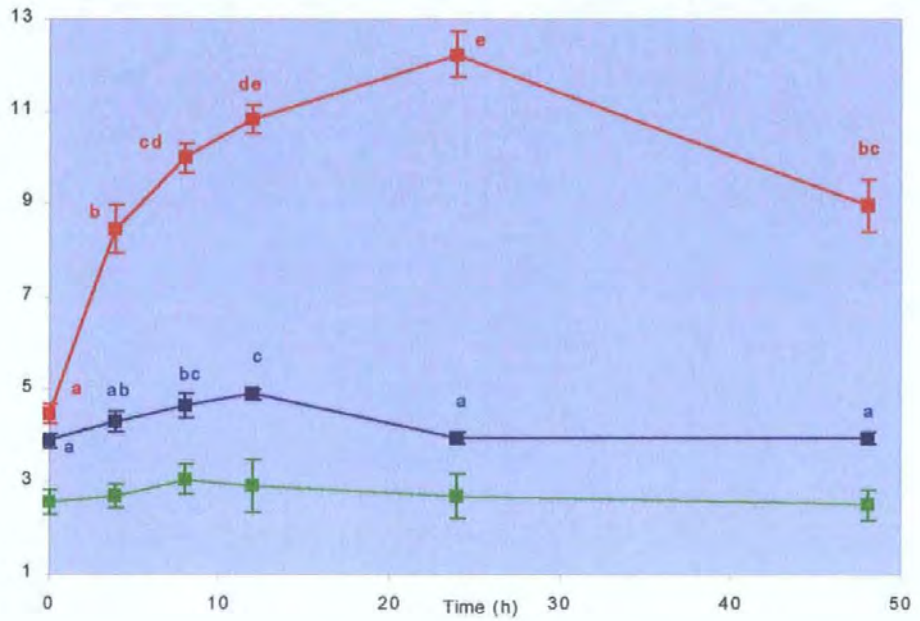


Figure 5.2.9 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed high carbohydrate (HC) diet.

Data points in each graph allocated different letters are significantly different from each other ($P< 0.05$). Bars denote ± 5 standard error of the mean.

5.2.4 DISCUSSION

In this investigation, rainbow trout, *Oncorhynchus mykiss* were fed three different levels (15.3, 32.2 and 43.5 %) of extruded wheat meal as the principal carbohydrate source in order to study gastric evacuation, appetite return and postprandial plasma circulating nutrient levels.

The time required for clearance of 95 % stomach content was estimated to be approximately 44.8, 37 and 35.4 hours for LC, MC and HC diets, respectively according to the square root equations. The decrease in evacuation rate in the LC fish could be explained by the higher digestible energy (DE) value of the LC diet (20.2 MJ kg^{-1}) compared to the MC (17.3 MJ kg^{-1}) and the HC (16.1 MJ kg^{-1}) diets. Similarly, digestible protein (DP) concentration of LC diet (43.4 %) was considerably higher than MC (34.0 %) and HC (30.7 %) diets. Probably negative feedback signals due to the transport of energy dense digesta into the upper intestine or amino acid receptors in the duodenum played as a regulatory factor as implied by Jobling (1986a) and Bromley (1987). These findings are in accordance with the common view that high energy diets are evacuated slower compared to low energy diets, however, no realistic quantification has been performed in order to understand the direct effect of energy concentration of the digesta on gastric evacuation rate.

First order and sigmoid relationships (Table 5.2.7) were employed for the return of appetite modelling. Since the explanation of the data provided marginally lower RSM and higher r^2 in sigmoid model, this equation was chosen for all appetite return determinations. Following the comparison of the fitted equations using the F-Test, It was detected that the instantaneous rate of appetite revival in LC group was significantly slower compared to other two groups. In a

similar manner to gastric evacuation rates, no significance was evident between the slopes of MC and HC treatments. The time required for 95 % of the return of appetite was calculated as 42.3, 38.2 and 38.2 hours for LC, MC, and HC groups, respectively. These results are in agreement with the previous findings (Chapter 4.2) and general view (i.e. Rozin & Mayer, 1961; Lee & Putnam, 1973; Fänge & Grove, 1979; Basimi & Grove, 1985; Ruohonen *et al.*, 1997) that fish increase their feed intake if the dietary energy concentration is diluted.

The similarity between gastric evacuation and return of appetite in trout fed three different levels of carbohydrate source was also explained statistically. Almost 100 % relationship (r^2= 1.0) was observed between these two parameters for all three groups following application of linear, exponential and square root equations (Table 5.2.10). This relationship also confirms the previous findings (Chapter 5.4.2) that the appetite revival in trout is mainly controlled by cardiac stomach fullness. In addition, emptying of the stomach contents is also likely to be regulated by the amount of food in the stomach or the distension of the stomach wall (Brodeur, 1984; Basimi & Grove, 1985; Bromley, 1994).

These claims are supported by Grove *et al.* (1978, 1985) and Sims *et al.* (1996) who declared that the cardiac stomach is likely to be the primary organ with respect to the regulation of voluntary feed intake in fish.

It is apparent that complex interrelationships exist both at the physiological and biochemical levels which determine the effect of diet composition on gastric evacuation and return of appetite profiles in salmonids. Since the appetite of trout under laboratory conditions returned before complete evacuation of the meal, it may be proposed that the main determinant regulating appetite in this species is the degree of stomach fullness and

emptying rate as demonstrated by Windell & Norris (1969), Bret (1971), From & Rasmussen (1984) and the author (Chapter 4.2). As the feed intake terminates before absorption is completed, meal size will likely be dependent on the signals from the gastro-intestinal tract. In this context, Rayner (1992) pointed out the importance of dietary factors and feeding regime, and concluded that animals receive more information from the gastro-intestinal tract than they need and are able to integrate information as required in relation to former experiences. The amount of digesta in the cardiac stomach and the degree of stomach wall distension might indicate further physiological mechanisms that modulate the emptying pattern. Signals from stretch receptors may be conducted to the central nervous system by abdominal afferent neurons in the initial stages of feeding.

The chemical composition of the diet is also an important factor determining gastric motility, however the exact mechanisms (via neurons and endocrine cells) which are modulating the effect remain unclear (Grove & Holmgren, 1992). These workers have proposed that distension of the stomach causes a reflex activity via cholinergic and serotonergic nerves. Somatostatin then suppresses rhythmic contractions whilst VIP (vasoactive intestinal polypeptide) induces gastric relaxation.

Plasma glucose, protein and triglyceride profiles all displayed a characteristic pattern during the postprandial sampling phase. For the HC diet, the tansient increase in glucose level (Figure 5.2.9) was sustained 48 hours post feeding as previously reported (Cowey *et al.*, 1977a; Bergot, 1979; Walton, 1986). The scope of this prolonged hyperglycemia was consistent with the carbohydrate level. Rainbow trout did not show any lack of appetite throughout the study therefore it could be suggested that plasma glucose level did not affect appetite of trout from the viewpoint of feed regulation. Similarly, Peter *et al.* (1976)

suggested that plasma glucose is not a significant metabolite in the appetite control of Goldfish, *Carassius auratus*. The initial rate of protein absorption appears to be suppressed by increasing dietary carbohydrate (Figure 5.2.7-5.2.9). Postprandial triglyceride levels (Figure 5.2.7-5.2.9) were also unaffected by elevated carbohydrate or decreased dietary lipid concentration. However, these plasma nutrient interactions did not appear to have greatly influenced return of appetite in the rainbow trout as previously shown in channel catfish, *Ictalarus punctatus* (Lovell, 1979)(cited in Fletcher, 1984) and dogfish, *Scyliorhinus canicula* (Sims, 1994).

In this study, secondary feed intake was determined by incorporating different size of radio-opaque beads as previously used by Koskela *et al.* (1993). In this connection, it may be implied that the method employed for the quantification of return of appetite in the present study was valid since a number of points were taken into consideration. Some of these are minimum stress because of handling with utmost care, a good prediction of recovery time and ensuring the complete clearance of ballotini from the gastrointestinal tract. Indeed a considerable inter-individual feed intake difference was confronted, but it was resolved by using a large number of uniform sized fish per X-radiography session.

As far as the serial slaughter technique is concerned, Talbot (1985) and Bromley (1994) have advocated that it is not a practical method to sacrifice large numbers of fish for such studies. However, stomach evacuation results derived from Experiment 3 (Chapter 4.2) using X-radiography were not scientifically representative as previously demonstrated on arctic charr, *Oncorhynchus kisutch* (Jorgensen & Jobling, 1988) and Atlantic cod, *Gadus morhua* (Dos Santos & Jobling, 1991).

In conclusion, it can be suggested that gastric evacuation rate may have been the major factor controlling the return of appetite, irrespective of carbohydrate level in the diet. Gastric evacuation in the LC group was different from their counterparts due to their different DE intake. Gastric evacuation of MC (322 g extruded wheat kg^{-1} diet) and HC (435 g extruded wheat kg^{-1} diet) were not significantly different whilst they exhibited different plasma glucose patterns. Under normal experimental conditions trout (as a sight feeder) feed for gastric fullness in the short term in order to reach their maximum growth potential (conservation of body weight). However body energy stores might interact in the longer term.

Plasma nutrients apparently do not have a major role for appetite regulation as observed in Chapter 4.2. Glucose in particular did not appear to influence homeostatic regulation and modulate feed intake response in the current investigation. It is quite interesting to know whether the appetite response of rainbow trout is influenced by simple sugars such as D-glucose and maltose compared to more complex starch polymers typically in cereals. Defining the gastric evacuation patterns of trout fed carbohydrates of varying level and complexity will be useful in this context. Finally, it is advantageous to investigate the effects of such dietary components on growth performance and nutrient utilization in balanced diet formulations. In this manner, it may be possible to characterise the protein sparing potential of carbohydrates compared to dietary lipid for rainbow trout. Therefore the next series of experiments were designed to evaluate those interaction outlined above and on the basis of the data reported in this present chapter (Chapter 5.1 & 5.2) and Chapter 4.1 & 4.2., respectively.

CHAPTER 6

THE INFLUENCE OF DIETARY CARBOHYDRATE COMPLEXITY ON FEED INTAKE, NUTRIENT UTILIZATION, GASTRIC EVACUATION AND RETURN OF APPETITE IN RAINBOW TROUT, *Oncorhynchus mykiss*.

EXPERIMENT. 6

6.1 EFFECT OF DIETARY CARBOHYDRATE COMPLEXITY ON FEED INTAKE AND NUTRIENT & ENERGY UTILIZATION IN RAINBOW TROUT, *Oncorhynchus mykiss.*

6.1.1 INTRODUCTION

One of the significant factors influencing carbohydrate utilization in rainbow trout is the degree of complexity or polymerization in relation to the rate of digestion and further metabolic interactions (Steffens, 1989; Wilson, 1994).

It has been fairly well established that trout generally utilize cooked starch and dextrin efficiently within compounded feeds. It has been demonstrated (Chapter 5.1) that extruded wheat meal was utilized for energy and the protein was spared for growth as previously demonstrated by Kaushik & Oliva-Teles (1985), Kaushik *et al.* (1989) and Pfeffer *et al.* (1991) in rainbow trout.

It is also known that simple sugars or highly digestible carbohydrates are utilized less well for carnivorous fish compared to native or gelatinized complex polysaccharides (Akiyama *et al.*, 1982). The possible reason for this could be that appetite of fish could be suppressed at a metabolic level due to a variety of possible reasons (see Chapter 1.6) such as low glucokinase activity, and it may thus result in reduction of feed utilization. On the contrary, it was proposed that feed efficiency of trout fed simple sugars eg: mono and di-saccharides

was superior compared to complex carbohydrates such as starches. Then it may be asked how apparent net protein and energy utilization are influenced with reference to the degree of complexity of carbohydrate sources in the diet.

There are also a large number of studies investigating the effect of carbohydrate complexity on proximate composition of carcass in fish. However, inadequate analysis of data has led to some misinterpretations such as the view that body protein was decreased in fish fed high levels of native carbohydrate forms (Reinitz, 1983; Beamish & Medland, 1986).

It is well established that elevated levels of carbohydrate may result in an increased liver size due to hepatic glycogen deposition (Cowey *et al.*, 1977a; Hilton *et al.*, 1987). The effect of liver size in relation to glycogen deposition on relative feed intake remains to be investigated from both a metabolic and physiological standpoint.

Since high oil feeds have been implicated in excessive lipid deposition in the fillet resulting in flesh quality problems (Takeuchi *et al.*, 1978; Cho & Kaushik, 1990) dietary carbohydrates remain to be the only alternative sources of energy in diets for rainbow trout.

Therefore, the aims of this study were to evaluate the effect of carbohydrate complexity on feed intake, growth performance, energy and nutrient utilization and proximate carcass and muscle composition in rainbow trout, *Oncorhynchus mykiss*.

6.1.2 MATERIALS AND METHODS

6.1.2.1 Experimental Fish and Holding Facilities

Fish and experimental facilities were as detailed in Chapter 2.1.1, 2.1.2 and 3.2.1.

6.1.2.2 Feeding and Performance Indicators

Six experimental diets including approximately 300 g kg^{-1} DM glucose (GLU), maltose (MAL), dextrin (DEX), native wheat starch (NWS), native corn starch (NCS) and pregelatinized corn starch (PCS) were formulated. The formulations and chemical compositions of the diets are given in Table 6.1.1. The manufacture of the diets was as described in Chapter 2.2.2.

Fish were fed to apparent satiation three times daily (09.00, 13.00 and 17.00 h) and feed provision was recorded every day throughout the 84-day-trial. One day starved trout (without being anaesthetized) were weighed individually every two weeks. Parameters relevant to growth and feed utilisation efficiency were calculated as outlined in Chapter 2.7.

6.1.2.3 Sampling and Analytical Procedures

Fish sampling and all analytical procedures were as explained in 4.1.2.3.

6.1.2.4 Statistical Analysis

Statistical analysis used for the interpretation of the experimental data was as outlined in Chapter 2.10.1 and applied in Chapter 3, Chapter 4.1. & Chapter 5.1., respectively.

Table. 6.1.1 Dietary formulation and chemical composition of experimental diets

Ingredients	GLU	MAL	DEX	NWS	NCS	PCS
LT Fish Meal[1]	49.0	49.0	49.0	49.0	49.0	49.0
Blood Meal[2]	3.0	3.0	3.0	3.0	3.0	3.0
Poultry Meat Meal[3]	5.0	5.0	5.0	5.0	5.0	5.0
D-Glucose[4]	30.0	-	-	-	-	-
Maltose[4]	-	30.0	-	-	-	-
Dextrin[4]	-	-	30.0	-	-	-
Native Wheat Starch[5]	-	-	-	30.0	-	-
Native Corn Starch[5]	-	-	-	-	30.0	-
Pregelatinized Corn Starch[5]	-	-	-	-	-	30.0
Fish Oil[6]	7.0	7.0	7.0	7.0	7.0	7.0
Vitamin/Mineral Premix[7]	2.5	2.5	2.5	2.5	2.5	2.5
α- Cellulose[4]	0.5	0.5	0.5	0.5	0.5	0.5
Cr_2O_3[4] (Dietary marker)	0.5	0.5	0.5	0.5	0.5	0.5
Binder[4] (CMC*)	2.5	2.5	2.5	2.5	2.5	2.5
Nutrient Analysis						
Protein (% DM)	43.2	43.1	43.4	42.8	42.5	42.8
Lipid (% DM)	11.3	11.6	11.4	11.7	11.6	11.4
Ash (% DM)	9.6	9.9	9.3	9.4	9.5	9.2
Carbohydrate (% DM)	29.6	28.9	30.6	31.1	31.1	30.8
Digestible Protein (DP) (%)	41.5	40.0	41.1	40.4	40.5	38.1
Digestible Energy (DE) ($MJ\ kg^{-1}$)	20.4	19.0	18.3	18.3	18.3	18.6
DP/DE Ratio (g DP/ $MJ\ kg^{-1}$ DE)	20.3	21.0	22.4	22.0	22.1	20.5

1. Low Temperature Fish Meal, Norsea Mink, LT 94. Donated by Trouw Aquaculture, Wincham, Cheshire, UK.

2. Int. Feed Number, 5-00-381, Trouw Aquaculture, Wincham, Cheshire, UK.

3. Int. Feed Number, 5-03-798, " " " " "

4. Sigma Chemical Company, Poole, Dorset, UK. 5. Roquette Freres, Lestrem, France.

6. Int. Feed Number, 7-01-994, Boost Oil, Cod liver oil, Seven Seas, Hull, UK.

7. (Closed Formulation). Trouw Aquaculture, Wincham, Cheshire. *, Carboxy methyl cellulose

6.1.3 RESULTS

Following the feeding trial, apparent digestibility coefficients of dry matter, protein, energy, lipid and carbohydrate for each treatment were determined (Table 6.1.2). All groups displayed high digestibility coefficients. Dry matter digestibility of GLU and MAL treatments was higher than that of DEX, NWS and NCS groups which also showed superior dry matter digestibility coefficients compared to PCS trout. Protein digestibility was between 95.2 % (NCS) and 89.0 % (PCS) and energy digestibility was between 94.1 % (GLU) and 82.1 % (DEX). Lipid digestibility displayed marginal fluctuations around 90.0 %.

