THE UTILIZATION OF DIETARY CARBOHYDRATES BY TROUT AND CARP

EDOCHIEM BEN CHIDI ONWUKA, B.Sc., MIBiol.

A Thesis submitted to the University of Aston in Birmingham for the degree of Docotor of Philosophy

NOVEMBER 1980

SUMMARY

THE UTILIZATION OF DIETARY CARBOHYDRATES BY TROUT AND CARP

Edochiem Ben Chidi Onwuka, B.Sc., MIBiol.
Ph.D. Thesis, University of Aston in Birmingham.

November, 1980.

The growth responses, nutrient utilization and tissue carbohydrase activities in Rainbow trout and Mirror carp when respectively fed 0-30% and 0-45% of cassava, rice, corn, potato or dextrin in isonitrogenous diets were investigated.

In trout, optimum growth and food utilization was at 20% dietary cassava or rice. Weight gain was positively correlated with dietary level of corn or potato. The cassava and rice diets produced better growth than the corn and potato diets. Dietary dextrin was less well utilized. Carbohydrate digestibility depended on the carbohydrate source, while protein digestibility did not, but was often over 75%.

Carp could tolerate higher levels of carbohydrates and less protein than trout. The 45% cassava, rice or dextrin, or 30% corn led to the best weight gain and food utilization in carp. Apparent digestibility of most of the carbohydrates was over 80%. Dietary carbohydrate did not seem to suppress protein digestibility, which was always over 65%.

In both species, the very low levels (<3.5%) of dietary hydrolysable carbohydrate greatly suppressed carbohydrate digestibility. No group manifested significant liver damage, or persistent hyperglycaemia. α-amylase and α-glucosidase activities increase initially with increase in dietary dextrin, and is higher in Carp than in Trout. 45% dextrin in the diets of carp produced the highest levels of α-amylase in the liver and α-glucosidase in the hind-gut. Carcass and liver composition were affected only marginally except for glucose/glycogen contents which showed positive correlation with the dietary digestible carbohydrate levels.

It is concluded that digestible carbohydrates enhance growth, the extent depending on carbohydrate type and level, when fed to Rainbow trout and Mirror carp, the latter being better adapted to utilizing high levels.

KEYWORDS: TROUT . CARP . NUTRITION

CARBOHYDRATES . CARBOHYDRASES .

ACKNOWLEDGEMENTS

I would like to thank Professor Allen Matty and Dr. Albert Tacon for their close supervision, keen interest and encouragement during the conduction of this research, and during the writing up of this thesis. To Dr. Andrew Jackson who read the manuscripts and made vital suggestions, I remain very grateful.

I would also like to thank Dr. Niall Bromage for his help and for procuring the research materials, and Drs. Terry Atkins and Cliff Bailey for useful pieces of advice.

I am grateful to Mr. Bob Burns, the departmental Superintendent, and all the departmental technical staff for their friendliness and help; in particular, I should like to mention Mrs Sue Taylor, Mrs. Pauline Faulkner, Messrs. Tony Watson, Steve Howitt and Jim Berwick.

I would also like to thank my colleagues of Aston Fish Culture Unit, for their comradeship, Mr. Dan Okoli for tracing the graphs, and Mrs. Linda Perkins for typing this thesis.

In addition, I would like to express my gratitude to University of Jos for sponsoring this research, and Professor Chiweyite Ejike for both stimulating my interest in Animal Physiology and for his contribution in making this research possible.

Finally, I would like to express my indebtedness to my late mother and father for encouraging their children unto high academic pursuits, to my late brother, Dr. Golie T. Onwuka for his financial and moral support to me during the first year of this research, and to my wife, Chinyelu, for the love, understanding and encouragement.

E.B.C.O.

TO THE SWEET MEMORY OF MY

LOVING MOTHER AND FATHER

&

TO MY DARLING WIFE FOR

MAKING LIFE WORTHWHILE

CONTENTS PAGE

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1

CHAPTER 1

GENERAL INTRODUCTION

1.1. DEFINITION AND STRUCTURE

According to the Henneberg and Stohmann, Weende Food Analysis Scheme, (1868), carbohydrates are classified into nitrogen-free extract (NFE) and crude fibre. The former consists of monosaccharides, oligosaccharides and polysaccharides that are insoluble, or according to Van Soest and McQueen (1973), partially, but variably soluble in dilute acid and alkali. The difficulty in giving a specific definition for such a heterogeneous group as carbohydrates has been observed by Hawk, (1965). Hawk (1965) however, attempted defining carbohydrates as polyhydroxyaldehydes or ketones, and derivatives of them. Oxidation, reduction or substitution of one or more of the functional groups can result in compounds which are also classified as carbohydrates.

The most important carbohydrates may be classified as follows :

1. Monosaccharides or simple sugars

This consists of (a) trioses with the formula, ($C_3H_6O_3$), (b) tetroses ($C_4H_8O_4$), (c) pentoses ($C_5H_{10}O_5$), (d) hexoses ($C_6H_{12}O_6$), and (e) heptoses ($C_7H_{14}O_7$).

II Oligosaccharides

This consists of (a) reducing sugars ($C_{12}H_{22}O_{11}$), which are maltose and lactose (b) Non-reducing disaccharides such as sucrose, (c) Trisaccharides, which are raffinose, gentianose and melezitose.

III Polysaccharides

The definition for polysaccharides in most text-books is still unsatisfactory. They may be roughly classified as (a) Homopolysaccharides such as starch, dextrins, glycogen, cellulose, inulin, and chitin (glucosamine, and acetic acid), (b) Heteropolysaccharides, This is mainly mycopolysaccharides, and is divided into the following major groups: hyaluronic acid, chondroitin sulphate A, chondroitin sulphate B, α-heparin, blood group substances, and blood serum mucoids.

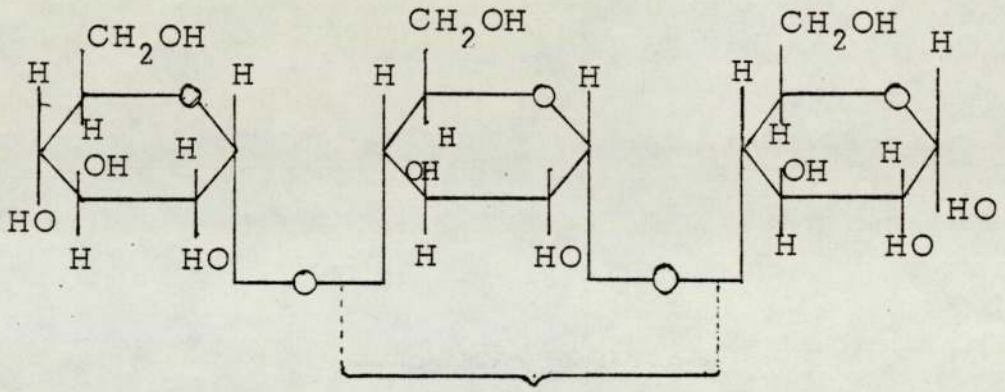
IV Derived carbohydrates

This consists of oxidation products, reduction products, amino sugars and deoxysugars.

Starch which constitutes the bulk of most digestible plant carbohydrates is made up of two distinct polysaccharides, amylose and amylopectin. Amylose constitutes 20 - 30% total in most plant starches. However, in waxy maize and waxy sorghum and in some

other starch amylose is almost absent. Contrarily, some starches such as wrinkled pea starch (Lloyd, et. al., 1978) and probably cassava and rice contain mainly amylose.

Amylose as shown in the diagram below comprises of hundreds of glucose units linked by α-1, 4 -linkages.



GLUCOSE UNIT

Little or no branching of the chain occurs. In contrast, amylopectin consists of several hundred units of 20 - 25 α-1, 4-linked glucose residues joined by α- 1, 6-glucosidic linkages (Bourne, 1951). It is highly branched and some α- 1, 3-linkages have infact, been observed in it.

1.2. AVAILABILITY OF CARBOHYDRATES

In nature, carbohydrates are the most abundant of all food items. Unfortunately, numerous difficulties have arisen when these substances have been encountered in attempts to incorporate them into diets for fish.

Cassava, rice and corn are among the most abundant sources of carbohydrate foods in the tropics, while in most temperate regions, potato seems to be the most common carbohydrate containing food eaten. If one looks at the cost of various carbohydrate sources and for example, compares those carbohydrate containing products normally consumed in Nigeria and in England, results similar to that shown in Table 1 would be obtained.

TABLE 1 - Average costs[1] of some carbohydrate food sources in Nigeria and England in July 1980 (price per kilogram)

	NIGERIA	ENGLAND
Cassava	50 kobo	Not available
Rice	91 kobo	40p
Corn (dried)	54 kobo	42p
Potato	80 kobo	10p

[1] 1.00 Nigerian kobo ≙ 0.79 English penny (p)

Maclean, et al., (1978) on analysising rice for experimental feeding to human infants discovered that his rice had between 7.1% and 11.4% protein, and 76.2% to 81.6% hydrolysable carbohydrate. Corn starch has been shown to contain about 100% carbohydrate, (Maclean, et al., 1978) and is well utilized by Rainbow Trout (Koops et al., 1974).

1.3 DIGESTIBILITY STUDIES

One of the earliest investigations in the digestion and utilization of feed by fish was carried out at the end of the last century by Kanuthe, (1898), (in Black et al., 1961). Since then, a great deal of research has been undertaken on the digestibility of various carbohydrates and proteins sources by fish. Further studies on the effect of dietary carbohydrate on the digestibility of fish feed have also been documented (Kitamikado and Tachino, 1960; Kitamikado et al., 1964; Singh and Nose, 1967).

1.3.1. CHROMIC OXIDE INDEX

The use of chromic oxide (Cr_2O_3) as an index substance in digestibility studies for fish was reported by Furakawa and Tsukahara in 1966. Since then a great deal of accurate determination using their technique for the digestibilities of dietary ingredients by fish has been carried out (Smith and Lovell, 1973; Windell et al., 1978 a, & b; Austreng , 1978). Some controversy as to the

accuracy of the chromic oxide index technique arose at the end of the last decade. Bowen (1978), outlined some precautionary measures to be taken and the pitfalls that could occur in digestibility studies when fish are fed with a high percentage of chromic oxide in the diet. Foltz (1979) criticised Bowen's recommendations though, later, Bowen (1979) stressed his trend of reasoning. However, from these and other investigations, to be discussed in subsequent parts of this chapter, a maximum of 1% chromic oxide in the test diet of fish is generally accepted as having no deleterious effects on the fish. Hence the incorporation of 0.5% of chromic oxide in the diets for this research is believed to be adequate.

1.3.2. DIGESTIBILITY AND UTILIZATION OF FEEDS

The rate of gastric evacuation has been used to speculate on gastric responses of Rainbow trout (Windell et al., 1969, a & b, 1972, 1978 a, & b). Using chromic oxide as an index substance, a number of fish digestibility studies have been carried out in recent times. Regardless of carbohydrate, lipid and energy contents of carp diets, the digestibilities of protein and carbohydrate have been shown to be constant at 95% and 85% respectively, for this fish (Takeuchi, et al., 1979 a). Increase of dietary lipid from 5% to 15% which led to an increase of digestible energy from 320 to 460 KCal/100g diet did not lead to better growth or Net Protein Utilization (NPU). However, when fed a diet containing less than 310 Kcal/100 g digestible energy, the values for both Protein Efficiency Ratio

(PER) and NPU in Carp were found to be significantly lowered (Takeuchi, et al., 1979b). These workers also reported that with a protein level of less than 23%, the growth rate and feed conversion of Carp was quite low. They also reported that increase in dietary cellulose led to a decrease in digestibility of energy.

Using an indirect method of protein digestibility estimation, Rychly and Spannhof (1978) showed that both true and apparent protein digestibility in Rainbow trout was over 97%. They stated that the digestibility of their whole diet decreased significantly with increasing the carbohydrate and decreasing the protein level in trout diet. The important effects of factors such as fish size, temperature and amount fed on nutrient digestibility by Rainbow trout (Windell, et al., 1978), and the adaptation of digestive enzymes in Roach and Rudd (Niederholzer & Hofer, 1979) has been elucidated. However, the cellulase activity detected in the gut of some carnivorus fish has been shown to be brought about by cellulase producing micro-organisms in the fish (Spataru 1978; Spararu & Korn 1978; Niederholzer & Hofer, 1979; Lindsay & Harris, 1980), and is probably the source of the cellulase reported in herbivorous fish and some other omnivorous cyprinids (Stickney & Shumway; 1974; Prejs & Blaszczyk, 1977; Mair, 1977; Dyke & Sutton, 1977). The rate of absorption of digested carbohydrates in yearling carp has been shown to depend on both their concentration in the gut and also on the type or molecular structure of the specific carbo-

hydrate (Erman, 1969). Thus; mannose and xylose were shown by Erman to be slowly absorbed than uronic acids, galactose and glucose. There is however, insufficient information on the coefficient of digestibility of specific diet ingredients (Hastings, 1967, 1969; Nose, 1967; Phillips, 1969; Smith & Lovell, 1971, 1973; Hastings & Dickie, 1972; Lee & Sinnhuber, 1972; Furukawa, 1976; Halver, 1976; Smith, 1971, 1976). Moreover, there is no available reference on tests of digestibilities of cassava, corn and rice in Rainbow trout and Mirror Carp fed different levels of these dietary ingredients in iso-nitrogenous diets. Rain bow trout and Mirror Carp are similar to the Alligator in not only requiring low energy for protein digestion (Smith et al., 1978) but also for amino acid absorption and high energy for protein synthesis, (Coulson & Hernandez, 1979), and if so, to what extent this could possibly affect carbohydrate utilization by these fish is problematical.

1.4. ASPECTS OF CARBOHYDRATE METABOLISM

1.4.1. CARBOHYDRATES IN FISH DIETS

Apart from the digestibility of dietary carbohydrate and the effect of dietary carbohydrate concentration on the digestibility of protein, some other aspects of carbohydrate metabolism in Rainbow trout and Mirror Carp have been investigated in the past. The extent of the abilities of the different species of fish to metabolize carbohydrate however, remains a controversial topic.

Claude Bernard (1876), was the first person to discover the relationship of liver glycogen to blood sugar and was the first to report on the glycogen content of fishes. Many workers have since then looked at various aspects of carbohydrate metabolism in fish (Kilborn & MacLeod, 1919; Macpherson, 1932; Cordier & Cordier, 1957; Ono & Nagayama, 1957). Phillips, et al., (1948) using glucose, maltose, sucrose, lactose, cooked corn starch and raw corn starch as dietary carbohydrate sources for trout reported high glycogen in livers of trout fed these carbohydrate diets, and this led to high mortalities. They then recommended a maximum of 9% and later between 9 - 12% (Phillips, et al., 1956) of dietary digestible carbohydrate for trout. Poor utilization of dietary carbohydrate by trout and the dependency of its metabolism on environmental temperature has been observed (Luquet, et al., 1975, 1976; Leger et al., 1975, 1976). Studies with chinook salmon have shown that this species of fish is capable of utilizing higher quantities of carbohydrate (Buhler & Halver, 1961). Comparable results have in recent times been obtained for Rainbow trout (Luquet, 1971; Furuichi & Yone, 1971, Lin, et al., 1977; 1979; Bergot, 1979).

Edwards, et al., (1977) and Austreng, et al., (1977), using diets which contained 17%, 25% and 38% of their digestible energy as carbohydrate, and which when analysed contained 31.9%, 35.5% and 43.6% respectively of digestible carbohydrate (NFE) showed the

best growth, condition factor and food conversion efficiencies in the group receiving 31.9% of NFE. They however, reported a healthy condition in all their fish. On the other hand, carcass weight gain in Rainbow trout was reported to be supressed by sucrose containing diets (Leger, et al., 1975; 1976).

The capability of Rainbow trout to utilize corn starch incorporated in diets has been reported (Koops, et al., 1974; Abel, et al., 1979). However, the diets used by Koops and his colleagues (1974) contained varying levels of crude fat, while that of Abel and his colleagues (1979) contained varying levels of protein. The values of corn starch recommended for Rainbow trout by these and some other workers could be misleading due to the fish utilizing the bulking agent or growth retardation brought about by unbalanced diets (Buhler & Halver, 1961, 1966). However, Abel and his colleagues (1979) reported that dietary starch, more than sucrose, promoted glucokinase activity and decreased phosphoenolpyruvate carboxylkinase in trout livers.

When compared to Rainbow trout much less work has been carried out on the ability of Mirror carp to utilize dietary carbohydrates.

Shimeno, et al., (1978), reported poor growth and feed efficiency in fish fed diets containing 10 - 20% digestible

carbohydrate when compared to the control fish receiving no carbohydrate. When compared to their control, carp receiving the carbohydrate diets contained less activity of gluconeogenic enzymes but higher activities of glycolytic and pentosephosphate cycle enzymes. Body, liver and blood constituents were otherwise similar in all groups. They also showed that the 40% carbohdyrate diet led to glucose intolerance, decrease in enzyme activities and digestibilities of carbohydrate and protein, which culminated in growth retardation. Such growth retardation and low feed efficiency was also observed with 40% dextrin in a carp diet by Furuichi and Yone (1979). Using different natural sources of carbohydrate, Svobodova(1976) showed that the type of feed influenced glycogen storage levels in carp tissue when fish received limited or basal quantities of feed.

The earlier observations of Buhler and Halver (1961) of the shortcomings in the preparation of the Salmonoid experimental diets led them to feeding chinook salmon with 36% casein-gelatin mixture which was supplemented with arginine and methionine, the indespensible amino acids. However, there might still be some essential amino acids for fish which were lecking in their purified protein source and which calls for the use of some better protein source for practical diets in nutritional studies. The protein source in such studies is indeed of great importance as the investigations in the effects of type and level of fat and/or protein in

test diets have shown (Phillips & Brockway, 1958; Phillips, et al., 1966; Sin, 1973 (a); Malevski, et al., 1974; Viola, 1977; Watanebe, 1977; Matty & Smith, 1978; Poston, et al., 1978; Reinitz, et al., 1978 (a) & (b); Ketola, 1978; Austreng, 1979; Cowey & Sargent, 1979; Yu & Sinnhuber, 1979; Tacon, 1979).

1.4.2. CARBOHYDRASES AND RELATED ENZYMES

Altered nutritional states in animals has been observed to evoke compensatory changes in systemic levels of several hormones (Klain, 1977). Klain's observation in the rat is similar to the situation in Salmon (Oncorhynchus kisutch (Walbaum)) (Lin, et al., 1977), though not exactly the same. Lin, et al., (1977) observed that a short term (less than six days) fasting did not affect the concentration of tissue carbohydrases. On fasting the Salmon for 23 days, the activities of fatty acid synthetase, citrate cleavage enzyme, malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase decreased. Changing the diet from a high-carbohydrate to a high-fat had only a minute influence on hepatic lipogenic enzyme activities, after seven to fourteen days. Long term study showed a depression in hepatic lipogenic enzyme activity resulting from a change from a high-carbohydrate to a high-fat diet.

The incidence of strong hyperglycaemia in carp when injected with mammalian glucagon has been observed by several

workers (Murat, 1976 b; Murat & Plisetskaya 1977; Murat et al., 1978). Murat (1976 b) also observed that 2U/100g of insulin injection induced a steep drop in liver glycogen of carp accompanied by an increase in α-glucosidase (γ-amylase) activity in the liver. However, it has been observed that when starved for a long period of time, carp maintained for several months a high glycogen level in tissues (Wittenberger &Vitca 1966; Murat, 1976 (a)). Tissue proteins in carp are readily metabolised and most probably supply most of the energy requirements during starvation (Gas, 1972; Creach & Serfaty, 1974). Besides, unlike in mammals, liver glycogen is not significantly affected during hyperglycaemia (Murat & Serfaty, 1975). However, in the eel (Anguilla anguilla), exogenous insulin, delivered intraperitoneally has been found to produce hypoglycaemia, depletion of hepatic glycogen and an initial increase in muscle glycogen formation (Lewander, et al., 1976). The exogenous insulin also affected lipid metabolism, but its effect on protein and plasma inorganic ions were only marginal. Glucose tolerance test conducted on red sea bream (Furuichi & Yone, 1971) tends also to support the impression that this animal and perhaps fish in general are inferior in carbohydrate utilization. Simon & Rosselin (1979) showed that intermittent feeding increases insulin sensitivity of target tissues and also modifies the β-cell sensitivity to glucose in the chicken. In all the work reported, the glucagon and insulin used in testing glucose tolerance in fish have been of mammalian origin, not of fish origin. The source could

probably affect the mode of action of the enzyme on fish tissue.

The key enzyme in carbohydrate breakdown in fish is α-amylase. This enzyme randomly attacks α-1, 4-glucosidic linkages which are prevalent in large glucose polymers. This enzyme has little or no effect on α-1, 6-linkages and is incapable of splitting β-1, 4 and β-1,-6-glucosidic linkages. The product of α-amylase breakdown which is maltose is further degraded in the fish by α-glucosidase which attacks mainly the α-1, 6-glucosidic linkages left by α-amylase (Hastrin, et al., 1955; Fishman, 1955; Bernfield, 1955; Barrington, 1962; Nagayama, et al., 1967; Nagayama & Saito, 1978). Other but less important carbohydrases have also been identified in Rainbow trout. (Nagayama & Saito, 1978).

The synthesis of glycogen in vitro by the action of purified enzyme was first achieved by Cori et al., (1939). The discovery in 1957 by Leloir and Cordine of an enzymatic activity in liver which catalyzes the transfer of glucose from UDP-D-glucose[1] to glycogen (α-glucan-UDP,D-glucose glucosyltransferase) changed the view on the role of phosphorylase in glycogen metabolism. The formation of α-1, 4-glucosidic bonds are much more favoured in fish by synthetase reaction than by phosphorylase reaction. Hence, since α-amylase attacks α-1, 4-glucosidic bonds in suitable

[1] UDP: Uridine-diphosphate.

situations, there is thermodynamic equilibrium between synthetase and α- amylase activities in the tissues. It is the equilibrium constant for this reaction that would govern the rate or extent of carbohydrate utilization.

The only known pathway for the synthesis of UDP-D-glucose is via the nucleotide sugar-pyrophosphorylase reaction :

$$UTP^{1} + \text{glucose-1-phosphate} \rightleftharpoons \text{UDP-D-glucose} + \text{pyrophosphate (PPi)}$$

$$PPi + H_2O \longrightarrow 2Pi \quad \text{(Pyruvate)}$$

This is the key reaction by which glucose enters the intermediate biosynthetic pathway to glycogen. This important reaction sequence was first discovered by Truco (1951) and was confirmed by Kalckar, et al., (1953).

Figure 1 gives a summary of tissue metabolism of carbohydrate (After-Shimeno, et al., 1978).

It is obvious that the isomerases are important in interconversions which in the long-run are consequential to gluconeogenesis or glycogenolysis.

---

[1] UTP : Uridine triphosphate

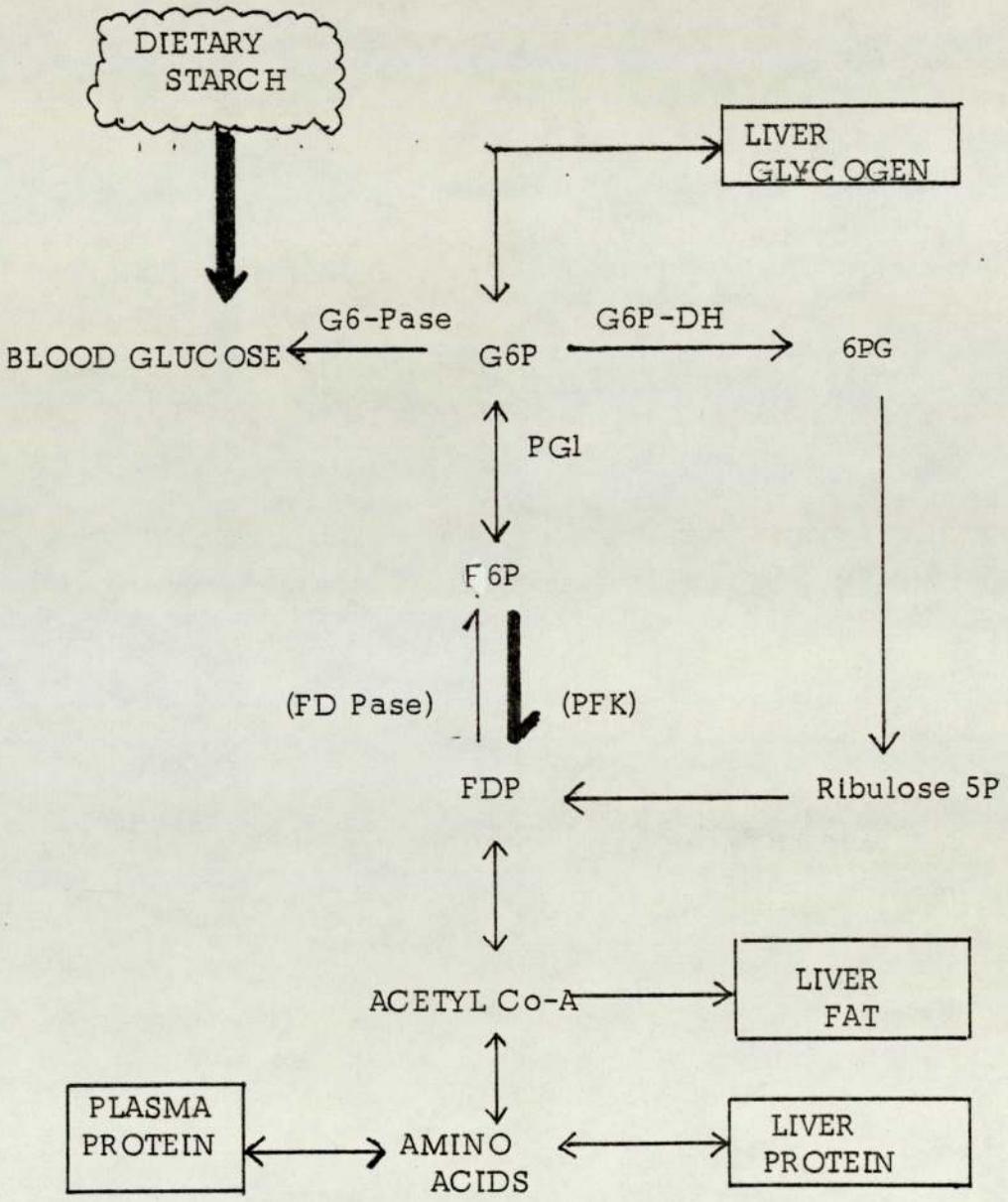


Fig. 1. METABOLIC ADAPTATION TO DIETARY STARCH IN FISH[1]
(After Shimeno et al., 1978)

1 KEY

1. G.6Pase: Glucose- 6 phosphatase
2. 6PG : 6-phosphogluconate
3. G6P : Glucose-6-phosphate
4. G6P DH : Glucose-6, phosphate dehydrogenase
5. F6P : Fructose - 6, phosphate
6. PGl : Phospho-glucoisomerase
7. PFK : Phospho-fructokinase
8. FD Pase : Fructose-diphosphatase
9. PGDH : Phospho-glucodehydrogenase

Besides D-glucose, it has been reported that D-galactose, D-glucosamine and D-fructose are transferred to glycogen (Peat, et al., 1952; Nordin & Hansen, 1963; Maley et al., 1966). Tissue synthesis of glycogen can be summarised by the reaction cascade illustrated in Fig.2.

Glycogen synthetase exists in 2 isomeric forms : the Independent or I form and the Dependent or D synthetase. The latter depends on glucose-6-phosphate for its activity while the former does not. Glucose-6-phosphate concentration thus affects the rate of glycogen deposition. The concentration of glucose-6-phosphate is affected by the presence of some bivalent ions such as Mg^{2+}. Physiological state of the fish such as stress also affect the glucose-6-phosphate concentration. The lethal concentration

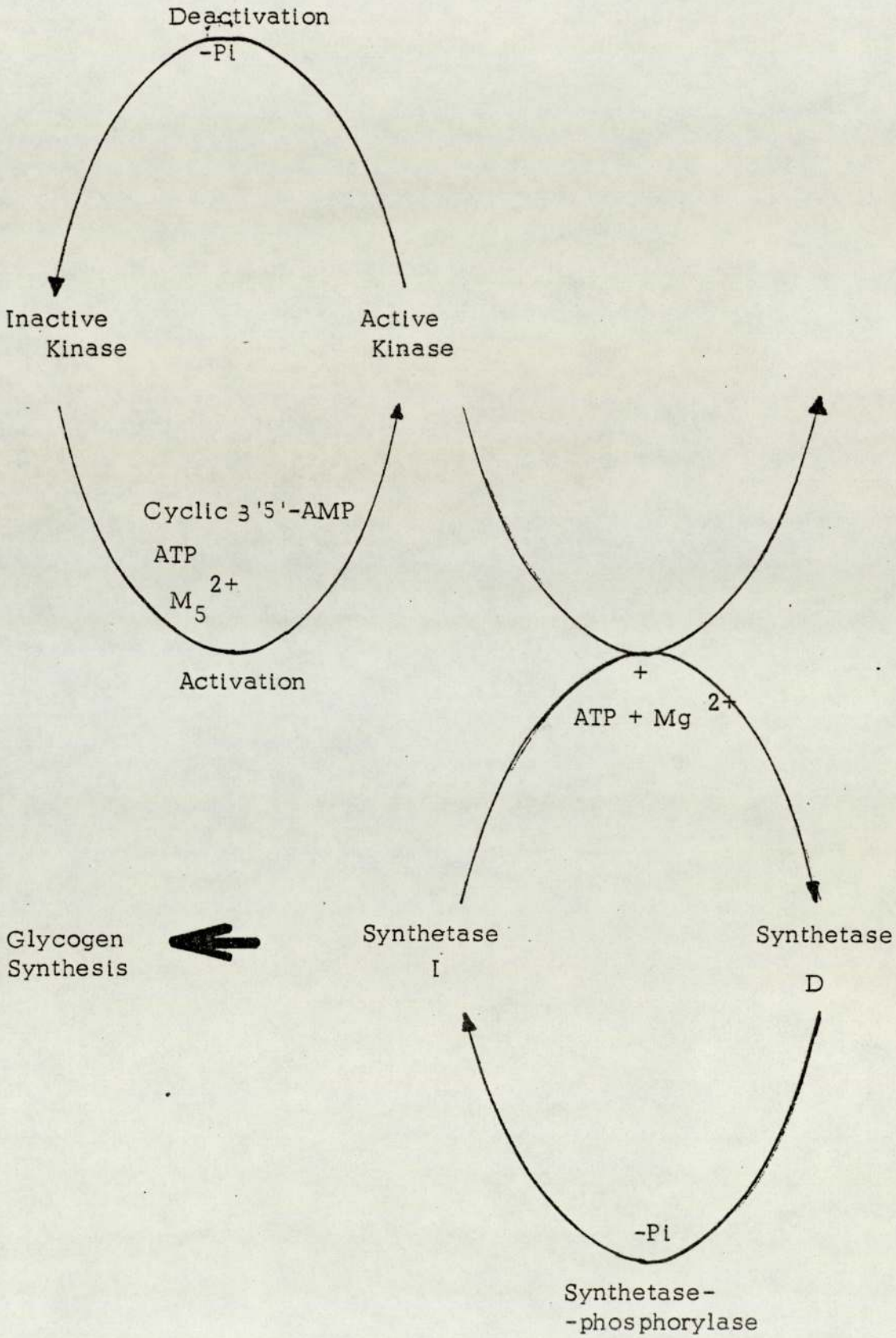


FIG.2. Reaction cascade for the activation of glycogen synthetase (After Helmreich & Cori, 1969)

of glucose-6-phosphate still needs to be established in Rainbow trout and Mirror carp before conclusive statements can be made on the utilisation of carbohydrate by these species.