Table 6.1.2 Digestibility coefficients (%) of dietary nutrient components*

	GLU	MAL	DEX	NWS	NCS	PCS
Dry Matter	91.9	89.5	84.1	84.7	85.6	79.7
Protein	94.0	92.8	94.6	94.3	95.2	89.0
Energy	94.1	92.6	82.1	83.2	83.0	86.2
Lipid	90.1	90.8	90.5	90.6	90.8	90.3
Carbohydrate	98.1	93.6	73.4	77.1	76.2	82.8

* Coefficients based on pooled sample material from each dietary treatment (n=3).

The most substantial difference was observed in carbohydrate digestibility. This showed an expected trend that maximum values were determined in GLU (98.1 %) and MAL (93.6 %) treatments. PCS group followed with a 82.8 % carbohydrate digestibility. DEX (73.4 %), NWS (77.1 %) and NCS (76.2 %) demonstrated lower values compared to simple sugars. However, no possible statistical conclusion can be drawn since the faecal material was pooled for each

treatment although above mentioned trends in digestibility of all nutrients (except lipid) and energy are evident.

Results for relative feed intake are presented in Table 6.1.3. It was observed that feed intake reduced with increase in carbohydrate digestibility. There was considerable appetite suppression in GLU and MAL groups between second and fourth weeks of feeding trial. Feeding response of these groups (GLU and MAL) returned to initial level between the sixth and eighth weeks. Then, an important reduction was detected again after the eighth weeks of the experiment. Although some fluctuations were observed in other treatments but not as much as GLU and MAL fed trout.

Table 6.1.3 Relative feed consumption of rainbow trout (g 100 g^{-1} biomass)

Time (Weeks)	GLU	MAL	DEX	NWS	NCS	PCS
0-2	2.1	2.0	2.2	2.2	2.3	2.0
2-4	1.3	1.3	2.3	2.2	2.0	1.9
4-6	2.2	1.8	2.7	2.5	2.5	2.4
6-8	2.0	1.9	2.4	2.5	2.7	2.1
8-10	1.4	1.9	2.2	2.5	2.2	1.7
10-12	1.6	1.7	2.2	2.3	2.0	1.8
Mean F. I.	1.8^{a}	1.8^{a}	2.3^{b}	2.4^{b}	2.3^{b}	2.0^{ab}

When the overall mean feed intake is taken into consideration, GLU and MAL fish displayed an identical results which was significantly lower ($P<0.05$) compared to DEX, NWS and NCS groups while mean feed intake of PCS was not significantly different ($P>0.05$) than the other five treatments (Table 6.1.3).

Although the feeding behaviour of DEX, NWS and NCS was quite similar ($P>0.05$) throughout the 84-day-feeding study, final mean weight of NCS (Table 6.1.4) was significantly inferior compared to that of DEX and NWS fed trout. DEX and NWS groups showed superior growth ($P<0.05$) than GLU, MAL and NCS treatments whilst the growth performance of fish fed PCS diet was only significantly higher ($P<0.05$) than that of GLU treatment. Growth of fish fed GLU, MAL and NCS diets was observed similar ($P>0.05$).

Feed efficiency of GLU, MAL and PCS groups exceeded 100 % however it was significantly ($P<0.05$) higher in MAL than DEX, NWS and NCS groups. This parameter was lowest in NCS (81 %) and significantly inferior compared to PCS, GLU and MAL fed trout.

Digestible protein (DP) utilized per kg^{-1} growth was calculated between 395 (MAL) and 528 g (NCS). It was predicted that only MAL and PCS treatments utilized less protein per kg^{-1} growth in this study. In a similar style, Digestible Energy (DE) utilized per kg^{-1} growth lay between 17.4 (MAL) and 19.5 (NCS) MJ per kg^{-1} growth.

Apparent Net Protein Utilization (ANPU) of DEX, NWS and NCS fish was lower compared to that of GLU, PCS and MAL fish. MAL group demonstrated the best ANPU (41.8 %). Apparent net energy utilization (ANEU) was also observed as accordance with the ANPU parameter. GLU, PCS and MAL trout displayed the higher values compared to DEX, NWS and NCS trout. ANEU of MAL fed fish was also 10.3 and 16.8 % higher than that of GLU and PCS fed trout, respectively.

Table 6.1.4. Growth performance of rainbow trout fed different carbohydrate diets for 84 days.

	GLU	MAL	DEX	NWS	NCS	PCS	±SEM*
Initial mean wt (g)	30.5	30.4	30.5	30.4	30.6	30.5	0.56
Final mean wt (g)	120.5^{a}	135.2^{ab}	156.0^{c}	152.0^{c}	129.4^{ab}	145.2^{bc}	5.81
Specific growth rate (% day^{-1})	1.6	1.8	2.0	1.9	1.7	1.8	0.11
Feed efficiency (%)	101.0^{bc}	110.0^{c}	92.0^{ab}	88.0^{ab}	81.0^{a}	101.0^{bc}	0.05
Feed intake (% bw)	1.8^{a}	1.8^{a}	2.32^{b}	2.4^{b}	2.3^{b}	2.0^{a}	0.10
DP utilized kg^{-1} growth (g)	476	395	486	494	528	429	3.89
DE utilized kg^{-1} growth (MJ)	19.1	17.4	20.8	21.6	22.6	18.3	0.41
Apparent Net Protein Utilization (%)	37.2	41.8	32.6	32.3	31.7	38.7	0.45
Apparent Net Energy Utilization (%)	41.7	46.0	33.6	35.1	34.8	39.4	0.74
Condition Factor	1.23^{a}	1.32^{b}	1.31^{b}	1.27^{a}	1.22^{a}	1.24^{a}	0.02
Dress Out (%)	86.1^{a}	87.7^{b}	87.7^{b}	87.6^{b}	87.1^{b}	87.9^{b}	0.25
Hepatosomatic Index (%)	2.1^{c}	1.5^{b}	1.2^{a}	1.1^{a}	1.1^{a}	1.3^{ab}	0.06

* ± standard error of the pooled means Values in each row allocated common superscripts or without superscripts are not significantly different from each other ($P > 0.05$)

Condition factor (CF) of MAL and DEX trout was significantly higher ($P<0.05$) compared to other treatments. Dress Out (%) of all groups displayed similar values, however this parameter of GLU fed trout was significantly higher than other treatments. No significance was evident in Dress out (%) of MAL, DEX, NWS, NCS and PCS groups. Hepatosomatic Index (HSI) decreased significantly ($P<0.05$) with increasing carbohydrate complexity. HSI in DEX, NWS and NCS fish was not different ($P>0.05$) and these three group displayed

minimum HSI. PCS group showed higher HSI compared to DEX, NWS and NCS groups, but no significance ($P>0.05$) was detected. On the other hand, HSI of GLU was also significantly higher than that of MAL fed trout.

The estimation of dietary energy partitioning (Table 6.1.5) demonstrated that the faecal loss of GLU and MAL fed fish was considerably lower than other groups. This high digestibility resulted in higher retained energy in the carcass of trout fed GLU and MAL diets. On the other hand non-faecal energy losses were generally similar apart from MAL treatment which displayed minimum non-faecal energy loss (27.8 % of GE).

Table 6.1.5 Estimation of dietary energy utilization by rainbow trout fed different carbohydrate diets.

Gross Energy (%)	GLU	MAL	DEX	NWS	NCS	PCS
Gross Energy (GE)	100	100	100	100	100	100
Faecal Energy (FE)	5.9	7.4	17.9	16.8	17.0	13.8
Digestible Energy (DE)	94.1	92.6	82.1	83.2	83.0	86.2
Non-faecal Energy (ZE + UE + HiE)	33.9	27.8	34.4	34.2	33.9	30.9
Net Energy (NE)	60.2	64.8	47.7	49.0	49.1	55.2
Maintenance Energy	18.5	18.8	14.1	13.9	14.3	15.9
Retained Energy (RE)	41.7	46.0	33.6	35.1	34.8	39.4

Carcass and muscle proximate compositions of fish are presented in Table 6.1.6 and Table 6.1.7, respectively. It appears that carcass and muscle protein percentage of GLU was lower compared to other groups. However, there is no significant difference in carcass or muscle components as is seen in Table 6.1.8.

Table 6.1.6 Proximate composition of the pooled carcasses of rainbow trout presented as a percentage of the whole fish.

	Initial	GLU	MAL	DEX	NWS	NCS	PCS	± SEM*
Moisture	71.8	70.1	69.9	71.1	70.4	69.1	70.3	0.57
Protein	14.8	15.2	16.1	15.8	16.0	16.2	16.0	0.23
Lipid	10.4	11.8	11.7	10.4	11.2	12.0	11.5	0.51
Ash	2.3	2.4	2.2	2.3	2.2	2.4	2.2	0.08

*± standard error of the pooled means (n=10). Values in each row are not significantly different from each other (P > 0.05) (see Table 6.1.8)

Table 6.1.7 Proximate composition of pooled muscle of rainbow trout presented as a percentage of the muscle.

	Initial	GLU	MAL	DEX	NWS	NCS	PCS	± SEM*
Moisture	77.6	73.3	71.2	71.6	71.3	72.8	73.4	0.46
Protein	16.5	17.2	18.6	18.9	19.0	18.9	18.9	0.27
Lipid	3.0	8.0	9.1	8.2	8.6	7.1	6.5	0.42
Ash	2.2	2.0	1.9	1.9	2.0	2.0	1.97	0.04

*± standard error of the pooled means (n=10). Values in each row are not significantly different from each other (P > 0.05) (see Table 6.1.8).

Table 6.1.8 Allometric analysis of carcass and muscle components of rainbow trout.

	Log (body protein)= a + b* Log (wt) R^2 = 0.99		Log (body lipid)= a + b* Log (wt) R^2= 0.92		Log (body ash)= a + b* Log (wt) R^2= 0.94		Log (muscle pro.)= a +b* Log (wt) R^2= 0.95		Log (muscle lipid)= a + b* Log (wt) R^2= 0.82		Log (muscle ash)= a + b* Log (wt) R^2= 0.93	
	a	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**	**a**	**b**
GLU	-0.85	1.02	-1.12	1.08	-1.45	0.91	-0.86	1.05	-1.43	1.18	-1.52	0.90
MAL	-0.85	1.02	-1.12	1.08	-1.45	0.91	-0.82	1.05	-1.39	1.18	-1.52	0.90
DEX	-0.85	1.02	-1.12	1.08	-1.45	0.91	-0.83	1.05	-1.45	1.18	-1.52	0.90
NWS	-0.85	1.02	-1.12	1.08	-1.45	0.91	-0.80	1.05	-1.42	1.18	-1.52	0.90
NCS	-0.85	1.02	-1.12	1.08	-1.45	0.91	-0.83	1.05	-1.49	1.18	-1.52	0.90
PCS	-0.85	1.02	-1.12	1.08	-1.45	0.91	-0.82	1.05	-1.54	1.18	-1.52	0.90
	NS f= 2.22	NS f= 0.82	NS f= 2.2	NS f= 1.43	NS f= 1.58	NS f= 0.7	S f= 3.32	NS f= 0.64	S f= 5.84	NS f= 1.43	NS f= 1.38	NS f= 2.02

(s; significant, ns; nonsignificant)

6.1.4 DISCUSSION

This feeding trial demonstrated that 30 % inclusion of highly digestible carbohydrates (glucose, maltose and pregelatinized corn starch) influenced the relative feed intake in the long term for growing rainbow trout. Fish fed the GLU and MAL diets decreased their feed intake after two weeks into the study, however they recovered their feed intake after four to eight weeks. PCS trout reduced their relative feed intake after the eighth week, whilst voluntary feed intake of DEX (dextrin), NWS (native wheat starch) and NCS (native corn starch) was similar (2.3, 2.4 and 2.3 % bw^{-1} respectively). Therefore, superior growth response was observed in DEX, NWS and PCS trout compared to GLU, MAL and NCS fish. Similarly, the specific growth rates (SGR) of DEX, NWS and PCS (2.0, 1.9 and 1.8 day^{-1} respectively) were higher than those of GLU, MAL and NCS (1.6, 1.7 and 1.7 day^{-1} respectively). On the contrary, feed efficiency of MAL, GLU and PCS (110.0, 101.0 and 101.0 % respectively) was higher than that of DEX, NWS and NCS treatments (92.0, 88.0 and 81.0 % respectively). This better feed efficiency could be attributed to the higher apparent carbohydrate digestibility values of GLU, MAL and PCS diets, respectively for the experimental fish.

The dress out (%) of the fish were similar and in support of carcass and muscle composition data. However, Dress out (%) of the GLU group was significantly higher probably because the hepatosomatic index (HSI) (%) of GLU fed fish was significantly higher (2.1 %) compared to the other treatments.

The apparent lipid and protein digestibility was not affected by incorporation of different sources of dietary carbohydrate as previously shown in trout (Takeuchi *et al.*, 1990; Bergot, 1993).

Carbohydrate digestibility was observed to be higher in GLU, MAL and PCS (98.1, 93.6 and 82.8 % respectively) than DEX, NWS and NCS (73.4, 77.0 and 76.2 % respectively). Glucose (GLU) digestibility determined in this study was in agreement with that reported earlier by Singh & Nose (1967) and Hilton *et al.* (1987).

Digestibility coefficients of dextrin (DEX) was also in accordance with the results of Singh & Nose (1967) who determined 77.2 % and 74.8 % digestibility in the rainbow trout diets containing 20 and 30 % dextrin respectively. The relatively low digestibility of dextrin and starch by rainbow trout might be due to the absorption of amylase by starch and the inhibition of hydrolysis of the starch (Spannhof & Plantikow, 1983). Significantly higher voluntary feed intake of DEX, NWS and NCS treatments ($P<0.05$) apparently could be explained by acceleration of the chyme transport through the intestine in order to obtain more digestible energy, thus reducing the magnitude for hydrolysis and digestion (Bergot & Breque, 1983). High digestibility of glucose (98.1 %) and maltose (93.6 %) suggests that mono and di-saccharides are absorbed quickly, whilst the digestibility of polysaccharides showed the inferior ability of rainbow trout to convert polysaccharides to di-saccharides. Starch digestibility of PCS fed fish (82.8 %) was similar to that (79.7 %) determination by Kim & Kaushik (1992) for rainbow trout.

The digestible energy (DE) utilised per kg weight gain of trout varied between 17.4 MJ (MAL) and 22.6 MJ (NCS). Apart from MAL treatment, these values are higher than those of Kim & Kaushik (1992) who reported values between 17.4 and 17.6 MJ kg^{-1} This could be because of the different feeding regimes applied in these studies. Fish were fed three times to satiation daily in this study compared to twice satiation in the study of those latter workers. It has been

suggested that reducing the level of feeding increased the digestibility of protein and carbohydrate (Bergot & Breque, 1983; Pfeffer *et al.*, 1991). Similarly, digestible protein (DP) utilised per kg weight gain of trout was calculated from 395 g for MAL to 528 g for NCS treatments. This may also suggest that fish offered low energy diets might eat for maximum stomach capacity to obtain enough nutrient and energy which may consequently reduce digestion efficiency.

Although DP/DE ratios in this study varied between 20 to 22 g DP/ MJ DE as Cho (1992) recommended, growth performance and nutrient utilisation were significantly affected by carbohydrate complexity. The overall pattern of feed intake indicated that despite a possible compensatory short-term modulation of appetite (i.e: a depression in feed intake for mono and di- saccharides), in the long term, highly digestible carbohydrates are able to influence voluntary feed intake.

The hepatosomatic index (%) of GLU and MAL was significantly different than other treatments probably because of hepatic glycogen deposition (Phillips *et al.*, 1948, Lee & Putnam, 1973; Refstie & Austreng, 1981; Hilton & Atkinson, 1982; Kim & Kaushik, 1992). Therefore, the affect of liver glycogen on feed intake could be more important than stomach capacity in the long term. It has been reported that 250 g kg^{-1} extruded starch in the rainbow trout diet caused intracellular damage due to excess deposition of glycogen (Baeverfjord, 1992; Hemre *et al.*, 1996). Anderson *et al.* (1984) suggested that glucose might inhibit the amino acid transport at specific absorption sites on the gut membrane and consequently impair growth performance and protein retention since glucose and di-saccharides are rapidly assimilated across the gut and polysaccharides must be hydrolysed by enzymes before assimilation. Kim & Kaushik (1992)

also observed a maximum specific growth rate (SGR) (2.06 day^{-1}) when they included 38 % carbohydrate in the rainbow trout diet at 18 °C.

The present investigation clearly demonstrated that 30 % inclusion of dextrin, native wheat starch or pregelatinized corn starch did not retard the growth in the rainbow trout as suggested by Bergot (1979) and Kaushik & Oliva-Teles (1985). However, Bergot (1979) claimed that the inclusion level of digestible carbohydrate including D-glucose can be raised up to 30 % without any adverse effect on growth and health conditions, but growth performance of GLU and MAL fed fish was not as good as DEX, NWS and PCS fish. This investigator also indicated that 15 and 30 % glucose diets increased fat deposition in the viscera of trout. This is probably because the natural diet of rainbow trout ingests little carbohydrate in nature (Steffens, 1989) and these fish would not be expected to have developed mechanisms to metabolise high level of digestible carbohydrates efficiently (Cowey *et al.*, 1977a; Cowey & Walton, 1989).

Proximate composition of carcass (Table 6.1.6) and muscle (Table 6.1.7) did not differ significantly amongst treatments. Allometric analysis (Multiple Regression) of carcass and muscle protein, lipid and ash also indicated that dietary carbohydrate complexity does not influence the body component composition when including 30% in the diet for rainbow trout (Table 6.1.8).

In contrast to Austreng *et al.* (1977), Austreng & Reftsie (1979), Hilton & Atkinson (1982), Beamish *et al.* (1986), Kim & Kaushik (1992) and Mazur *et al.* (1992) who declared an increase in carcass protein and decrease in lipid concentration in the fish fed

high carbohydrate diets, carcass and muscle proximate composition of all treatments in this trial exhibited no significance following allometric analysis as outlined by Shearer (1994).

Dietary interactions unarguably influence growth performance and feed utilization, but body protein and ash content can be observed to be controlled endogenously when the weight of fish and actual protein amount in the body are taken into consideration. The utilization of processed carbohydrates such wheat starch (Henrichfreise & Pfeffer, 1992) as dietary fillers or energy components of practical diets for salmonids have obvious physiological and biochemical implications with respect to appetite and feed utilization in these species. It is evident that more defined experiments are required in order to evaluate maximum digestion and absorption characteristics for carbohydrate enhanced feeds for trout. These dietary energy sources are relatively less expensive and could provide a useful dietary substitute for lipid in feed formulations.

From the results of this study, it was observed that appetite of trout was suppressed when offered carbohydrates in the form of simple sugars. However, it is not certain whether such appetite suppression was due to the elevated plasma glucose or concentration of other nutrients. Furthermore it was detected that DEX and NWS fish performed a similar feed intake and growth performance. However, it is not known if they ate for stomach fullness in order to obtain energy and nutrients available for growth. As a completion of the present experiment, further physiological research was necessary. Therefore the next investigation was conducted in order to evaluate the gastric evacuation and return of appetite rates with postprandial plasma nutrients in rainbow trout fed the same dietary formulations.

EXPERIMENT 7

6.2. EFFECTS OF CARBOHYDRATE COMPLEXITY ON GASTRIC EVACUATION, RETURN APPETITE AND PLASMA NUTRIENT CONCENTRATION IN RAINBOW TROUT, Oncorhynchus mykiss.

6.2.1 INTRODUCTION

Considering the importance of dietary macro nutrient interactions in the regulation of feed intake, the inclusion level and complexity of dietary carbohydrates might play a regulatory role from dietary energy and metabolic effect standpoints. A few studies (Cowey *et al.*, 1977a, 1977b; Hilton & Atkinson, 1982; Bergot & Breque, 1983; Walton, 1986) related to carbohydrate nutrition have indicated a physiological effect of carbohydrates on voluntary feed intake or appetite.

Carbohydrate complexity influences their degree of absorption and assimilation with respect to the nutrition of carnivorous fish species. It was also reported (Hemre *et al.*, 1995, 1996; Arnesen & Krogdahl, 1996) that fish such as trout and salmon can absorb glucose and have the capacity to digest polysaccharides such as starches but have limited abilities to metabolise glucose effectively as an energy source (Wilson, 1994).

However, a significant proportion of diet formulation for farmed fish contains carbohydrate in the form of native or cooked in different cereal and pulse based ingredients (Lovell, 1989; NRC, 1993). In fact, the degree of gelatinization resulting from a variety of processing methods such as extrusion and expansion may greatly influence the physico-chemical

characteristics of these components (Pieper & Pfeffer, 1980; Takeuchi *et al.*, 1990; Pfeffer, 1995). These carbohydrates may exist in a variety of forms extending from simple hexose type monomer sugars to intermediate dextrin like polysaccharides to even more complex starches in raw ingredients.

In this connection, the influence of the level of extruded wheat meal (approximately 15, 30 and 45 % of the diet) on gastric evacuation, appetite revival and blood nutrients in rainbow trout were assessed in Chapter 5.2. A consistent relationship was observed between appetite return and stomach emptying. Although there was a transient hyperglycemia in fish fed the high level carbohydrate diet, this was not observed to influence voluntary feed intake. Likewise plasma protein and triglyceride concentrations also did not display a major role in appetite regulation in this investigation.

Effects of carbohydrate complexity on feed intake, nutrient utilization and proximate body composition were demonstrated in the first part of this Chapter (Experiment, 6). It appeared that the feed intake of rainbow trout offered simple sugars such as D-glucose and maltose was suppressed, whilst complex carbohydrates were found not to influence feed intake of trout in the long term. Therefore, it is imperative to examine how such differences in carbohydrate structure can influence the feed intake, circulating nutrients, gastric evacuation rates and consequent return of appetite in trout. Also, the role of available digestible energy (DE) from different carbohydrate sources remains to be evaluated under practical conditions (Smith, L., 1989).

During feed intake (appetite revival) measurements conducted in Experiment 5 (Chapter 5.2), only one size of X-ray dense marker was employed. However, the first

consecutive meal eaten by fish may not be exactly quantified since it does not contain any marker. In order to measure first meal intake as well as second meal intake, two different sizes of X-ray dense marker were used in the present study. Therefore, the same experimental diets used in Chapter 6.1 were manufactured with different sizes of ballotini and fed to rainbow trout in order to assess the effect of carbohydrate complexity on gastric evacuation, return of appetite and postprandial plasma nutrients under laboratory conditions.

6.2.2 MATERIALS AND METHODS

6.2.2.1 Experimental Fish and Holding Facilities

Experimental fish and conditions were as outlined in Chapter 2.1.2, 4.2.2.1 and 5.2.2.1.

6.2.2.2 Test Diets

Formulation and chemical composition of experimental diets are presented in Table 6.2.1 and 6.2.2. 3.8 % small size ballotini (0.65-0.90 mm) in weight was added in the first batch of test diets (Table 6.2.1) whilst 9.3 % large size ballotini (1.16-1.40 mm) in weight was incorporated into the second batches of test diets (Table 6.2.2). The numbers of marker "ballotini" in known weights of diet were determined by X-radiography to ensure even distribution. The relationship between the weight of feed (FW) and the number of bead (N) was linear as outlined in Table 6.2.3.

Table 6.2.1 Dietary formulation and chemical composition of experimental diets with small ballotini.

Ingredients	**GLU**	**MAL**	**DEX**	**NWS**	**NCS**	**PCS**
LT Fish Meal[1]	47.6	47.6	47.6	47.6	47.6	47.6
Blood Meal[1]	2.9	2.9	2.9	2.9	2.9	2.9
Poultry Meat Meal[1]	4.9	4.9	4.9	4.9	4.9	4.9
D-Glucose[1]	29.2	-	-	-	-	-
Maltose[1]	-	29.2	-	-	-	-
Dextrin[1]	-	-	29.2	-	-	-
Native Wheat Starch[1]	-	-	-	29.2	-	-
Native Corn Starch[1]	-	-	-	-	29.2	-
Pregelatinized Corn Starch[1]	-	-	-	-	-	29.2
Fish Oil[1]	6.8	6.8	6.8	6.8	6.8	6.8
Vitamin/Mineral Premix[1]	2.4	2.4	2.4	2.4	2.4	2.4
Glass beads (ballotini)[2]	3.8	3.8	3.8	3.8	3.8	3.8
Binder[1] (CMC)	2.4	2.4	2.4	2.4	2.4	2.4
Nutrient Analysis						
Protein (% DM)	42.0	41.9	42.2	41.6	41.3	41.6
Lipid (% DM)	11.0	11.3	11.1	11.4	11.3	11.1
Ash (% DM)	9.3	9.6	9.0	9.1	9.2	8.9
Carbohydrate (% DM)	28.8	28.1	29.7	30.2	30.2	29.9
Digestible Protein (DP) (%)	40.3	38.9	39.9	39.3	39.4	37.0
Digestible Energy (DE) (MJ kg^{-1})	19.8	18.5	17.8	17.8	17.8	18.1
DP/DE Ratio (g DP/ MJ kg^{-1} DE)	20.4	21.0	22.4	22.1	22.1	20.4

1. Same ingredient as given Table 6.1.1.
2. Size: 0.65-0.90 mm (Jensons Ltd. UK)

Table 6.2.2 Dietary formulation and chemical composition of experimental diets with large ballotini.

Ingredients	GLU	MAL	DEX	NWS	NCS	PCS
LT Fish Meal[1]	45.6	45.6	45.6	45.6	45.6	45.6
Blood Meal[1]	2.8	2.8	2.8	2.8	2.8	2.8
Poultry Meat Meal[1]	4.7	4.7	4.7	4.7	4.7	4.7
D-Glucose[1]	27.9	-	-	-	-	-
Maltose[1]	-	27.9	-	-	-	-
Dextrin[1]	-	-	27.9	-	-	-
Native Wheat Starch[1]	-	-	-	27.9	-	-
Native Corn Starch[1]	-	-	-	-	27.9	-
Pregelatinized Corn Starch[1]	-	-	-	-	-	27.9
Fish Oil[1]	6.5	6.5	6.5	6.5	6.5	6.5
Vitamin/Mineral Premix[1]	2.3	2.3	2.3	2.3	2.3	2.3
Glass beads (ballotini)[2]	9.3	9.3	9.3	9.3	9.3	9.3
Binder[1] (CMC)	0.9	0.9	0.9	0.9	0.9	0.9
Nutrient Analysis						
Protein (% DM)	40.2	40.1	40.4	39.8	39.5	39.8
Lipid (% DM)	10.5	10.8	10.6	10.9	10.8	10.6
Ash (% DM)	8.9	9.2	8.6	8.7	8.8	8.6
Carbohydrate (% DM)	27.5	26.9	28.5	28.9	28.9	28.6
Digestible Protein (DP) (%)	38.6	37.2	38.2	37.6	37.7	35.4
Digestible Energy (DE) ($MJ\ kg^{-1}$)	19.0	17.7	17.0	17.0	17.0	17.3
DP/DE Ratio (g DP/ $MJ\ kg^{-1}$ DE)	20.3	21.0	22.5	22.1	22.2	20.5

1. Same ingredients as given Table 6.1.1.
2. Size: 1.16 1.40 mm (Jensons Ltd UK)

Table 6.2.3 Relationship between the weight of feed and the number of beads in diets.

Linear Regression[1]	Diet 1			Diet 2		
	a	b	r^2	a	b	r^2
GLU	-	0.0243	0.96	-	0.0396	0.96
MAL	0.29	0.0396	0.96	0.08	0.0372	0.90
DEX	-	0.0221	0.95	-	0.0420	0.98
NWS	0.07	0.0220	0.93	0.32	0.0329	0.92
NCS	0.06	0.0213	0.94	0.04	0.0416	0.90
PCS	0.07	0.0239	0.90	-	0.0486	0.91

[1] Coefficient derived from the fitted model y= (a+b*x), where 'y' is the amount of diet and 'x' is the number of ballotini (n=20)

6.2.2.3 Return of Appetite Determinations

Methods used for X-Radiography and fish sampling for the determination of gastric emptying rate and plasma nutrients level were as outlined in Chapter 2.8 and 2.10, respectively. In this experiment, a protocol (Table 6.2.4) was designed to allow each diet to be assayed at set time intervals (t = 0, 4, 8, 12, 24, 30 and 36 h) so that no fish was X-rayed more than once in a 144 h period.

Following a –72- hour starvation period, fish were fed diets with small ballotini (Table 6.2.1) until all fish reached apparent satiation. This was determined by monitoring the bottom of the tanks where a small amount of uneaten feed remains. After removing uneaten feed, fish were starved until the second meal was applied. The second meal with large ballotini was offered to each respective group according to the protocol (Table 6.2.4) until all fish reached satiation. The level of re-alimentation at the specified

time interval was equal to the extent of appetite return. Subsequently, 10 fish were anaesthetised (Benzocaine, Sigma Chemical Co. Ltd., Poole, UK; 1 g dissolved in 100 ml of ethanol, this added to fresh water at a concentration of 5 ml l^{-1}), weighed and X-rayed using a portable Phillips Practix X-ray unit with light beam diaphragm attachment. Then X-rayed fish were transferred to their original tank after which a second 10 fish were removed from this tank. These second 10 fish were also anaesthetised, weighed, X-rayed and returned to the tank where the first X-rayed fish were recovered. This same procedure was repeated for all groups.

Table 6.2.4 X-Radiography protocol for return of appetite determinations.

Day	GLU	MAL	DEX	NWS	NCS	PCS
1	T= 4h	T= 8h	T= 12h			
2				T= 24h	T= 30h	T= 36h
7	T= 8h	T= 12h	T=4h			
9				T= 30h	T= 36h	T= 24
13	T= 12h	T= 4h	T= 8h			
16				T= 36h	T= 24h	T= 30h
20	T= 24h	T= 30h	T= 36h			
22				T= 4h	T= 8h	T= 12h
27	T= 30h	T= 36h	T= 24h			
28				T= 8h	T= 12h	T= 4h
34	T= 36h	T= 24h	T= 30h			
35				T= 12h	T= 4h	T= 8h

The recovered group of fish were then maintained on the same diet for 2 days and deprived of food for three days before beginning further appetite revival measurements. During the X-radiographic studies, no fish vomited or died. The X-radiographic pictures of rainbow trout were viewed on a light table (PLH Scientific Ltd, UK) and glass beads were counted. Weight of feed consumed by each fish was calculated according to the calibration formula and expressed in weight specific terms (g kg^{-1} body weight). Return of appetite of fish for each set time interval was expressed as a percentage of the mean feed intake of fish at time = 0. The X-radiography technique employed was as described in Chapter 2.8.

6.2.2.4 Gastric Evacuation Study and Fish Sampling

After completing return of appetite measurements, the fish used for the return of appetite experiment and reserved for gastric evacuation study were pooled. 60 fish were placed in each of the six tanks and conditioned to the respective diets prior to sampling for one week.

Fish were starved for 72 h to ensure that the last meal had been completely evacuated. Then each group of fish was fed with diets without ballotini (Table 6.1.1) until all fish reached apparent satiation. Fish from each of the three treatments were removed at selected time intervals: time= 0 (as soon as feeding is completed), 6h, 12h, 18h, 24h, 30h and 36h. On each occasion, eight fish were sacrificed following prolonged immersion in ethyl p-amino benzoate (Benzocaine), weighed and measured individually. 2.0 ml blood was withdrawn from the caudal vein of each trout. Then, each fish was weighed and paper plugs were placed in the buccal cavity of the trout to prevent regurgitation of digesta. Sampled fish were then placed in a freezer (-20 ^{o} C) for a

period of up to 12 hours so as to solidify stomach contents and facilitate removal without loss. Sampled blood was centrifuged (6500rpm) for 5 minutes to obtain clear plasma. The supernatant of each sample of blood was pipetted into a clean, labelled tube and kept frozen at -70 ° C until plasma was analysed and frozen (-80 ° C) for further analysis. Finally, stomach contents were removed into separate aluminium dishes and liver weight and gut weight of sampled fish were determined. Stomach contents were accurately weighed and dried at 105 °C until a constant dry weight was obtained. All stomach contents were expressed as a percentage of the initial dry weight of the feed. With respect to blood samples, plasma glucose, protein and triglyceride reagents were supplied from Sigma Diagnostics (Sigma Chemical Co. Ltd., Poole, Dorset, UK) and spectrophotometric assays performed according to the protocol outlined in Chapter 2.6.

6.2.2.5 Statistical Analysis

Statistical analysis and modelling of return of appetite and gastric evacuation data in the present study was as explained in Chapter 2.11 and used in Chapter 4.2 and Chapter 5.2, respectively.

6.2.3 RESULTS

6.2.3.1 Gastric Evacuation and Return of Appetite Rates

Following a sequence of comparative analysis for the gastric evacuation data, linear and square root models gave a better fit for the data set under the examination. These two models were fitted to each data set and slopes of equations were compared by multiple regression analysis. In order to choose the best fit, minimum residual mean sum of squares (RMS), intercepts nearest to 100 and consequently highest r^2 were taken into account. Consequently, the linear fit for the evacuation of the GLU, MAL and PCS diets, and the square root model for the gastric evacuation of DEX, NWS and NCS diets were applied. The comparisons of slopes for linear and square root models are presented Table 6.2.5 and Table 6.2.6, respectively. According to linear or square root fit for evacuation of all six diets, no significant difference ($P>0.05$) was evident (Table 6.2.7).

Moreover, the comparison of slopes of GLU, MAL and PCS treatments in a linear form (Table 6.2.5) did not indicate any considerable difference ($P>0.05$). In a similar manner, the comparison of slopes of DEX, NWS and PCS groups in a square root model (Table 6.2.6) also displayed no significant difference ($P>0.05$). First order and sigmoid equations were employed for return of appetite modelling (Table 6.2.8). Although both models fitted well, first order equations resulted in a marginally better fit due to the lower residuals mean sum of squares.

Table 6.2.5 Statistical summary of comparison of the fitted gastric evacuation slopes in linear form for GLU, MAL and PCS treatments.

		Regression[1]			Multiple Regression Analysis[2]		
Linear	**RMS[3]**	**S_0**	**k**	**r^2**	**F**	**d.f.**	**P**
GLU	46.18	102.2	2.61	0.92	0.16	3:108	>0.05
MAL	49.31	102.0	2.55	0.92			
GLU	46.18	102.2	2.61	0.92	1.0	3:108	>0.05
PCS	72.48	101.0	2.77	0.90			
MAL	49.31	102.0	2.55	0.92	1.82	3:108	>0.05
PCS	72.48	101.0	2.77	0.90			

[1] Coefficients derived from the fitted linear model.