Some studies on carbohydrase activity in fish tissues tend to suggest the presence of amylase and α-glucosidase in the digestive organs of Rainbow trout and Mirror carp, and the absence of α-galactosidase, β-galactosidase, β-h-fructosidase and cellulase (Kitamikado & Tachino, 1960). Similarly, the distribution of these carbohydrases has been observed in the other tissues and organs of fish (Shimeno et al., 1978); though Nagayama and Saito (1978) detected the presence of both α-amylase and α-glucosidase as well as β-glucosidase, β- galactosidase and β-glucouronidase in some parts of the digestive system of Rainbow trout, carp and some other cyprinids. Shimeno, et al., (1978) and Nagayama & Saito (1978) also observed the dependency of the enzyme activities on the level of dietary carbohydrate, higher carbohydrase activities occurring in carp than in trout. Shimeno et al., (1978) observed that there was higher activities of glycolytic and pentosephosphate cycle enzymes in the liver of fish receiving the carbohydrate diets. At dietary carbohydrate level of over 40% they noticed a drop in carbohydrate tolerance by the fish demonstrated by poor growth, decrease in some enzyme activities and digestibilities of carbohydrate and protein. Further implication of protein in carbohydrate metabolism in fish tissues has been

demonstrated by Raffin and Levay (1979) who discovered that the proteases are implicated in AMP *1 deaminase activation in the gill of Rainbow trout.

1.5. ECOSYSTEM, RATION AND GENETICS

Several environmental factors could affect the ability of Rainbow trout and Mirror carp to utilize dietary carbohydrate. Such factors include temperature, photoperiod, stocking density, water quality, ration size, feed size, feeding frequency, fish size and genetic imprint in the fish.

1.5.1. TEMPERATURE AND PHOTOPERIOD

Temperature is an important environmental factor that affects not only the survival of the fish but also its appetite. A lot of work has been done to determine the optimum temperature of Rainbow trout and Mirror carp; (Kwain, 1975; Hokanson, et al., 1977; Leatherland, et al., 1977; McCauley, et al., 1977; Kaya, 1978; Keading & Kaya, 1978; Muller-Feuga et al., 1978; Spigarelli & Thommes, 1979).

Under reciprocal (light/dark ratio opposite that of natural) photoperiod, higher ATPase [1] activity was observed than during

---

*1 AMP : Adenosine-5'-monophosphate

1 ATPase : Adrenosine triphosphatase

simulated natural photoperiods in Atlantic Salmon (Saunders & Henderson, 1978). The influence of temperature, season, and diet on amylase activity in Roach (Rutilus rutilus) and Rudd (Scardinius erythrophthalmus) has been investigated (Hofer, 1979 (a)).

Protease activity in Roach and Rudd has been shown to be controlled alternately by endogenous and by environmental factors (Hofer, 1979 (b)). From the vast literature surveyed on temperature effect on carbohydrate utilization by fish, from which only the most important have been cited, optimum temperature for best growth in Rainbow trout is around 12°C while that of Mirror carp is approximately 25°C.

1.5.2. WATER QUALITY

Poor water quality has adverse effect on the nutrition and metabolism of fish (Shireman, et al., 1977). Exposure of Rainbow trout to over 320 ppm lignosulphonates leads to significant suppression of amylase activities and growth performance (Roald, 1977). Bleached kraft pulp mill effluent has also been shown to have deleterious consequences on salmonoid fishes (McLeay & Brown, 1975; McLeay, 1977). Minimum oxygen concentration in water for normal life activities of grass carp is 4 mg/l (Shireman et al., 1977) and 70 - 80 mm Hg (45-55%) for carp (Itazawa & Ikeda, 1979).

1.5.3. FISH AND RATION SIZE AND FEEDING FREQUENCY

Knowledge of growth and food conversion ratios of fish of various sizes is vital in fish farming (Gerking, 1972; Brett & Shelbourn, 1975). The effect of size on the growth of fish fed ad libitum has received considerable attention and is reasonably well understood (Brown, 1946; Menzel, 1959; Pandian, 1967; Brett & Shelbourn, 1975; Hamada et al., 1975; Elliot, 1975(a)). On the contrary, the observed effect of size on fish fed restricted rations have been contradictory (Brown, 1946; Lee, 1969; Gerking, 1971; Kelso, 1972; Niimi & Beamish, 1974; Brett & Shelbourne, 1975; Elliot, 1975 b). Wurtsbaugh & Davis (1976) discovered that with rations near maintenance level, growth rate of Rainbow trout dropped with increase in temperature. With increase in feeding rate, temperature effect was ameliorated. Wurtsbaugh & Davis (1977) using Rainbow trout that weighed between 0.6 and 5.2 g also found out that when fish were kept at low ration levels, the growth rates and gross efficiencies of food conversion increased with increase in fish size. As ration level increased, the effect of fish size was ameliorated. At very high ration levels, (9-12% body weight/day), gross efficiency values for large fish began to drop. In addition to the effect of ration level on growth and feed efficiency, the variations in growth rate and feed efficiency brought about by differences in fish size, feeding frequency and age of Rainbow trout and carp have been documented (Paloheimo & Dickie, 1966; Kono &

Nose, 1971; Huisman, 1976; Weatherley, 1976). Similar studies on other fish species such as Winter Flounder, (Tyler & Dunn, 1976), and Juvinile Penaeus merguiensis De Man (Sedgwick) have yielded identical results with those of trout and carp. Thus, Tyler & Dunn (1976) found that with decrease in feeding frequency, winter flounder ate less food per month but more food per meal. This was an attempt by the fish to compensate for lower food supply available to the fish.

Method of feeding has also been shown to have some effect on the growth performance of fish. Thus Pfeffer (1976) showed that Rainbow trout fed by hand had better feed efficiency, higher growth rate and better energy utilization than their counterparts fed with the automatic feeding device. Losses of feed from the self-feeder most probably caused the inferior feed efficiency as Pfeffer observed.

1.5.4 POPULATION DENSITY AND GENETIC INHERITANCE

Two variables that could influence recruitment to fish stocks are food and population density, both of which could influence growth and fecundity, (Bagenal, 1971). In the Juvenile fathead minnows (Pimephales promelas), high population density appeared to limit growth and gamete development regardless of food abundance (Smith et al., 1977). Similar depression of growth at high stocking densities have been reported in Rainbow trout (Refstie, 1980; Kilambi, et al., 1979), channel catfish, (Kilambi, et al.,

1979), and grass carp (Shireman, et al., 1977). Stocking density and feed consumption rate were found to be correlated with oxygen levels, and when oxygen levels dropped below 4 mg/l, consumption dropped by approximately 40%. (Shireman, et al., 1977). The need therefore, arises in nutritional studies to maintain an oxygen level above the lethal shortage level, and to balance out the stocking density, water flow rate, tank size, feeding rate and level fed.

Even when the discussed environmental factors are taken into account, genetic factors have also been shown to influence the growth of Rainbow trout (Reinitz, et al., 1978); Refstle, 1980), and almost certainly Mirror carp. Variations in body composition (Reinitz, 1977; Reinitz, et al., 1979), in swimming speed (Ware, 1975), and in Thermal resistance (Kaya, 1978) have been shown to be associated with different genetic strains.

The conduction of growth experiments with fish of the same family, bred at the same time is essential to avoid errors that are inherent in using fish of different strains. This is difficult to implement in long term nutritional studies with limited tank facility.

1.6. NUTRITIONAL PATHOLOGY

A high proportion of the fish hepatic and haematological pathological

conditions have been attributed to nutritional annomalies.

1.6.1. HEPATIC AND OTHER ORGAN DAMAGE

The influence of nutrition on chemical carcinogenesis has been reviewed (Clayson, 1975). Dietary protein reportedly affects the toxicity and carcinogenicity of several chemical carcinogens in animals, possibly by altering the activities of enzymes involved in their activation and/or detoxification (McLean & Magee, 1970; Swann & McLean, 1971). Diets deficient in protein have been observed to increase the susceptibility of mammals to acute Aflatoxin - B_1 (AFB) toxicity and the induction of cancer. (McLean & Magee, 1970; Madhavan & Gopalan, 1965; Madhavan & Gopalan, 1968; Rogers & Newberene, 1971; Sisk & Carlton, 1972; Todd et al., 1968). Increase in dietary protein has been reported to have led to increase in the carcinogenic activity of AFB fed to rats (Madhavan & Gopalan, 1968), and Rainbow trout (Lee, et al., 1978), though an increase in good quality dietary protein level has been observed to result in Rainbow trout converting larger amounts of AFB to the non-toxic Aflatoxicol (Stott & Sinnhuber, 1978, 1979). Therefore, since the incidence of trout hepatoma is believed to be connected with the ration supplied (Halver, 1967; Halver & Mitchell 1967; Ghittino, 1976; Scarpelli, 1976; Loveland et al., 1978) the need for a high quality protein source to be incorporated into Rainbow trout and Mirror carp carbohydrate diets has to be satisfied. The inadequacy of using a purified protein source such as casein has

been demonstrated (Lee & Wales, 1973). Lee & Wales (1973) also emphasised the shortcomings inherent in nutritional research, in relying solely on growth as a criterion for acceptability of diets, with minimal attention being paid to the effects of diet on histology. Observed postmortem bile damage to Rainbow trout liver (Hendricks et al., 1976) could adultrate identifications of dietary heapatic disorders reported by some workers.

Apart from the nature of the diet, certain environmental factors such as temperature (Heidinger & Crawford, 1977) could affect the condition of the liver. Within a given feeding regime, Heidinger & Crawford (1979) found that increased temperature lowered the liver-somatic index of Rainbow trout.

Rancid diets which are deficient in Vitamins C and E are known to induce growth depression, microcytic anaemia and liver lipoid degeneration in Rainbow trout (Smith, 1979).

1.6.2. BLOOD CHEMISTRY

The incidence of hyperglycaemic conditions resulting from oral glucose administration to fish (Palmer & Ryman, 1972), and the production of a haemoconcentration, elevated blood lactate, increasedglucose concentrations and alterations in the plasma electrolyte balance in brackish- and freshwater pike, Esox leucius L., (Sotvoi& Oikori, 1976), by stress have been documented. Such

increases in blood glucose concentration resulting from decrease in glucose utilization and shifts in plasma metabolites has been observed in the fasting dog (Brady, et al., 1977).

There is no available data on the digestibilities of cassava, rice, corn and potato starch by Rainbow trout and Mirror carp. Though some work has been done on some carbohydrases of Rainbow trout and Mirror carp, mainly α - amylase and α-glucosidase, this area of carbohydrate metabolism is still not conclusively investigated. The findings on the availability of digestible carbohydrates in the diets of Rainbow trout and Mirror carp still do not conform with one another completely. The four crude carbohydrate sources investigated in this work are cheap when compared to protein or even to some other carbohydrate sources. If these carbohydrates could improve the growth of Rainbow trout and Mirror carp receiving a minimal quantity of protein, then they would be very useful in the fish-feed industry.

These experiments were therefore, designed to investigate

the ability of Rainbow trout and Mirror carp to digest and utilize dietary protein and carbohydrates when fed diets containing different levels of cassava, rice, corn and potato starch. A further set of experiments was also conducted to compare the tissue carbohydrase activities as well as the digestibilities and feed utilization in these two fish species when they are fed with diets containing different levels of dextrin.

CHAPTER 2

CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 EXPERIMENTAL FISH AND QUARANTINE PROCEDURE

Two fish species were employed during this investigation, Rainbow trout (Salmo gairdneri) and Mirror carp (Cyprinus carpio). Rainbow trout were obtained from fish farms within the West Midlands region of England.

The Mirror carp were obtained from supplies already present within Aston Fish Culture Unit, these fish having been produced by induced spawning of brood-stock of Mirror carp, Cyprinus carpio.

Fish which had been collected from sources other than the Aston Fish Culture Unit hatchery were subjected to a vigorous quarantine procedure prior to experimentation. The quarantine procedure consisted of a three-week close observation and treatment with specified drugs.

During the first 10 days in quarantine, fish were fed a commercial, trout diet (Edward Baker Limited, Sudbury, Essex), containing bifuran antibiotic (ICI Ltd), at a concentration of 1 tablet/[1] 5kg of fish. After the first 5 days, fish were flushed with 100 ppm of formalin. After the first 10 days, fish were flushed for 1 hour with a 2 ppm solution of malachite-green. This was repeated after 2 days, and again after a further 2 days. At the completion of the

[1] Each tablet contained units of bifurin activity.

quarantine period, fish were then transferred to their experimental facilities within the main hatchery building. All fish were allowed to acclimate for a period of one week during which time they were fed commercial trout diet. All through the 2 weeks of quarantine holding fish were fed 1% of their body weight per day, but were starved on the days when they were subjected to formalin or malachite green treatment.

2.2. EXPERIMENTAL TANKS

Four different arrangements of experimental tanks were employed during the course of this research.

2.2.1. SYSTEM 1

This consisted of 7 white plastic experimental tanks with an arrangement for water re-circulation (Plate 1). Each tank held 20 experimental fish and was continuously supplied with water from the header tank, through the inflow tap (Fig. 3) at the rate of 3 l/min.

Water, on leaving the experimental tanks flowed into a biological filter via two faecal traps. The faecal traps were cleaned every alternate day. Two Rainbow trout, each weighing approximately 100 g. were placed in the water space above the filter to act as scavenging sweepers. These fish helped keep the top of the filter free of faeces and algae.

The biological filter which had a total volume of 600 l contained approximately 400 l of gravel chips, supported on a corrugated plastic plate with a number of perforations. The filtered water was pumped from the bottom of the filter with a Beresford PV52 pump (James Beresford & Sons Limited, Birmingham) into the header tank at an approximate rate of 42 l/minute. The force with which this water was pumped onto the surface of the water in the header tank helped aerate the water. An in-line filter fitted between the biological filter and the pump acted as a sludge trap.

The header tank which consisted of a 300 l double layered fibre-glass tank was supplied with make-up water from a tap flowing at a rate of 300 ml/minute. This replaced any water lost through evaporation or splashing. From the header tank, water flowed at a rate of approximately 20 l/minute to the white tanks containing the experimental fish. The rate of flow to each experimental tank was regulated with clips at 3l/minute. Excess water from the header tank over-flowed into the biological filter.

The header tank was supplied with an immersion cooling coil, controlled from a central refrigeration unit. A contact thermometer that regulated the action of the cooling coil was immersed in the

PLATE 1 - Recirculating system used for Experiments 1,2,3, & 4

PLATE 2 - Recirculating system used for Experiments 5 & 6

faecal trap. The filter, header tank and lid for the header tank were of double-layered fibre-glass (Supaglass, White Lund, Morecambe) with a 2 cm. cavity filled with polyurethane foam.

The presence of a stand-pipe at the middle of each fish tank (Fig. 3) and the angle at which water was spurted into the fish-tanks from the inflow taps helped to circulate the water within the tanks and also aided the self-cleaning action of the tanks.

A number of water quality parameters were measured twice weekly throughout the experimental periods. The values recorded are shown in Table 2.

TABLE 2 WATER QUALITY RECORD OF "SYSTEM 1"

Temperature	$12.0 \pm 1.0\ ^{\circ}C$
Dissolved oxygen	> 6 mg/l
Total Nitrate/Nitrite	< 51.0 mg/l
Total Ammonia	< 0.1 mg/l
pH	6.8 - 7.2

The system described was used in conducting experiments 1 and 3.

2.2.2. SYSTEM 2

The plan of this system is essentially the same as that of experimental system 1 (2.2.1), the only difference being the temp-

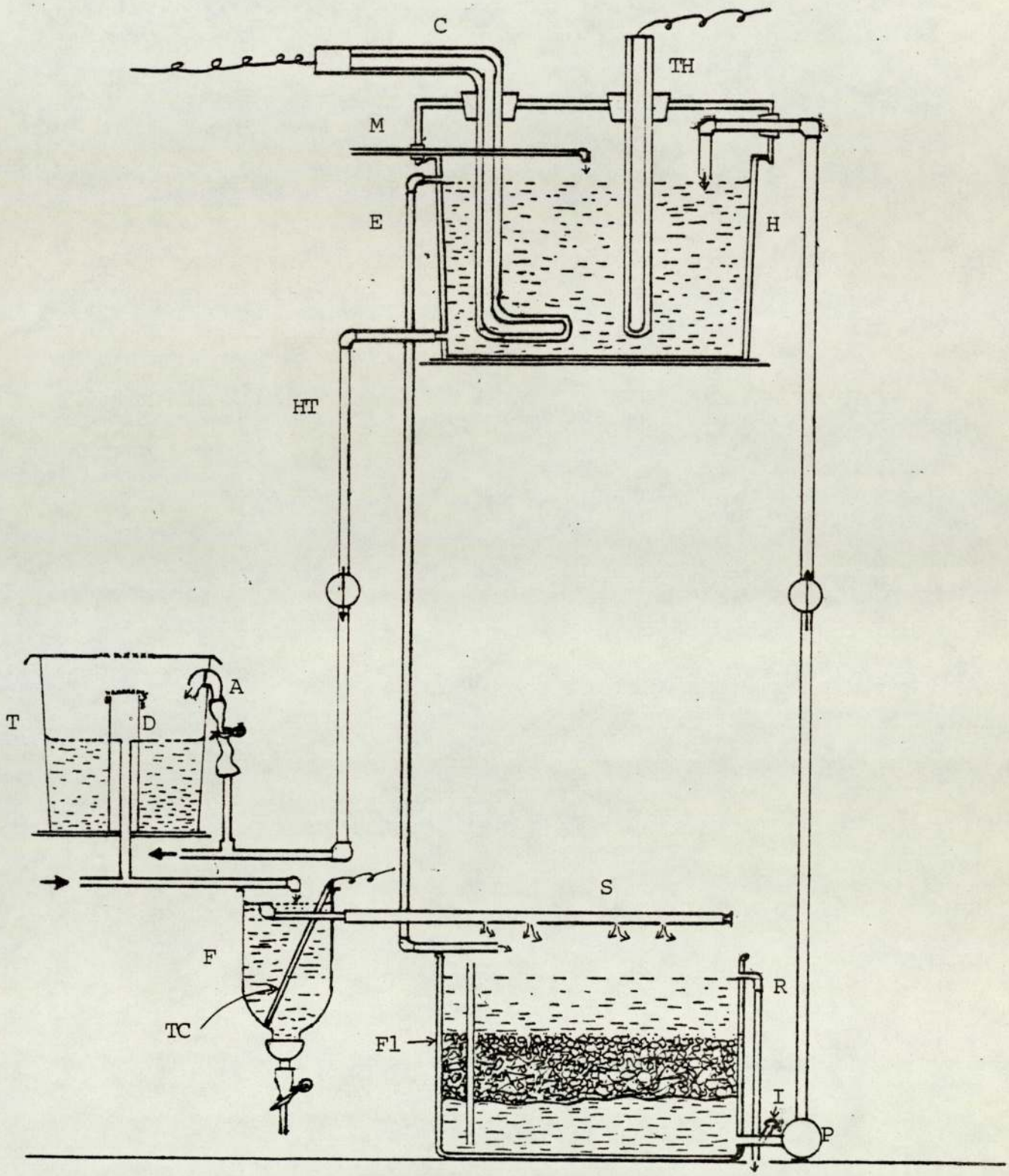


Fig. 3 Cross-sectional diagram of water circulation system used in the Experiments

A - Inflow Tap; D - Stand pipe; T - Fish tank; F - Faecal trap; TC - Thermocouple (for cooler); Fl - Filter; S - Spray pipe; R - Filter overflow pipe; I - Inline filter; P - Pump; H - Header tank; TH - Thermometer; C - Heating/Cooling coil; E - Header tank overflow pipe; HT - Flow pipe from header tank to fish tank ; M - Tap for make-up water Arrows indicate direction of water-flow

erature control device employed.

The header tank of "System 2" was fitted with a 3 Kw heater (Buntings Titanium Limited, West Bromwich) and a Gallenkamp contact thermometer (Gallenkamp & Co. Limited, London) graduated from 0 to 50°C. The heater was connected to the mains electric current supply via an electronic relay (EC 980, Gallenkamp & Co. Limited, London). The temperature of this system was set at 25°C. during the experimental period. Two Mirror carp, each weighing about 100g were placed within the filter to act as scavengers.

The water quality of this experimental system which was measured twice weekly is reproduced in Table 3.

TABLE 3 - WATER QUALITY RECORD OF "SYSTEM 2"

Temperature	25.0 ± 0.5°C
Dissolved oxygen	> 5 mg/l
Total Nitrate/Nitrite	< 70.0 mg/l
Total ammonia	< 0.1 mg/l
pH	6.9 - 7.3

"System 2" was used in conducting experiments 2 and 4.

2.2.3. SYSTEM 3

This experimental unit comprised of a 3 circular fibreglass tanks.

(Plate 2). Each tank held a constant volume of water, 250 l, and was continuously supplied with water from the header tank through the inflow tap (Fig. 3) at the rate of 5l/minute. Because of the high stocking density in the experiments with this system, the inflow tap was perforated so that the inflow water sprayed into the fibre-glass tanks, thereby aiding aeration. As in System 1, (2.2.1) faeces were collected in a faecal trap, and further aeration of water was enhanced by the "spray pipes" (Fig. 3).

The filter design and size was exactly the same as that described for System 1 (2.2.1). However, the pump (which was the same make as that described in Section 2.2.1) pumped water into the header tank from the bottom of the filter at a rate of 30.0 l/minute. The inline filter, header tank, and the cooling device were the same as described in Section 2.2.1. The rate of water flow from the header tank to the pipe supplying the 3 tanks was 15.0 l/minute and the rate of flow from the header tank via the header over-flow pipe into the filter was 15.0 l/minute. The flow-rate of the make-up water into the header tank was maintained at 500 ml/minute.

The temperature of this system was maintained at 12.0^{o}C. Water quality which was measured twice weekly is shown in Table 4.

This system was used for conducting experiment 5.

TABLE 4 - WATER QUALITY RECORD OF SYSTEM 3

Temperature	12.0 $\pm$ 1.0°C
Dissolved oxygen	$>$ 8 mg/l
Total Nitrate/Nitrite	$<$ 50 mg/l
Total Ammonia	$<$ 0.1 mg/l
pH	7.0 - 7.1

2.2.4. SYSTEM 4

This experimental system was similar to "System 3" (2.2.3). It differed from "System 3" in the following ways :

(i) Instead of a cooling coil and thermocouple, a 3Kw heater (Buntings Titanium Limited, West Bromwich) and the bulb of a Gallenkamp contact thermometer graduated from 0 - 50°C (Gallenkamp & Co. Limited, London) were immersed in it.

(ii) The water temperature was maintained at 25°C throughout the experimental period.

(iii) Two Mirror carp, each weighing about 120 g were placed in the filter to act as scavengers.

The water quality of System 4 which was measured twice weekly is shown in Table 5.

TABLE 5 WATER QUALITY RECORD OF SYSTEM 4

Temperature	25.0 ± 0.5°C
Dissolved Oxygen	> 5 mg/l
Total Nitrate/Nitrite	< 75 mg/l
Total ammonia	< 0.1 mg/l
pH	6.9 - 7.0

This system was employed in the conduction of Experiment 6.

2.3. DIET PREPARATION AND PRESERVATION

The crude carbohydrate sources used in this research, namely, cassava (from Malaya), rice (Overseas Trading Co. Bradford) corn (Sandhar & Kang Limited, Birmingham) and potato flour were milled in a Hobart hammer mill (Hobart Limited, London) where necessary. They were then analysed (Table 6). The potato was obtained in the form of semi-purified starch (Sigma Chem. Co. St. Louis, USA). Dextrin was obtained in the form of soluble powder (Sigma Chem. Co., St. Louis, USA) while the white fish-meal was obtained fresh from Edward Baker (Edward Baker Limited, Essex). The potato starch, dextrin and white fish meal were equally analysed for their food constituents (Table 6). The advantage of using "White" fish meal as opposed to herring meal was that its high protein content made it possible for a limited amount to be used in preparing the diets, thus leaving enough room for large quantities

TABLE 6 COMPOSITION OF CARBOHYDRATES AND FISHMEAL USED IN FORMULATING EXPERIMENTAL DIETS; AND OF THE COMMERICAL DIET (% weight)

Proximate Composition (mean values)	FOOD ITEMS						
	Cassava	Rice	Corn	Potato	Dextrin	White Fishmeal	Commercial Diet [1]
Moisture	9.54	10.85	8.11	14.12	10.11	8.60	8.37
Fat	1.90	1.29	2.99	2.98	0.49	9.50	8.64
Protein	3.56	5.85	4.50	1.13	1.89	68.10	44.10
Hydrolysable carbohydrate	74.45	73.99	74.85	75.09	76.95	0.35	31.72
Ash	3.81	0.73	0.58	0.66	2.54	13.50	6.94
Sub-total	93.26	92.71	91.03	93.98	91.98	100.05	99.77
Crude Fibre [2]	6.74	7.29	8.97	6.02	8.02	0.00	0.23

[1] From Edward Baker Limited, Sudbury, Essex.

[2] Obtained by substracting the sub-total from 100.

of carbohydrate to be added so that the carbohydrate effect could be readily observed.

The carbohydrates, caseinand fish-meal were accurately weighed, (correlations being made for their moisture contents), placed in the bowl of a Hobart A 200 (Hobart Limited, London) food mixer and thoroughly blended for three minutes. The mineral supplement (Table 7), Vitamin Premix (Table 8) (B.P. Nutrition) and oil were then added.

Blending was continued at low gear for a further 5 minutes. Water was carefully added while still whisking the mixture round in the bowl. When just the right amount of water (from experience) had been added, the Hobart food mixer was switched tnto high speed for 30 seconds to give a final homogenization. The semi-moist paste thus obtained was extruded through the mincer of the food mixer using either a 2mm or a 3 mm die (depending on the size of the experimental fish to which the diet would be fed. Adding just the right amount of water during mixing allows for pelletisation immediately after extrution. This also produced pellets that were neither too hard for the fish nor too soft to stay in one piece when dropped into the fish tank during feeding. The diets were dried in a cabinet for 24 hours with an electric convector fan heater which blew warm air at 40^{o}C over the diets.

The dried diets were sieved to eliminate granular pellets,

TABLE 7 COMPOSITION OF MINERAL SUPPLEMENT

(From Test diet U440 Western Fish Nutrition Laboratory (NAC, 1973))

A combination of :-

Premix No. 5 (mineral) - (grams)

Aluminium chloride	0.015
Potassium iodide	0.015
Cuprous chloride	0.010
Manganese sulphate	0.080
Cobalt chloride	0.100
Zinc sulphate	0.300

and Salt Mixture No. 2 (grams)

Calcium biphosphate	13.58
Calcium lactate	32.70
Ferric citrate	2.97
Magnesium sulphate	13.20
Potassium phosphate	23.98
Sodium biphosphate	8.70
Sodium chloride	4.35
TOTAL	100.00

TABLE 8 COMPOSITION OF VITAMIN PRE MIX [1]

(mg/Kg of premix)

Vitamin A	0.012 m.i.u.
Vitamin D_3	0.0015 m.i.u.
Vitamin E	60
Vitamin K	15
Thiamine	10
Riboflavin	25
Pyridoxine	15
Biotin	60
Vitamin B_{12}	2
Nicotinic acid	150
Folic acid	4
Pantothenic acid	50
Choline chloride	1130
Vitamin C	60
Iron	20
Cabalt	200
Manganese	30
Copper	200
Zinc	50
Iodine	4500
BHT (antioxidant)	1000

1. Supplied by B.P. Nutrition (No information is available concerning the salts employed.

while rather long pellets were all broken into pieces which were 5 mm. long. The diets which for each experiment were prepared just before the start of the experiment were then sampled for proximate analysis, and the rest stored in sealed polythene bags at -20°C for the experimental period. From this frozen stock, daily rations were weighed and utilized.

2.4. ANESTHESIA, MARKING AND WEIGHING OF FISH

A great deal of work has been carried out to investigate the suitability of tricaine methane sulphonate (MS-222 Sandoz) as a narcotic agent for fish (McFarland, 1959; Marking, 1967; Schoettger & Julin, 1967; McErlean & Kennedy, 1968; Smit , et al., 1977). Ferreira, et al., (1979) compared the anesthetic potency of MS-222 with that of benzocaine hydrochloride, and observed that the latter was more effective. MS-222 has been found to produce a linear increase in haematocrit values of Rainbow trout (Reinitz & Rix, 1977), as well as in carp and some other fish species (Smit , et al., 1979, a, b, & c). These workers also observed more stress in unanesthetized than in anesthetized fish. Rainbow trout were more susceptible to stress by MS-222 than carp. Smit et al., (1979, a, b & c), also found that neutralised MS-222 produced more hematological disorders in fish than did ordinary MS-222.

A comparative study of the effects of anaesthesia with MS-222, neutralised MS-222 and benzocaine on the blood constituents

of Rainbow trout (Soivio, et al., 1977) showed that benzocaine caused the least hypoglycaemia during anaesthesia, while neutral MS-222 produced the most drastic hypoglycaemia.

Since 2-amino-4-phenylthiazole, a piscine anesthetic, (Suzuki & Sekizawa, 1979) is relatively novel and has not yet received sufficient research attention, benzocaine which is cheaper than MS-222, and which produces the least blood glucose change in fish was therefore, employed in anaesthetizing each fish before handling.

Approximately 0.5 g of the benzocaine powder,(which is insoluble in water) was dissolved in 1 ml absolute alcohol. This was then washed into 5 l of water contained in a plastic bucket, and which was at the same temperature as that contained in the tank from which fish were being sampled. Fish were anaesthetized in this solution until they just stopped active movement, but were still breathing (Stage III, plan 2: Klontz and Smith, 1968).

Fish were individually marked before the onset of each experiment, so as to identify each fish and monitor its progress. The choice of marking device was carefully carried out so as to minimize stress , and errors inherent in some marking procedures.

Tagging of fish for identification has proved problemmatical because the fish either lost the tags or had post-tagging infections or mortalities (Dickie, 1963; Beckett, 1971; Winters, 1977). Hot

branding of fingerling channel catfish weighing below 27g resulted in the retardation of the growth rate of these fish (Joyce & El-Ibiary, 1977). No deleterious consequences were observed by Joyce and El-Ibiary (1977) when fish weighing over 27g were branded. Cold branding has however, been more successfully used. The use of freon (-40°C) (Brock, 1977), and liquid nitrogen (-196°C) (Gunnes & Refstie , 1980) for the cold-branding of fish has been documented. When freon was used brand signs were still legible after one year, while with liquid nitrogen, fish needed rebranding only after two years. In this research therefore, fish marking was conducted by branding the fish with an L-shaped chrome-plated rod with one of the points mounted on a wooden handle.

The metal rod which was 1 mm. in diameter was cooled in liquid nitrogen and then used to brand each fish for 3 seconds just behind the operculum and above the lateral line. The signs used to mark each individual fish (Appendix 1) were a slight modification of Joyce and El-Ibiary (1977) hot brand signs. A repitition of these signs on the opposite side of another set of 10 fish, or the use of more than one sign per fish made it possible to mark and individually identify all the fish.

Fish were individually weighed (while under the effec t of anaesthesia) at the beginning of each experiment, and at regular fortnightly intervals. This was carried out using a Satorius 3719

MP top-pan balance. Liver weight when required was measured after severing the intact gall bladder from the liver.

2.5. BLOOD SAMPLING

A 500 units/ml solution of heparin was prepared by dissolving a vial of 20,000 units heparin (Sigma Chem. Co. St. Louis, USA), in 40 ml of distilled water. This solution was used in rinsing a 2 ml Gillette syringe fitted with a Gillette sterile hypodermic needle (Sigma Co., St. Louis, USA). The drop of heparin solution adhering unto the needle and syringe was sufficient to prevent blood clotting and was small enough to produce only negligible dilution of the blood sample. Blood was obtained by puncturing the cuverian duct as described by Lied, et al., (1975). The blood was then gently ejected from the syringe into an LP3 test-tube (Sigma Co., St. Louis, USA), centrifuged and the plasma withdrawn immediately for assay.

2.6. FAECAL SAMPLING

Faeces was collected from each anesthetized fish. Faecal collection was carried out by gently stripping the rectal region of the fish antero-posteriorlly (Windell, et al., 1978b). Faeces from all fish on each diet were pooled fortnightly, dried at 105^{o}C for 24 hours and stored in air-tight bottles for subsequent chemical analysis.

2.7 CHEMICAL ANALYSES

Problems arise in the storage of fish blood and other tissues for subsequent glycogen or glucose content estimation. At low temperatures the blood of Rainbow trout was found to be more stable than at high temperatures (Nomura & Kawatsu, 1977). The substrates in the blood are unaffected by temperature but the enzymes are affected (Warner, et al., 1978).

During frozen storage, minute enzymatic activities still take place in fish blood, and frozen sample can keep for only about 3 weeks (Reinitz, 1976). However, blood storage in liquid nitrogen (-196^{o}C) improved the longevity of the blood, resulting in upto 90% recovery of cod erythrocyte stored for 18 months (Chao & Birkbeck, 1978). For maximum accuracy, blood, liver and muscle samples were therefore analysed fresh immediately after sampling for carbohydrates and carbohydrases where appropriate.

2.7.1. MOISTURE

The moisture content of diets, fish carcass, liver and faeces were determined by drying the samples in an oven pre-set at 105^{o}C for 24 hours. Cutting the fish open enhanced drying.

2.7.2. SUGARS AND HYDROLYSABLE CARBOHYDRATES

The glucose content of the plasma was estimated within 30 minutes of sampling to avoid errors due to deterioration during cold

storage (Renitz, 1976; Nomura & Kawatsu, 1977). Glucose was estimated at 37°C with Beckmans Glucose Analyser, (Beckmans Instrument Inc., USA) using Beckmans Glucose oxidase reagent (Beckmans Instruments Inc., Ireland), and 10 Ul aliquot of sample. A glucose standard containing 150 mg/100 ml of glucose in water (Beckmans Instruments Inc., Ireland) was used to standardize the machine.

The Association of Official Analytical Chemists (AOAC) method for carbohydrate determination (AOAC, 1975) is lengthy and indirect. The Somogyi micro copper colorimetric and titrimetric procedures, (Hodge & Davis, 1952; Hodge & Hofreiter, 1962) are equally cumbersome. The automated method for determining the carbohydrate in foodstuffs gave values which were less than those obtained when using the manual method (Hudson, et al., 1976). The rapid colorimetric method described by Sims (1978) which is a modification of the phenolsulphuric acid reagents method is recent and its reliability has not been confirmed. As a result of the simplicity, accuracy and availability of equipment the method of Murat & Serfaty (1974) which involved the splitting of the α-1, 4- and α-1, 6-glucosidic bonds in starch and glycogen to give glucose was used. The enzyme employed for the hydrolysis was amyloglucosidase (glucoamylase, α-1, 4-glucohydrolase) from Rhizopus sp (Sigma Chem.Co., St. Louis, USA). The glucose released was then estimated with a Beckmans Glucose Oxidase Machine as described

above for blood sugar. For conformity (Johnson et al., 1976) red muscle sample was taken from the same location on the dorsal aspect of the pectoral region of each fish that was analysed. This method gave results 5 to 10% higher than the classic KOH-ethanol method (Murat & Serfaty, 1974) perhaps due to more thorough hydrolysis.

For the determination of hydrolysable carbohydrate content of dry samples, (diets and faeces), a slight modification of the method of Murat & Serfaty (1974) was employed. This involved using between 10 and 20 mg of sample and correspondingly smaller quantities of buffer and enzyme. No sodium fluoride was added as this was considered unnecessary.

2.7.3. CRUDE FAT

Total fat was determined by the soxhlet petroleum ether extraction method (AOAC, 1975). Using the mean of the loss in weight of the extraction thimbles and contents, and the gain in weight of the extraction flask, as the fat-content of the sample, was discovered to be a more accurate approximation than relying only on the gain in weight of the extraction flask.

2.7.4. KJELDAHL PROTEIN

Crude protein was determined by the micro-kjeldahl method for determination of total nitrogen (AOAC, 1975). The factor, 6.25 was used to multiply the total nitrogen to obtain total crude protein.

2.7.5. TOTAL ASH

Ash content was determined by ashing known quantities of samples contained in procelian crucibles in a muffle furnace (Gallenkamp Instruments Inc.,) at 450°C for 24 hours.

2.7.6. CRUDE FIBRE

This was determined by substracting total ash, protein, crude fat, digestible carbohydrate and moisture from 100.

2.7.7. CHROMIC OXIDE CONTENTS

Chromic oxide contents of diets and faeces was determined using the wet acid digestion technique of Furukawa and Tsukahara, (1966).

2.7.8. TOTAL ENERGY IN DIET

The gross energy of each diet was determined by burning a known weight of the dry diet in a Gallenkamp Ballistic Bomb Calorimeter (Gallenkamp Instr. Limited, England) which was previously standardised by burning 1 g of benzoic acid (BDH Chemicals Limited). The energy in the diet was expressed as kilojoules per gram of diet.

2.8. CARBOHYDRASE ACTIVITIES IN TISSUES

The activities of two of the key enzymes generally accepted to be affected by the nature of the fish diet (Nagayama & Saito, 1978) namely, α-amylase and α-glucosidase were investigated. The tissues surveyed for the enzyme activities were the dorsal muscle, liver, fore-gut and hind-gut and blood.

2.8.1. α-AMYLASE The amylase activity was determined by a process based on the colorimetric analysis of maltose liberated from starch (Bernfeld, 1955).

The gut used was slit open and the contents rinsed out with 1% saline solution from a wash-bottle. In Trout, the fore-gut was severed from the hind-gut just posterior to the digestive caecae. In Carp, the point of severing was at the first "V" fold of the alimentary canal.

A known weight of tissue was homogenized in 5 ml of 0.02M phosphate buffer, pH 6.9 (prepared by mixing 50 ml of 0.02 M solution of sodium hydrogen orthophosphate and 40.91 ml of 0.02M potassium dihydrogen orthophosphate). 5 ml of 1% potato starch (Sigma Chem. Co., St. Louis, USA) solution was added rapidly to the homogenate, and the reaction mixture was incubated in a water bath at 37°C for 30 minutes (with constant shaking). Reaction was stopped by adding 10 ml of amylocolor reagent (made by dissolving 10 g of 3,5-dinitrosalycylic acid and then 300g Rochelle salt in 200 ml of 2N sodium hydroxide at 30°C, and diluting the solution to 1 litre). The mixture was then placed in a boiling water bath for 5 minutes, and cooledunder running cold tap water. The resultant precipitate was filtered off, and the optical density of the filtrate was read at 540mU against a blank prepared by mixing 5 ml of tissue homogenate with 10 ml of amylo-color reagent before adding 5 ml of 1% starch solution, heating in a boiling water bath for 5 minutes and filtering. Using a standard curve the enzyme activity was expressed in U moles of maltose liberated per minute per gramme of moist tissue at 37°C.

2.8.2. α-GLUCOSIDASE

The substrate used for α-glucosidase activity was 0.01M p-nitrophenyl-α-D-glucoside. The method employed is a slight modification of that of Hestrin, et al., (1955), for β-galactosidase. A known weight of tissue was homogenised in a mixture of 3.5 ml of 0.07 M acetate buffer pH, 45 (prepared by mixing 114 ml and 86 ml of 0.07M solutions of acetic acid and sodium acetate, respectively), and 1 ml distilled water. To the tissue homogenate, 0.5 ml of 0.01 M freshly prepared substrate solution was added and the reaction mixture was incubated in a water bath at 37°C for 30 minutes with constant shaking. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid (TCA). The precipitate was filtered and 4 ml of the filtrate was mixed with 1 ml of 0.5M Na_2CO_3 in 0.5N NaOH solution. A blank was prepared in much the same way except that the TCA was added to the tissue homogenate before the addition of the substrate. The optical density was then read against the blank at 420 MU. The enzyme activity was expressed, using a standard curve as U-moles of p-nitrophenol liberated per minute per gramme of moist tissue, at 37°C.

2.8.3. STANDARD CURVES

A calibration curve for α-amylase activity in the tissues was constructed by dissolving 0 - 20 mg of maltose from potato starch (Sigma Co. Ltd, St. Louis, USA) in 10 ml distilled water

and adding 10 ml amylo-color reagent. This was heated in a boiling water bath for 5 minutes and the colours developed were read against the sample with zero mg maltose as blank. A curve of concentration of the maltose solutions against spectrophotometric readings was then plotted.

Using aliquots of a solution of 10 mg of p-nitrophenol dissolved in 10 ml of distilled water, solutions of p-nitrophenol containing between 0.02 mg/10 ml and 0.10 mg/10 ml were made up. To 4 ml of each of these solutions, 1 ml of 0.5M Na_2CO_3 in 0.5N NaOH was added, and the optical densities read at 420 mU, using distilled water as standard. A calibration curve was then plotted with the readings obtained for α-glucosidase activity.

All spectrophotometric readings for these enzyme experiments and for the chromic oxide estimation in diets and faeces were made with a Linear-Readout Ultraviolet spectrophotometer (Cecil Instruments CE272).

2.9 HISTOCHEMISTRY

Histological preparations of the liver were made using the standard histochemical procedure for Haematoxylin-Eosin staining as detailed by Culling (1963). Medium melting point (57°C) wax was used for embedding the tissue which were sectioned at 5U using a rotary microtome. Only representative sections were photographed.

2.10 ANALYSIS OF EXPERIMENTAL DATA

2.10.1. WEIGHT GAIN (%)

This was computed as the mean weight gain per tank during the experimental period.

2.10.2. SPECIFIC GROWTH RATE (S.G.R)

In the early stages of the life of a fish, growth under controlled conditions follows an exponential curve expressed by the equation :

$$W_2 = W_1 \cdot e^{g(T-t)}$$

Where W_1 is the initial weight (gram at time t) and W_2 is the final weight at time T; e is the base of natural logarithms and g is the specific growth rate (SGR).

Rearranging,

$$SGR(\%/day) = \frac{\log_e W_2 - \log_e W_1}{T - t} \times 100$$ (Brown, 1957).

2.10.3. PROTEIN EFFICIENCY RATIO (PER)

This is the efficiency with which fish utilize dietary protein, and is defined by the equation

$$PER = \frac{\text{Weight gain (g wet fish)}}{\text{Crude protein consumed (g)}}$$

(Osborne et al., 1919)

2.10.4 APPARENT NET PROTEIN UTILIZATION (NPU)

Protein Efficiency Ratio (PER) is a fair but not an absolutely reliable way of describing the efficiency of protein utilization by fish. This is because the assumption that the fish are of identical composition at the end of the experiment is made in deriving it. The efficiency of deposition of protein as body tissue, termed Net Protein Utilization (NPU) is a more adequate way of assessing the efficiency of protein utilization. As no correction was made for endogenous nitrogen losses in this research, the results are expressed as Apparent NPU (Bender & Miller 1953; Miller & Bender 1955). This is expressed by the formula :

Apparent NPU (%)

$$= \frac{\text{Carcass protein at end - Carcass protein at beginning}}{\text{protein fed}} \times 100$$

2.10.5. FOOD CONVERTION RATIO (FCR)

This was calculated using the formula :

$$\frac{\text{Weight of food consumed per fortnight (g)}}{\text{Weight gained by fish per fortnight (g)}}$$

2.10.6. HEPATO-SOMATIC INDEX (HSI)

The formula used for this was

$$\frac{\text{Weight of liver minus gall bladder}}{\text{Weight of whole fish (including liver)}} \times 100$$

2.10.7. PERCENTAGE DIGESTIBILITIES

Apparent digestibilities were computed using the formula of Maynard and Loosli (1969);

$$\% \text{ Apparent Digestibility} = 100 - \left[100 \times \frac{CD}{CF} \times \frac{NF}{ND}\right]$$

Where CD = Percentage Cr_2O_3 in diet

CF = Percentage Cr_2O_3 in faeces

NF = Percentage nutrient in faeces

and ND = Percentage nutrient in diet

2.10.8. BLOOD AND TISSUE GLUCOSE AND GLYCOGEN

Blood glucose is expressed as mg/100 ml. Total tissue glucose and glycogen is expressed as a percentage of the weight of tissue used. This is easily obtained using the simple formula :

$$G = \frac{Vr}{W}$$

Where V = Volume of citrate buffer used

r = Glucose-oxidase machine reading

W = Weight of tissue homogenized (mg)

and G = Percentage glucose/glycogen.

Using this same formula, percentage carbohydrate in diets and faeces were computed.

2.10.9 STATISTICAL METHODS

Statistical comparisons between means were made by multiple analysis of variance using Duncan's Multiple Range F-Test (Duncan, 1955). Standard Errors of Means ($\pm$ SEM) were calculated from the residual mean square in the analysis of variance.

CHAPTER 3

CHAPTER 3

EXPERIMENT ONE

THE UTILIZATION OF DIFFERENT LEVELS OF DIETARY CASSAVA AND RICE BY RAINBOW TROUT FED ISONITROGENOUS DIETS

3.1. INTRODUCTION

Cassava and rice probably constitute the major source of dietary carbohydrate in most tropical countries, and West Africa in particular. Unfortunately, some strains of cassava contain a cyanide toxin bound to glucose molecules as cyanogenic-glucoside, (Ketiku, & Onyenuga, 1968). However, the toxin can be readily removed mainly by Leuconostoc and to a lesser extent, yeasts and the bacterium, Corynebacterium species by fermentation during processing (Collard, 1963; Akinrele, 1964: Okafor, 1977).

A number of carbohydrates have been used in experimental trout rations. These include mainly monosaccharides and disaccharides. Refined starch as well as crude starch containing feedstuffs have been used in experimental trout diets, but the incidence of hyperglycaemia has occurred predominantly in situations where the animal is fed a diet containing simple sugars.

Investigations with high and low protein rice (MacLean, et al., 1978) has shown that weaning children are able to digest and utilize this carbohydrate food material. Since cassava, corn and rice are relatively

cheap carbohydrate sources, (Table 1) and since there is no available information on the ability of Rainbow trout to utilize them, there was the need to find out the possibility of using these carbohydrate sources in the manufacture of trout diets.

3.2. MATERIALS AND METHODS

3.2.1. EXPERIMENTAL ANIMALS AND SYSTEM

The 140 fingerling Rainbow trout used for this experiment were obtained from Midland Fisheries, Nailsworth, Gloucestershire. Fish were 28 - 36 g in weight, and 13 - 15 cm. in length. The Rainbow trout were subjected to quarantine treatment as described in Chapter 2.1, and were then distributed equally among the 7 tanks of experimental "System 1", (Chapter 2.2.1). Fish were branded with liquid nitrogen (Chapter 2.4) and were then given one week to acclimatize, during which time they were fed 2% of their body weight twice daily on commercial (Edward Baker, Sudbury, Essex) diet. Post-mortem examination of any fish that died during the experiment was conducted to preclude or conclude death possibly caused by diet.

3.2.2. PREPARATION OF EXPERIMENTAL DIETS

The cassava used in this experiment was a non-toxic variety obtained as dried chips from Malaysia. The rice was a long-grain prefluff (Overseas Trading Co., Bradford) packeted rice obtained

TABLE 9 COMPOSITION OF DIETS FOR EXPERIMENT I (g/100g diet)

COMPONENTS	DIETS DESIGNATIONS						
	CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7 (Control)
Cassava	10.00	20.00	30.00	–	–	—	–
Rice	–	–	–	10.00	20.00	30.00	–
White fish meal	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Casein	10.00	10.00	10.00	10.00	10.00	10.00	10.00
α- cellulose	20.00	10.00	–	20.00	10.00	—	30.00
Mineral mix [1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix [2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cod-liver oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Carboxy methyl cellulose (Binder)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
TOTALS	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] Composition given on Table 7

[2] Composition given on Table 8

locally. The cassava and rice were milled separately in a hammer mill, analysed (Table 6) and used for diet formulation. (Chapter 2.3) (Table 9). The diets were analysed as detailed in Chapter 2.7, results of the analyses being represented in Table 10.

3.2.3. FEEDING

In the present study, fish were fed 2% of their body weight twice daily at 10.00 a.m and 6.00 p.m. All fish showed good appetite, and the feeding period for each tank usually lasted for less than 5 minutes.

3.2.4. WEIGHING AND SAMPLING

Fish were weighed fortnightly (2.4), during which time faeces was obtained (2.6). Fish were not fed on the weighing day, weighing being carried out between 8.00 a.m. and 10.00 a.m. At the end of the ten week experimental feeding period, ten fish were withdrawn randomly from each tank. Each of these fish was sampled for blood (2.5) and their livers were excised and weighed for calculation of the Hepato-somatic Index (2.4). Five fish were analysed fresh for muscle and liver glucose and glycogen content (2.7.2). Each of the remaining five fish sampled was then analysed using the procedure described in Chapter 2.7.

3.2.5. HISTOCHEMISTRY

Histological preparations of the liver were made and studied

TABLE 10 PROXIMATE COMPOSITION OF EXPERIMENT 1 TEST DIETS FROM BIOCHEMICAL ASSAY (% weight)

INGREDIENTS	DIETS						
	CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7 (control)
Moisture	5.49	5.95	6.26	5.39	6.48	6.72	5.04
Protein	41.76	41.35	41.63	43.46	43.57	42.85	41.34
Fat	12.28	12.32	12.09	12.25	11.97	11.97	10.61
Carbohydrate [1]	11.15	18.25	26.62	9.18	19.51	26.01	1.34
Ash	9.86	10.02	10.38	9.01	9.07	9.41	8.76
Sub-totals	80.54	87.89	96.98	79.29	90.60	96.96	67.09
Fibre [2]	19.46	12.11	3.02	20.71	9.40	3.04	32.91
Cr_2O_3 [3]	0.50	0.49	0.51	0.53	0.52	0.53	0.50
Energy [4]	20.49	21.55	24.12	20.42	21.00	23.11	18.28

1. Hydrolysable carbohydrate
2. Computed as difference between subtotal and 100
3. Presented on dry-weight basis
4. Kilojoules/gram

as detailed in Chapter 2.9.

3.2.6. ANALYSES OF EXPERIMENTAL DATA AND STATISTICAL METHODS

These were performed as discussed in Chapter 2.10.

3.3. RESULTS

Although it was ensured that all fish consumed their feeds, the fish on zero or low carbohydrate diets were rather reluctant to feed. Fish on this ration were observed to be less active than their counterparts taking moderate to high dietary carbohydrate. Mortalities recorded during the experiment are shown in Table 12. Postmortem examination of all dead fish showed that death was due to an attack of Saproleagenales spp fungus.

3.3.1. GROWTH PERFORMANCE

The growth of Rainbow trout fed 0 - 30% cassava and rice are shown respectively in Figures 4 and 5. Comparison of the growth performance of the fish fed diets containing cassava with the control shows that the fish receiving 20% dietary cassava showed the fastest growth response. A 30% dietary cassava inclusion level significantly ($p < 0.05$) decreased the growth rate of the fish. However, 10% dietary cassava did not produce a significant ($p > 0.05$) difference in growth rate when compared to the control.

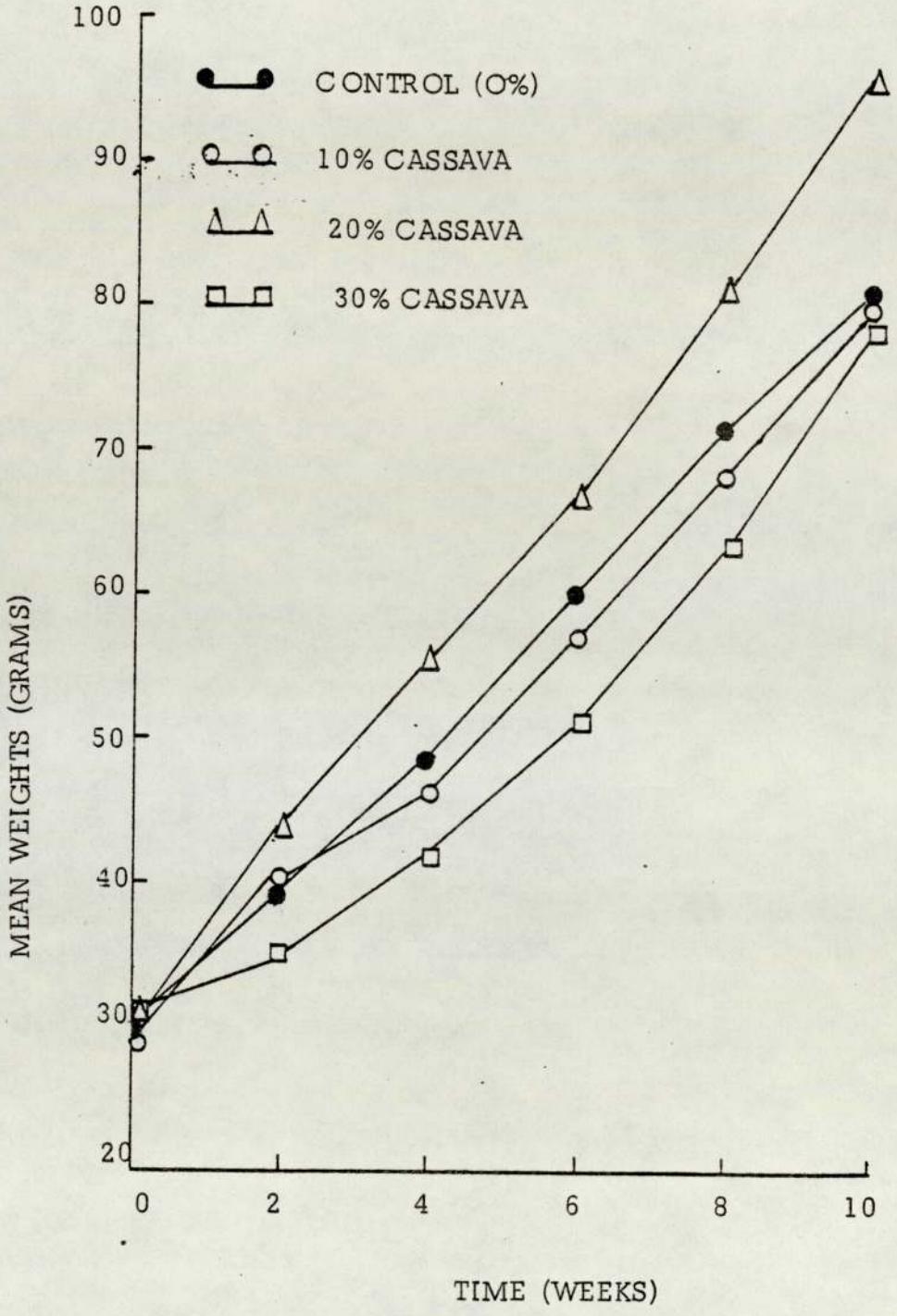


FIG. 4 Effect of different levels of dietary cassava on weight gain of Rainbow Trout

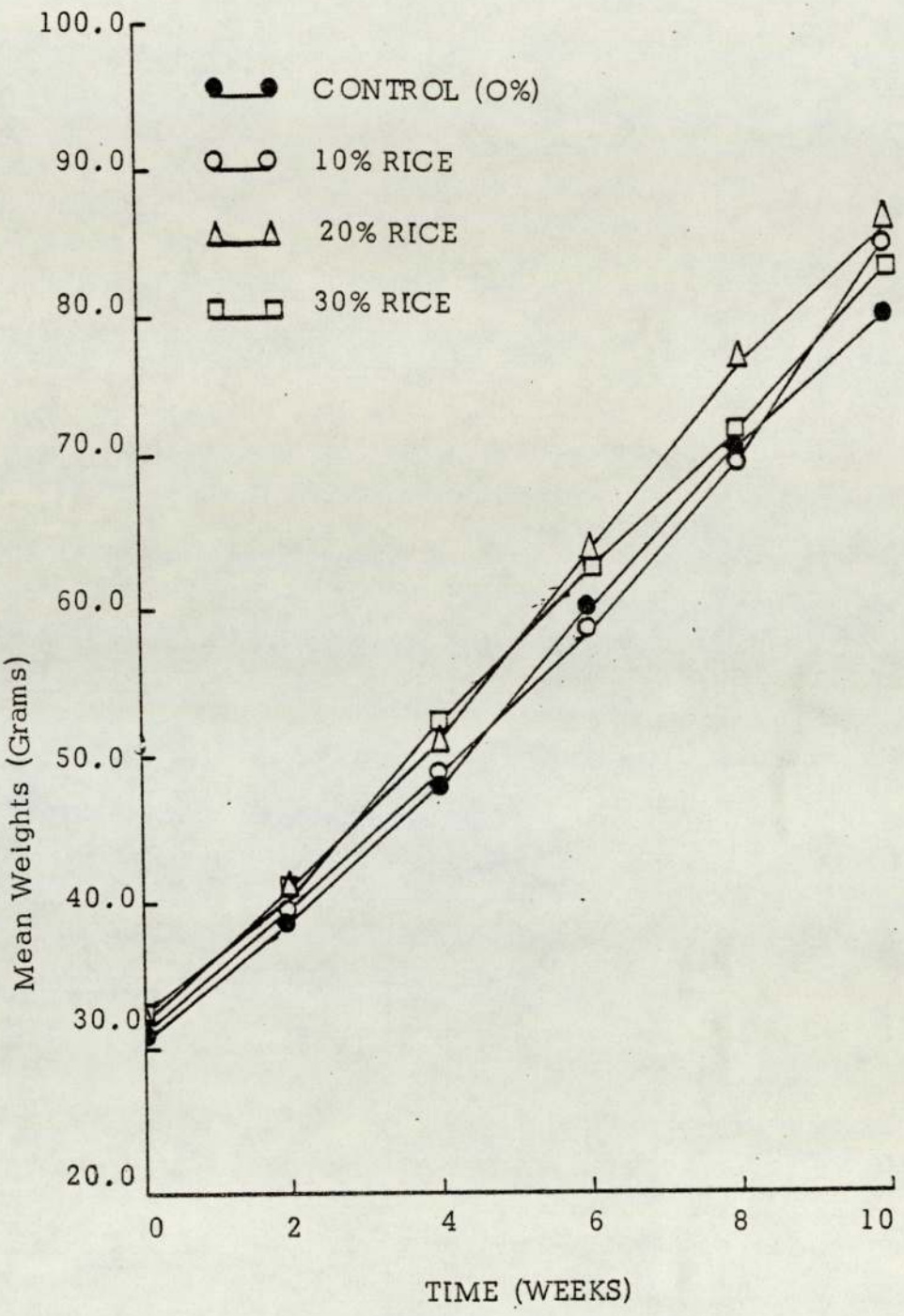


FIG.5 Effect of different levels of dietary Rice on weight gain of Rainbow Trout

TABLE 11 GROWTH AND FOOD UTILIZATION OF RAINBOW TROUT FED DIFFERENT LEVELS OF DIETARY CASSAVA AND RICE FOR 10 WEEKS

MEAN VALUES	DIETS							
	CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7	± SEM
Initial Weight (g)	28.75[a]	29.63[a]	31.06[a]	31.32[a]	32.17[a]	32.58[a]	30.86[a]	2.370
Final weight (g)	79.92[ab]	95.78[d]	78.86[a]	84.97[c]	85.88[c]	83.37[c]	80.12[b]	2.029
Weight gain (%)	177.80[d]	224.00[e]	138.60[a]	163.11[c]	151.24[b]	141.41[a]	142.85[a]	1.268
S.G.R.[1] (%)	1.12[a]	1.34[d]	1.36[d]	1.13[a]	1.23[c]	1.18[b]	1.24[c]	0.033
F.C.R.[2]	1.44[d]	1.14[a]	1.42[d]	1.35[c]	1.24[b]	1.30[bc]	1.27[b]	0.037
P.E.R.[3]	1.69[a]	2.00[d]	2.03[d]	1.74[b]	1.84[c]	1.78[b]	2.04[d]	0.028
Apparent NPU[4] (%)	34.60[b]	43.71[c]	35.95[b]	37.80[b]	35.62[b]	29.72[a]	46.10[c]	1.085

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

1. Specific growth rate 2. Food Conversion Ratio 3. Protein Efficiency Ratio
4. Net Protein Utilization

Upto the sixth week, there was no significant difference ($p>0.05$) between the weights of the fish receiving the rice containing diets. At the tenth week, the weight of the fish on 20% rice-containing diet was significantly ($p<0.05$) greater than those of the other groups.

The best percentage weight gain occurs in fish receiving 20% cassava while the best specific growth rate (S.G.R) occurs in the groups receiving 20% and 30% cassava. There is no significant difference ($p>0.05$) between the daily weight gain in the fish on 30% cassava, 30% rice and the control group. These groups of fish also showed the lowest daily weight gain while the lowest S.G.R. is observed in the fish receiving 10% cassava or rice. (Table 11)

3.3.2. FOOD CONVERSION

The Food Conversion Ratios (F.C.R's) were good for all fish, (Table 11), the best F.C.R. being obtained with 20% dietary cassava. The poorest F.C.R. was obtained at the 10% and 30% cassava, inclusion level (there was no significant difference ($p>0.05$) between these treatments). Similarly the F.C.R. of fish receiving 10% and 30% rice were not significantly different ($p>0.05$).