[2] Significant differences (P<0.05) in shape of slopes determined by multiple regression analysis

[3] Residual Mean sum of Squares

Table 6.2.6 Statistical summary of comparison of the fitted gastric evacuation slopes in square root form for DEX, NWS and NCS treatments.

		Regression[1]			Multiple Regression Analysis[2]		
Square Root	**RMS[3]**	**S_0**	**k**	**r^2**	**f**	**d.f.**	**P**
DEX	68.06	9.96	0.19	0.89	0.67	3:108	>0.05
NWS	84.33	10.28	0.196	0.87			
DEX					0.003	3:108	>0.05
NCS	36.83	10.14	0.19	0.94			
NWS					0.68	3:108	>0.05
NCS							

[1] Coefficients derived from the fitted square root model.

[2] Significant differences (P<0.05) in shape of slopes determined by multiple regression analysis

[3] Residual Mean sum of Squares

Table 6.2.7. Statistical summary of comparison of the fitted gastric evacuation slopes in linear or square root form for all treatments.

		Multiple Regression Analysis[1]			
	RMS[2]	r^2	F	d.f.	P
Linear	112	0.90	0.63	11:324	>0.05
Square Root	0.724	0.90	1.33	11:324	>0.05

[1]Insignificant relationship (P>0.05) in shape of slopes determined by multiple regression analysis, [2] Residual Mean sum of Squares

Gastric evacuation and return of appetite models for GLU, MAL, DEX, NWS, NCS and PCS treatments are presented in Figures 6.2.1, 6.2.2, 6.2.3, 6.2.4, 6.2.5 and 6.2.6, respectively. Primarily, a significant evacuation (P<0.05) was observed every 6 hours until the sampling time of 30h in MAL, DEX, NWS, NCS and PCS groups and no difference (P>0.05) was detected in evacuation rate between time 30h and time 36h. However, trout fed GLU diet (Figure 6.2.1) displayed an initial delay until time 6h, then a significant linear emptying pattern was observed. The evacuation time of 95 % of the digesta from the cardiac stomach was calculated as 37.2 hours for GLU, 38.1 hours for MAL and 36.7 hours for PCS fed trout (Table 6.2.9). 95 % clearance time of the DEX, NWS and NCS diets was 40.7, 41.1 and 41.6 hours, respectively.

Six first order equations described the appetite revival data of experimental groups were presented with gastric evacuation data in the same figures. There was always a significant feed intake (P<0.05) at time 4h in all groups of trout. And also feed intake of all groups at time 30h and 36h was not significantly different (P>0.05). However, appetite return patterns of groups displayed some variances. For instance, GLU, NCS

and PCS fish did not increase their feed intake significantly between time 8h and 12h. DEX and NWS fish responded to the feed in a similar manner that the feed intake between 4 and 8 hours was not considerably different (P>0.05).

The time required for 95 % of appetite return was predicted as 33.9, 26.7 and 24.5 hours for GLU, MAL and PCS treatments, respectively (Table 6.2.10) according to the fitted first order equations. The time needed for 95 % appetite revival was 28.4, 35.7 and 26.4 for DEX, NWS and NCS groups, respectively (Table 6.2.10).

Table 6.2.8 Fitted equations for the return of appetite.

Diets	Model[1]	a	b	k	r^2	RSM[2]
GLU	*First Order*	193.0	-	-0.02	0.80	279
	Sigmoid	0.009	0.074	-0.11	0.80	284
MAL	*First Order*	134.4	-	-0.046	0.83	394
	Sigmoid	0.0095	0.084	-0.196	0.80	400
DEX	*First Order*	106.3	-	-0.079	0.89	158
	Sigmoid	0.01	0.047	-0.164	0.87	185
NWS	*First Order*	101.6	-	-0.06	0.80	266
	Sigmoid	0.011	0.062	-0.17	0.78	284
NCS	*First Order*	126.2	-	-0.053	0.86	245
	Sigmoid	0.0093	0.055	-0.154	0.85	269
PCS	*First Order*	122.5	-	-0.061	0.86	269
	Sigmoid	0.009	0.043	-0.137	0.84	312

[1] Coefficients derived from the fitted first order and sigmoid relationships.

[2] Residual Mean sum of Squares

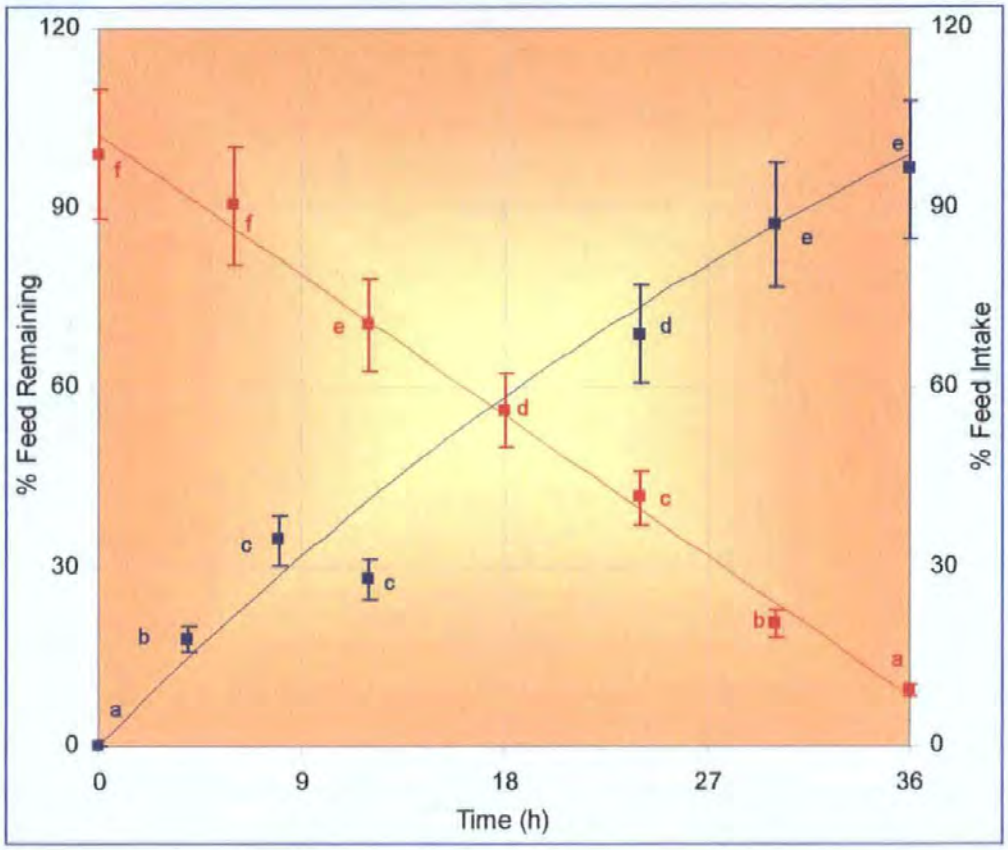


Figure 6.2.1 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed GLU diet.

Stomach evacuation rate was described by a linear model; $\mathbf{S_t = 102.2 - 2.61*t}$, $\mathbf{R^2 = 0.92}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$\mathbf{FI = 192.97* (1-e^{-0.02* t})}$, $\mathbf{R^2 = 0.80}$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

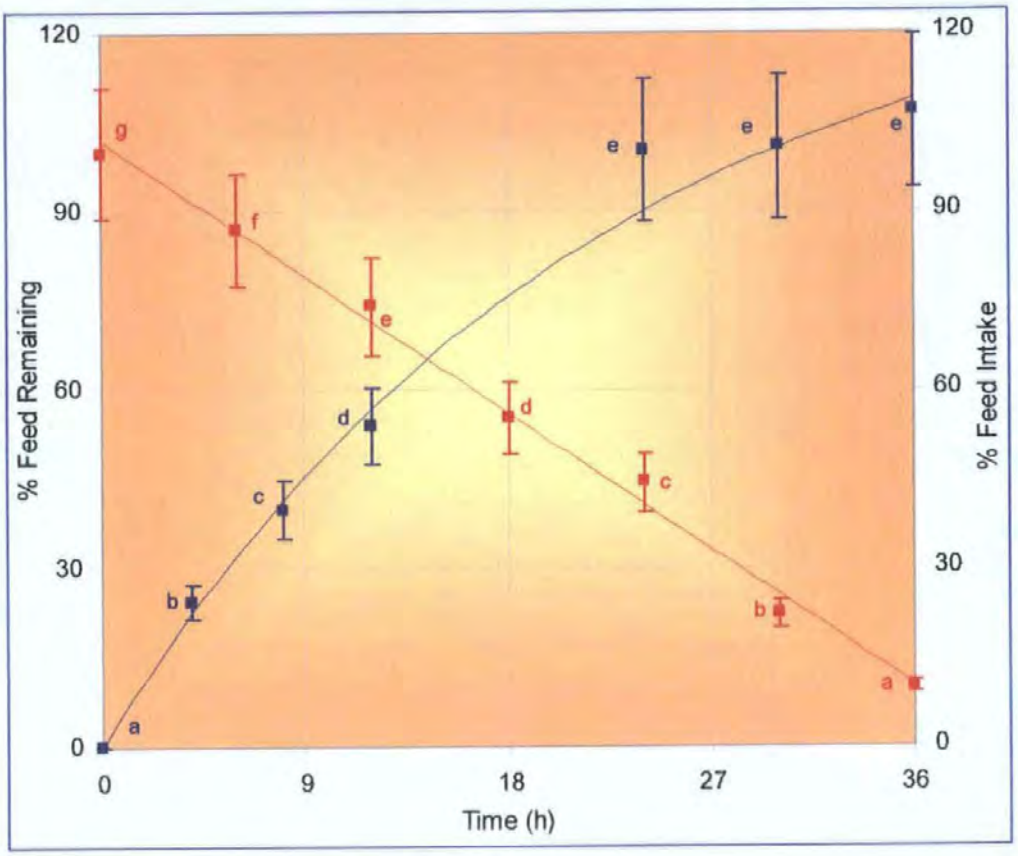


Figure 6.2.2 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed MAL diet.

Stomach evacuation rate was described by a linear model;

$S_t = 102.0 - 2.55*t$, $R^2 = 0.92$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$FI = 134.42* (1-e^{-0.046*t})$, $R^2 = 0.83$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

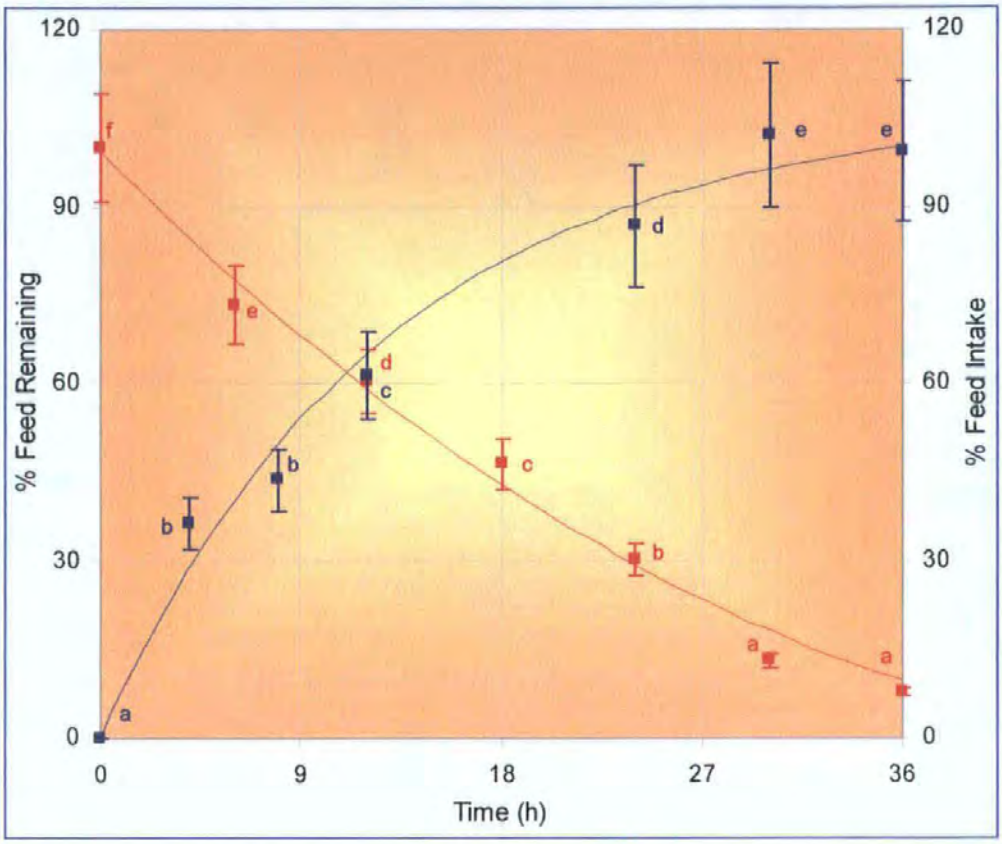


Figure 6.2.3 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed DEX diet.

Stomach evacuation rate was described by a square root model;

$\mathbf{S_t = (9.96-0.19*t)^2}$, $\mathbf{R^2 = 0.89}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$\mathbf{FI = 106.26* (1-e^{-0.079*t})}$, $\mathbf{R^2 = 0.89}$, Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

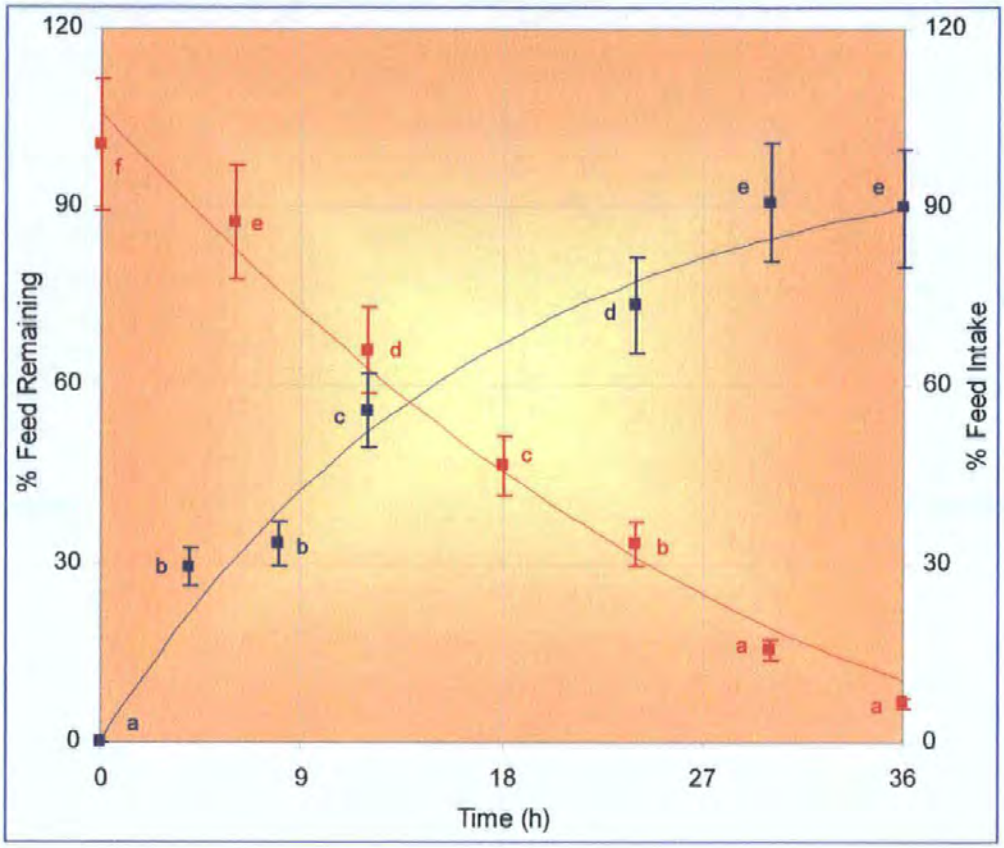


Figure 6.2.4 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed NWS diet.