3.3.3. PROTEIN UTILIZATION

The Protein Efficiency Ratio (PER) which is an indication of the efficiency or the ability of the fish to utilize dietary protein was

TABLE 12 PERFORMANCE OF RAINBOW TROUT FROM EXPERIMENT 1

	INITIAL	FINAL							
		CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	CC-7 (Control)	± SEM
Blood Glucose (mg %)	59.90 [b]	57.90 [b]	65.10 [c]	68.70 [c]	56.70 [b]	60.90 [b]	66.90 [c]	36.00 [a]	1.817
H.S.I [1]	1.58 [c]	1.43 [a]	1.61 [c]	1.81 [d]	1.60 [c]	1.40 [a]	1.61 [c]	1.48 [b]	0.016
% Moisture in faeces	-	83.94 [a]	85.79 [a]	84.54 [a]	84.56 [a]	82.44 [a]	83.73 [a]	82.69 [a]	2.405
Gross Mortalities [2]	-	1	1	3	2	1	1	2	

Figures in the same row having the same superscript are not significantly different ($p>0.05$)

[1] Hepato-somatic index

[2] All due to fungal infection

computed with the method described in Chapter 2.10.3. The fish receiving 20% cassava, and 30% cassava and the control group show no significant difference ($p > 0.05$) in their P.E.R's.

Apparent Net Protein Utilization (NPU) which is regarded as a better indication of the ability of the fish to utilize protein was calculated as shown in Chapter 2.10.4. (Table 11). The control fish showed the highest Apparent N.P.U. This is probably because this group of fish received only negligible quantities of digestible dietary carbohydrate, and thus had to utilize their protein maximally. After the control group, the fish on 20% cassava ranked next in Apparent N.P.U. value. No significant difference ($p > 0.05$) is observed in the Apparent N.P.U. of fish on 10% and 30% cassava and 20% rice, and the poorest Apparent N.P.U. occurs in the fish on 30% rice.

3.3.4. PATHOLOGY

The major parameters used in predicting the health condition of the fish were blood, liver and faeces conditions.

The blood shows a slight increase in glucose concentration with increase in dietary glucose level. (Table 12). The control

fish showed acute hypoglycaemia ($p < 0.05$) and this probably accounted for their poor disposition as observed above (3.3).

Fish fed 10% cassava and 20% rice as well as the control group show a significant decrease in Hepato-somatic Index (H.S.I), ($p<0.05$) when compared to the initial HSI values at the start of the experiment. The reason for this decrease in HSI of the fish fed 20% rice containing diet cannot be readily explained. However, the lower values obtained for the group on 10% cassava and the control group was probably due to relatively lower quantities of glucose and glycogen accumulation in their livers (Table 12). The relatively high HSI obtained for fish on 30% cassava is probably due to high liver glycogen resulting from high digestible carbohydrate diet.

Faecal moisture content showed no significant difference. All the faeces showed good consistency, and there was no evidence of diarrhoea resulting from the carbohydrate diets.

Histological examination of the livers showed no acute hepatomegaly, necrosis, excessive vacuolation or proliferation of parenchymal cells. (Plates 3 & 4).

3.3.5. CARCASS AND LIVER COMPOSITION

There is no significant difference between the moisture

TABLE 13 INITIAL AND FINAL CARCASS COMPOSITION OF RAINBOW TROUT FROM EXPERIMENT 1

(% wet weight basis)

	INITIAL (%)	FINAL (%)							
		CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7 (Control)	± SEM
Moisture	73.20[a]	74.15[a]	73.19[a]	73.86[a]	73.98[a]	73.19[a]	75.14[a]	71.34[a]	0.862
Carbohydrate[1]	0.70[b]	0.72[b]	0.72[b]	0.87[c]	0.74[b]	0.74[b]	0.65[b]	0.46[a]	0.038
Fat	4.85[a]	5.08[a]	4.87[a]	4.96[a]	4.24[a]	4.97[a]	4.74[a]	4.85[a]	0.184
Protein	18.45[c]	17.61[a]	19.17[e]	18.15[b]	18.69[d]	18.92[d]	17.40[a]	20.59[f]	0.080
Ash	2.86[a]	2.54[a]	2.39[a]	2.30[a]	2.56[a]	2.40[a]	2.25[a]	2.72[a]	0.569
Totals	100.06	100.10	100.34	100.14	100.21	100.22	100.18	99.96	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Total hydrolysable carbohydrate

TABLE 14 INITIAL AND FINAL COMPOSITION OF LIVER OF RAINBOW TROUT FROM EXPERIMENT 1

(% wet weight basis)

	INITIAL (%)	FINAL (%)							
		CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7	± SEM
Moisture	75.50^{a}	76.53^{a}	75.56^{a}	75.61^{a}	77.68^{a}	76.51^{a}	77.89^{a}	74.55^{a}	0.561
Carbohydrate [1]	7.80^{d}	6.02^{b}	6.95^{c}	7.80^{d}	5.59^{a}	7.50^{d}	6.80^{c}	5.55^{a}	0.093
Fat	0.65^{b}	0.94^{d}	0.89^{cd}	0.61^{ab}	0.83^{c}	0.66^{b}	0.54^{a}	0.93^{cd}	0.095
Protein	14.26^{a}	14.59^{a}	14.31^{a}	13.91^{a}	14.90^{a}	13.82^{a}	13.26^{a}	14.52^{a}	0.495
Ash	1.55^{a}	1.95^{a}	1.51^{a}	2.88^{b}	1.76^{a}	1.62^{a}	2.59^{b}	3.78^{c}	0.025
Totals	99.76	100.03	99.22	100.81	100.76	100.11	101.08	99.33	

Figures in the same row having the same superscripts are not significantly different ($p > 0.05$)

[1] Total glucose and glycogen

contents of the carcass and liver of fish receiving diets containing cassava or rice (Table 13 and 14). The control group however, contained less carcass and liver moisture. The carcass of the control group had a higher protein content than the other groups while the liver of the control contained more ash than each of the other groups. Inclusion of 30% cassava in the diet of the fish is seen to increase muscle glucose and glycogen level significantly while the control group had significantly lower muscle glucose and glycogen ($p < 0.05$). In the liver however, increase in dietary digestible carbohydrate level increased glycogen deposition, although the much reduced level of digestible carbohydrate in the control group caused only a slight decrease in liver glycogen. The carcass fat and ash contents of all groups do not vary significantly ($p > 0.05$). The liver fat however, shows erratic variations which might have been due to experimental errors resulting from some accidents with the fat extraction paper thimbles.

The ash contents of liver in the groups receiving 10% or 20% cassava or rice and that of the initial fish sample are not significantly different ($p > 0.05$). The almost total lack of dietary carbohydrate however, led to increase in liver ash content of the control group.

3.3.6. NUTRIENT DIGESTIBILITY

The digestibility of dietary carbohydrate (Fig. 6) is approximately 80% when fish are fed 10% or 20% cassava-containing diets. At 30% dietary cassava, the digestibility of dietary carbohydrate is significantly decreased ($p < 0.05$). The digestibility of dietary carbohydrate when fish are fed rice-containing diets is significantly poorer ($p < 0.05$) than when fish are fed on cassava containing diets. However, change in the level of dietary rice between 10% and 30% does not seem to produce any significant change in the digestibility of carbohydrate in Rainbow trout ($p > 0.05$). Lack of cassava or rice in the control group led to very low digestibility of dietary carbohydrate which was almost lacking in the diet of this group.

Apart from the group receiving 10% cassava which showed slightly lower digestibility of dietary protein, there appears to be no significant difference ($p > 0.05$) between protein digestibilities in all the other six groups of fish (Fig. 7). Protein digestibilities in all groups appear good, and range between approximately $82\% \pm 1.0\%$ and $87.5\% \pm 1.5\%$.

3.4. DISCUSSION AND CONCLUSIONS

It is seen from this study that Rainbow trout (Salmo gairdneri) is capable of tolerating limited amounts of cassava and rice in their

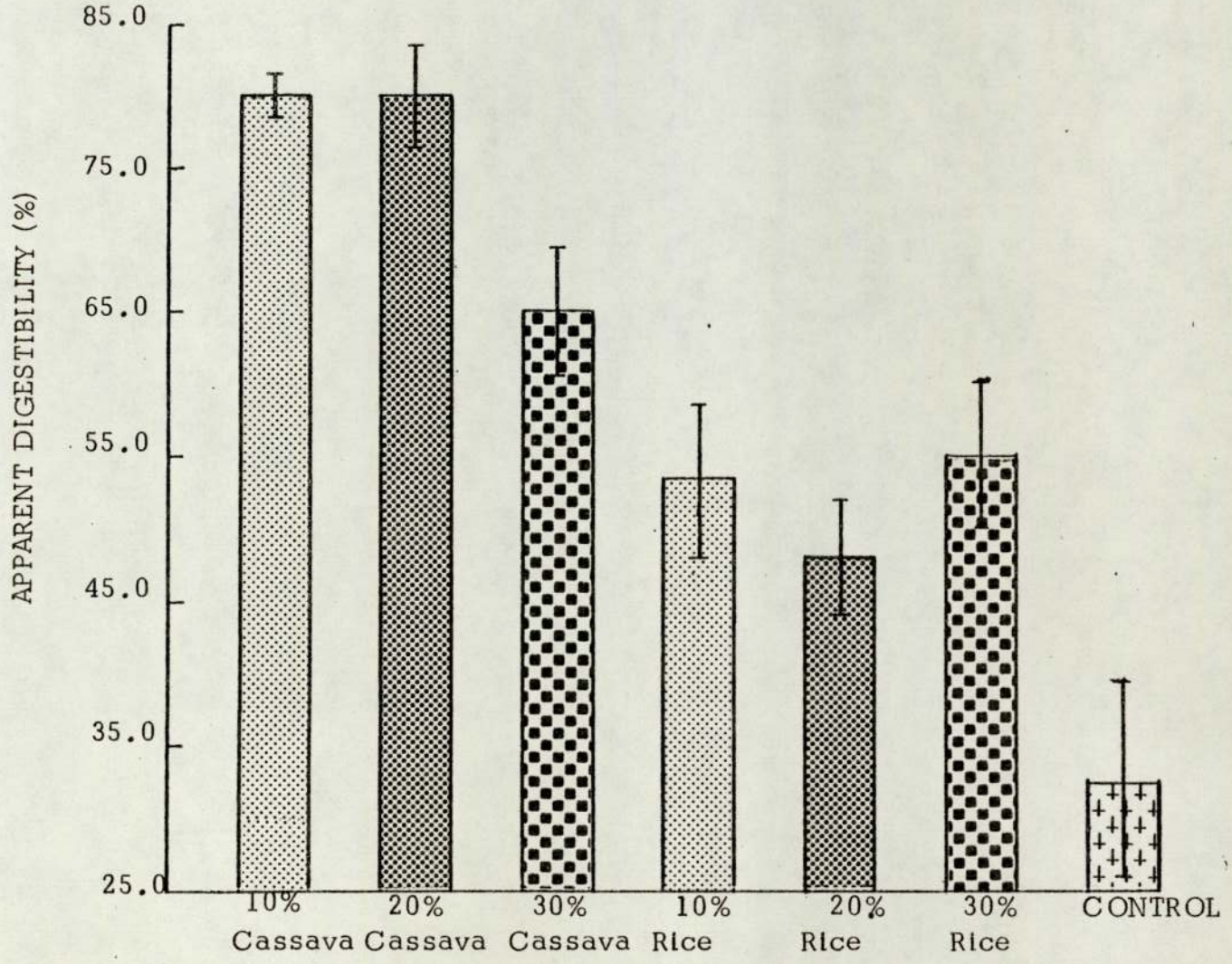


Fig. 6 Apparent digestibility of dietary carbohydrate in Rainbow trout from Experiment 1

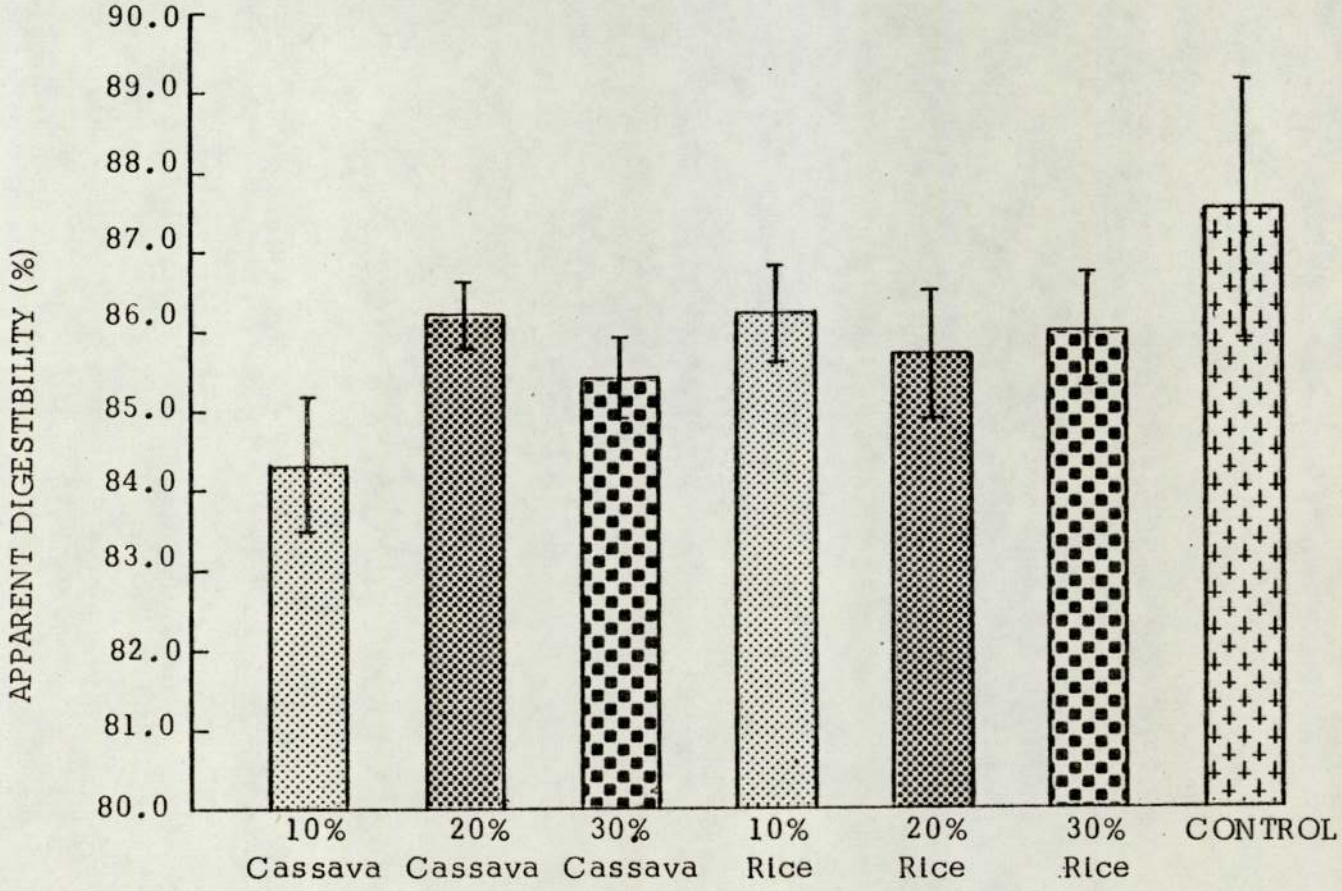


Fig. 7 Apparent digestibility of dietary protein in Rainbow Trout from Experiment 1

diets. The inclusion of 20% cassava in the diet of Rainbow trout led to faster growth, a higher Specific Growth Rate, lower Food Conversion Ratio and good Apparent Net Protein Utilization. As shown on Table 10, this diet contained 18.25% digestible carbohydrate, from proximate biochemical analysis. Phillip et al., (1948) suggested that about 9% digestible carbohydrate, but not more than that, was well tolerated by Rainbow trout. However, Bergot (1979) showed that under certain conditions, 30% dietary glucose produced better growth, feed and protein efficiency. As observed by Furuichi and Yone (1971) work on the utilization of carbohydrate by fish tend to give different results wit different workers. In this investigation, though many growth and food utilization parameters are affected by carbohydrate source, carcass composition, however, did not appear to be greatly affected by the carbohydrate source employed. Similar observations have been made by previous workers using other sources of carbohydrate (Furuichi & Yone, 1971; Edwards, et al., (1977)).

However, the chemical composition of the carcass has been observed to vary with the level of dietary carbohydrate (Austreng, et al., 1977), lower fat but higher protein and ash content being obtained in the carcass of fish receiving the higher carbohydrate diets. In this investigation however, there is no significant difference between the fat contents of the carcass ($p > 0.05$), and the highest level of tissue protein is recorded in the fish receiving 0% and 25% dietary cassava and rice. The observations of Austreng and his colleagues (1977) could probably be affected by the source

of dietary protein they used, (Capelin meal, torula yeast, soya-bean meal and wheat meal). In this work white fish meal which contained over 70% protein and possibly had a better essential amino acid profile for a carnivorous fish like Rainbow trout was used.

Low Hepato-somatic Index (H.S.I) was obtained in the fish receiving low cassava and rice diets. This is possibly indicative of poor growth as was concluded by Furuichi and Yone (1971). Austreng, et al., (1977), observed larger and discoloured livers in Rainbow trout receiving very high carbohydrate diets. In this work, the high H.S.I. obtained in the fish on 30% cassava or rice coupled with the rather high liver glycogen and glucose in their group shows that this level of cassava or rice is not well utilized by Rainbow trout. The corresponding low values of H.S.I. and liver glucose and glycogen in the fish on 10% cassava or rice appears to call for digestible carbohydrate over 10% in the diet of Rainbow trout.

From the results, 30% cassava or rice diets tend to produce poor growth, despite the fact that these diets have higher energy values than the rest. Since the fish receiving no cassava or rice did not have significantly ($p > 0.05$) lower growth rates than the group on 10% cassava or rice, it would appear that a threshold value of dietary digestible carbohydrate is required to trigger off the enzymes necessary for the catabolism of carbohydrate in Rainbow trout.

It is concluded from this study that the utilization of dietary carbohydrate by Rainbow trout depends not only on the level of the carbohydrate and energy content in the diet, but also on the carbohydrate source; perhaps on the nature of the glycosidic linkages. On the basis of this experiment the enzymatic metabolism of dietary and tissue carbohydrates is possibly at its optimum at 20% dietary cassava or rice, though cassava appears to be better utilized at this level than rice by Rainbow trout.

Subsequent experiments were therefore designed to test the ability of a tropical teleost, Mirror carp, to utilize these tropical carbohydrate sources, cassava and rice, and also to test the availability of corn and potatoe starch, which are present in both the tropics and the temperature regions, to Rainbow trout (Salmo gairdneri) and Mirror carp, (Cyprinus carpio).

CHAPTER 4

CHAPTER 4

EXPERIMENT TWO

THE UTILIZATION OF DIFFERENT LEVELS OF DIETARY CASSAVA AND RICE BY MIRROR CARP FED ISONITROGENOUS DIETS

4.1. INTRODUCTION

The importance of carbohydrate in the diets of carnivorous fish has been discussed (Chapter 1.5.1).

Carp have been shown to utilize higher levels of dietary carbohydrate than trout (Nagayama & Saito, 1978).

As with Rainbow trout, the majority of experiments on the utilization of carbohydrate by Carp have been conducted using refined carbohydrate sources. In spite of the relative abundance of cassava and rice in tropical countries, there is no available record of their use in the formulation of Carp diets.

Since the incorporation of 20% dietary cassava into the diet of Rainbow trout improved growth while the presence of rice between 0% and 30% in the diet of Rainbow trout did not seem to produce any significant difference in the growth rate, this research was carried out to see the extent to which Mirror Carp are capable of utilizing dietary cassava and rice for growth.

4.2. MATERIALS AND METHODS

4.2.1. EXPERIMENTAL ANIMALS AND SYSTEM

For this experiment, 140 Mirror carp spawned from the brood stock in Aston Fish Culture Unit were used. At the start of the experiment, fish weighed 4.0 - 4.3 g and measured 4.5 - 6.5 cm. Each of the fish tanks of system 2 (2.2.2) was stocked with 20 fish after the fish had been branded (2.4). The fish were given one week to acclimate during which time they were fed 5% of their body weight daily on a commercial diet (Edward Baker, Sudbury, Essex).

Post-mortem examination was conducted on the fish that died during the course of the experiment to find out the possible cause of death.

4.2.2. PREPARATION OF EXPERIMENTAL DIETS

The same cassava and rice used in Experiment One (3.2.2) were employed in the preparation of Mirror Carp diets. High protein white fishmeal (Table 6) was employed in the preparation of isonitrogenous diets (2.3) containing 0 - 45% cassava or rice (Table 15). This was then biochemically analysed (2.7), the results of the analyses being shown in Table 16.

TABLE 15 COMPOSITION OF DIETS FOR EXPERIMENT 2 (g/100 g)

INGREDIENTS	DIET DESIGNATIONS						
	CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7 (Control)
Cassava	15.00	30.00	45.00	-	-	-	-
Rice	-	-	-	15.00	30.00	45.00	-
White fish meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Casein	10.00	10.00	10.00	10.00	10.00	10.00	10.00
α-cellulose	30.00	15.00	-	30.00	15.00	-	45.00
Mineral mix [1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix [2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cod-liver oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Carboxy-methyl cellulose (binder)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
TOTALS	100.00	100.00	100.00	100.00	100.00	100.00	100.00

1 Composition given on Table 7

2 Composition given on Table 8

TABLE 16 PROXIMATE COMPOSITION OF EXPERIMENT 2 TEST DIETS FROM BIOCHEMICAL ASSAY (% weight)

COMPONENTS	DIETS						
	CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7
Moisture	4.32	6.22	6.53	6.48	7.04	7.42	8.70
Protein	30.35	28.72	30.67	30.48	30.50	28.36	27.97
Fat	11.55	11.51	11.87	11.43	11.96	11.50	11.32
Carbohydrate [1]	14.74	25.64	39.10	16.55	30.60	44.50	3.08
Ash	6.38	8.97	9.48	6.93	8.67	6.69	7.07
Sub totals	67.34	81.06	97.65	71.87	88.77	98.47	58.14
Fibre [2]	32.66	18.94	2.35	28.13	11.23	1.53	41.86
Cr_2O_3 [3]	0.50	0.49	0.51	0.49	0.50	0.50	0.51
Energy [4]	18.11	19.45	20.88	18.00	18.95	20.45	17.01

1 Hydrolysable carbohydrate

2 Computed as difference between subtotal and 100

3 Presented on dry weight basis

4 Kilojoules/gram

4.2.3. FEEDING

Fish were fed 5% of their body weight five times daily at 9.00 a.m., 11.30 a.m., 2.00 p.m., 4.30 p.m., and 7.00 p.m. Carp were found to be much slower feeders than Rainbow trout. Feeding period for each tank usually lasted for at least twenty-minutes, fish being fed small amounts of feed at a time until all the ration was consumed. Fish were found to have good appetite, and less than 1% mortality was recorded.

4.2.4. WEIGHING AND SAMPLING

Fish were individually weighed at the start of the experiment during which time five fish were sampled for analyses. Fish were thereafter weighed fortnightly (2.4) during which time faeces were collected (2.6). Because of the small size of the fish, only a small quantity of faeces could be collected during each weighing. Faeces for the ten weeks were therefore pooled for each tank for analysis. Ten fish were withdrawn randomly from each tank at the end of the ten week experimental period. Each of these fish were sampled for blood (2.5), and their livers were excised from the rest of the body. The gall bladder was severed from the liver and the liver was weighed and used for calculating Hepato-somatic Index. From five of the fish sampled carcass and liver samples were taken as soon as the fish were killed, for tissue glucose and glycogen estimation as detailed in Chapter 2.7.2. The rest of the ten fish sampled were then analysed for

proximate tissue composition (2.7).

4.2.5. HISTOCHEMISTRY

Histological preparations of the liver were made and studied as detailed in Chapter 2.9.

4.2.6. ANALYSES OF EXPERIMENTAL DATA AND STATISTICAL METHODS

These were performed as discussed in Chapter 2.10.

4.3. RESULTS

All fish were active and showed good appetite throughout the experimental period.

4.3.1. GROWTH PERFORMANCE

There was no significant difference ($p > 0.05$) between the initial weights of the seven groups of fish. The final weights however, showed for most of the groups, significant difference ($p < 0.05$) (Table 17). As shown in the growth curve (Fig. 8), the best growth occurred in the Mirror Carp receiving 45% cassava. When compared to the control group, the 15% and 30% cassava diets appeared to produce a significant ($p < 0.05$) depression in growth. When the effects of the cassava and rice diets on the growth rate of Mirror Carp are compared (Fig. 8 & 9), growth enhancement due to the addition of these carbohydrates is observed to run in the order : 45% rice > 30% rice > 45% cassava.

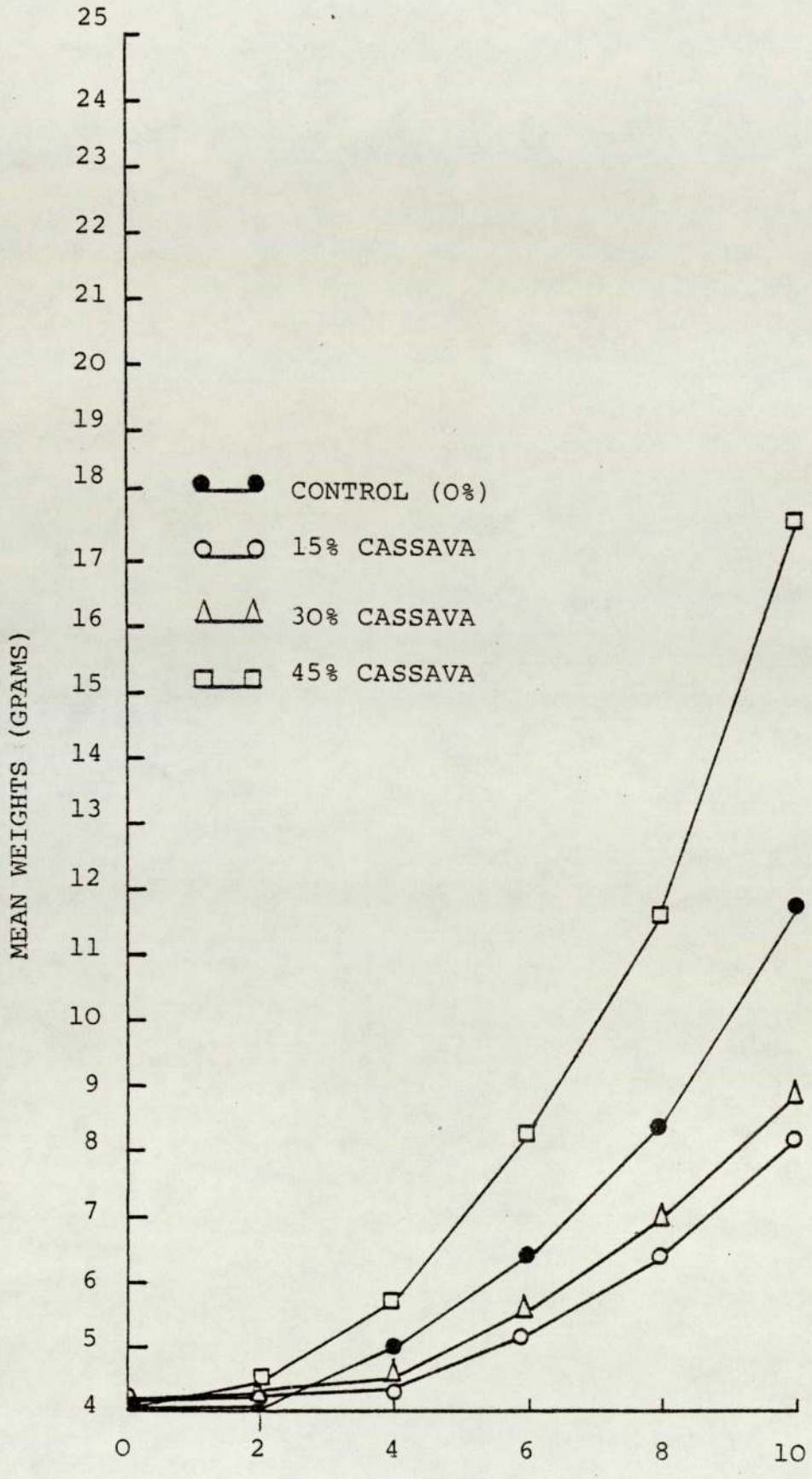


Fig. 8 Effect Of Different Levels Of Dietary cassava on weight gain of Mirror Carp

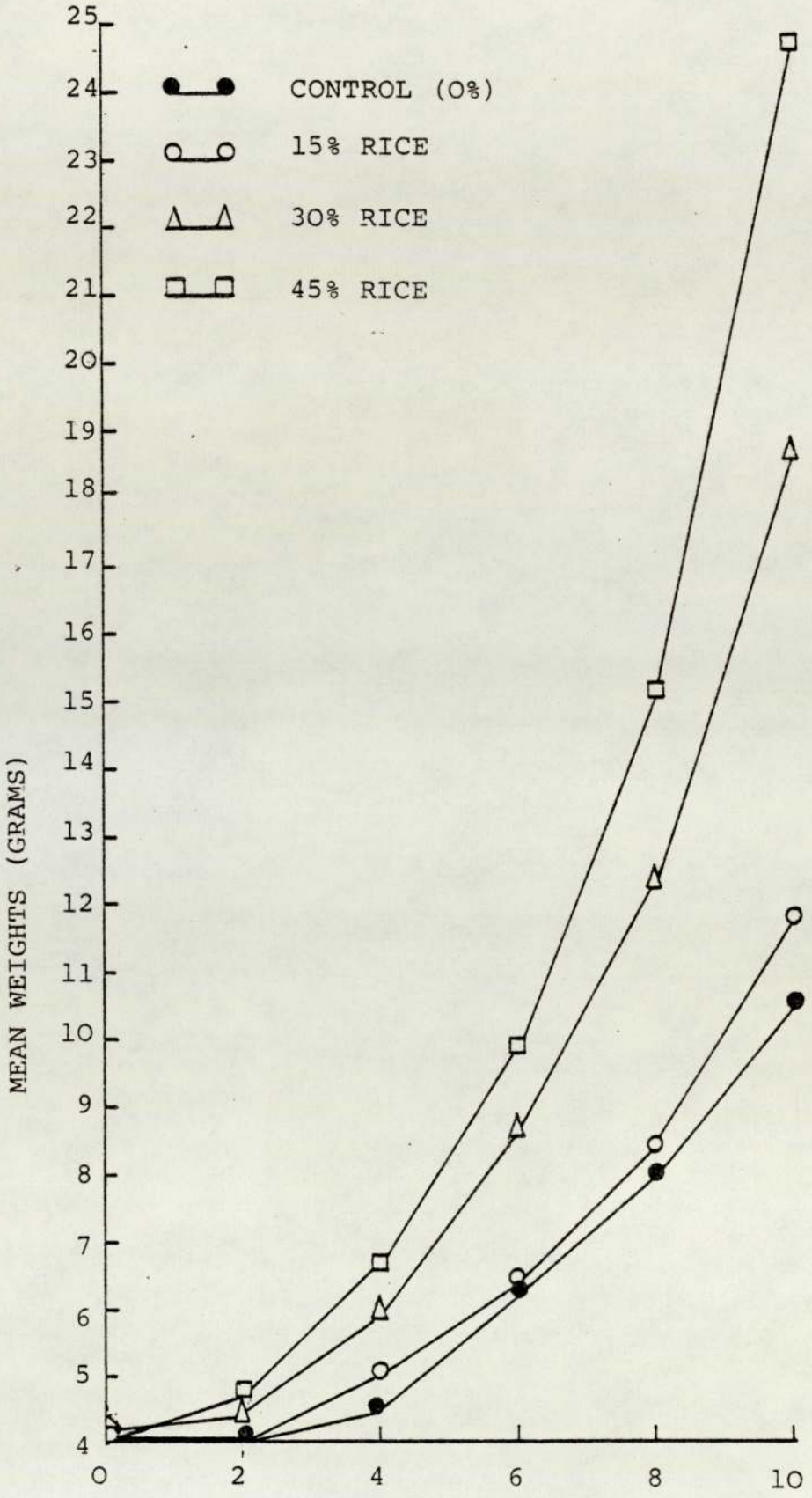


Fig. 9 Effect of Different Levels of Dietary Rice on Weight Gain of Mirror Carp

TABLE 17 GROWTH AND FOOD UTILIZATION OF MIRROR CARP FED DIFFERENT LEVELS OF DIETARY CASSAVA OR RICE FOR 10 WEEKS

MEAN VALUES	DIETS							
	CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7	± SEM
Initial weight (g)	4.24^{a}	4.16^{a}	4.13^{a}	4.09^{a}	4.23^{a}	4.09^{a}	4.14^{a}	0.122
Final weight (g)	8.21^{a}	8.96^{a}	17.56^{b}	10.48^{a}	18.67^{b}	24.71^{c}	11.75^{a}	2.142
Weight gain (%)	93.81^{a}	115.52^{a}	325.50^{c}	156.11^{b}	341.56^{c}	504.13^{d}	184.13^{b}	7.950
S.G.R.[1] (%)	0.94^{a}	1.10^{a}	2.07^{c}	1.34^{b}	2.12^{c}	2.57^{d}	1.49^{b}	0.080
F.C.R.[2]	3.50^{f}	3.06^{e}	1.51^{b}	2.53^{d}	1.51^{b}	1.17^{a}	2.15^{c}	0.032
P.E.R.[3]	0.94^{a}	1.13^{ab}	2.16^{d}	1.30^{b}	2.17^{d}	3.01^{e}	1.66^{c}	0.055
Apparent N.P.U.[4] (%)	11.34^{a}	15.02^{bc}	27.05^{d}	14.60^{b}	25.30^{d}	38.48^{e}	17.90^{c}	0.483

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

1 Specific growth rate 2 Food Conversion Ratio 3 Protein Efficiency Ratio

4 Net Protein Utilization

TABLE 18 PERFORMANCE OF MIRROR CARP FROM EXPERIMENT 2

	INITIAL	FINAL							
		DIETS							
		CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7	± SEM
Blood glucose (mg %)	64.44^{c}	55.64^{b}	66.56^{c}	63.39^{c}	39.40^{a}	66.93^{c}	64.28^{c}	48.63^{b}	1.304
H.S.I [1]	1.60^{c}	1.09^{a}	1.57^{c}	1.67^{d}	1.05^{a}	1.49^{b}	1.99^{e}	1.45^{b}	0.011
% Moisture in faeces		87.01^{a}	88.42^{a}	88.45^{a}	85.21^{a}	88.62^{a}	86.99^{a}	87.95^{a}	0.427
Gross Mortalities [2]		0	0	0	1	0	0	0	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$).

[1] Hepato-somatic Index

[2] Probably due to diet from post-mortem examination

4.3.2. FOOD CONVERSION

The efficiency with which the fish converted their food into flesh (Table 17) indicates a pattern similar to that obtained for the growth performance.

No significant difference ($p > 0.05$) exists between the Food Conversion Ratios (FCR's) of fish on 45% cassava and 30% rice. The poorest FCR is observed for each of the two digestible carbohydrates to be obtained with fish receiving 15% of the cassava or rice, while the best FCR is obtained with 45% dietary cassava or rice. However, no significant difference ($p > 0.05$) exists between the FCR's of the fish on 45% cassava and 30% rice.

4.3.3. PROTEIN UTILIZATION

The Protein Efficiency Ratio (PER) and Apparent Net Protein Utilization (NPU) values of the experimental fish show variations that tend to depend on the levels of dietary carbohydrate employed (Table 18) The best PER and Apparent NPU is obtained with 45% dietary rice. The PER and Apparent NPU values obtained with 30% rice and 45% cassava rank next, with no significant difference ($p > 0.05$) occurring between the respective two pairs of values.

The efficiency with which the fish utilize protein as shown by the PER and Apparent NPU values tends to be poorest when the dietary level of cassava is less than 30% or that of rice is less than

15%. The fact that the PER and Apparent NPU values obtained with the fish receiving no cassava or rice in their diets were not the poorest when compared to the other groups probably suggest, as in Experiment one, that there is a correlation between the ability of the fish to utilise carbohydrate, and the level of digestible dietary carbohydrate.

4.3.4. PATHOLOGY

There is no significant difference ($p > 0.05$) between the blood glucose levels of fish receiving 30% cassava or rice and over, when compared with results from the initial assay (Table 18). Contrarily, all fish receiving either 15% or less cassava, or rice showed hypoglycaemia.

The Hepato-somatic Index (HSI) tends to drop with decrease in dietary cassava or rice. No significant difference ($p > 0.05$) is obtained with 30% dietary cassava when compared to the HSI value from the pre-experimental assay.

No significant difference is observed ($p > 0.05$) between the moisture contents of the faeces of fish from the different treatments. No liver damage was observed in any of the histological preparations (Plates 5 & 6).

4.3.5 . CARCASS AND LIVER COMPOSITION

The moisture content of the carcass of fish receiving upto 30% cassava or upto 15% rice in diets show some increase when compared with the initial value before the feeding trial (Table 19), while the inclusion of dietary digestible carbohydrate increases the muscle glucose and glycogen content of Mirror Carp (Table 19). In the liver, increase in dietary cassava results in gradual decrease of liver moisture and an increase in liver glucose and glycogen. With the rice diets however, no significant variation is produced in the liver moisture and glucose / glycogen ($p > 0.05$). No significant difference ($p > 0.05$) occurs in carcass fat at 0% to 30% dietary cassava. At 45% dietary cassava, carcass fat increases. With the rice diet, there is a graded increase in carcass fat with increase in dietary rice. The liver in all the treatment groups show no significant difference ($p > 0.05$) with increase in dietary cassava or rice, except in the fish fed 45% cassava, which show a slight increase in liver fat accumulation. Dietary cassava and rice do not produce any significant difference ($p > 0.05$) in carcass protein and ash content in all groups. In the liver, however, protein content seems to be

TABLE 19 INITIAL AND FINAL CARCASS COMPOSITION OF MIRROR CARP FROM EXPERIMENT 2

Mean Values weight (%)	INITIAL	FINAL							
		CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7	± SEM
Moisture	76.65^{b}	79.39^{c}	79.10^{c}	74.74^{a}	78.57^{c}	76.21^{b}	73.85^{a}	78.92^{c}	0.705
Carbohydrate [1]	0.69^{bc}	0.49^{a}	0.60^{b}	0.73^{c}	0.36^{a}	0.78^{c}	0.96^{d}	0.42^{a}	0.071
Fat	6.95^{b}	4.77^{a}	4.09^{a}	8.98^{c}	5.57^{ab}	8.27^{c}	10.14^{d}	4.96^{a}	0.733
Protein	12.24^{a}	12.14^{a}	12.83^{a}	12.46^{a}	11.65^{a}	11.76^{a}	12.69^{a}	11.35^{a}	0.472
Ash	3.45^{a}	3.02^{a}	3,45^{a}	3.76^{a}	3.48^{a}	3.45^{a}	3.42^{a}	3.39^{a}	0.166
TOTALS	99.98	99.81	100.07	100.67	99.63	100.47	101.06	99.04	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

1 Total glycogen and glucose

TABLE 20 INITIAL AND FINAL LIVER COMPOSITION OF MIRROR CARP FROM EXPERIMENT 2

Mean Values (% by weight)	INITIAL	FINAL							± SEM
		CC-1	CC-2	CC-3	RC-4	RC-5	RC-6	OC-7	
Moisture	78.07^{cd}	76.87^{bc}	75.00^{b}	72.54^{a}	73.50^{a}	72.30^{a}	73.11^{a}	80.09^{d}	0.950
Carbohydrate [1]	3.73^{a}	6.89^{b}	7.83^{bc}	8.90^{c}	8.38^{c}	7.16^{b}	8.69^{c}	3.39^{a}	0.215
Fat	3.00^{a}	2.63^{a}	2.84^{a}	3.30^{a}	2.65^{a}	3.20^{a}	4.08^{b}	2.80^{a}	0.058
Protein	13.45^{ab}	12.81^{a}	14.22^{bc}	13.21^{ab}	13.67^{ab}	15.08^{c}	12.64^{a}	11.59^{a}	0.349
Ash	1.45^{a}	1.13^{a}	1.10^{a}	1.66^{ab}	2.15^{bc}	2.75^{c}	2.26^{bc}	2.65^{c}	0.054
TOTALS	99.70	100.33	100.99	99.61	100.35	100.49	100.78	100.52	

Figures in the same row lhaving the same superscript are not significantly different ($p > 0.05$)

[1] Total glucose and glycogen

highest with 30% cassava or rice and drops slightly with 15% or 45% cassava or rice.

4.3.6. NUTRIENT DIGESTIBILITY

The results of Apparent carbohydrate and protein digestibilities calculated as detailed in Chapter 2.10.8. are plotted in the histograms (Figures 10 and 11, respectively). As shown in Fig. 10, the inclusion of cassava or rice into the diets of Mirror Carp result in between 86% and 97% Apparent Digestibility of dietary carbohydrate. With no inclusion of cassava or rice, trace quantities (3.08%) (Table 17) of digestible carbohydrate exist in the diet, but this is only 17% digested. No significant difference ($p>0.05$) exists between the carbohydrate digestibilities at different levels of cassava and rice.

From the results shown in Fig. 11, inclusion of cassava or rice into the diets of Mirror Carp tend to improve protein digestibility significantly ($p<0.05$) from 76% to between 83.5% and 88%. The best protein digestibility is obtained with 45% rice in the diet. No significant difference ($p>0.05$) is obtained in protein digestibility of the groups receiving dietary cassava and the other dietary rice groups.

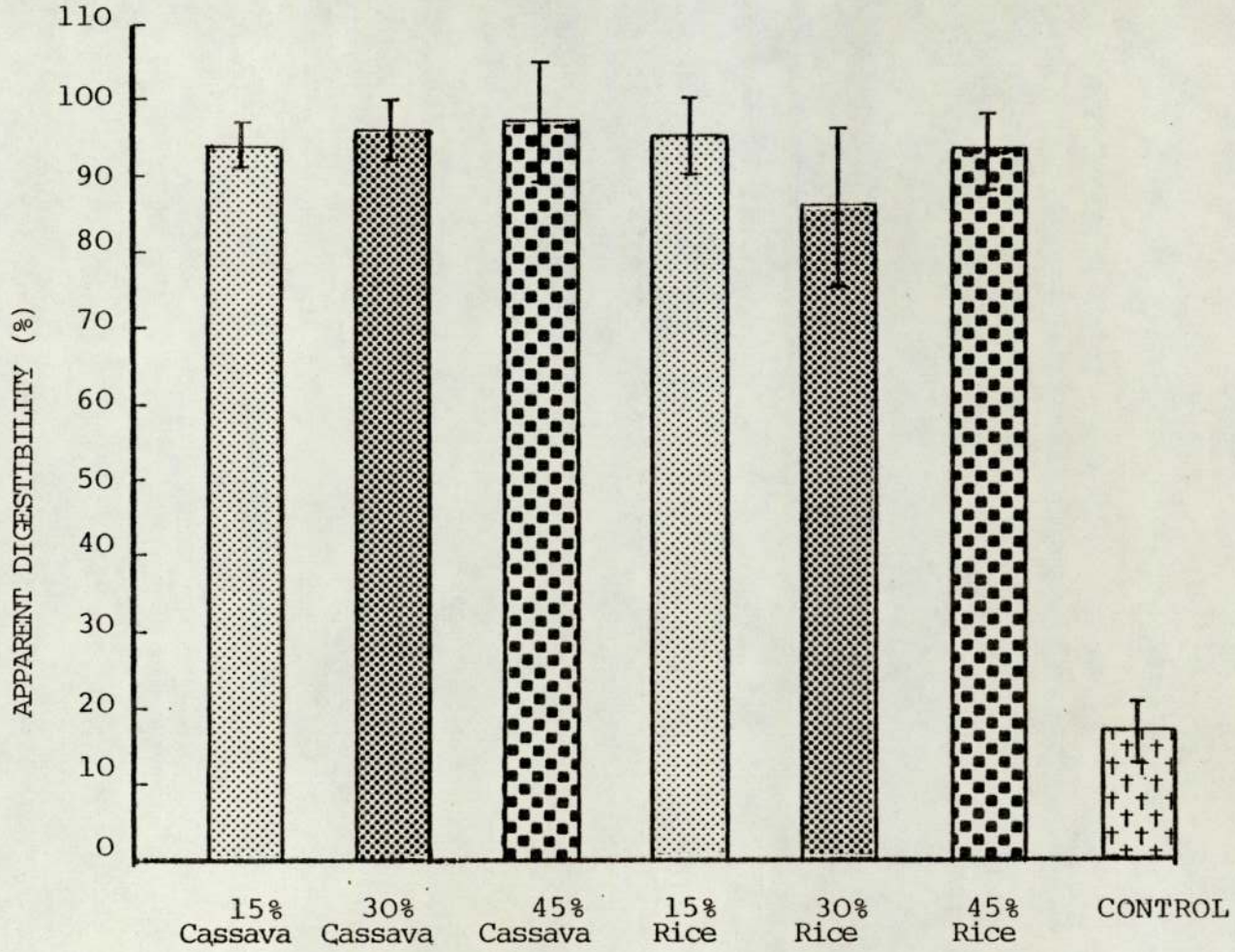


FIG. 10 Apparent digestibility of dietary carbohydrate in Mirror Carp from Experiment 2

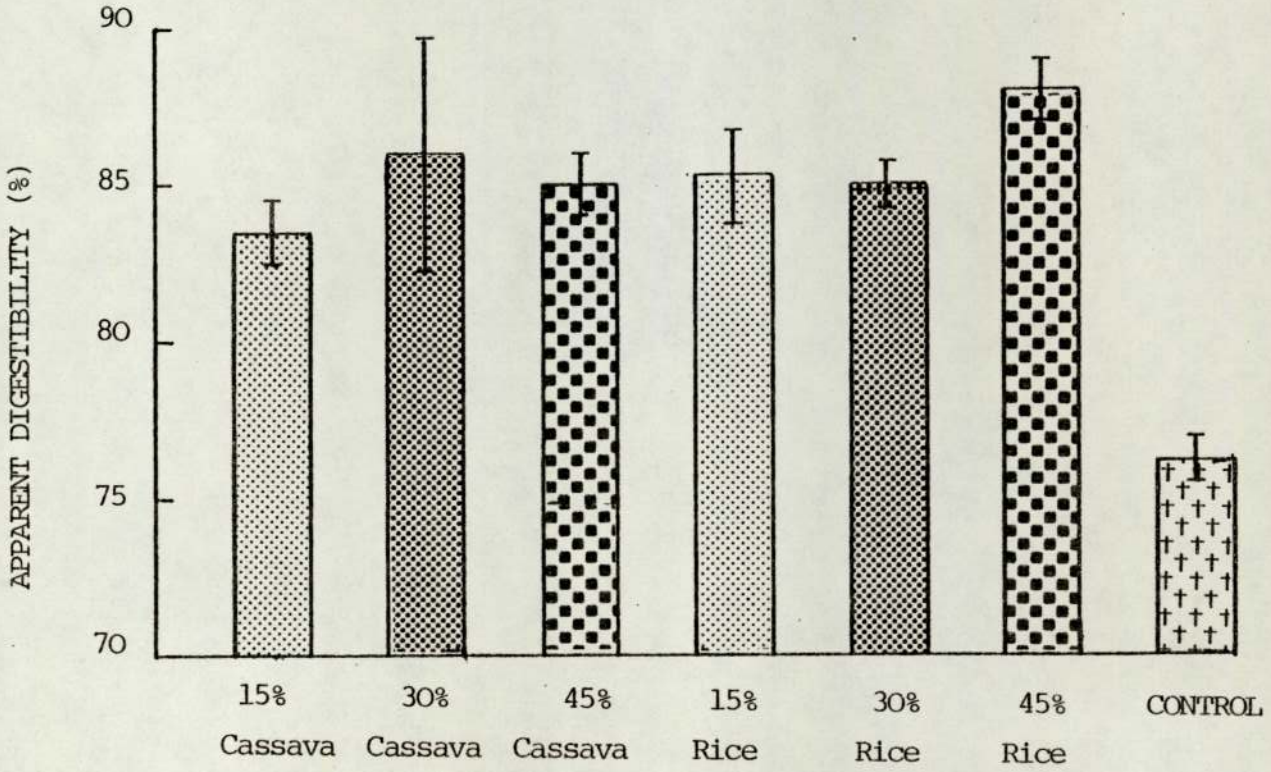


FIG. 11 Apparent digestibility of dietary protein in Mirror Carp from Experiment 2

4.4. DISCUSSION AND CONCLUSIONS :

From the investigation presented in this Chapter, it is seen that Mirror Carp, (Cyprinus carpio) is capable of metabolising dietary carbohydrate as cassava and rice. There is no available literature on the efficiency of the utilization of these two carbohydrate sources by Mirror Carp, though the work on Rainbow trout (Chapter 3) as well as previous studies, have shown that the absorption of carbohydrates and the growth of fish show variation with the type of carbohydrate fed (Phillips, et al., 1948; Buhler & Halver, 1961).

The results obtained in this investigation show that the rice containing diets are better utilized by Mirror Carp than the cassava diets. 45% dietary rice resulted in a Specific Growth Rate (SGR) of 2.57% while the best S.G.R. with the cassava diet was 2.07% achieved with 45% cassava. With 30% dietary rice, a better growth rate than that obtained with 45% dietary cassava was achieved.

The usefulness of cassava and rice containing diets is further shown by the hypoglycaemic condition of the blood of fish receiving below 15% of these carbohydrates. The protein source and level used by Shimeno, et al., (1978) could have affected their observations of intolerance of the 40% carbohydrate diets by Mirror Carp, but their observation of poor growth and feed efficiency in Carp receiving between 10% and 20% of digestible carbohydrate when compared with their control receiving no digestible carbohydrate is repeated in this

investigation.

Except for the fish receiving 15% cassava or rice who had comparatively low Hepato-somatic Index (HSI) (1.09 and 1.05) respectively), all the other values for HSI lie between 1.45 and 2.00 which are similar to the initial values.

Svobodova (1976) observed that the glycogen content of Carp tissues was influenced by the type of feed when the fish received natural sources of carbohydrates. In this work, no pronounced correlation between the levels of tissue glycogen/glucose and source of digestible carbohydrate is observed (Tables 19 and 20). Hoever, some relationship exists between the levels of carbohydrate in the diets and in the tissues. The inclusion of cassava or rice in the diets at all levels tend to improve the digestibility of carbohydrate and protein by Mirror Carp, the digestibilities of carbohydrate and protein being significantly inferior ($p > 0.05$) in the control group. The energy values of the different diets vary between 17.01 and 20.88 KJ/g, a graded increase in dietary energy being obtained with increase in dietary digestible carbohydrate. The different parameters used in measuring the utilization of the diets by carp do not vary in conformity with the dietary energy levels. Provided the dietary energy level is not too low, it is unlikely that this should be the over-ruling factor in adaptability of Mirror carp to carbohydrate diets.

It is concluded that Mirror carp is capable of metabolising dietary cassava and rice at upto 45% dietary inclusion. Mirror carp tend to grow better on rice diets (of 45% or 30%) than on cassava diet (of 45%).

Like in Rainbow trout, there seems to be dependency of the ability of Mirror carp to metabolise dietary carbohydrate on the carbohydrate source. Two further carbohydrates, corn and potato starch which are equally cheap were therefore fed to Rainbow Trout and then Mirror Carp to see if this dependency is persistent.

CHAPTER 5

CHAPTER 5

EXPERIMENT THREE

THE UTILIZATION OF DIFFERENT LEVELS OF DIETARY CORN AND POTATO STARCH BY RAINBOW TROUT FED ISONITROGENOUS DIETS

5.1. INTRODUCTION

Low quantities of corn starch have been shown to be well utilized by Rainbow trout (Phillips, et al., 1948; Koops, et al., 1974; Abel, et al., 1979). However, while the diets of Koops and his colleagues had varying levels of fat, those of Phillips et al., (1948), and Abel, et al., (1979) contained varying levels of protein.

Since potato is very cheap in temperate countries, and corn is relatively cheap in the tropics, (Table 1) the toleration of high quantities of these carbohydrates could help in the production of low cost Rainbow trout feed. This experiment was therefore conducted to find out the effect of high and low quantities of corn and potato starch in Rainbow trout fed isonitrogenous diets.

5.2. MATERIALS AND METHODS

5.2.1. EXPERIMENTAL ANIMALS AND SYSTEM

The 140 fingerling Rainbow trout used in this experiment were obtained from Burwarton Fish Farm, Cleobury North, Bridgenorth in Shropshire. Fish weighed between 44 g and 48 g and measured

between 15 cm and 17 cm at the start of the experiment. Fish were subjected to the usual quarantine treatment (2.1) and were then distributed equally among the seven tanks of experimental "System 3". After branding with liquid nitrogen (2.4), fish were fed 2% of their body weight twice per day on commercial (Edward Baker, Essex) diet for one week, during which time they acclimatized.

5.2.2. PREPARATION OF EXPERIMENTAL DIETS

Dry corn (Sandhar & Kang Limited, Birmingham) was milled in a hammer mill, analysed (Table 6) and was employed in the formulation (2.3), of diets NC-1, NC-2 and NC-3 (Table 21). The potato starch (from Sigma Chem. Co., St. Louis, USA) was equally analysed and then used in the preparation of diets PC-4, PC-5 and PC-6 (Table 21). A control diet was equally formulated but contained neither corn nor potato starch. Rather, it contained α-cellulose as a bulking agent. The diets were then analysed, (2.7), results from the analyses being presented in (Table 22).

5.2.3. FEEDING

Fish were fed 2% of their body weight twice daily, at 10.00 a.m. and 6.00 p.m. Feeding usually lasted for about five minutes, care being taken, to ensure that the fish in each experimental tank consumed all their meal.

5.2.4. WEIGHING AND SAMPLING

Fish were weighed at the start of the experiment during which time five fish were sampled for proximate analysis. Fish were thereafter weighed fortnightly, between 8.00 a.m. and 10 a.m. on the weighing day (2.4). Fish were usually starved on the weighing day. During weighing, faeces was collected from each fish and then pooled for each tank (2.6). At the end of the ten week experimental period, ten fish were randomly withdrawn from each tank, sampled for blood (2.5) and their liver removed and weighed for the calculation of Hepato-somatic Index (2.4). Five fish were analysed fresh for muscle and liver glucose and glycogen (2.7.2). Each of the remaining five fish sampled was then analysed as detailed in Chapter 2.7.

5.2.5. HISTOCHEMISTRY

Histological preparations of the liver were made and studied as detailed in Chapter 2.9.

5.2.6. ANALYSES OF EXPERIMENTAL DATA AND STATISTICAL METHODS

These were performed as detailed in Chapter 2.10.

5.3. RESULTS

All the fish ate voraciously during the ten-week experimental period. A small number of fish were lost during this experiment, probably as a result of *Saprolegniales* spp fungal infection. Other-

TABLE 21 COMPOSITION OF DIETS FOR EXPERIMENT 3 (g/100g)

INGREDIENTS	DIETS DESIGNATIONS						
	NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7(Control)
Corn	10.00	20.00	30.00	-	-	-	-
Potato	-	-	-	10.00	20.00	30.00	-
White fish meal	45.00	45.00	45.00	45.00	45.00	45.00	45.00
Casein	10.00	10.00	10.00	10.00	10.00	10.00	10.00
α-cellulose	20.00	10.00	-	20.00	10.00	-	30.00
Mineral mix [1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix [2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cod-liver oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Carboxy-methyl Cellulose (binder)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
TOTALS	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1] Composition given on Table 7

[2] Composition given on Table 8

TABLE 22 PROXIMATE COMPOSITION OF EXPERIMENT 3 TEST DIETS FROM BIOCHEMICAL ASSAY (% weight)

INGREDIENTS	DIETS						
	NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7
Moisture	4.94	4.58	5.68	4.98	5.43	7.02	4.96
Protein	43.88	43.55	44.42	43.82	44.56	43.58	42.97
Fat	12.64	13.25	13.04	12.14	12.19	13.89	13.48
Carbohydrate [1]	8.93	17.44	26.56	9.89	17.00	27.42	1.58
Ash	7.83	7.90	8.72	8.50	8.04	7.83	8.05
Subtotal	78.22	86.72	48.42	79.33	87.22	99.74	71.04
Fibre [2]	21.78	13.28	1.58	20.67	12.78	0.26	28.96
Cr_2O_3 [3]	0.54	0.50	0.49	0.51	0.50	0.50	0.51
Energy [4]	17.51	18.29	21.49	18.51	19.55	21.24	17.01

[1] Hydrolysable carbohydrate

[2] Computed as difference between subtotal and 100

[3] Presented on dry weight basis

[4] Kilojoules/gram

wise the health of the fish appeared good.

5.3.1. GROWTH PERFORMANCE

There is no significant difference ($p > 0.05$) between the initial weights of the fish in the seven experimental groups. The final weights of the fish were significantly different ($p < 0.05$) (Table 24). There appears to be a graded improvement in growth (Figs. 12 and 13), resulting from stepwise increase in the level of dietary corn or potato starch.

The percentage daily weight gain and Specific Growth Rate (S.G.R) also show corresponding increases with increase in dietary corn or potato starch, the poorest values being obtained with the control diet which contained neither corn nor potato starch. The values obtained tend to suggest that corn is better utilized by Rainbow trout than potato.

5.3.2. FOOD CONVERSION

The Food Conversion Ratios (FCR's) were improved with increase in the percentage of corn or potato starch added to the diets (Table 24). The best FCR's were 1.56 obtained with 30% corn and 1.80 obtained with 30% potato; the poorest was obtained at 10% potato and the control. No significant difference ($p > 0.05$) exists between the former two or the later two values. From the results on FCR, it is evident that Rainbow trout was better adapted to met-

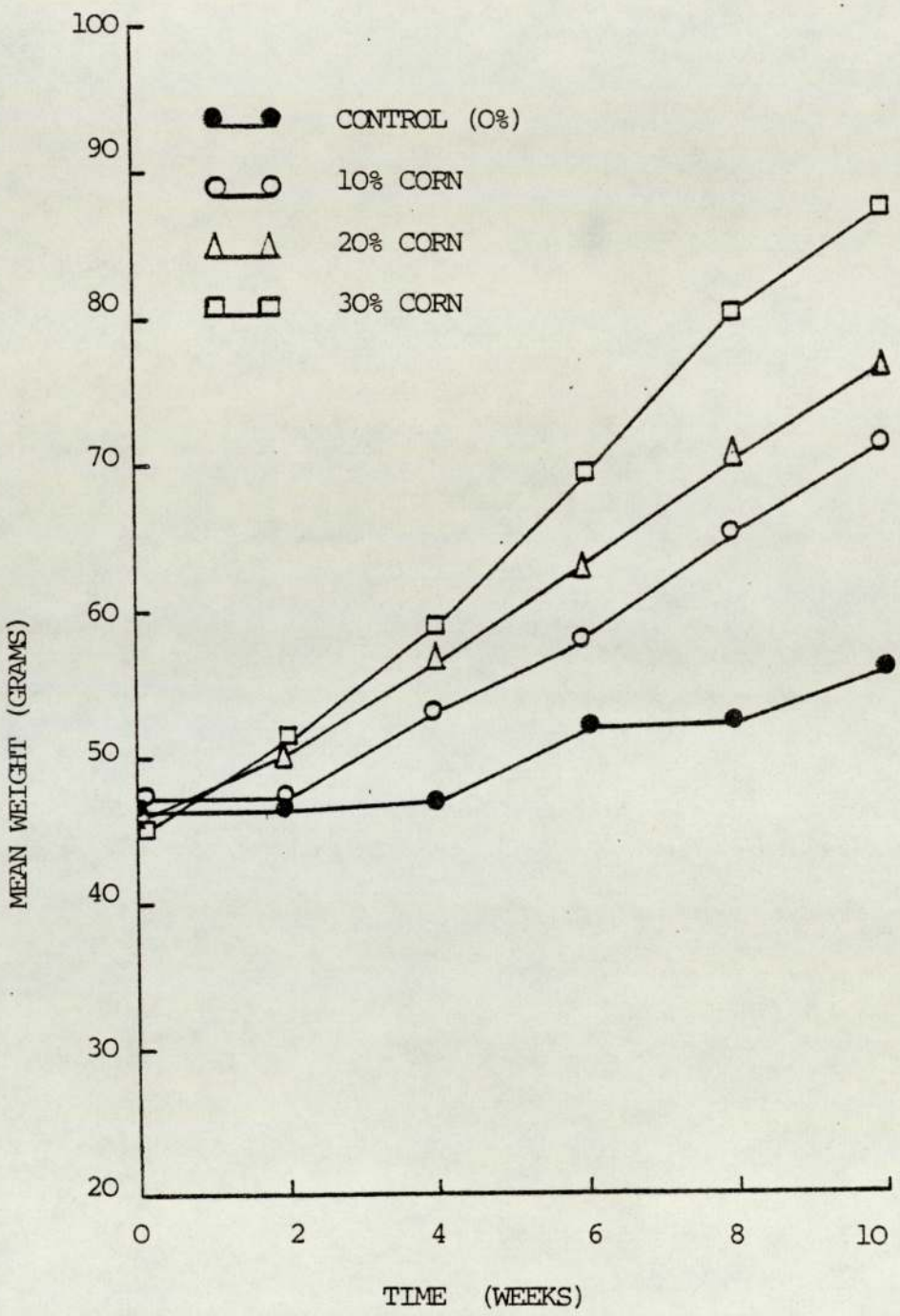


Fig. 12 Effect of different levels of dietary corn on weight gain of Rainbow Trout