Stomach evacuation rate was described by a square root model;

$\mathbf{S_t = (10.28-0.196*t)^2}$, $\mathbf{R^2 = 0.87}$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$\mathbf{FI = 101.56* (1-e^{-0.06* t})}$, $\mathbf{R^2 = 0.80}$ Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

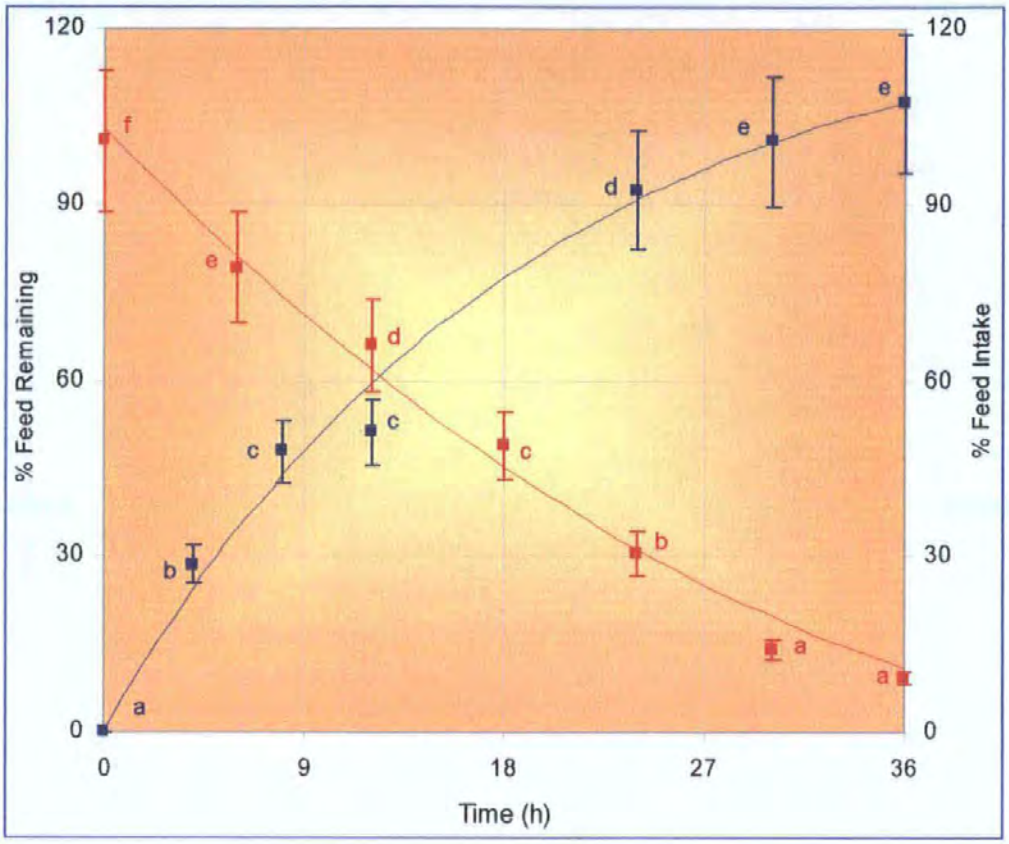


Figure 6.2.5 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed NCS diet.

Stomach evacuation rate was described by a square root model;

$S_t = (10.14-0.19*t)^2$, $R^2 = 0.94$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$FI = 126.24* (1-e^{-0.053* t})$, $R^2 = 0.86$ Where, 'FI' represents percentage feed intake or appetite return at time 't', n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

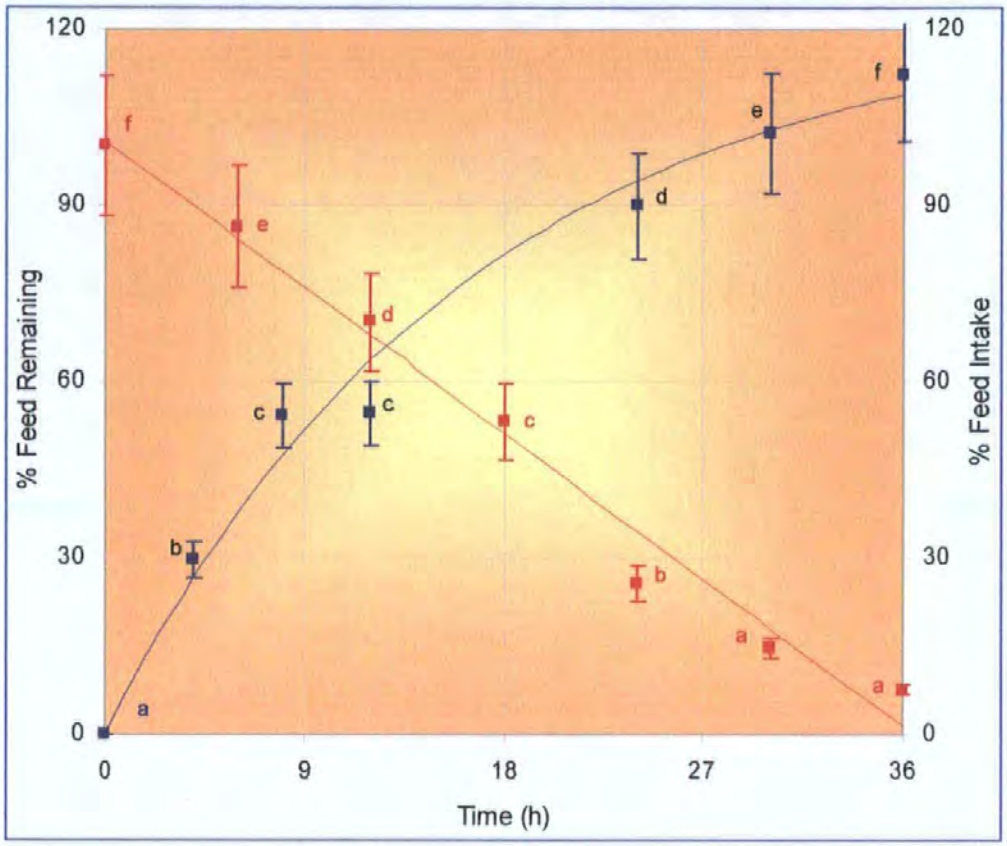


Figure 6.2.6 Percentages of stomach evacuation (■) and return of appetite (■) in trout fed PCS diet.

Stomach evacuation rate was described by a linear model;

$S_t = 102.98 - 2.77*t$, $R^2 = 0.90$, Where, 'S_t' denotes percentage stomach content at time 't', n = 56.

Non-linear regression model for return of appetite (First Order);

$FI = 122.52* (1-e^{-0.061*t})$, $R^2 = 0.86$, Where, 'FI' represents percentage feed intake or appetite return at time t, n = 20.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$). Bars denote ± 5 standard error of the mean.

Table 6.2.9 Predicted gastric evacuation times[1].

Model	Treatments	Calculated times (h) for gastric evacuation (%) 25	50	75	95
Linear	GLU	**10.4**	**20.0**	**29.6**	**37.2**
	MAL	**10.6**	**20.4**	**30.2**	**38.1**
	DEX	**7.1**	**17.0**	**26.8**	**34.7**
	NWS	**9.2**	**18.3**	**27.5**	**34.8**
	NCS	**8.4**	**18.0**	**27.5**	**35.1**
	PCS	**9.4**	**18.4**	**27.5**	**36.7**
Square Root	GLU	**9.4**	**18.5**	**29.6**	**46.1**
	MAL	**9.6**	**19.0**	**31.3**	**47.6**
	DEX	**6.9**	**15.2**	**26.1**	**40.7**
	NWS	**8.3**	**16.4**	**27.0**	**41.1**
	NCS	**7.8**	**16.2**	**27.1**	**41.6**
	PCS	**8.5**	**16.6**	**27.1**	**41.1**

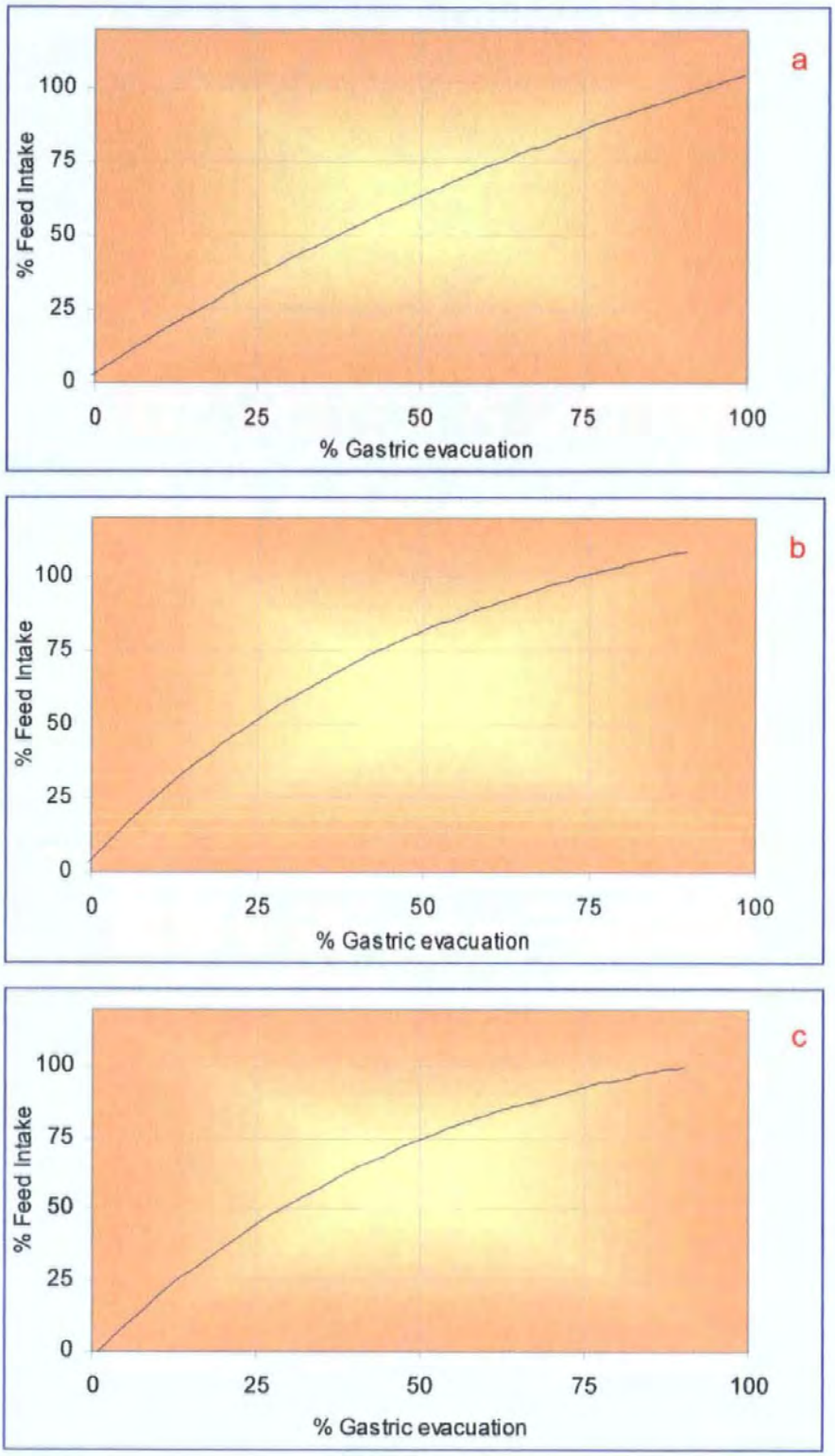
1. Calculations are based on the fitted linear and square root models.

Table 6.2.10 Comparison of predicted return of appetite times[1].

Model	Treatments	Calculated times (h) for appetite revival (%) 25	50	75	95
First Order	GLU	**7.0**	**15.0**	**24.6**	**33.9**
	MAL	**4.5**	**10.1**	**17.8**	**26.7**
	DEX	**3.4**	**8.1**	**15.6**	**28.4**
	NWS	**4.7**	**11.3**	**22.4**	**35.7**
	NCS	**4.2**	**9.5**	**17.0**	**26.4**
	PCS	**3.8**	**8.6**	**15.5**	**24.5**
Sigmoid	GLU	**7.9**	**17.2**	**25.6**	**35.0**
	MAL	**5.2**	**10.6**	**15.8**	**22.5**
	DEX	**2.7**	**9.4**	**16.1**	**27.4**
	NWS	**4.5**	**11.4**	**19.3**	**28.3**
	NCS	**3.8**	**10.6**	**17.0**	**24.7**
	PCS	**2.4**	**10.0**	**16.8**	**24.4**

1. Calculations are based on the fitted first order and sigmoid models given in Table 6.2.8.

Irrespective of the models applied, very strong relation was calculated between appetite revival and gastric evacuation rates in rainbow trout fed GLU, MAL, DEX, NWS, NCS and PCS diets. These relationships are presented in Figure 6.2.7 for GLU (a), MAL (b), DEX (c), NWS (d), NCS (e) and PCS (f) groups, respectively. Also estimated equations are tabulated in Table 6.2.11.



See legend in Figure 6.2.7.

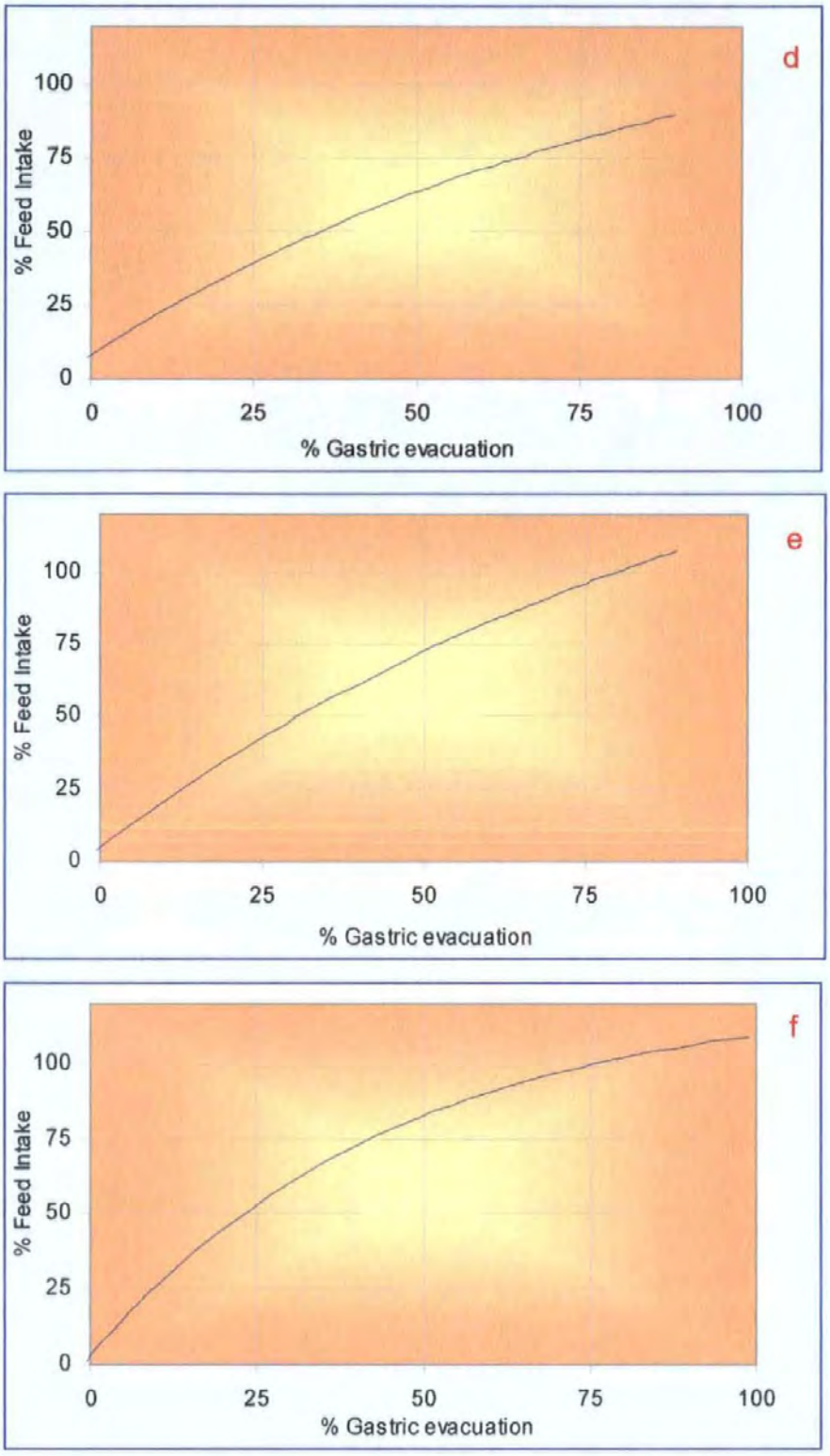


Figure 6.2.7. Relationship between return of appetite (% Feed Intake) and gastric evacuation (%) in rainbow trout fed GLU (a), MAL (b), DEX (c), NWS (d), NCS (e) and PCS (f) diets.

Table 6.2.11 Fitted equations for the relationship between return of appetite and gastric evacuation[1].

Diet	Model[1]	a	b	R^2	RMS
GLU	*First Order*	169.04	-0.01	1.0	1.41
	Linear	9.27	1.02	0.99	10.47
	Square Root	3.47	0.08	0.90	24.89
MAL	*First Order*	127.28	-0.02	1.0	2.80
	Linear	18.38	1.14	0.96	47.87
	Square Root	4.28	0.08	0.83	1.07
DEX	*First Order*	127.46	-0.02	1.0	0.42
	Linear	15.68	1.03	0.95	40.16
	Square Root	4.03	0.08	0.83	0.93
NWS	*First Order*	115.68	-0.016	0.99	10.01
	Linear	14.57	0.9	0.98	14.03
	Square Root	3.81	0.07	0.88	0.64
NCS	*First Order*	161.37	-0.01	1.0	2.64
	Linear	12.34	1.13	0.99	13.34
	Square Root	3.77	0.083	0.89	0.69
PCS	*First Order*	120.28	-0.024	1.0	0.68
	Linear	22.58	1.02	0.93	78.55
	Square Root	4.63	0.07	0.79	1.3

6.2.3.2 Plasma Nutrients

Postprandial plasma nutrients of rainbow trout fed varying source of dietary carbohydrate are presented in Figure 6.2.8, 6.2.9, 6.2.10, 6.2.11, 6.2.12 and 6.2.13, respectively. Circulating protein (mg dl^{-1}) concentration of all treatments generally displayed an increase following feeding, however this was only significantly different ($P<0.05$) from the initial level in fish fed GLU and NCS diets. No significantly difference ($P>0.05$) in the protein concentration of MAL, DEX, NWS and PCS fed trout was evident throughout the sampling.