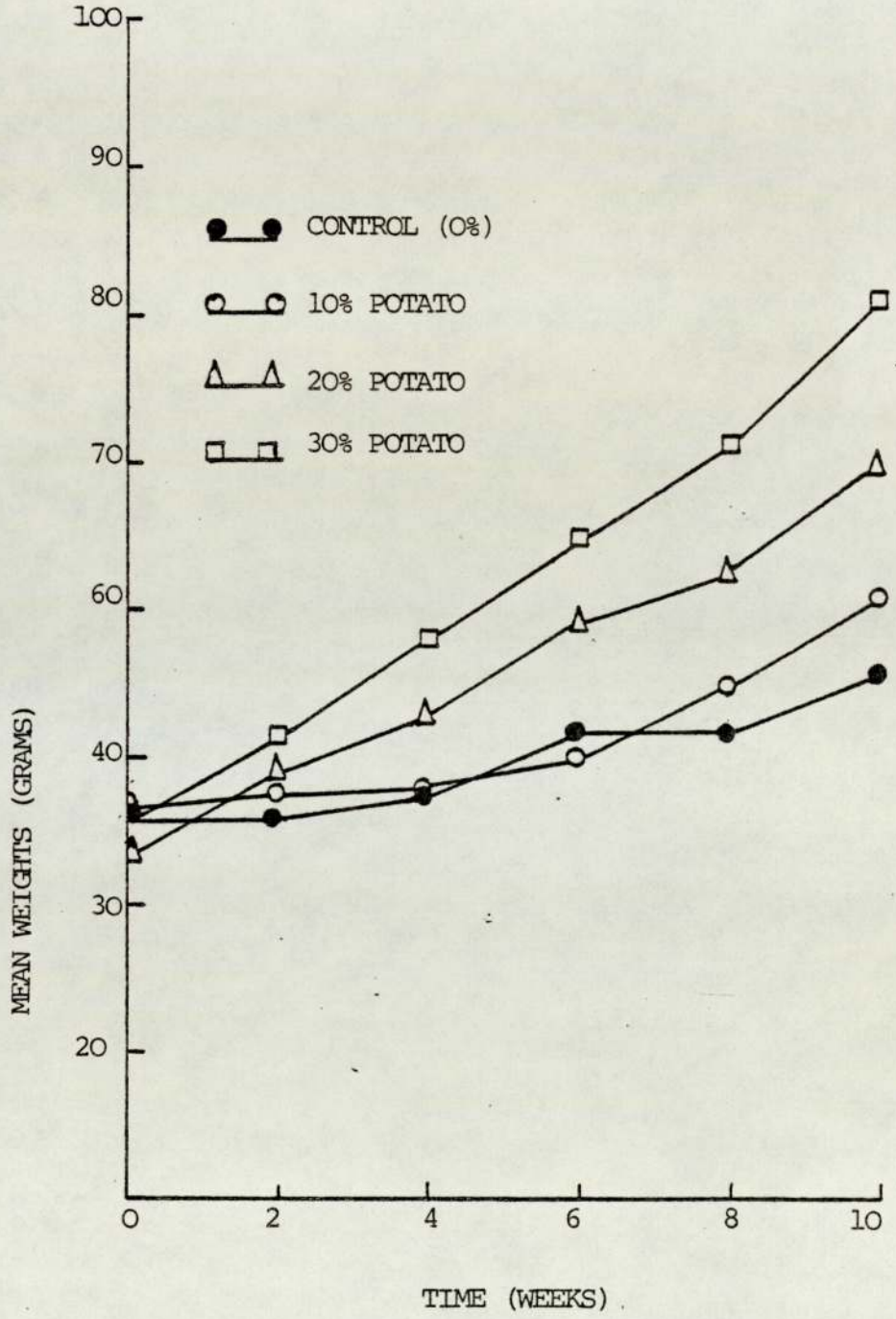


Fig. 13 Effect of different levels of dietary potato on weight gain of Rainbow Trout

TABLE 23 GROWTH AND FOOD UTILIZATION OF RAINBOW TROUT FED DIFFERENT LEVELS OF DIETARY CORN AND POTATO FOR 10 WEEKS

MEAN VALUES	DIETS							± SEM
	NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	
Initial weight (g)	47.19^{a}	46.33^{a}	44.93^{a}	46.24^{a}	44.37^{a}	46.15^{a}	46.11^{a}	0.831
Final weight (g)	71.25^{c}	76.41^{d}	86.80^{f}	61.20^{b}	70.10^{c}	80.71^{e}	55.58^{a}	2.791
Weight gain (%)	51.11^{c}	65.10^{d}	93.12^{f}	32.20^{b}	58.11^{c}	74.92^{e}	20.31^{a}	1.083
S.G.R^{1} (%)	0.59^{c}	0.72^{d}	0.94^{f}	0.40^{b}	0.65^{c}	0.80^{e}	0.27^{a}	0.010
F.C.R.^{2}	2.13^{c}	2.06^{c}	1.56^{a}	4.00^{e}	2.39^{d}	1.80^{b}	3.89^{e}	0.067
P.E.R.^{3}	0.97^{b}	1.15^{c}	1.47^{e}	0.66^{a}	1.02^{b}	1.31^{d}	0.56^{a}	0.025
Apparent N.P.U^{4} (%)	15.88^{b}	18.45^{b}	24.10^{c}	11.29^{a}	16.55^{b}	23.63^{c}	10.98^{a}	1.842

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

1 Specific growth rate 2 Food Conversion Ratio 3 Protein Efficiency Ratio

4 Net Protein Utilization

abolise corn than potato starch.

5.3.3. PROTEIN UTILIZATION

With increase in the levels of dietary corn and potato, the Protein Efficiency Ratios (PER's) increase. The poorest PER is obtained with the control diet. The difference between the PER's obtained with 10% corn and 20% corn is not significant, ($p > 0.05$). Similarly, the difference in PER of fish on the control diet and those on 10% potato diet is not significant ($p > 0.05$).

The Apparent Net Protein Utilization (NPU) of all fish follow much the same pattern as the PER. Increase in dietary corn or potato increases the Apparent NPU. Thus, there is evidence of protein sparing by the carbohydrate in the diets.

5.3.4. PATHOLOGY

There is no significant difference ($p > 0.05$) between the blood glucose values at the start and at the end of the experiment, the values ranging between 69.60 mg % and 76.70 mg %. The Hepato somatic Index similarly shows no significant difference ($p > 0.05$).

However, some livers from fish fed the 30% potato starch ration showed some discoloration. Histological examination of sections of the liver however, did not reveal any enlargement of nuclei of the liver cells (megalocytotic hepatocytes), excessive fat vacuolation or necrosis of hepatocytes (Plates 3 & 4).

No significant difference ($p > 0.05$) occurred in the moisture contents of faeces of fish on 10% to 30% corn, 10% potato and the control (Table 24). No significant difference ($p > 0.05$) occurs between the moisture in the faeces of fish fed 30% corn and 20% potato; nor between fish fed 20% potato and 30% potato, this last group of fish having the largest faecal moisture content. This last group of fish also showed some evidence of diarrhoea during the first four weeks on the potato meal diet.

A small number of fish (maximum of two per tank) died during the course of the experiment. Death was probably due to fungal infection.

TABLE 24 PERFORMANCE OF RAINBOW TROUT FROM EXPERIMENT 3

	INITIAL	FINAL							
		NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	± SEM
Blood glucose (mg %)	75.45[a]	72.25[a]	74.50[a]	75.56[a]	76.70[a]	69.60[a]	75.75[a]	71.52[a]	2.826
H.S.I.	1.20[a]	1.56[a]	1.18[a]	1.16[a]	1.41[a]	1.21[a]	1.02[a]	1.45[a]	0.360
Moisture in faeces (%)		82.38[ab]	77.02[a]	86.14[ab]	84.38[ab]	87.48[b]	88.67[b]	83.83[ab]	1.352
Gross Mortalities [2]		2	1	0	2	0	0	1	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Hepato-somatic index

[2] Probably due to fungal infection

TABLE 25 INITIAL AND FINAL CARCASS COMPOSITION OF RAINBOW TROUT FROM EXPERIMENT 3

Mean Values (% by wet weight)	INITIAL	FINAL							± SEM
		NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	
Moisture	76.74[a]	73.90[a]	73.41[a]	72.98[a]	73.71[a]	72.76[a]	72.13[a]	73.50[a]	2.795
Carbohydrate [1]	0.44[a]	0.46[a]	0.50[a]	0.69[c]	0.48[a]	0.57[b]	0.75[d]	0.47[a]	0.038
Fat	4.74[a]	7.90[b]	9.26[d]	9.13[d]	8.58[c]	9.51[d]	9.20[d]	9.00[cd]	0.518
Protein	14.39[a]	15.04[b]	15.06[b]	15.36[b]	15.06[b]	15.08[b]	15.97[c]	15.09[b]	0.144
Ash	3.11[b]	2.49[a]	2.19[a]	2.24[a]	2.60[a]	2.29[a]	2.33[a]	2.49[a]	0.099
TOTALS	99.42	99.79	100.42	100.40	100.43	100.21	100.38	100.55	

Figures in the same row having the same superscript are not significantly different ($p>0.05$)

[1] Total glycogen and glucose

TABLE 26 INITIAL AND FINAL LIVER COMPOSITION OF RAINBOW TROUT FROM EXPERIMENT 3

Mean Values (% by weight)	INITIAL	FINAL							
		NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	± SEM
Moisture	76.68[a]	76.98[a]	76.62[a]	74.16[a]	76.89[a]	76.13[a]	76.16[a]	76.12[a]	1.859
Carbohydrate [1]	3.98[a]	4.15[a]	4.11[a]	4.67[b]	4.51[b]	4.03[a]	4.98[c]	4.05[a]	0.112
Fat	2.90[b]	2.73[b]	2.92[b]	2.95[b]	1.39[a]	1.59[a]	1.63[a]	2.01[a]	0.413
Protein	15.03[a]	14.75[a]	15.23[a]	16.97[a]	16.01[a]	16.64[a]	15.65[a]	16.25[a]	0.948
Ash	1.54[a]	1.48[a]	1.28[a]	1.34[a]	1.27[a]	1.55[a]	1.43[a]	1.52[a]	0.185
TOTALS	100.13	100.09	100.16	100.09	100.07	99.94	99.85	99.95	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Total glucose and glycogen

5.3.6. CARCASS AND LIVER COMPOSITION

There was no significant difference ($p > 0.05$) between the carcass and liver moisture content of fish at the end of the 10-week feeding trial.

The highest muscle glucose/glycogen content is obtained with 30% dietary potato (Table 25). The highest liver glycogen/glucose content is also obtained with 30% potato (Table 26). The significant ($p < 0.05$) fall in liver glycogen (Table 26) of fish receiving 20% potato when compared to fish receiving 10% dietary potato cannot be readily explained. The inclusion of between 20% and 30% corn or potato in the diets led to no significant ($p > 0.05$) increase in carcass fat when compared to the control. 10% corn or potato however, led to a drop in carcass fat. No significant change ($p > 0.05$) is observed in the carcass protein of fish receiving less than 30% corn or potato, or in the carcass ash of all groups.

In the liver, dietary potato significantly ($p < 0.05$)

caused decrease in liver fat content. Carbohydrate source or level does not seem to cause any significant change ($p < 0.05$) in the level of liver protein or ash.

5.3.7. NUTRIENT DIGESTIBILITY

The digestibility of dietary potato is very poor at levels between 10% and 20% dietary inclusion. At 30% dietary potato, the digestibility rises to only 34% (Fig. 14). With corn as dietary carbohydrate, the digestibility of the carbohydrate ranges between 43% and 53%. In the control group, carbohydrate digestibility of the trace (1.58%) dietary carbohydrate is only 4.5%.

At 20% and 30% corn or potato in diet, protein digestibility is between 89.6% and 90.1% (Fig. 15). Lack of dietary corn or potato starch in the diet noticeably reduces protein digestibility.

5.4. DISCUSSION AND CONCLUSIONS

The results obtained from this investigation indicate that

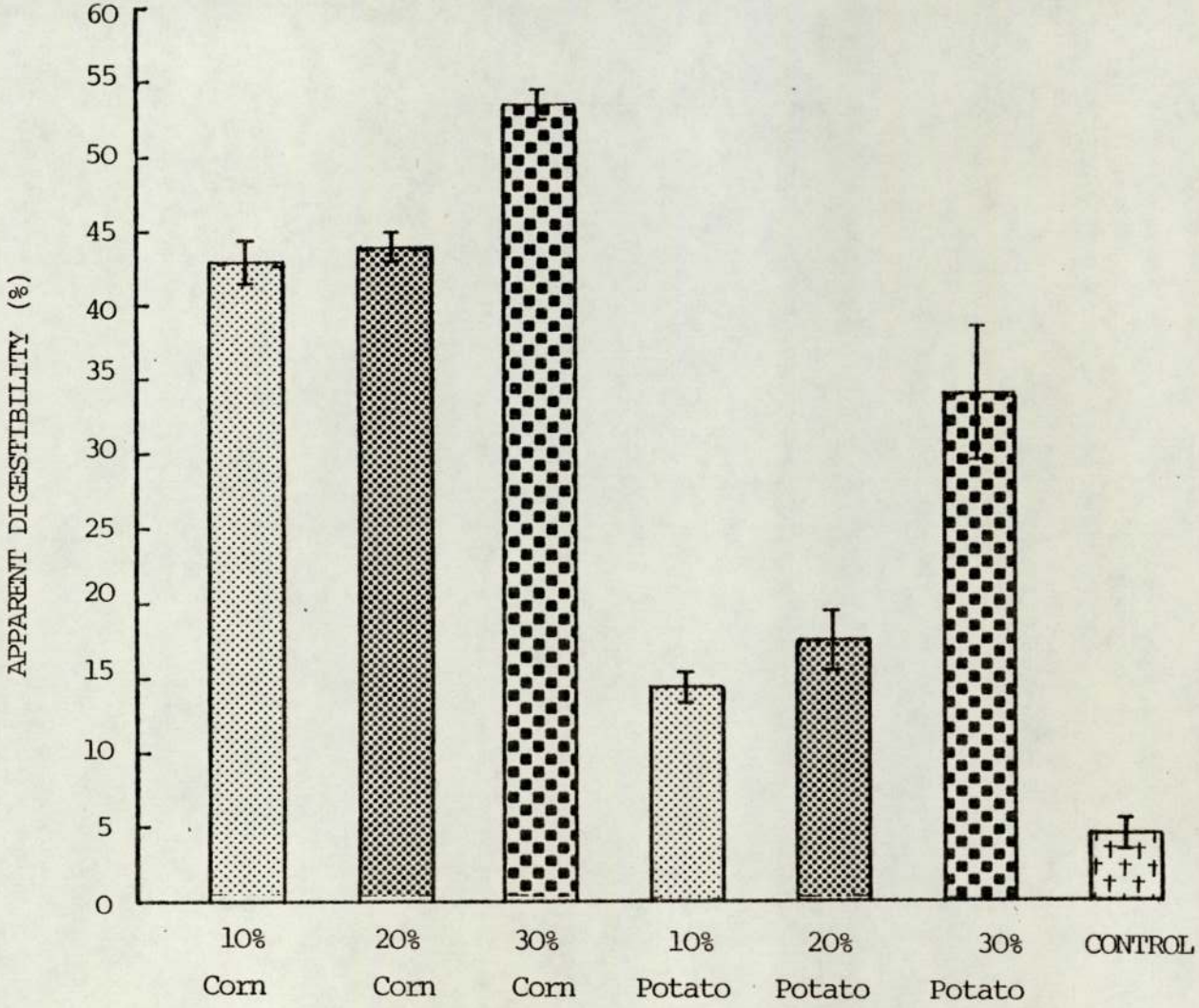


Fig. 14 Apparent digestibility of dietary carbohydrate in Rainbow Trout from Experiment 3

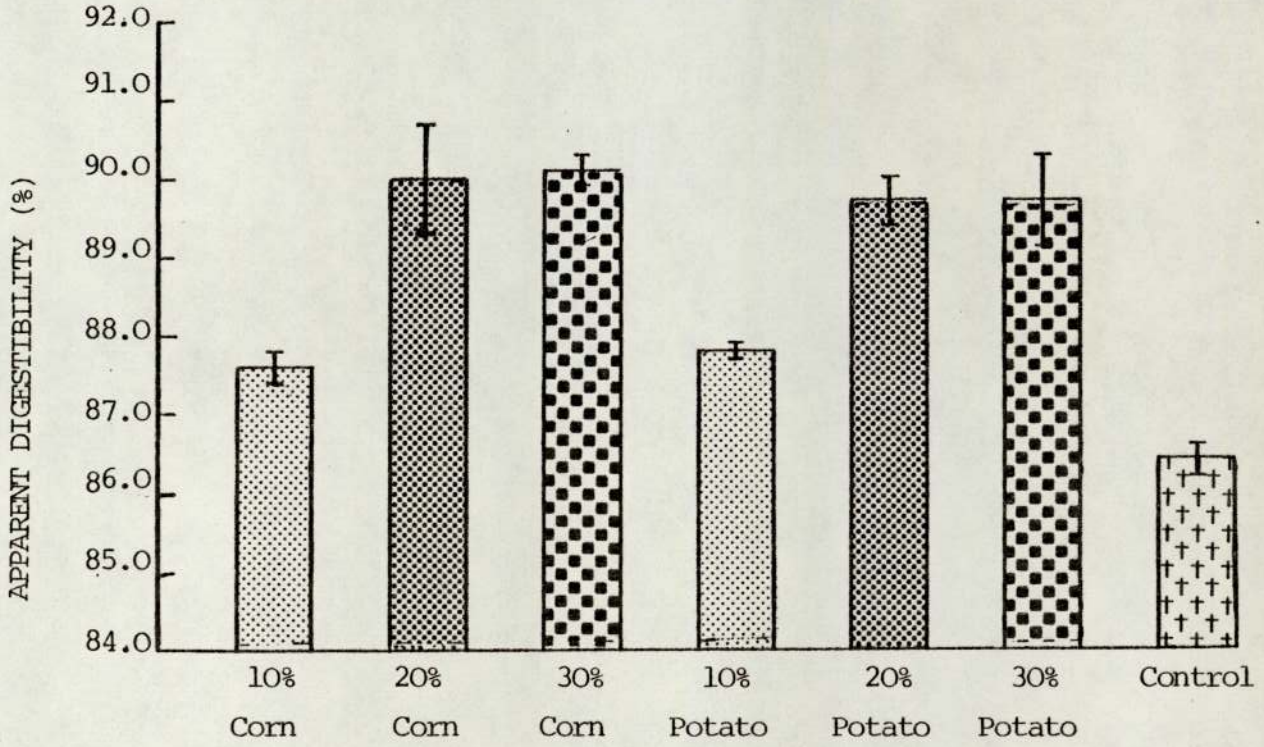


Fig. 15 Apparent digestibility of dietary Protein in Rainbow trout from Experiment 3

Rainbow trout is capable of utilizing dietary corn and potato starch. However, it is evident that 30% dietary potato probably caused an increase in liver glycogen deposition (Table 25).

The best Apparent NPU was obtained with 30% corn or potato. However, when the Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Protein Efficiency Ratios obtained in this experiment are compared with the values obtained for Rainbow trout in Experiment 1, it would appear that the efficiency of utilization of the cassava and rice diets by Rainbow trout is better than their efficiency in corn and potato utilization. Furuichi and Yone (1971), working on Red Sea bream concluded that they were inferior in potato starch utilization. However, when the values of growth rate and nutrient utilization of the control fish are compared it appears, that the better SGR, PER, FCE and Apparent NPU obtained with most of the cassava and rice diets over and above the corresponding values obtained with corn and potato is very much due to genetic factors or to some unobserved material released from the diets into the water and which probably produced deleterious effects on the fish. The water quality parameters for this experiment (Table 2) were however good. The genetic constitution of the fish which has been shown to be capable of affecting the growth and nutrition of Rainbow trout (Reinitz, et al., 1978a, Refstie, 1980) could therefore, have been a major contributing factor as the two sets of fish for the two

experiments are from two different stocks.

There is some evidence from Table 25 that 30% corn or potato in the diet of Rainbow trout leads to protein sparing. No drastic alterations in the carcass and liver composition result from the dietary corn or potato starch.

There is evidence from the digestibility studies that the level of corn or potato starch influenced protein and carbohydrate digestibilities, (Table 27; Figs. 14 & 15). The fibre content of the diets which affects gut passage time of gut contents (Takeuchi, et al., 1979b) probably has some influence on digestibility. Though there is no apparent difference in protein digestibilities produced by carbohydrate type (Fig. 15), the only difference being caused by carbohydrate level there is evidence (Fig. 14) of dependency of carbohydrate digestibility on carbohydrate source. This observation when compared with those of Abel, et al., (1979), that gelatinised maize starch, more than sucrose, increased the activity of glucokinase and decreased that of phosphoenolpyruvate (PEP) carboxykinase in trout liver, demands for further work on carbohydrase activities in trout.

It was essential therefore, before making conclusive statements on the utilisation of dietary carbohydrate by Rainbow trout to investigate the carbohydrase activities in the tissues of this fish in relation to carbohydrate source and concentration

CHAPTER 6

CHAPTER 6

EXPERIMENT FOUR

THE UTILIZATION OF DIFFERENT LEVELS OF DIETARY CORN AND POTATO STARCH BY MIRROR CARP FED ISONITROGENOUS DIETS

6.1. INTRODUCTION

The ability of Mirror carp to tolerate high quantities of cassava and rice in diets has been shown (Chapter 4). Mirror Carp fed on the diet containing 45% cassava, or 30% or 45% rice grew faster; had better FCE, PER, NPU, and generally appeared more healthy than the groups receiving lower quantities of cassava or rice in their diets and the group receiving diets devoid of cassava or rice (Chapter 4). The efficiency with which Mirror carp or Rainbow trout utilized the carbohydrate diets has been shown to be affected by the carbohydrate type (Chapters 3, 4 & 5). Since corn and potato are relatively cheap carbohydrate sources (Table 1), they could be useful in the formulation of carp diets. This of course depends on the ability of carp to digest and utilize the corn and potato diets. This study was therefore conducted in order to establish the adaptability of Mirror carp to diets containing corn and potato as digestible carbohydrate sources.

6.2 MATERIALS AND METHODS

6.2.1. EXPERIMENTAL ANIMALS AND SYSTEM

The fish for this experiment were six months old fingerling Mirror Carp (Cyprinus carpio) hatched from the brood stock in Aston University Fish Culture Unit. The fish weighed between 17 g and 22 g and measured between 8 cm. and 11 cm. at the start of the experiment. Each tank of Experimental "System 4" (2.2.1) was stocked with 20 fish, with the weights being equally balanced among the seven tanks. The fish were branded with liquid nitrogen (2.4). The fish were then fed 3% of their body weight thrice daily for one week on commercial diet (Edward Baker, Sudbury, Essex), during which time they were allowed to acclimatize to the tanks.

6.2.2. PREPARATION OF EXPERIMENTAL DIETS

The corn and potato starch used in this experiment were obtained from the same source as those for Experiment 3 (5.2.2). Diets NC-1, NC-2 and NC-3 contained 15%, 30% and 45% respectively of corn while diets PC-4, PC-5 and PC-6 contained 15%, 30% and 45% respectively of potato starch (Table 27). Diet CC - 7 which was the control contained neither corn nor potato but rather had α-

cellulose as a bulking agent. The diets were prepared using the method described in Chapter 2.3, analysed (2.7) (Table 28), frozen and utilized when required.

6.2.3. FEEDING

The experimental fish were fed 3% of their body weight thrice daily at 9.00 a.m., 1.00 p.m. and 5.00 p.m. All the fish showed good appetite. Feeding time lasted for at least twenty minutes during each feeding period, care being taken to ensure that the fish consumed all the feed they were given per meal.

6.2.4. WEIGHING AND SAMPLING

Fish were weighed at the start of the experiment during which time five fish were taken for initial assay (2.6). Fish were weighed fortnightly thereafter, (2.4), during which time faeces were collected as detailed in Chapter 2.6. Weighing was carried out between 8.00 a.m. and 9.00 a.m., and the fish were not fed on the weighing day. To prevent an electric fire hazzard which would have resulted from inevitable flooding, this experiment was terminated on the eighth week instead of the tenth. At the termination of the experiment, fish were sampled for analyses as detailed in Chapter 3.2.4.

6.2.5. HISTOCHEMISTRY

Histological preparations of the liver were made and studied

TABLE 27 COMPOSITION OF DIETS FOR EXPERIMENT 4 (g/100g diet)

INGREDIENTS (% weight)	DIETS DESIGNATIONS						
	NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7 (Control)
Corn	15.00	30.00	45.00				
Potato				15.00	30.00	45.00	
White fish meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Casein	10.00	10.00	10.00	10.00	10.00	10.00	10.00
α-cellulose	30.00	15.00		30.00	15.00		45.00
Mineral mix [1]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix [2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cod-liver oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Corn oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Carboxy-methyl cellulose (binder)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
TOTALS	100.00	100.00	100.00	100.00	100.00	100.00	100.00

1 Composition given on Table 7 2 Composition given on Table 8

TABLE 28 PROXIMATE COMPOSITION OF EXPERIMENT 4 TEST DIETS FROM BIOCHEMICAL ASSAY (% weight)

COMPONENTS (% weight)	DIETS						
	NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7
Moisture	5.34	5.45	5.96	5.22	5.95	6.36	5.00
Protein	30.65	31.36	31.35	31.85	32.50	32.57	31.35
Fat	12.18	12.33	12.18	11.99	12.04	12.25	11.67
Carbohydrate [1]	15.51	25.95	36.60	13.40	27.20	37.10	1.24
Ash	8.72	8.79	8.69	6.99	7.93	8.54	8.67
Subtotals	72.40	83.88	94.78	69.45	85.62	96.82	57.93
Fibre [2]	27.60	16.12	5.22	30.55	14.38	3.18	42.07
Cr_2O_3 [3]	0.50	0.50	0.51	0.52	0.52	0.51	0.50
Energy [4]	18.59	20.45	22.56	18.42	19.84	20.99	16.41

[1] Hydrolysable carbohydrate

[2] Computed as difference between subtotal and 100

[3] Presented on dry-weight basis

[4] Kilojoules/gram

as detailed in Chapter 2.9.

6.2.6. ANALYSES OF EXPERIMENTAL DATA AND STATISTICAL METHODS

These were carried out as discussed in Chapter 2.10.

6.3. RESULTS

The health of all fish appeared good. Fish fed actively all through the eight-week experimental period. Results obtained from the growth data food utilization, nutrient digestibility and other growth parameters are presented.

6.3.1. GROWTH PERFORMANCE

There was no significant difference ($p>0.05$) between the initial weighs of the experimental fish (Table 29). Inclusion of 30% dietary corn or potato starch significantly ($p<0.05$) improved growth (Table 29). At 45% dietary corn, no further increase in growth or specific growth rate occurs. At 45% dietary potato, there is growth retardation as evidenced by the values for the final weight, daily percentage weight gain and specific growth rate. (Table 29). The largest weight gain during the 8 week feeding period is obtained with 30% corn or potato (Figs. 16 & 17).

6.3.2. FOOD CONVERSION

The efficiency of conversion of feed into flesh referred to as Food Conversion Ratio (FCR) (Chapter 2.10.6) was calculated and is represented in Table 29. The poorest FCR occurs in fish fed 15% dietary corn. Mirror Carp readily respond to increase in dietary

TABLE 29 GROWTH AND FOOD UTILIZATION OF MIRROR CARP FED DIFFERENT LEVELS OF CORN AND POTATO STARCH FOR 8 WEEKS

MEAN VALUES

DIETS

	NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	± SEM
Initial weight (g)	18.52^{a}	18.13^{a}	17.69^{a}	17.62^{a}	18.66^{a}	18.26^{a}	17.65^{a}	0.41
Final weight (g)	27.66^{a}	34.34^{c}	34.42^{c}	32.05^{bc}	34.52^{c}	32.02^{bc}	30.26^{ab}	0.442
Weight gain (%)	49.28^{a}	89.60^{d}	94.64^{e}	81.76^{c}	85.12^{cd}	75.60^{b}	71.68^{b}	1.303
S.G.R [1] (%)	0.72^{a}	1.14^{d}	1.19^{e}	1.07^{cd}	1.10^{d}	1.00^{bc}	0.96^{b}	0.011
F.C.R [2]	3.00^{e}	1.61^{b}	1.33^{a}	1.71^{b}	1.63^{b}	1.99^{d}	1.83^{c}	0.029
P.E.R [3]	1.09^{a}	1.98^{e}	2.40^{f}	1.84^{cd}	1.89^{d}	1.54^{b}	1.75^{c}	0.050
Apparent NPU [4] (%)	17.25^{a}	28.32^{c}	28.67^{c}	25.73^{b}	27.24^{bc}	28.44^{c}	27.92^{bc}	0.449

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Specific Growth Rate [2] Food Conversion Ratio [3] Protein Efficiency Ratio

[4] Net Protein Utilization

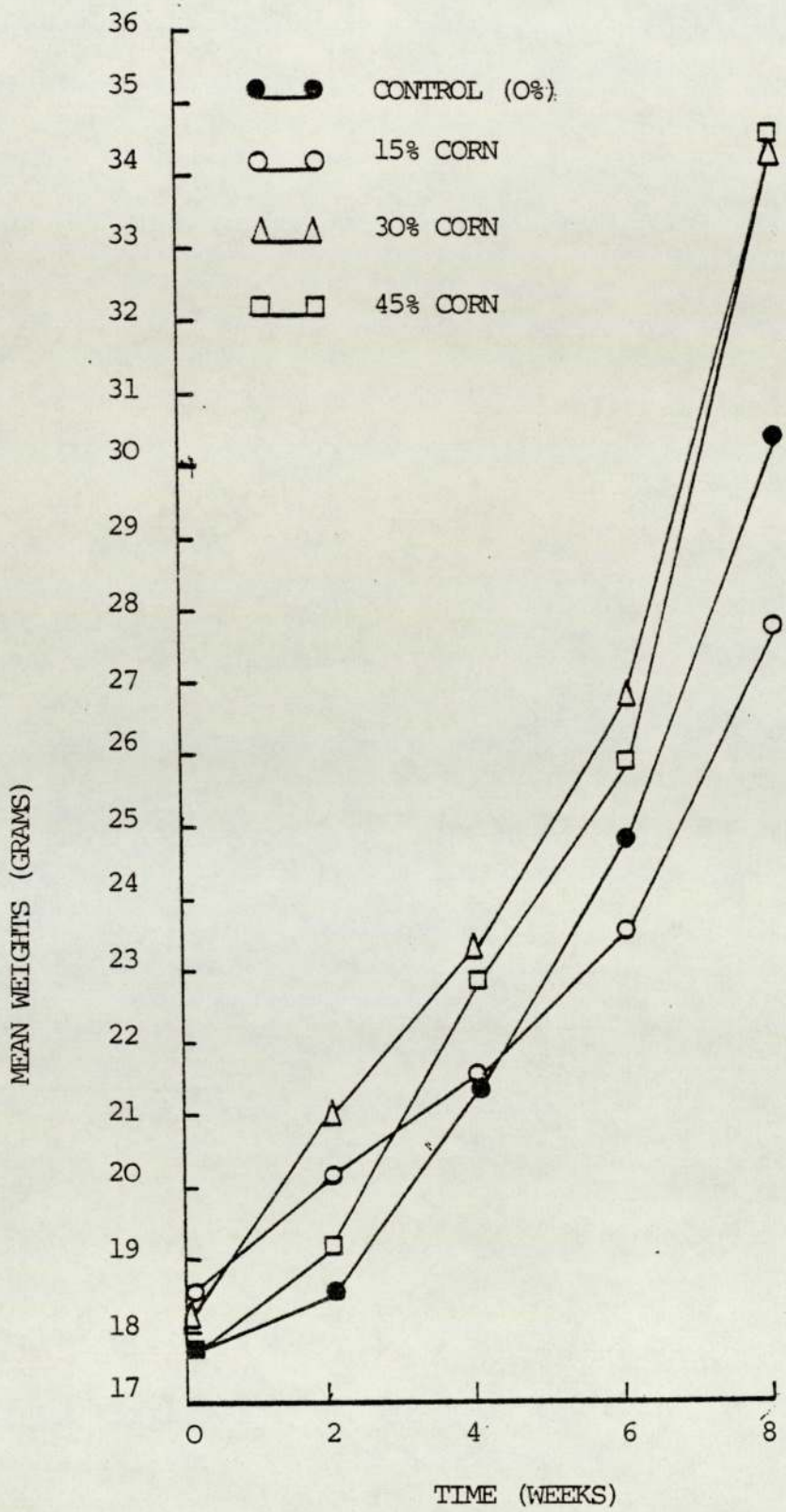


FIG. 16 Effect of different levels of dietary corn on weight gain of Mirror Carp

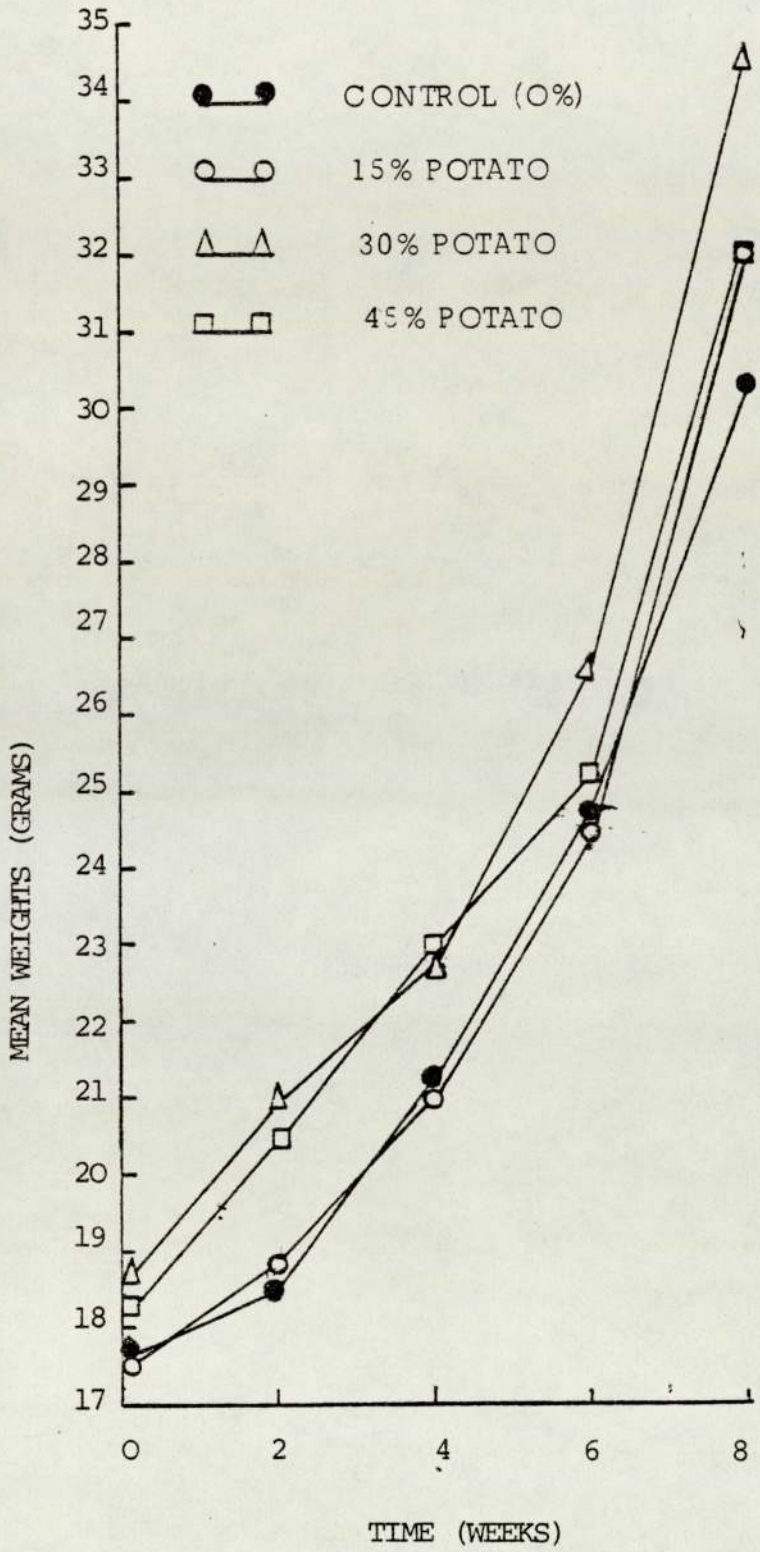


FIG. 17 Effect of different levels of dietary potato on weight gain of Mirror Carp

corn, the best FCR being obtained at 45% dietary corn. With the potato diets however, the best FCR is obtained at 30% dietary potato, the 45% potato diet leading to a poorer FCR than the 15% potato diet.

6.3.3. PROTEIN UTILIZATION

Increase in dietary corn increases the Protein Efficiency Ratio (PER) upto the high value of 2.40 for 45% dietary corn. There is no significant difference ($p > 0.05$) between the PER's at 15% and 30% dietary potato. At 45% dietary potato, the PER drops to 1.54. The PER at 15% dietary corn is the poorest, in all the seven experimental groups, and has a value of 1.09.

The Apparent Net Protein Utilization (NPU) shows a pattern very similar to that obtained for PER's. No significant difference ($p > 0.05$) exists between the Apparent NPU's at 30% and 45% dietary corn nor between the fish on 30% dietary corn, 30% and 45% potato and the control group.

6.3.4. PATHOLOGY

There was slight hypoglycaemia in fish receiving 15% or less of corn or potato starch. (Table 30).

When compared to the initial assay, a significant ($p < 0.05$) drop in Hepato-somatic Index occurred in the control fish as well as those receiving 15% dietary corn or potato. The faeces of fish receiving the 15% corn or potato diets was more consistent than those of the remaining five groups. There is however, no significant difference ($p > 0.05$) between the moisture contents of fish faeces on 30% and 45% corn and potato starch, and the control, and there was no evidence of diarrhoea except in the fish fed 45% dietary potato. There was less than 1% mortality during the eight-week experimental period. Histological examination of the liver showed no liver damage (Plates 5 & 6).

6.3.5. CARCASS AND LIVER COMPOSITION

The composition of the carcass and liver from proximate analysis are shown in Tables 33 and 34. With increase in age and size, carcass moisture content decreased by an average of about 3%. Carcass moisture content does not depend on the level of dietary corn or potato. No such size or age effect on liver moisture content is observed. However, the control group possess slightly more liver moisture than most of the other fish groups. Only at 45% dietary

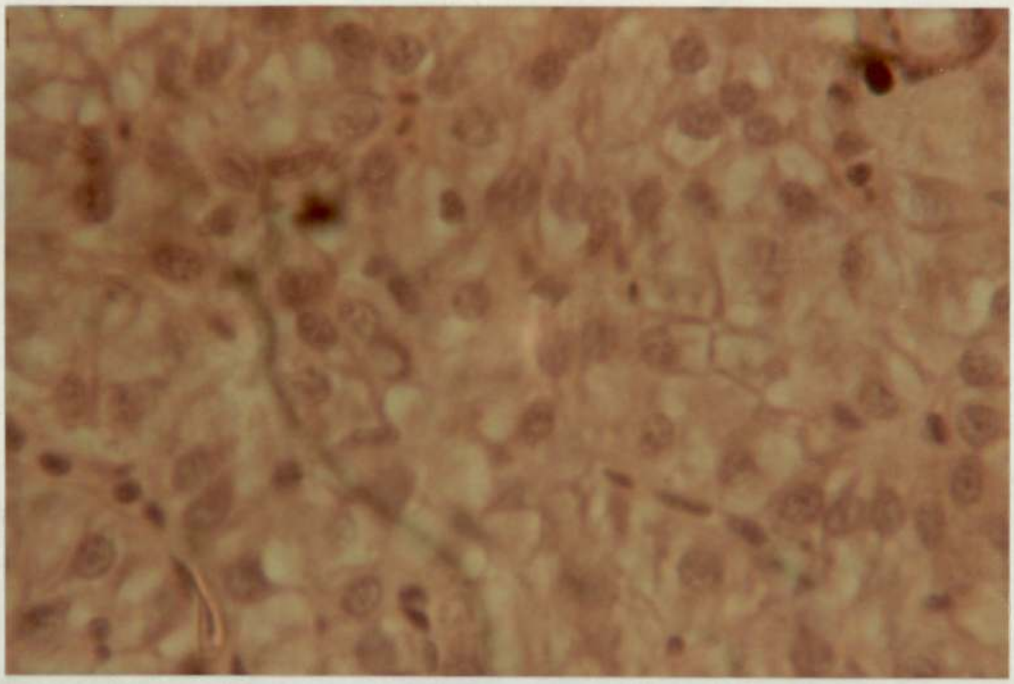


PLATE 3 Cross-section of the liver of Rainbow trout before Experimental feeding (x 250)

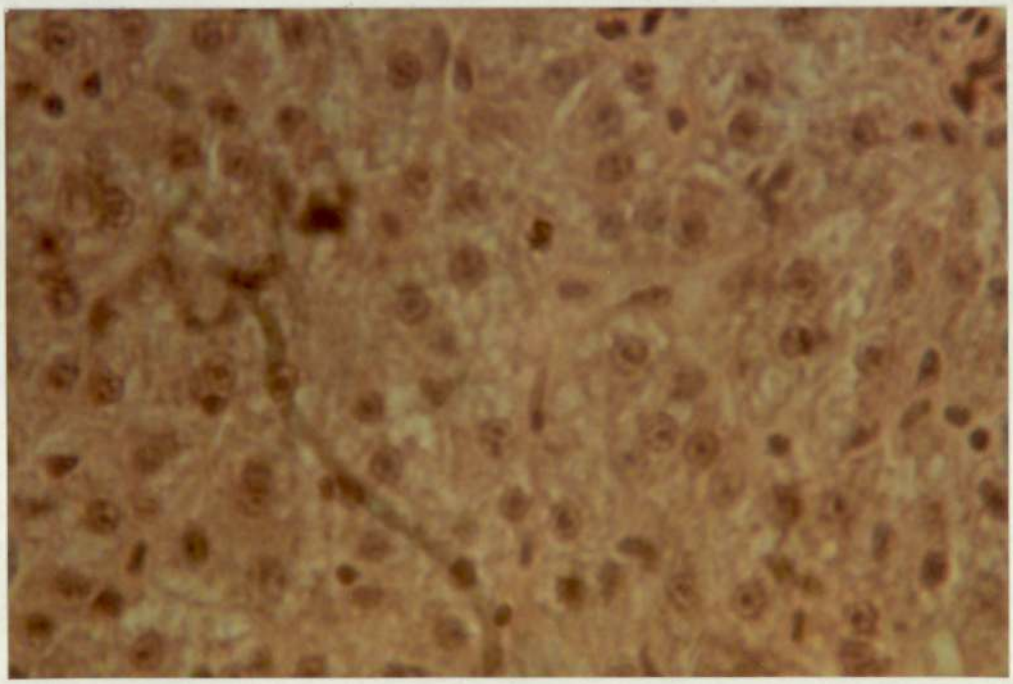


PLATE 4 Cross-section of the liver of Rainbow trout after 10 weeks of feeding 45% dietary potato

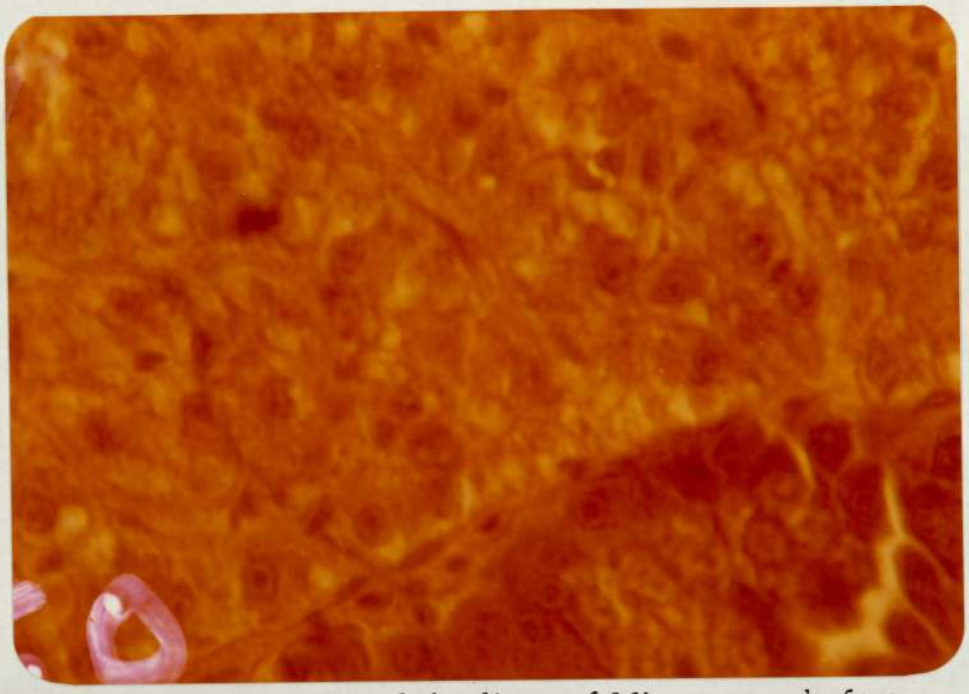


PLATE 5 Cross-section of the liver of Mirror carp before experimental feeding (x 250)

PLATE 6 Cross-section of the liver of Mirror carp after 10 weeks of feeding a 45% dietary potato

TABLE 30 PERFORMANCE OF MIRROR CARP FROM EXPERIMENT 4

	INITIAL	FINAL						
		NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	± SEM
Blood glucose (%)	55.65^{b}	51.33^{a}	65.66^{b}	68.01^{b}	58.83^{a}	59.50^{b}	59.00^{b}	55.00^{a}	2.013
HSI [1]	1.50^{c}	0.98^{a}	1.49^{c}	1.54^{c}	0.94^{a}	1.15^{b}	1.98^{d}	1.04^{a}	0.040
Moisture in faeces (%)		77.88^{a}	89.37^{bc}	92.21^{bc}	83.69^{ab}	91.00^{bc}	94.76^{c}	93.32^{c}	3.145
Gross mortalities [2]		1	0	0	0	0	0	0	

Figures in the same row having the same superscript are not significantly different ($p>0.05$)

[1] Hepato-somatic Index

[2] Died from overdose of anesthesia

TABLE 31 INITIAL AND FINAL CARCASS COMPOSITION OF MIRROR CARP FROM EXPERIMENT 4

MEAN VALUES (% weight)	INITIAL	FINAL							± SEM
		NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	
Moisture	80.25^{b}	77.75^{a}	76.85^{a}	77.51^{a}	76.67^{a}	77.44^{a}	75.47^{a}	77.83^{a}	1.958
Carbohydrate [1]	0.56^{b}	0.63^{b}	0.61^{b}	0.72^{c}	0.70^{c}	0.68^{bc}	0.91^{d}	0.45^{a}	0.021
Fat	4.42^{a}	5.88^{b}	5.83^{b}	6.80^{c}	5.46^{b}	5.06^{ab}	5.63^{b}	4.38^{a}	0.082
Protein	11.11^{a}	12.70^{b}	12.60^{b}	11.53^{a}	12.41^{b}	12.61^{b}	14.27^{c}	13.14^{b}	0.599
Ash	3.40^{bc}	2.99^{ab}	3.12^{b}	2.57^{a}	3.09^{b}	3.40^{bc}	3.28^{b}	3.74^{c}	0.049
TOTALS	99.74	99.95	99.01	99.13	98.33	99.19	99.56	99.54	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Total glycogen and glucose

TABLE 32 INITIAL AND FINAL LIVER COMPOSITION OF MIRROR CARP FROM EXPERIMENT 4

	INITIAL	FINAL							
MEAN VALUES (% weight)		NC-1	NC-2	NC-3	PC-4	PC-5	PC-6	OC-7	± SEM
Moisture	78.88^{a}	79.82^{a}	79.16^{a}	80.78^{a}	82.14^{ab}	80.22^{a}	79.56^{a}	84.50^{b}	1.095
Carbohydrate [1]	4.55^{b}	4.35^{b}	4.33^{b}	4.57^{b}	5.57^{c}	4.91^{bc}	6.74^{d}	3.54^{a}	0.246
Fat	4.24^{a}	3.95^{a}	4.00^{a}	3.82^{a}	3.64^{a}	4.51^{ab}	3.52^{a}	5.36^{b}	0.655
Protein	10.51^{c}	10.29^{c}	11.21^{c}	10.95^{c}	8.36^{b}	8.85^{b}	8.69^{b}	6.04^{a}	0.521
Ash	1.62^{d}	1.62^{d}	1.41^{c}	1.01^{b}	1.02^{b}	0.99^{b}	0.78^{a}	1.01^{b}	0.015
TOTALS	99.80	100.03	100.11	101.13	100.73	99.48	99.29	100.45	

Figures in the same row having the same superscripts are not significantly different ($p>0.05$)

[1] Total glucose and glycogen

potato does the carcass and liver glucose/glycogen show any significant ($p < 0.05$) increase. The liver glucose/glycogen content of the control group is however, lower than those of the experimental groups. Carcass fat at 30% dietary corn is significantly greater than the rest of the experimental groups ($p < 0.05$) though there is no significant difference ($p > 0.05$) in liver fat of fish receiving dietary corn or those on the potato starch diet.

The potato diet tends to lead to more protein sparing. There is a higher carcass ash content in the fish fed the control diet. In the liver, increase in dietary corn or potato generally tends to lead to a graded decrease in ash content.

6.3.7. NUTRIENT DIGESTIBILITY

The results obtained for the digestibility of dietary carbohydrate and protein are represented in the histograms (Figs. 18 & 19). The improvement in carbohydrate digestibility with increase in dietary corn or potato is only eminent in the fish consuming the potato diets. The digestibility of carbohydrate in corn-fed Mirror Carp is not significantly affected by the level of dietary corn and was found to vary between 86% and 95%. In the fish fed on the control diet, dietary digestible carbohydrate content was only about 1.24%, and its digestibility was about 34%. Lack of dietary corn or potato equally depresses apparent digestibility of dietary protein

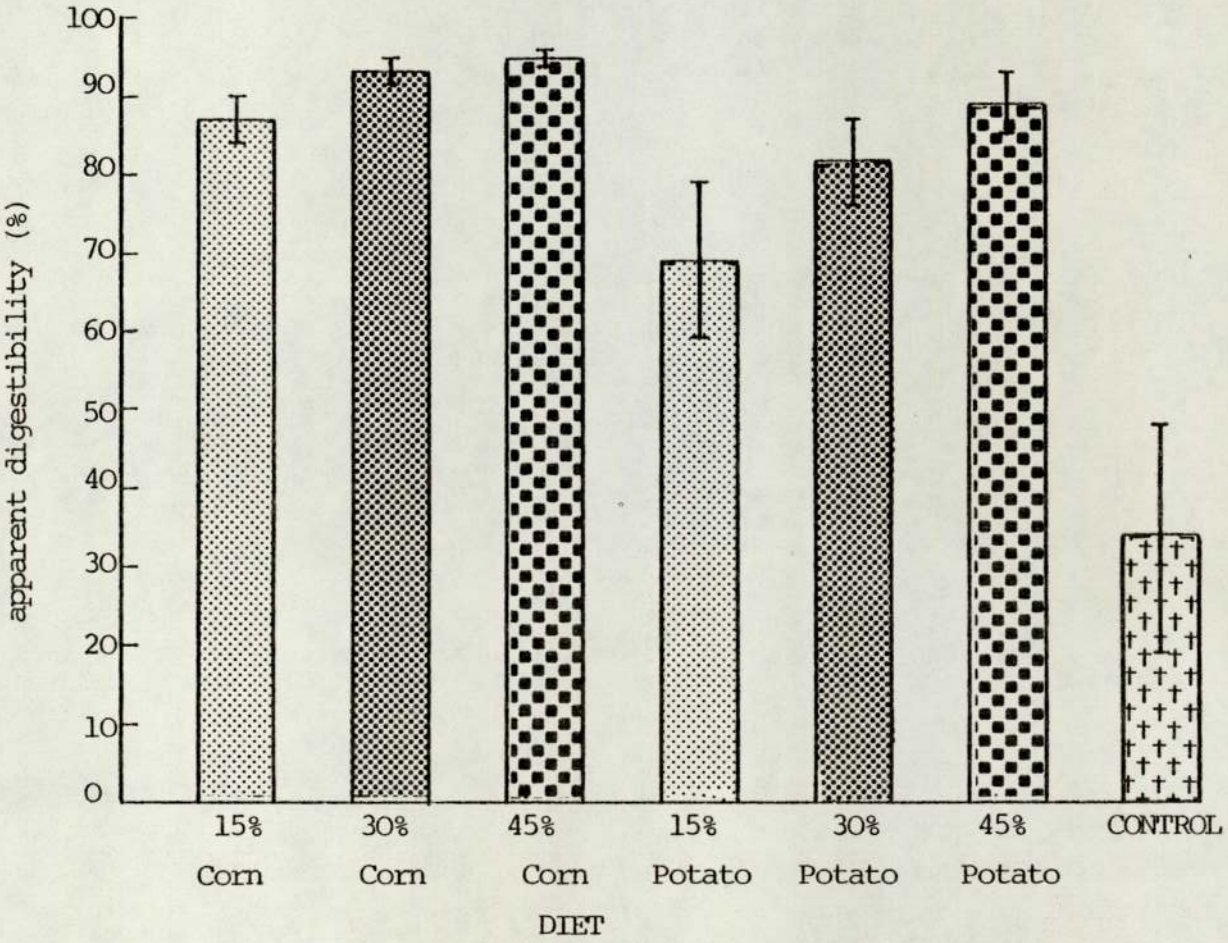


FIG. 18 Apparent digestibility of dietary carbohydrate in Mirror Carp from Experiment 4

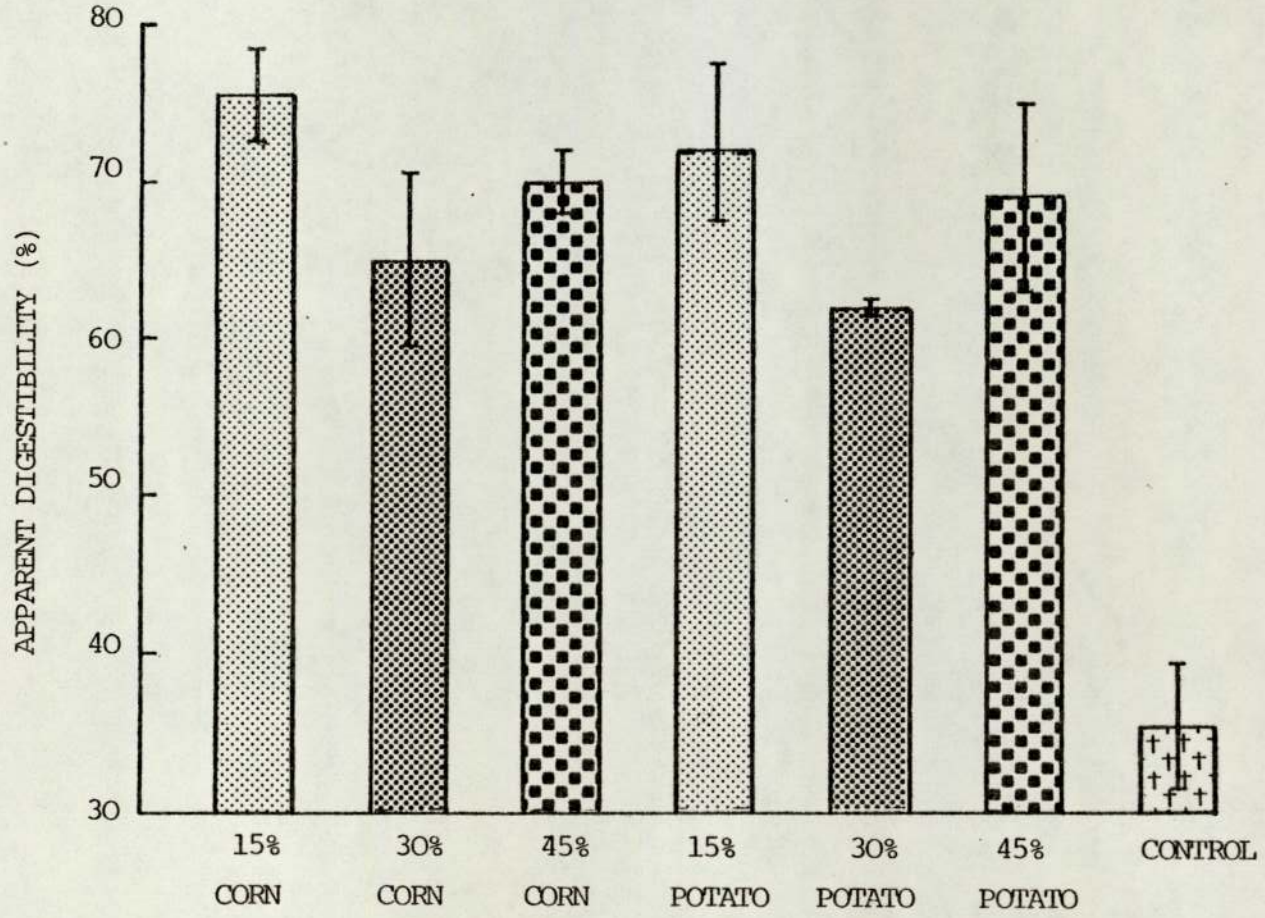


FIG. 19 Apparent digestibility of dietary protein in Mirror Carp from Experiment 4

in Mirror Carp to about 36%. With the inclusion of corn or potato however, protein digestibility is improved and ranges between about 63% (for 30% cassava diet) and about 75.5% (for 15% corn diet) (Fig. 19).

6.4. DISCUSSION AND CONCLUSIONS

From the results of the experiment reported in this Chapter, there is evidence that Mirror Carp are capable of utilizing limited amounts of dietary corn and potato. From the values of the weight gain, Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), and Protein Efficiency Ratio, (PER), it is obvious that Mirror Carp are best adapted to metabolise dietary corn rather than dietary potato starch. At a 30% dietary corn inclusion, the best growth and feed utilization was achieved. Dietary potato at 45% level of inclusion in diet led to comparatively poor growth, FCR and PER but, surprisingly good Apparent NPU. There was however, evidence of diarrhoea probably due to the diet, and appreciably higher Hepato-somatic Index. There was no evidence of persistent hyperglycaemia.

Results from carcass analysis show a slight increase in carcass fat of fish on 45% corn, with a corresponding slight drop in carcass protein. The protein content of the liver of fish on the potato diet were significantly ($p < 0.05$) less than those of fish on the

corn diet. The liver glucose/glycogen content of the fish on the potato diets were equally higher than those of the fish on the corn diets. At identical levels of inclusion in diet, corn and potato starch tend to have no significant ($p > 0.05$) effect on protein digestibilities. However, at 15% and 30% dietary corn and potato the digestibility of corn starch is significantly higher ($p < 0.05$) than potato starch digestibility. There is increase in dietary energy content with increase in the level of corn and potato. The performance of the fish did not however, portray such a corresponding graded rise. Thus though the slight differences in dietary energy level must have affected the growth of the fish, the acceptability of the carbohydrates must be an over-riding factor. For similar reasons, the increase in gut mortility resulting from an increase in dietary fibre content must have produced only a marginal effect on the nutrient digestibility.

It is concluded that Mirror carp are better adapted to utilize corn than potato starch in diets. Inclusion of between 30% and 45% dietary corn or 30% dietary potato into the diet of Mirror Carp improved growth, feed utilization, protein utilization, protein sparing and nutrient digestibilities.

CHAPTER 7

CHAPTER 7

EXPERIMENT FIVE

SOME ASPECTS OF CARBOHYDRATE METABOLISM IN RAINBOW TROUT FED DEXTRIN IN ISONITROGENOUS DIETS

7.1 INTRODUCTION

Some crude carbohydrate sources have been shown to be well utilized by Rainbow trout (Chapters 3 & 5). The incorporation of 10% crude carbohydrate into isonitrogenous trout diets tended generally to decrease growth, digestibility of dietary protein, and some other aspects of diet utilization (Chapters 3 & 5). However, with the increase of dietary carbohydrate concentration, growth and the utilization of dietary ingredients tended to improve at first, but deteriorated with very high levels of dietary carbohydrate (of 30%).

It is possible that there is a threshold level of dietary carbohydrate that would trigger off adequate utilization of dietary carbohydrate, while the apparent growth retardation at very high dietary carbohydrate levels could be the result of insufficient carbohydrases to metabolise the carbohydrate. This speculation is in agreement with those discussed in Chapter 1.

To obviate the possible advantageous or deleterious effect that a crude carbohydrate source could have on carbohydrate utilization in Rainbow trout tissues, an experiment was

designed to study the short-term effect of feeding diets containing different levels of ∝-dextrin[1] (Sigma Chem. Co., St. Louis, USA) on the growth, feed conversion, protein utilization, carbohydrate utilization, blood glucose level, nutrient digestibilities and some carbohydrase activities in Rainbow trout tissues.

7.2. MATERIALS AND METHODS

7.2.1. EXPERIMENTAL ANIMALS AND SYSTEM

For this experiment, 150 fingerling-Rainbow trout weighing 58.00 g $\pm$ 2.17 g and measuring 16.58 cm $\pm$ 0.17 cm. were used. Fish were collected from Midland Fisheries, Nailsworth, Gloucestershire. The fish were subjected to quarantine treatment (2.1), and were distributed evenly by number, and by weight, in the three tanks of Experimental "System 5". Fish were not branded as samples were randomly taken fortnightly for analysis. Before the onset of the experiment, fish were allowed to acclimatize for one week, during which time they were fed a total of 2% of their body weight twice daily on commercial (Edward Baker, Sudbury, Essex) diet.

7.2.2. PREPARATION OF EXPERIMENTAL DIETS

Soluble dextrin (Sigma Chem. Co., St. Louis, USA) was analysed (Table 6), and then incorporated in isonitrogenous diets containing white fish-meal (Edward Baker, Sudbury, Essex) as the major protein source. The three diets, DC-1, DC-2, and

[1] White dextrin from potato

DC-3 contained 0%, 15% and 30% of dextrin, (Table 33). Diets were prepared with the method described in Chapter 2.3., analysed as described in Chapter 2.7., (Table 34), and were then frozen and aliquots were weighed out and used when required.

7.2.3. FEEDING

The experimental fish were fed 2% of their body-weight twice daily at 10.00 a.m. and 5.00 p.m. All the fish fed actively.