Postprandial plasma glucose concentration (mmol l^{-1}) of GLU, MAL and PCS treatments increased sharply ($P<0.05$) following feeding and returned to their initial concentrations at time 24, 12 and 24 hours following alimentation, respectively. Glucose level in DEX fish was reduced at first 4 hour then increased significantly ($P<0.05$) at time 8h and returned to initial value at time 12h. This plasma nutrient in NCS fed trout was increased at first 4 hours than reduced significantly ($P<0.05$) until the sampling time of 12h and a significant increase was observed until time 24h. Glucose concentration of NWS treatment did not display any significant difference ($P>0.05$) between any time of sampling.

No significant relationship ($P>0.05$) was detected in triglyceride level (mmol l^{-1}) of DEX, NWS and NCS fed trout, an initial elevation was detected in the triglyceride concentration of these treatments. However, this nutrient in fish fed GLU, MAL and PCS diets increased significantly ($P<0.05$) 12 hours after feeding.

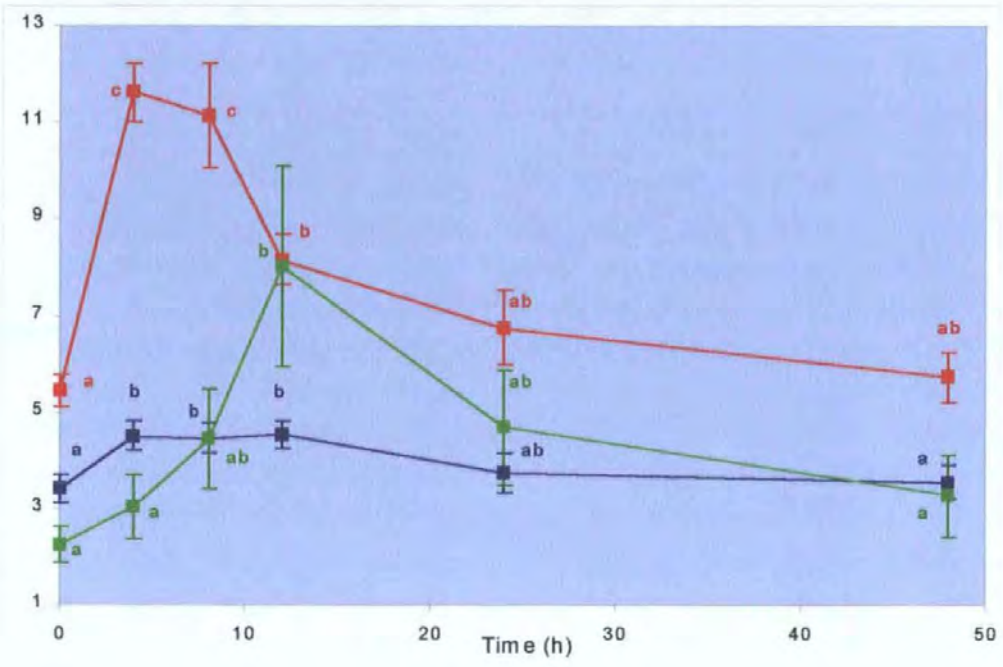


Figure 6.2.8. Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed GLU diet.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

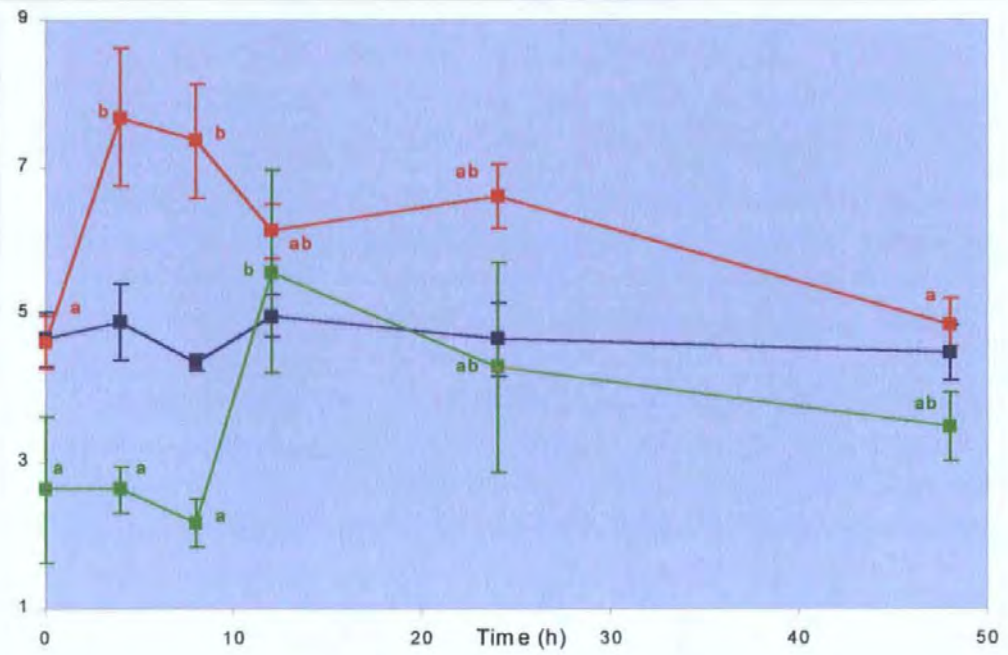


Figure 6.2.9. Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed MAL diet.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

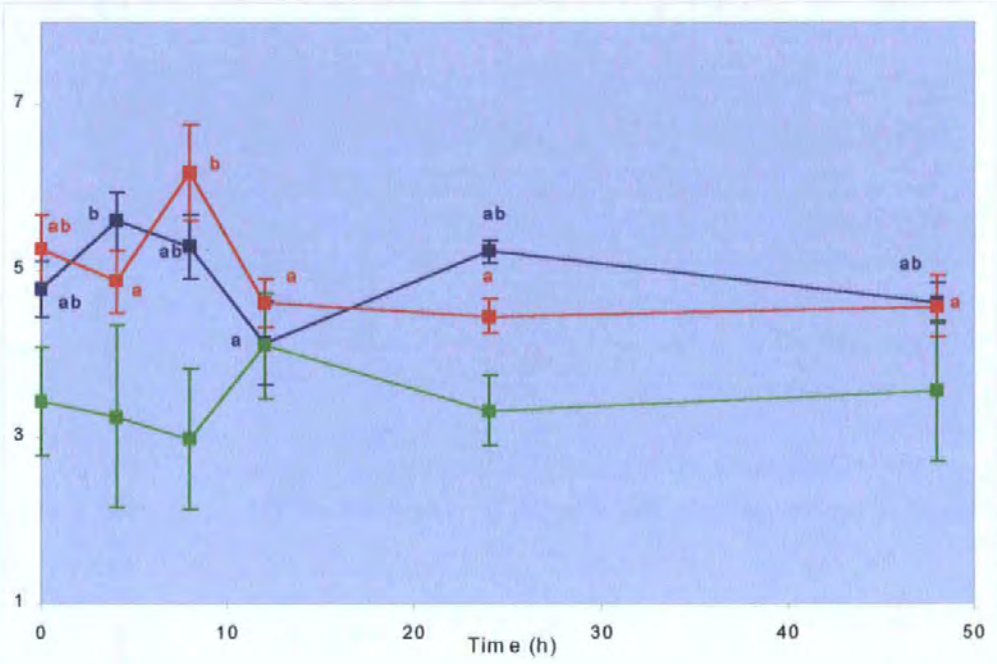


Figure 6.2.10. Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed DEX diet.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

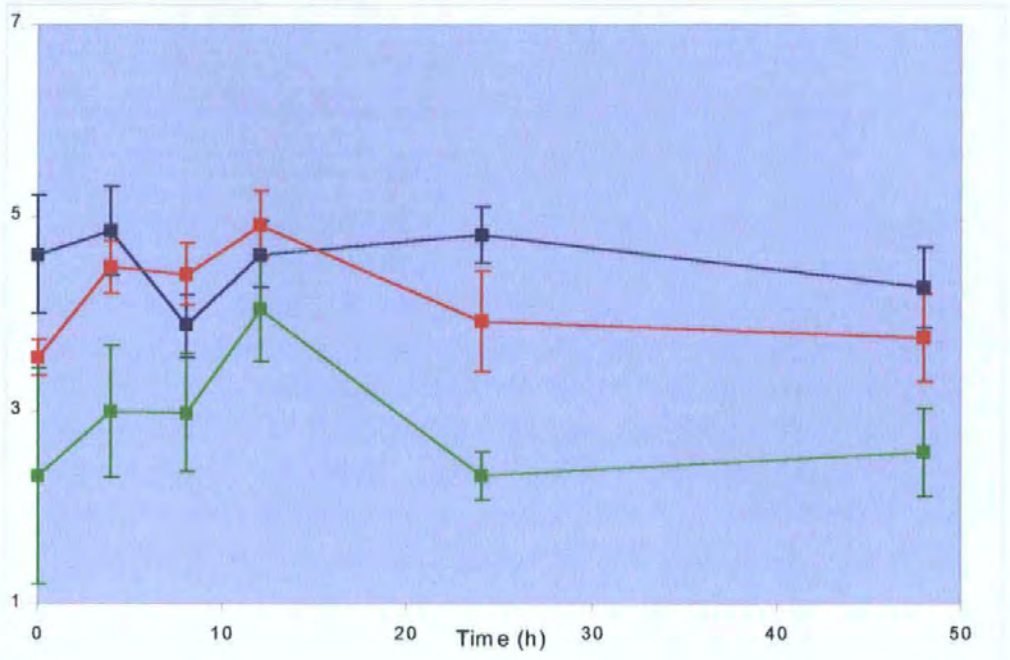


Figure 6.2.11 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed NWS diet.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

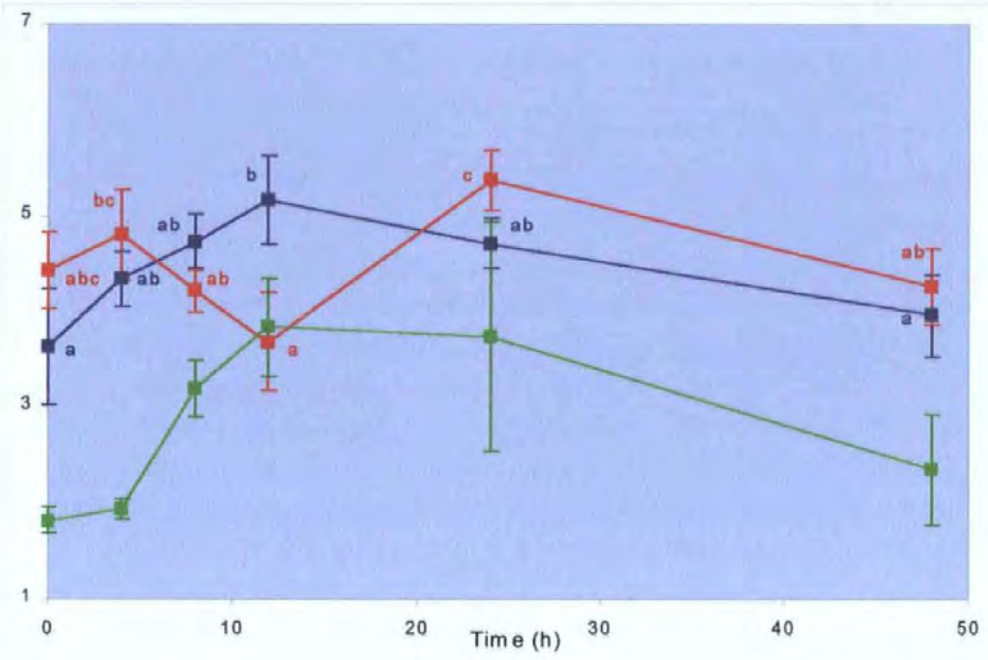


Figure 6.2.12 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed NCS diet.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

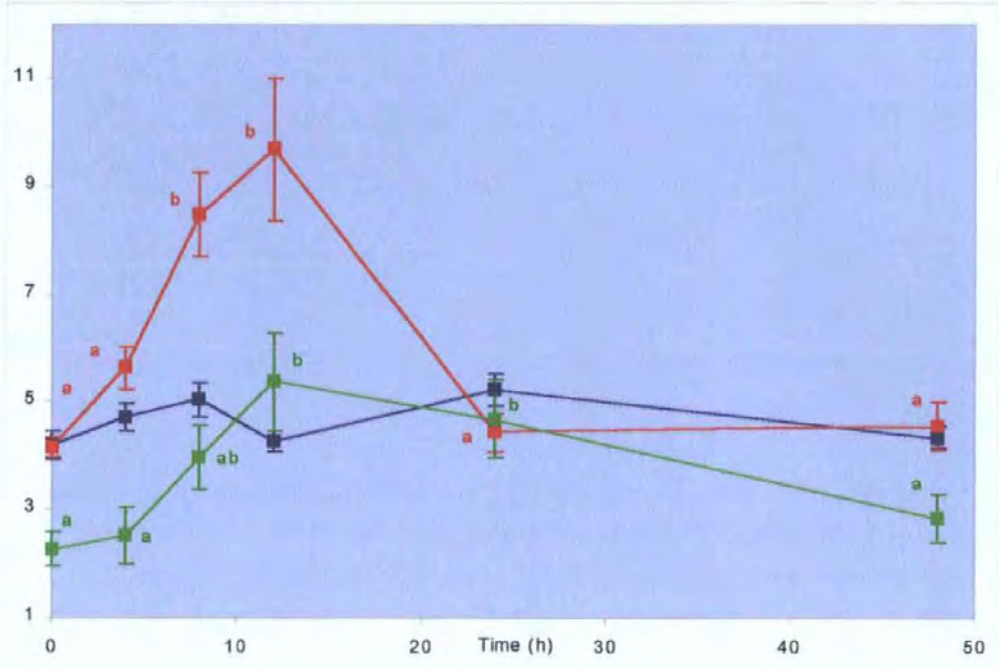


Figure 6.2.13 Postprandial plasma protein (mg dl^{-1}) (■), glucose (mmol l^{-1}) (■) and triglyceride (mmol l^{-1}) (■) concentration in the rainbow trout fed PCS diet.

Data points in each graph allocated different letters are significantly different from each other ($P < 0.05$) at a confidence level of 95 %. Bars denote ± 5 standard error of the mean.

6.2.4 DISCUSSION

Rainbow trout, *Oncorhynchus mykiss* were fed diets including approximately 30 % of D-glucose (GLU), maltose (MAL), dextrin (DEX), native wheat starch (NWS), native corn starch (NCS) or pregelatinized corn starch (PCS), respectively. This was in order to assess the influence of carbohydrate complexity on gastric evacuation & return of appetite rates and plasma circulating nutrients under given experimental conditions.

The efficacy of X-Radiography in order to quantify individual feed intake has previously been reported (Ross & Jauncey, 1981; Talbot & Higgins, 1983; Jobling *et al.*, 1993; McCarthy *et al.*, 1993a). Johnston *et al.* (1994) suggested using different coloured feed as a method to differentiate sequential feeding rate and claimed that it would not be possible to distinguish different meals using X-Radiography. However, X-Radiography was successfully utilised in this experiment for return of appetite determinations incorporating two different sizes of glass beads (0.65-0.90 mm and 1.16-1.40 mm) in the diets in order to quantify first and second meal intake. Although the incorporation level of marker "ballotini" may affect the chemical compositions of diets, no appetite suppression was observed in fish fed diets containing ballotini. The same level of small or large marker was added to each respective diets since the aim was to compare the instantaneous rates of appetite return in trout fed different sources of carbohydrate diets. Gastric evacuation and return of appetite profiles (Figures 6.2.1, 6.2.2, 6.2.3, 6.2.4, 6.3.5 and 6.2.6) and postprandial plasma nutrient concentrations (6.2.8, 6.2.9, 6.2.10, 6.2.11, 6.2.12 and 6.2.13) reveal that there seem to be complex interactions both at the physiological and bio-chemical level in relation to the diet composition.

The gastric evacuation rates (GER) of GLU, MAL and PCS treatments were described by three linear equations where as square root model gave better fit for the GER of DEX, NWS and NCS groups. Square root model shares the common characteristic that emptying is initially fast and slows down with time as the digesta in the stomach declines (Grove, 1986; Bromley, 1987; Mayer, 1994). On the other hand, digesta in the cardiac stomach decreased proportionally with time according to the linear equations. However, it can not be stated that these two models are exclusive for the treatments mentioned earlier. Because, the differences between the RSM (residuals of mean square) and r^2 of the linear and square root models were marginal (i.g: RSM, 46.18 and 68.24; r^2, 0.92 and 0.89 in linear and square root description of gastric evacuation of GLU group). Hence when the evacuation slopes of treatments were compared both models were applied. In the case of employing a linear model for all evacuation data, there was no significant difference ($P>0.01$) in the shape of slopes (Table 6.2.5). Similarly, no considerable difference ($P>0.05$) was evident between the slopes of all groups following the application of square root equations (Table 6.2.5). The same multiple regression comparisons were made between groups in order to support this analysis and the gastric evacuation slopes for any groups compared were not found to be important ($P>0.05$). The predicted times for the clearance 95 % of digesta also displayed a similar phenomenon. They were estimated between 34.7 hours (DEX) and 38.1 hours (MAL) in linear models, and between 41.1 hours (NCS and PCS) and 47.6 hours (MAL) according to the fitted square root equations.

The insignificant differences in gastric evacuation rates were despite a 10 % difference in the digestible energy of GLU (19.8 MJ kg^{-1}) and DEX (17.8 MJ kg^{-1}), NWS or NCS treatments. On the contrary, there was a significant difference in the gastric evacuation

slopes of low carbohydrate (LC) (DE: 20.2 MJ kg^{-1}) and medium (MC) (DE: 17.3 MJ kg^{-1}) or high (HC) (DE: 16.4 MJ kg^{-1}) diets (see Chapter 5.2). The DE of LC was 17 and 23 % higher compared to DE of MC and HC diets, thus it can be suggested that there may have to be a certain difference in digestible energy concentration of the diets in order to observe any considerable difference in the digestion rate. Another consideration is that the digestible protein (DP) content of the diets used in the present study was approximately identical whereas DP concentration of low carbohydrate (LC) diets was 28 and 42 % higher than medium carbohydrate (MC) and high carbohydrate (HC) diets, respectively (Chapter 5.2). Therefore a determined significant difference in the gastric evacuation rates could also be attributed to the different protein densities of the diets (Chapter 5.2). Conversely, the similar DP levels of the test diets could explain the insignificant gastric evacuation rates observed in the present experiment.

Basically, the time for 95 % appetite revival of GLU fed trout was the longest in both models. This finding could bring more light in to the data on feed consumption of rainbow trout presented in Chapter 6.1 (Table 6.1.3) that GLU fed fish displayed minimum feed consumption (1.4 % bw) following the 84-day- feeding trial. On the contrary, appetite return time for MAL treatment was quite shorter compared to that of GLU trout although the RFC of MAL group showed a similar feeding behaviour to GLU fish as presented in Table 6.1.3 (Chapter 6.1). A very high relationship (r^2 approximately 1.0) between gastric evacuation and return of appetite was found following plotting the data according to first order, linear and square root equations (Table 6.2.11 and Figure 6.2.7). This high correlation confirms the previous findings (Chapter 4.2 and 5.2) that the stomach fullness is likely to be a prime important factor in the regulation of appetite in rainbow trout. Since the rate at which the digested meal passes out of the stomach is dependent on the quantity

of food in the stomach in trout, metabolic demands may be coupled to intestinal absorption by regulation of the gastric volume. De Silva & Owoyemi (1983) reported that gastric emptying pattern may be differed by the density (specific gravity) of the ingested material. However, density or viscosity of diets (especially PCS diet) did not affect the gastric emptying shape in trout.

Postprandial circulating nutrient levels (Figure 6.2.8- 6.2.13) for each dietary treatment did not display a characteristic pattern during the sampling phase. For the GLU and MAL diet, the transient increase in glucose was sustained 24 hours post feeding and returned to a normal level after 48 hours (Figure 6.1.8). Such a prolonged hyperglycaemia has been reported extensively in rainbow trout (Cowey *et al.*, 1977a; Bergot, 1979; Walton, 1986). Hilton *et al.* (1987) suggested that poor glucose tolerance and prolonged hyperglycaemia induced by diet containing a high level digestible carbohydrates may affect glucostatic receptors in the trout resulting in suppression of appetite or feeding. However, no short-term effect of high levels of glucose on voluntary feed intake was monitored in this feeding trial. The plasma glucose level might have played a role. In this context; perhaps an elevated level of plasma glucose may decrease hexokinase activity due to feedback-inhibition by glucose-6-phosphate (Wilson, 1994), but appetite seems more likely to be regulated by other factors. For instance, the appetite of DEX, NWS and NCS fish was high throughout the feeding trial, indicating that physical capacity of the gut was probably the controlling factor in these treatments.

On the contrary, plasma glucose levels of DEX and NWS did not show any significant changes ($P>0.05$). Similarly, total plasma protein concentrations for all treatments were not significantly different probably due to similar dietary protein content of the diets.

However, postprandial triglyceride level increased sharply in GLU treatment after 12 h whilst plasma triglyceride levels of DEX and NWS fish remained consistent.

In contrast to a high relationship between the gastric evacuation and return of appetite of GLU treatment, the significant increase in plasma glucose level may not influence feed intake. However, it is difficult to draw a perfect conclusion due to an individual difference in meal consumption of fish (McCarthy *et al.*, 1993a; Jobling *et al.*, 1995). When the feed intake data in Figure 6.2.1 is revised, it can be observed that the mean appetite rate in GLU fish at 12 hours following first alimentation was reduced. This could be explained by a significant increase in triglyceride concentration (time 12; 8.02 mmol l^{-1}) (Figure 6.2.8). Thus, different metabolites can play more important roles at varying time intervals. From the standpoint of plasma triglyceride; H. Peres (pers. comm., 1998) observed that plasma triglyceride level was increased after a glucose solution (1g kg^{-1} live weight) was injected in to sea bream, *Sparus aurata*. Furthermore, G. Corraze (pers. comm., 1998) suggested that activity of lipogenesis enzymes were stimulated by an increase in carbohydrate intake in the same species. All these results combined with the increase in triglyceride concentration in GLU treatment indicate that plasma glucose may be converted into triglyceride by glycolysis and lipogenesis in the liver (Cowey & Walton, 1989) but the metabolic pathway is unclear. Cowey *et al.* (1977a) showed that rainbow trout fed a high carbohydrate diet reduced the rate of gluconeogenesis. Shimeno *et al.* (1993) also reported that high level carbohydrate intake elevated glycolysis and lipogenesis in *Oreochromis niloticus*. Walton & Wilson (1986) showed a postprandial increase in amino acids in the plasma rather than in the liver of rainbow trout. Despite the insignificance of postprandial total protein concentration on VFI in the present study, it cannot be ignored.

Systemic nutrients did not appear to reach a level which causes satiety in the brain via hepatic efferent signals. The liver is one of the most important organs in the regulation of appetite since it contains the enzymes necessary for the synthesis and degradation of glucose, glycogen and lipid (McGarry *et al.*, 1987) and supports the energy requirements of the brain and periphery (Novin, 1983). Thus, liver glycogen or HSI (Hepatosomatic index) might be an indication for the significance of this organ in appetite control. In this context, the highest and significantly different HSI of GLU fish may be the main reason for the decline of feed intake in this group probably because high level of glycogen deposition might have damaged liver membranes (Baeverfjord, 1992). Postabsorptive factors (especially plasma glucose and triglyceride levels) were more pronounced on meal frequency in GLU fish (after 8 and 12 hours respectively) probably because of the metabolic status of the liver. Postabsorptive factors have also been proven to modulate feeding frequency in mammals (Deutsch & Gonzales, 1981; Le Magnen & Devos, 1984; Cook *et al.*, 1997).

As far as the higher animals are concerned, Russek (1981) hypothesised that hunger appears after a decline in the carbohydrate reserves signalled to the brain by discharges from the hepatocytes in the liver. He proposed that the system operates as follows: "Some metabolites of the glycolytic sequence (i.e. pyruvate), related both to liver glycogen content and glucose input of the hepatocytes, have a hyperpolarizing effect on their membranes, perhaps through an increase in the sodium pump. Thus hunger would normally appear when intestinal absorption and liver glycogen (and liver pyruvate) decrease to a certain critical level" (Russek, 1981).

In conclusion, this investigation has confirmed the general view of Windell & Norris (1969), Grove *et al.* (1978) and Chapter 4.2 and 5.2 that stomach evacuation rate is an

important feature in the modification of feeding behaviour of rainbow trout. Furthermore, circulating nutrients in rainbow trout fed complex carbohydrates did not affect voluntary feed intake. However, a high dietary level of simple sugars may suppress the appetite by elevating the high plasma glucose and/or triglyceride concentration. It appears that stored carbohydrate within the liver and circulating pancreatic hormones interact (Matty & Lone, 1985a, 1985b; Sundby *et al.*, 1991) in the long-term control of feed intake as suggested for higher animals (Novin, 1983; Stricker & Verbalis, 1990; Walsh, 1994). We could focus our discussion on two factors which may provide considerable information about regulation of appetite in rainbow trout; gastric distension and the postabsorptive delivery of utilisable energy to the liver. Gastric (stretch and chemoreceptors) and postabsorptive signals are generated after a meal (Fletcher, 1984; Jobling, 1986a). Gastric distension is probably the main factor in the short-term satiety of trout whilst overall energy density of the meal may be less significant.

Both short-term and long-term factors influencing feed intake should be investigated using different quality diets. Sheridan & Mommsen (1991) suggested that Coho salmon, *Oncorhynchus kisutch* regulate body energy balance within short and long-term strategies which may be achieved by pancreatic hormones such as insulin, glucagon and glucagon-like peptide. Nutritional history studies should be organised such as those investigating the metabolic effect of feeding different quality diets after 24 and 48 hours starvation.

CHAPTER 7

7. GENERAL DISCUSSION

Aspects relating the complex dietary macro-nutrient interactions involved in feed consumption and appetite regulation together with energy & nutrient utilization were examined for (*Oncorhynchus mykiss*). A series of laboratory test diets were formulated to assess effects of varying nutrient and energy densities on various nutritional parameters. These included feed intake, growth performance, feed utilization efficiency and changes in proximate body composition at the end of each investigation.

The initial set of experimental feeding trials demonstrated the importance of presenting rainbow trout a balanced diet formulation at an optimum ration size. Such a feeding strategy resulted in maximum growth and feed efficiency with a consequent fish composition in accordance with a desired product quality. The results of these studies also revealed that a number of interactions may exist which are linked to the physical and chemical constituents of the diet and the digestive physiology of trout.

The relationship between appetite revival and gastric evacuation rate was a prime consideration in developing the further investigations. This has been deemed to be a dominant factor with respect to the control of feed intake in mono-gastric animals including fish. These experiments also incorporated measurements of postprandial plasma nutrient concentrations as possible modulating factors in the regulation of feed intake. This was the author's first attempt to determine the association between physiological and biochemical factors influencing appetite response of rainbow trout.

It should be mentioned that most previous investigators have employed commercial diets which have only a limited nutrient specification, usually standard commercial feeds for salmonids. In the current investigations, it was the practice to examine the effects of diet composition which therefore required alteration of the nutrient profiles. The effect of overall nutrient dilution at similar protein/energy ratios was evaluated together with diets of differing energy levels either as oil or carbohydrate based.

The role of carbohydrates in trout nutrition has always been controversial, especially as a potential source of useful energy for growth and metabolism. For these reasons, emphasis on the function of carbohydrate as an influencing factor on feed consumption and digestive physiology was particularly applicable. As a consequence, attention was directed to establish whether the degree of complexity associated with the carbohydrate component of the diet could alter the feeding response of rainbow trout. This work involved the integration of data obtained from previous feeding trials with more complex investigations including gastric emptying rate measurements, appetite return and systemic response to metabolites. Therefore the general discussion of the present research program can be focused into two main categories:

- "Feed consumption, growth performance and nutrient utilization" from practical feed formulations and perspectives relating to feeding strategy.
- The regulation of appetite in the rainbow trout with respect to physiological and biochemical factors.

7.1 Feed Consumption, Growth Performance and Nutrient Utilization

It has been a common view amongst many researchers that fish tend to eat in order to satisfy their energy requirements for both maintenance and growth. However, it must now be considered that other nutrients may also be involved in determining the level of dietary intake for fish. In this respect, more complex macro- and micro nutrient interactions are probably involved and the concept of energy being the primary basis for appetite regulation needs to be verified. Indeed, fish especially carnivorous species such as trout and salmon depend on protein as a major energy supply. Protein is therefore an important nutrient which most probably influences satiety control mechanisms in such fish. The association between energy concentration of the diet and allowances for major nutrients such as protein (amino acid), vitamin and mineral requirements has been stated for a number of animals. The situation for fish is unclear but this poses a challenging issue for the diversity of cultured fish species.

The second question that needs to be answered is whether rainbow trout have the capacity to regulate their feed intake in the short term according to dietary energy and nutrient levels. It is difficult to provide a clear answer to this, however some indications were obtained from the Experiment 1 (Chapter 3) and Experiment 2 (Chapter 4.1) where rainbow trout were fed different nutrient and energy dense diets. Rainbow trout were able to regulate their feed intake, however this was not evident immediately. Although fish achieved almost identical growth performance, they consumed different amounts of digestible energy whilst dry matter intake remained quite similar. These findings indicate that gastric capacity is a major factor and that physical constraint limits meal consumption. This questions the logic of offering fish very high-energy diets on a satiation basis. It has

been suggested that maximum growth demands high-energy diets, thus placing fish on a higher anabolic plane. Maximum feed intake (especially with energy dense diets) as previously proposed by Vahl (1979), Brett & Groves (1979), Talbot (1993) in order to obtain maximum growth has not always been relevant due to a reduction of feed efficiency following an *ad libitum* feeding regime (Elliott, 1976 and 1982; From & Rasmussen, 1984; Jobling, 1986b; Cho, 1992 and Kaushik & Medale, 1994).

High-energy diets unarguably help to reduce pollution and environmental impact resulting from organic matter discharge and associated nitrogen and phosphorus loading. Also dietary lipids assist in reducing dust and stabilizing the pellets during manufacture as well as increasing the palatability of feeds. However, the use of high energy diets with an *ad libitum* regime may lead to a decline in digestibility of 5-10 % as a result of overloading the digestive tract with consequent metabolic disturbances following excessive fat accumulation (Jobling, 1986b). The solution therefore would be to employ a restricted feeding strategy. In the case of feeding fish isoenergetically with different energy-dense diets, high-energy diets offer a reduction in feed volume and improve feed conversion efficiency. However, if the feeding protocol is restricted, then there will be competition among fish causing social hierarchy and variations in fish size. In this respect, diets of average energy concentrations containing a highly digestible carbohydrate would be more beneficial in order to produce homogenous fish populations under farming conditions.

It was shown in Experiment 4 (Chapter 5.1) that dietary carbohydrate (i.e. extruded wheat) can be used for energy and consequently some protein may be spared for growth in rainbow trout. Therefore dietary lipid could be replaced by digestible carbohydrate to the extent that maximum growth is achieved. This was tested in Experiment 6 (Chapter 6.1)

and demonstrated that different sources of carbohydrates (dextrin, native wheat starch and pregelatinized corn starch) in the level of 300 g kg^{-1} DM can be used in practical feed formulations for rainbow trout since they achieved high growth rates (approximately SGR= 2.0 g day^{-1}) and feed efficiency (100 %).

On the other hand, it has been demonstrated that the discharges from fish farms cause an increase in the effluent concentrations of ammonia, orthophosphates and suspended solids. The reduction of this pollution load can be coped within various ways by the farmer, commercial feed manufacturer and the nutrition consultant. In the first instance, the regulation of the stocking density and the nutritional characteristics of the feed become particularly important (Lanari *et al.*, 1993).

From a series analysis of whole carcass and muscle components of rainbow trout fed with different nutrient and energy dense diets, it is suggested that carcass lipid concentration is likely to be directly related to the dietary lipid level. It was observed that dietary lipid level of 250 g kg^{-1} DM or higher concentrations affected carcass lipid level significantly ($P<0.05$). However, muscle lipid concentration does not seem to be affected by dietary lipid. Similarly, it can be proposed that protein and ash content of whole carcass or fillet are independent of diet composition and endogenously controlled. Therefore misinterpretations of body proximate composition data should be avoided, and all researchers should strictly consider differences in fish size prior to comparisons with body constituents of experimental fish fed different dietary formulations.

From a commercial point of view, the most important objective is to strike two targets, simultaneously: maximum feed efficiency and maximum growth. In order to achieve this

under commercial fish production conditions, there is a need for accurate feeding tables applicable to specific conditions such as water temperature and fish size. The procedure proposed by Cho (1992) is based on the principle that feed intake should meet the fundamental nutrient requirements of fish. Thus, taking into account the daily energy needs of fish as defined by growth rate, size and water temperature together with data on the digestible energy density of the diet, it is feasible to develop feeding charts adapted to specific conditions.

Protein sources are the most expensive ingredients in commercial feed formulation. Therefore one should tend to avoid protein use for energy in order to maximise the efficiency of its use in tissue protein synthesis. In this context, alternative nutrients could be used as an energy source to spare the fate of protein. The optimal level of protein in fish diets is governed basically by promoting the correct protein to energy ratio, amino acid composition and digestibility of dietary protein.

In summary, it can be recommended for the growing rainbow trout that dietary partitioning of nutrients could be given as 40 % protein, 20 % lipid and 25 % carbohydrate with a range of 16-18 MJ DE kg^{-1} DM and DP/DE ratio of 22-24 g DP/MJ^{-1} DE as proposed by Cho (1992) and Kaushik & Medale (1994). This should be employed with a restricted feeding regime to promote maximum growth and feed efficiency, simultaneously. However, it should also be cautioned that nutrient allowances based on concentrations within the diet are often subjective due to the different feeding levels used by workers. In this respect, nutrient allowances should be ideally expressed as a function of fish size (kg^{-1} body weight) or biomass gain.

As was outlined by Cho et al. (1994), these points could be defined:

- careful selection of feed ingredients based open digestibility
- balanced feed formulation to ensure maximum nutrient and energy utilization
- controlled feeding regime.

7.2 Appetite Regulation

Voluntary feed intake might be regulated in either the short-term or long-term considering the nutritional history of the animal concerned. It is apparent that hunger and satiety mechanisms are ultimately regulated by the requirement to modulate a balance between energy input and output and also needs for specific nutrients. The amount of feed consumed voluntarily in a meal is largely dependent to the nutritional state of fish under defined environmental conditions. A sequence of events occurs involving chemo-reception beginning in the mouth, digestion, absorption and transport with the attendant production of enzymes, hormones, neurotransmitters and these are all involved in the regulation of feed intake.

The multi-factorial control of appetite has been proposed in higher vertebrates by Kissileff & Van Itallie (1982), Forbes (1994), Mayer (1994), Figlewicz *et al.* (1996). Extensive studies on feed intake regulation in *Oncorhynchus nerka* (Brett, 1971), *Salmo trutta* (Elliott, 1972), *Oncorhynchus kisutch* (Vahl, 1979), *Limanda limanda* (Fletcher, 1982), *Salvelinus alpinus* (Jobling & Wandsvik, 1983), *Scopthalmus maximus* (Grove *et al.*, 1985) and on elasmobranch *Scyliorhinus canicula* (Sims, 1994) have also been put forward on the multi-factorial regulation of feed intake.

In Vahl's proposal, a simplified model was employed to demonstrate the relationship between possible factors acting in appetite regulation. As a combination of a broad literature and personal observations, it was envisaged that a similar model could emerge from this data to better describe the mechanism governing the regulation of appetite and therefore the feeding response for production size of rainbow trout. Therefore a simplified

flow diagram showing the fate of the ingested meals and the regulation of appetite can be proposed (Figure 7.1).

According to this model, the appetite centre is likely to be informed by six main sites either for satiety or hunger. Search for feed is initiated when certain regions of the brain are activated by disinhibition of its plain activity as a result of inhibitory information from either the blood stream or cerebrospinal fluid or elevated excitation from neural or humoral actions from the liver. These inputs influence the brain cells by means of specific neurotransmitters. The same brain cells are also influenced in their activity by direct or indirect detection of fat stores (Kissileff & Van Itallie, 1982).

Once the feed is ingested, the stomach or the foregut distends accordingly to accommodate the meal. Food is then dispersed to smaller particles by the combination of enzymatic action in an acidic environment and rhythmic contractions of smooth muscle in the stomach wall (Grove, 1986; Bromley, 1994). Following these initial stages, the stomach or the foregut initiates the process of disrupting and expelling the digesta into the duodenum, pyloric sphincter and the intestine where primary nutrient absorption occurs. The rate at which the digesta leaves the stomach displays a characteristic gastric emptying pattern.

After ingestion of a meal, the stomach wall of a rainbow trout distends by a reflex relaxation in order to increase receptive capacity (Grove & Holmgren, 1992). The degree of fullness of the stomach is monitored by the stretch mechano-receptors in the stomach. The fullness at any time is dependent on the evacuation rate, which in turn is proportional to the mass of food remaining in the stomach. The information carried to the

hypothalamus by stretch receptors contains the information that there is free volume available in the stomach (Site 1)

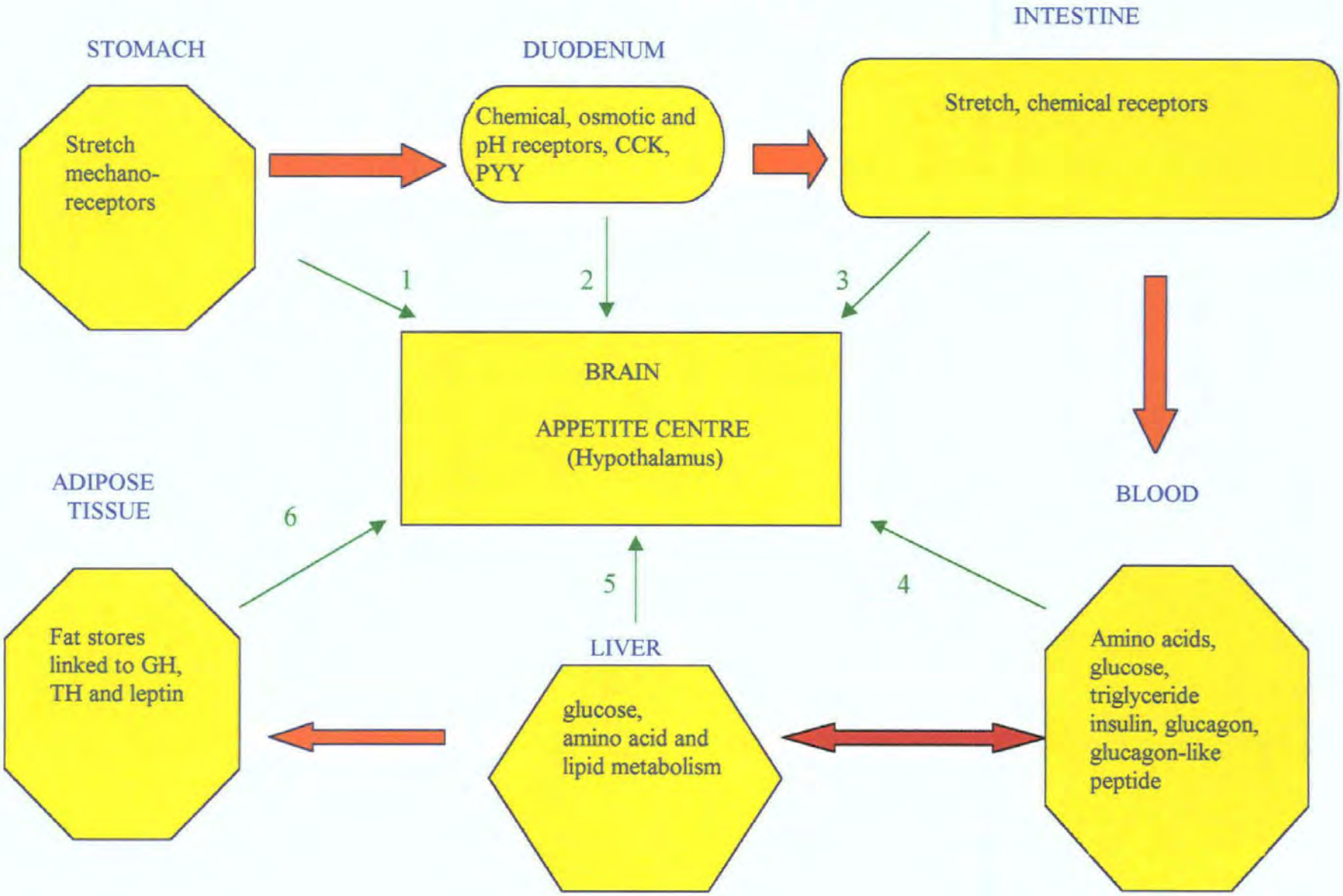
During the first hour of feeding, the effect of chemo-receptors and any mechanisms or receptors monitoring the energy content of the digesta in the cardiac stomach is not evident since rainbow trout fed either low or high energy diets until their stomach is full. Stretch receptors are likely to be one of the primary carriers of satiety information to the appetite centre in the brain. On the other hand, it is unlikely that the all of the information from these receptors is sufficient for the central nervous system to gain a complete picture of the quantities of nutrients ingested in order to balance intake with output.

Following ingestion of pelleted food in rainbow trout there is a lag phase before digesta starts moving through the rest of the alimentary tract. This is largely due to the time for liquidification of food and decreasing the diameter of nutrient particles in order to pass via the duodenum. This process is unlikely to play any role during the first feeding period. Assuming an equal and unlimited opportunity to feed, the satiation time recorded in the present research program is in approximate agreement with those of other salmonids. Satiation time for salmonids have been reported between 45 and 60 minutes (Brett, 1971; Ishiwata, 1968; Windell *et al.*, 1969, Elliott, 1972, 1975a; Grove *et al.*, 1978; Nagata, 1989; personal observation) following a 72-hour- starvation. A trout ingesting a full meal of pellets containing approximately 95 % dry matter consumes more than six-fold the amount contained in a meal of oligochaetes representing only 16 % dry matter (Windell *et. al*, 1969).

Figure 7.1 Schematic diagram of a model of feed intake regulation in rainbow trout. Six sites which are likely play a significant role in control of feed intake are demonstrated.

is the flow of nutrients and energy

is the flow of information


STOMACH
DUODENUM
INTESTINE
Stretch mechano-receptors
Chemical, osmotic and pH receptors, CCK, PYY
Stretch, chemical receptors
1
2
3
BRAIN
APPETITE CENTRE
(Hypothalamus)
BLOOD
ADIPOSE TISSUE
6
5
4
Fat stores linked to GH, TH and leptin
LIVER
glucose, amino acid and lipid metabolism
Amino acids, glucose, triglyceride insulin, glucagon, glucagon-like peptide

Some evidence (Dos Santos & Jobling, 1988) has also been reported which suggest that the size of the food particle affects the rate of gastric evacuation. However, since the diets used in the present investigations generally dissociate in a short time in the stomach to particles of a similar size (personal observation), the role of particle size in the gastric evacuation of rainbow trout was likely to be minimal.

In summary, Site 1 is the primary consideration with respect to the modulation of appetite. Stomach fullness following starvation to the extent that there is no digesta residuals in the gastro-intestinal tract is an important mechanism. This factor could be accepted as the first short-term regulatory mechanism of feed intake since the phenomenon of regulation of gastric emptying in trout depends upon the volume of gastric contents. As long as sufficient food is present in the stomach to stimulate the stretch receptors, peristalsis occurs at a fixed rate. When a small amount of food is present, the gut wall is not stretched and peristalsis is either not initiated or weakly initiated. This may be an adaptive mechanism for processing small amounts of food more efficiently and thereby allowing time for greater assimilation.

The picture of the first hour following meal consumption is quite easy to discuss compared to the rest of the digestion and assimilation processes. However the situation becomes more complex once nutrients are translocated past the duodenum. At this region, a number of chemo- and osmo-receptors and several hormones like substances (CCK, PYY) are involved (Site 2).

Regulating feed intake according to the energy content of the diet in fish may also be associated with the gastric emptying time (GET). Typically, diets with a higher energy

content are emptied more slowly than low energy feeds in fish (Grove *et al.*, 1978; Jobling, 1980). These delays in digestion times tend to lower overall feed intake (Grove, 1986; Jobling, 1986a).

The volume of the diet determines the gastric emptying time of a meal. Once a meal has emptied from the stomach, a vagally mediated signal initiates the demand for a new meal. A large meal predicts a long inter-meal interval but a long inter-meal interval does not predict that the next meal will be large (Mayer, 1994). Concentration of chemicals initially entering the small intestine will also determine the onset of feedback inhibition (Site 3). The duration of the initial emptying phase appears to be dependent on the rate and extent by which chemo and mechano-receptors in different parts of the intestine are activated by gastric contents exiting the stomach. Therefore a delivery of nutrients to the intestine in a meal might play a critical role in the onset of satiety after fish are offered a second meal. Application of second meal causes more complex interactions since it will affect the digestive physiology of fish which have already eaten. Intestinal mechanisms may control gastric emptying of energy and thereby regulate energy intake via mechanisms sensitive to gastric distension. While the digesta moves through the intestines, the stomach content decreases and amino acids, glucose and triglyceride absorption begin. At this point absorptive signals are generated from the liver as a result of delivery of utilisable energy to this organ.

When food is absorbed and processed further it will give rise to a higher level of metabolites in the blood (Site 4). The level will be determined by the size and composition of the meal and by the time elapsed after ingestion. Since the specific dynamic action (SDA) reaches a maximum value some time after ingestion, there is a time lag between the

ingestion and the contribution of the meal to the level of metabolites. The extent of the time lag depends on the rates of stomach evacuation, absorption and processing of the absorbed materials.

The liver is the first point at which most of the absorbed nutrients can be monitored by a single organ but even then lipids are absorbed via the lymphatic system and bypass the liver. The general circulation transports nutrients between organs and also is the medium whereby hormones, secreted from endocrine organs, pass to their target organs. Many of the hormones have metabolic functions and have been implicated in the control of feed intake. Liver glycogen and/or lipid stores may act as a first line of the maintenance of plasma glucose homeostasis and as the early warning signal of metabolic changes, and thereby signal the need for feed intake (Site 5).

Both gastric (stretch or chemical receptors) and absorptive signals (delivery of utilizable energy to the liver) of satiety are generated by a meal. Meal frequency is dependent on postabsorptive and metabolic factors as well as gastric capacity. Termination of a meal may not be controlled by a number of factors. However, the products of the meal can be more accurately monitored during the subsequent inter-meal interval and used to determine the onset of the next meal.

Signals that can play a major role in short-term regulation of feed intake can provide inputs to long term regulatory system that allow the adjustment of energy expenditure and subsequent feeding. In the longer term, fat depots can be a driving factor since increasing accumulation of fat in adipose tissue will decrease the abdominal space available for accommodation of feed by the stomach. Therefore it can be suggested that the amount of

body adipose stores in rainbow trout is regulated within a relatively narrow range over long periods of time (Site 6). Some of the body weight regulatory hormones could be insulin, glucagon and glucagon-like peptides as proposed by Harmon & Sheridan (1992a; 1992b). In this context, the approach of Sheridan & Mommsen (1991) could be a useful model to comprehend the regulation of feed intake in the short term and long term basis. These latter authors reported that Coho Salmon, *Oncorhynchus kisutch*, regulated their body reserves on a short- and long-term basis by pancreatic hormones (insulin, glucagon and glucagon-like peptide). Adipose tissue continually undergo lypolysis and lipogenesis, and plasma free fatty acid levels are approximately proportional to body lipid stores (Sargent *et al.*, 1989).

According to the above proposal, some feedback signals associate adaptively in their effect on feed intake rather than being jointly exclusive. Therefore we need to generate models based on hourly sampling intervals which serve to provide information on the minute by minute events underlying the processing of feed. Hopefully, this will help to establish realistic predictions of the daily feed intake response of trout in relation to growth and feed utilization efficiency.

Ultimately any physiological models that can be used to predict feed consumption in cultured fish will enable us to present fish with optimum feed formulations whilst sustaining maximum allowable growth. These will obviously have significant implications to both the fish farmer and consumer. It is now a topical issue that fish must attain a specified threshold with respect to fat content and flesh quality to meet consumer demand.

7.3 CONCLUSIONS

On the basis of the current programme of investigations, it can be stated that rainbow trout may eat to satisfy their nutrient and energy requirements. However, it cannot be suggested that rainbow trout are able to regulate their feed intake precisely; especially when offered an energy-dense diet formulation. The regulation does not appear to occur in the short term in rainbow trout fed such high-energy diets (i.e. < 20.0 MJ kg^{-1} DE) probably due to the increased palatability of fish oils.

The direct effect of a feeding strategy (restricted or satiation) on feed assimilation efficiency in salmonids fed energy dense diets (i.e: <200 g lipid per kg DM) should be studied using different experimental conditions (i.e: different fish sizes and temperature ranges).

Inappropriate feeding practices in aquaculture may lead to feed being wasted or to insufficient feed being provided, resulting in higher production costs and contamination of the aquatic environment. Clearly these are undesirable issues of major importance in fish production systems.

The appropriate balance of dietary nutrients such as amino acids, fatty acids and oligosaccharides which compete for the active transport sites in the gastro-intestinal epithelium, may improve assimilation efficiency. Possibly, the rate of gastro-intestinal activity could be modulated by incorporating stimulants and attractants in the diets of farmed fish in order to obtain the most efficient rate of feed utilization and consequently

assist the anabolic capacity of the growing fish (Windell *et al.*, 1969; Fänge & Grove, 1979).

Further, the use of X-Radiography techniques should be widened in order to quantify sequential meals which have a likely role in consecutive appetite regulation. Also choice feeding practices can be established towards better understanding of the response of fish to diets differing in quality. Such a basis for allowing feed selection is quite commonly employed for terrestrial domestic animal production.

The physico-chemical characteristics of diets tested and the nutritional history of experimental fish should be made prior to any nutritional physiology experiments.

A complete understanding of both neural and humoral regulation of appetite control in fish must be evaluated for a more complete comprehension. With this information, we will be able to generate predictive models for the endocrine effects associate with the manipulations of diet quality, quantity or feeding time. These models may provide a means for modulating endocrine function via the diet as well as a guide for the formulation of feeds which promote rapid, lean growth and deposition.

Finally, control of feed intake is a complex matter involving the interaction of many factors to initiate and terminate feeding in fish. Therefore multi-factorial experiments (Holmgren *et al.*, 1983) should be designed including determination of gastric evacuation & return of appetite rates, postprandial plasma hormones & nutrients, hepatic enzymes and the changes in specific neural centres following feeding. In turn, these types of investigations should be repeated using the same fish species fed sequentially under

similar laboratory conditions. These investigations will serve to establish the major factors modulating appetite for different fish species under varying environmental conditions.

Finally, I would like to share my last feelings with Prof. Forbes (1994) of Leeds University:

" It will be necessary to use artificial intelligence techniques in future models, but such applications are only just beginning. It may well be that intake control is so complex that it will not be fully understood until we have models as complex as animals themselves."

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