7.2.4. WEIGHING AND SAMPLING

At the start of the experiment, ten fish were randomly picked from the pool for analysis. All 50 fish in each tank were then individually weighed (2.4). Thereafter, fish were individually weighed fortnightly during which time faeces was collected (2.6), and samples taken for analyses as detailed in Chapter 2.7. Weighing was done between 8.00 a.m. and 9.00 a.m., and the fish were not fed on the weighing day.

7.2.5. CARBOHYDRASE ACTIVITIES

7.2.5.1. STANDARD CURVES

Standard curves for α-amylase and α-glucosidase activities were plotted as detailed in Chapter 2.8.3. From a standard curve equation derived from the relationship between the two axes in each of the curves, (Figs. 23 & 24) a further equation for interpreting the spectrophotometric readings for the

TABLE 33 COMPOSITION OF DIETS FOR EXPERIMENT 5 (g/100 g diet)

INGREDIENTS	DIETS DESIGNATIONS		
	DC-1 (control)	DC-2	DC-3
Dextrin	–	15.00	30.00
White fish meal	45.00	45.00	45.00
Casein	10.00	10.00	10.00
∝-cellulose	30.00	15.00	–
Mineral mix [1]	4.00	4.00	4.00
Vitamin mix [2]	2.00	2.00	2.00
Cod-liver oil	3.00	3.00	3.00
Corn oil	5.00	5.00	5.00
Chromic oxide	0.50	0.50	0.50
Carboxy-methyl cellulose (Binder)	0.50	0.50	0.50
TOTALS	100.00	100.00	100.00

[1] Composition given on Table 7

[2] Composition given on Table 8

TABLE 34 PROXIMATE COMPOSITION OF EXPERIMENT 5 TEST DIETS FROM BIOCHEMICAL ASSAY (% weight)

COMPONENTS (% weight)	DIETS		
	DC-1 (Control)	DC-2	DC-3
Moisture	4.54	5.48	6.16
Protein	43.91	43.55	43.00
Fat	10.98	8.99	10.80
Carbohydrate [1]	1.25	15.88	31.00
Ash	7.58	7.88	7.64
Subtotals	68.26	81.78	98.60
Fibre [2]	31.74	18.22	1.40
Cr_2O_3	0.51	0.49	0.50
Energy [4]	20.14	21.94	23.00

[1] Hydrolysable carbohydrate

[2] Computed as difference between subtotal and 100

[3] Presented on dry weight basis

[4] Kilojoules/gram

different enzyme reaction products as U-moles of liberated substance is then derived.

7.2.5.2. α -AMYLASE AND α-GLUCOSIDASE ACTIVITIES

The α-amylase and α-glucosidase enzyme activities are estimated in the reddorsal muscle, liver, stomach, intestine and blood as detailed in Chapters 2.8.1. and 2.8.2., using known weights of tissues. Using the standard curve equations (Figs. 23 and 24) the α-amylase and α-glucosidase enzyme activities were then computed.

7.2.6. ANALYSES OF EXPERIMENTAL DATA AND STATISTICAL METHODS

These were performed as detailed in Chapter 2.10.

7.3. RESULTS

Fish fed actively during the six-week experimental period. All the fish appeared healthy, and there were no mortalities.

7.3.1. GROWTH PERFORMANCE

The fortnightly weights of the Rainbow trout from this experiment is shown in the graph (Fig. 20). There is no significant difference ($p > 0.05$) between the initial weights of the fish. The final weight, daily percentage weight gain and the Specific Growth Rate (SGR) were best in the Rainbow trout

fed on 15% dextrin (Table 35). Though the final weight and the percentage weight gain of the fish on the 30% dextrin diet were slightly better than those of the control fish whose diet was devoid of dextrin, the SGR in these two groups are not significantly different ($p > 0.05$) (Table 35).

7.3.2. FOOD CONVERSION

The Food Conversion Ratios (FCR's) was good in all the three groups. The best FCR of 1.00 was obtained with 15% dietary dextrin. With 30% dextrin, the FCR was only slightly better than that obtained with the control diet.

7.3.3. PROTEIN UTILIZATION

The Protein Efficiency Ratio (PER) is highest with 15% dietary dextrin. There is no significant difference ($p > 0.05$) between the PER's of the control fish and those receiving diets containing 30% dextrin.

The Apparent Net Protein Utilization (NPU) is presented for this investigation in Table 35. Values of Apparent NPU of 37.76% were achieved with 15% dextrin diet. The poorest Apparent NPU is obtained with the control diet. Thus, the best protein sparing action is obtained with 15% dietary dextrin.

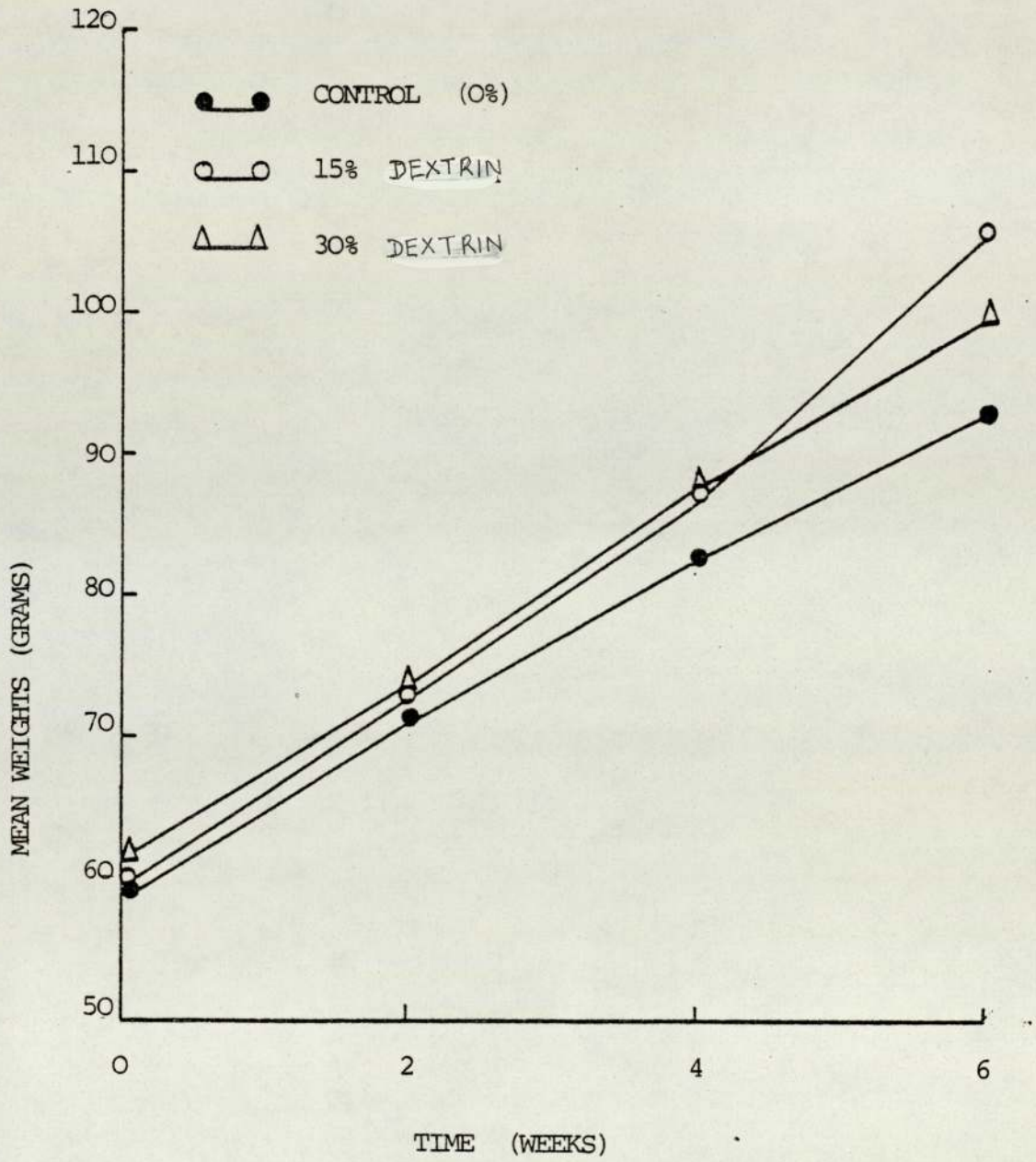


FIG. 20 Effect of different levels of dietary dextrin on weight gain of Rainbow trout

TABLE 35 GROWTH AND FOOD UTILIZATION OF RAINBOW TROUT FED FOR 6 WEEKS ON DIFFERENT LEVELS OF DIETARY DEXTRIN

MEAN VALUES	DIETS			
	DC-1 (Control)	DC-2	DC-3	± SEM
Initial Weight (g)	59.38 [a]	60.39 [a]	61.74 [a]	1.971
Final Weight (g)	93.03 [a]	105.60 [b]	100.15 [ab]	2.109
Weight gain (%)	48.72 [a]	65.50 [c]	52.50 [b]	1.500
S.G.R. [1] (%)	1.07 [a]	1.40 [b]	1.15 [a]	0.125
F.C.R. [2] (%)	1.35 [c]	1.00 [a]	1.26 [b]	0.016
P.E.R. [3]	1.74 [a]	2.30 [b]	1.88 [a]	0.070
Apparent N.P.U. [4] (%)	26.35 [a]	37.76 [b]	35.67 [b]	1.067

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Specific Growth Rate [2] Food Conversion Ratio [3] Protein Efficiency Ratio

[4] Net Protein Utilization

TABLE 36 PERFORMANCE OF RAINBOW TROUT FROM EXPERIMENT 5

	INITIAL	FINAL			
		DC-1	DC-2	DC-3	± SEM
Blood glucose (mg %)	65.20 [a]	61.75 [a]	65.25 [a]	66.50 [a]	2.525
H.S.I [1]	1.39 [a]	1.41 [a]	1.35 [a]	1.54 [b]	0.114
% Moisture in faeces	—	87.83 [a]	87.20 [a]	88.42 [a]	0.754
Gross mortalities	—	0	0	0	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Hepato-somatic Index

TABLE 37 CHANGE IN CARCASS COMPOSITION OF RAINBOW TROUT FROM EXPERIMENT 5

(% weight)

	Week 0	Week 2			Week 4			Week 6			
DIETARY DEXTRIN		0%	15%	30%	0%	15%	30%	0%	15%	30%	± SEM
Moisture	75.20^{a}	74.17^{a}	74.78^{a}	74.36^{a}	74.98^{a}	75.52^{a}	74.49^{a}	75.80^{a}	75.25^{a}	75.33^{a}	1.234
Carbohydrate[1]	0.45^{bc}	0.49^{c}	0.49^{c}	0.52^{cd}	0.34^{a}	0.40^{b}	0.58^{d}	0.45^{bc}	0.50^{c}	0.47^{c}	0.050
Fat	7.43^{a}	7.67^{a}	7.20^{a}	7.57^{a}	6.88^{a}	6.30^{a}	7.07^{a}	6.32^{a}	6.66^{a}	6.11^{a}	0.986
Protein	14.55^{a}	14.50^{a}	14.98^{a}	15.77^{a}	15.07^{a}	15.50^{a}	16.11^{a}	14.87^{a}	15.35^{a}	16.34^{a}	1.835
Ash	2.51^{d}	2.83^{e}	1.81^{a}	1.94^{a}	2.21^{c}	2.15^{c}	1.81^{a}	1.93^{a}	2.03^{b}	1.80^{a}	0.075
Totals	100.14	99.66	99.26	100.16	99.48	99.87	100.06	99.37	99.79	100.05	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Total glucose and glycogen

TABLE 38 CHANGE IN LIVER COMPOSITION OF RAINBOW TROUT FROM EXPERIMENT 5

(% weight)

	Week 0	Week 2			Week 4			Week 6			
DIETARY DEXTRIN		0%	15%	30%	0%	15%	30%	0%	15%	30%	± SEM
Moisture	78.91^{a}	80.27^{a}	78.81^{a}	79.96^{a}	78.51^{a}	78.28^{a}	76.83^{a}	79.98^{a}	76.67^{a}	76.23^{a}	2.055
Carbo-hydrate [1]	2.48^{a}	2.55^{ab}	2.60^{ab}	3.01^{b}	2.44^{a}	2.67^{ab}	3.61^{bc}	2.01^{a}	3.54^{bc}	4.00^{c}	0.071
Fat	2.07^{a}	2.09^{a}	3.12^{a}	2.26^{a}	3.08^{a}	3.00^{a}	2.36^{a}	2.66^{a}	2.40^{a}	2.24^{a}	0.824
Protein	14.46^{ab}	13.70^{a}	14.40^{a}	14.54^{ab}	14.42^{ab}	15.42^{bc}	16.27^{cd}	13.59^{a}	15.10^{b}	16.57^{d}	0.512
Ash	1.48^{de}	1.38^{cd}	1.48^{de}	0.76^{a}	1.10^{b}	1.57^{e}	1.31^{c}	0.68^{a}	1.53^{e}	1.41^{cd}	0.031
TOTALS	99.40	99.99	100.41	100.53	99.55	100.94	100.38	98.92	99.24	100.45	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Total glucose and glycogen

7.3.4. PATHOLOGY

As shown in Table 36, there is no significant ($p > 0.05$) change in the blood glucose level at the 6th week when this is compared to the initial value.

Only the fish receiving 30% dietary dextrin have a significantly ($p < 0.05$) larger Hepato-somatic Index (HSI) when compared to the other two groups. The moisture contents of the faeces of all the fish is however, not significantly different ($p > 0.05$).

7.3.5. CARCASS AND LIVER COMPOSITION

The carcass composition of the fish measured fortnightly over the six-week period is shown in Table 37. No significant difference ($p > 0.05$) occurs in the moisture, fat, and protein content of the carcass of all the groups. In the first two fortnights there was a slight decrease in the ash content of the fish receiving

dietary dextrin. Such a decrease is significant ($p < 0.05$) in the sixth-week only in the fish fed on 30% dextrin diet. Only in the fourth week is there a significant ($p < 0.05$) decrease in muscle glucose/glycogen content of the control fish, and an almost equivalent rise in the muscle glucose/glycogen content of the fish on 30% dietary dextrin.

In the liver, there is no significant ($p > 0.05$) change in moisture content. Liver glycogen however, increases gradually and steadily in the groups receiving dextrin diets. There is no significant difference ($p > 0.05$) between the liver fat content over the six-week period. However, a gradual decrease in liver fat was observed (at $p < 0.05$) while the liver protein tends to significantly ($p < 0.05$) increase with increase in dextrin.

The fish receiving 15% dietary dextrin tend to maintain the level of their liver ash at the status quo while the liver ash content of the control fish and those on 30% dietary carbohydrate show some decrease, that obtained with 30% dextrin diet being more drastic.

7.3.6. NUTRIENT DIGESTIBILITY

The Apparent digestibility of dietary carbohydrate is between 42.5% and 45.5% at 15% and 30% dietary dextrin; and is very close to zero (Fig. 21) when the diet is devoid of dextrin. Apparent protein digestibility is not significantly ($p > 0.05$) improved with the addition of dextrin to the diet and is observed to be between 85% and about 89.7%. (Fig. 22).

7.3.7. CARBOHYDRASE ACTIVITY

7.3.8.1. α -AMYLASE

The α-amylase activity in the dorsal muscle, liver, stomach, intestine and blood were investigated and results obtained by multiplying the spectrophotometric readings with the standard curve equation (590.1282 x U moles). Results are presented in Table 39 as U moles of maltose liberated/minute/gram moist tissue at $37^{o}C$.

It is seen from the results that the highest α-amylase activity in the tissues studied was in the liver, the highest activity being obtained in the liver of fish on 15% dietary dextrin. (Table 39).

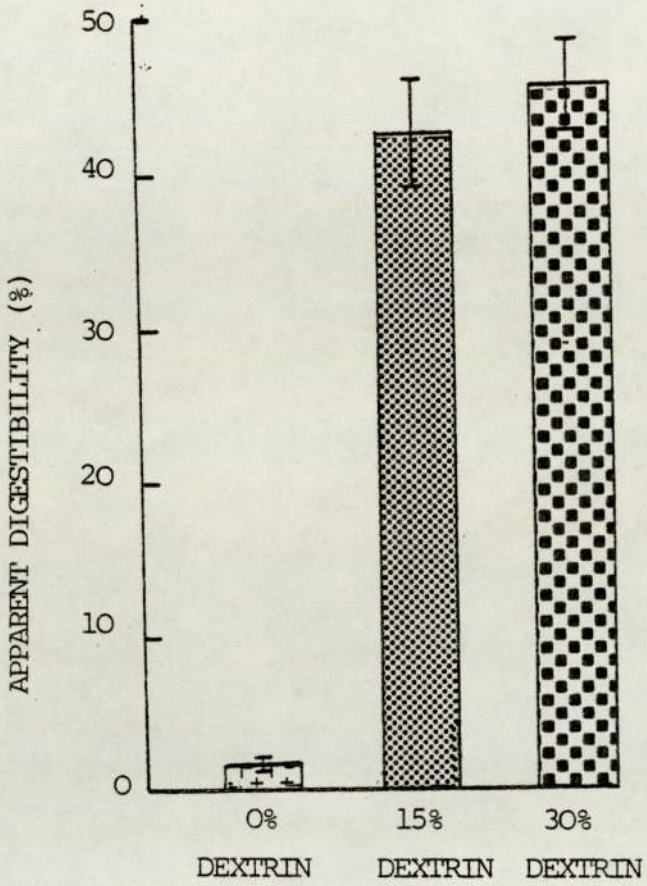


FIG. 21 Apparent digestibility of dietary carbohydrate in Rainbow Trout from Experiment 5

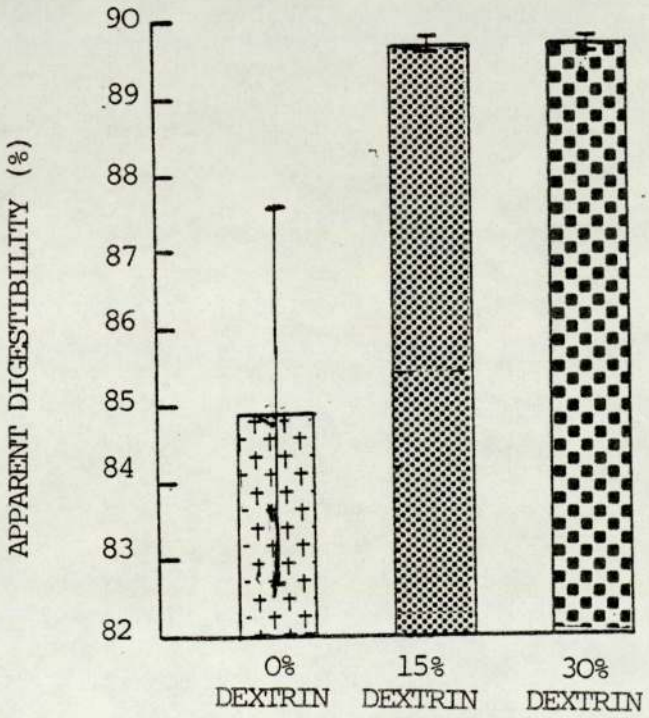


FIG. 22 Apparent digestibility of dietary protein in Rainbow trout from Experiment 5

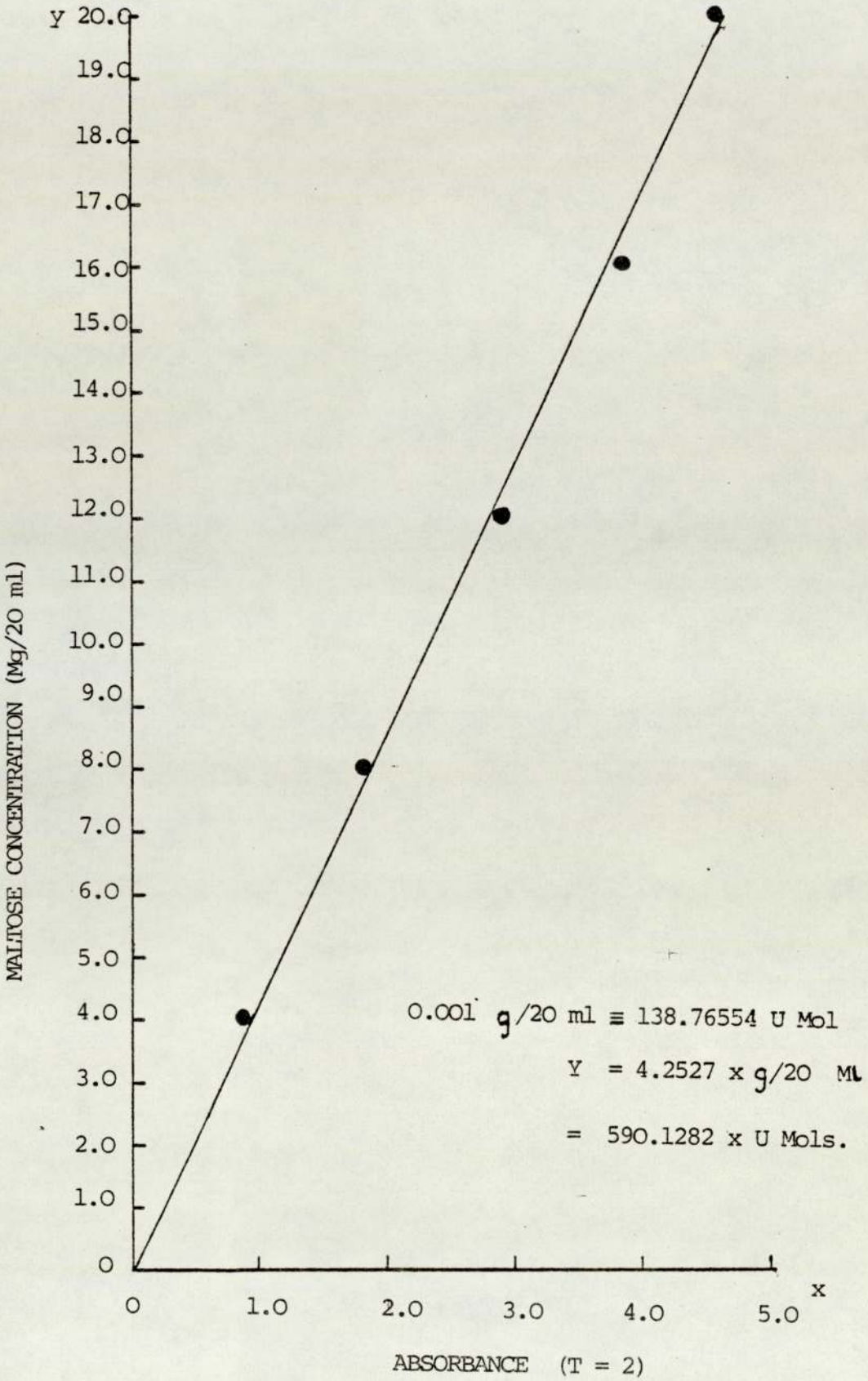


FIG. 23 Calibration curve for Maltose concentration
(α- amylase activity)

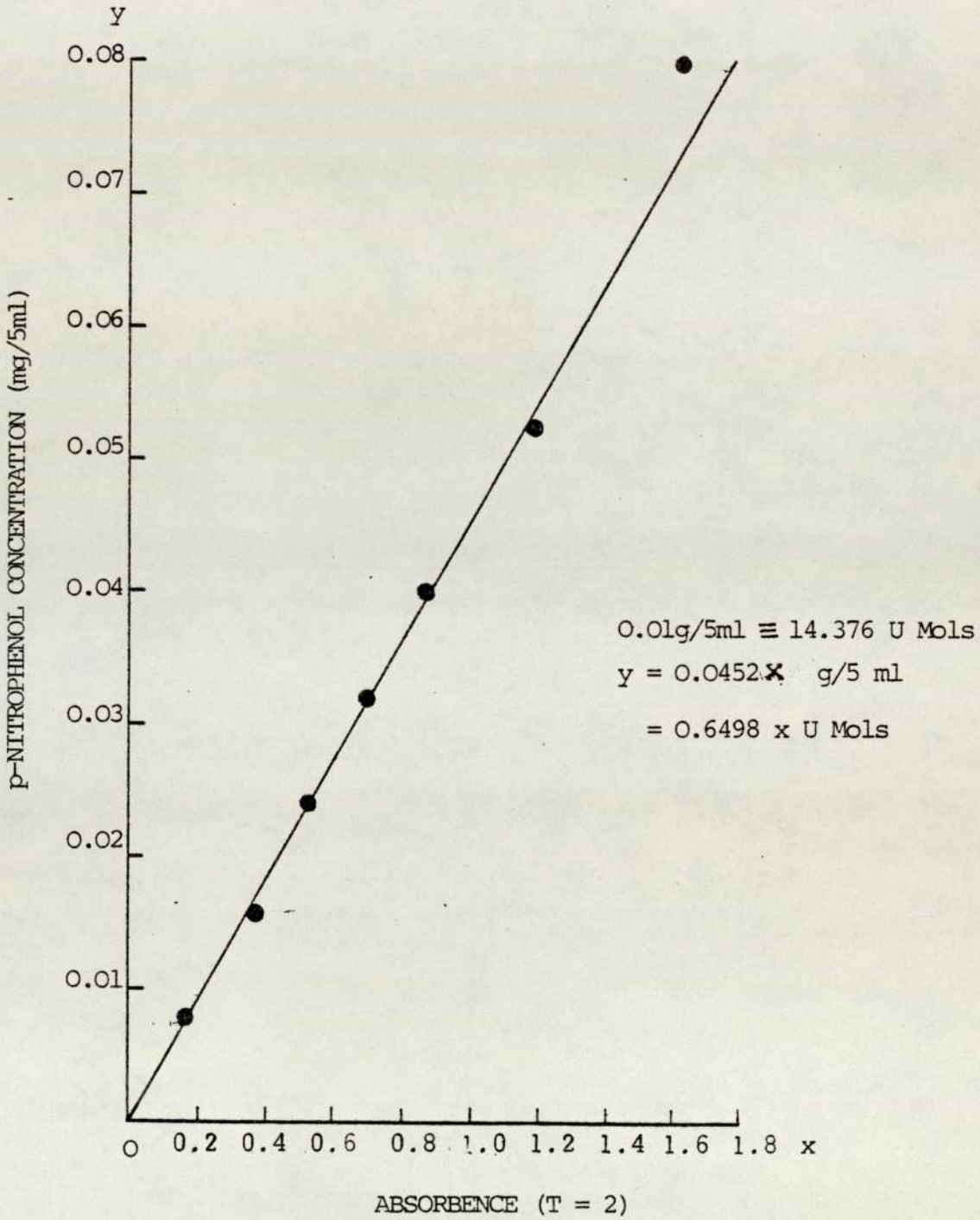


FIG. 24 Calibration curve for p-nitrophenol concentration
(α-glucosidase activity)

There is correlation between the α-amylase activity in the liver and the length of time the fish is kept on the dextrin diet. For the fish on the 0% dextrin diet, there is an initial drop in the α-amylase activity in the second week. By the fourth week, the activity rises to the status quo. By the sixth week however, there is a further rise in α-amylase activity in the liver of the control fish. For fish on 15% and 30% dietary dextrin, the α-amylase attains its maximum activity by the end of the second week. In the dorsal muscle, the tendency is for α-amylase activity to rise sharply in the second week and then drop sharply thereafter. Apart from the greater α-amylase activity in the stomach of fish fed 15% and 30% dextrin diets over and above the activity in the control group by the end of the second week, no significant difference caused by diet ($p > 0.05$) is observed in the subsequent weeks. However, a peak stomach α-amylase activity is achieved in all groups in the second week. The highest α-amylase activity in the intestine by the end of the second week occurs in the control fish. By the fourth week however, the α-amylase activity in the intestine of fish on 0% and 15% dextrin diets has dropped significantly. No detectable α-amylase activity was recorded for the blood.

7.3. .2. α-GLUCOSIDASE

From the α-glucosidase activity table (Table 40) it was observed that the enzyme activities represented as U mole of

p-nitrophenol liberated/minute/gram moist tissue at 37°C showed variations which had some correlation with the level of dextrin in diet and with time, and with the tissue type.

During the fourth week, there was a significant drop in α -glucosidase activity in all the tissues examined. The highest glucosidase activity of 0.214 U Mole of p-nitrophenol liberated/ minute/gram moist tissue is obtained at the end of the second week in Rainbow trout fed on 30% dextrin diet.

Generally, the intensity of α-glucosidase activity decreases in the order, liver > intestine > stomach > dorsal muscle > blood. In some of the cases (Table 40) order of magnitude of the α-glucosidase activity in the intestine, stomach and dorsal muscle are either reversed or are not significantly different ($p > 0.05$).

7.4. DISCUSSION AND CONCLUSIONS

From the results of the investigation reported in this Chapter, it is evident that though the inclusion of dextrin to the diet of Rainbow trout tends to improve growth and food utilization, a 30% dietary dextrin level when compared to the 15% dietary dextrin level causes growth retardation, and decrease in Food Conversion Ratio, (FCR), Protein Efficiency Ratio (PER), and Apparent Net Protein Utilization (NPU). The slight variation in the energy of the diets (Table 34) does not seem

TABLE 39 α-AMYLASE ACTIVITY [1] IN SOME TISSUES OF RAINBOW TROUT FROM EXPERIMENT 5

Tissue	Week 0	Week 2			Week 4			Week 6			± SEM
		0%	15%	30%	0%	15%	30%	0%	15%	30%	
Dorsal muscle	2.759^{c}_{a}	5.016^{d}_{b}	14.458^{f}_{c}	9.265^{e}_{b}	1.488^{b}_{a}	4.290^{d}_{b}	8.262^{e}_{a}	0.466^{a}_{a}	0.520^{a}_{a}	1.199^{ab}_{a}	0.665
Liver	11.366^{b}_{d}	3.541^{a}_{a}	28.326^{c}_{d}	8.852^{b}_{b}	10.528^{b}_{b}	53.702^{e}_{d}	41.309^{d}_{c}	25.538^{c}_{c}	48.840^{e}_{c}	39.854^{d}_{c}	2.021
Stomach	3.140^{a}_{b}	5.901^{b}_{b}	8.262^{c}_{b}	13.573^{e}_{c}	10.624^{d}_{b}	9.442^{cd}_{c}	10.622^{d}_{b}	5.339^{b}_{b}	5.316^{b}_{b}	5.645^{b}_{b}	0.511
Intestine	4.394^{bc}_{c}	8.557^{d}_{c}	3.246^{b}_{a}	5.311^{c}_{a}	1.180^{a}_{a}	1.475^{a}_{a}	11.803^{e}_{b}	0.912^{a}_{a}	0.678^{a}_{a}	1.128^{a}_{a}	0.412
Blood	ND [2]	ND	ND	ND	ND	ND	ND	ND	ND	ND	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

Figures in the same column having the same subscript are not significantly different ($p > 0.05$)

[1] Activity figures are in UMoles of maltose liberated/minute/gram moist tissue at 37°C

[2] Not Detectable

TABLE 40 α-GLUCOSIDASE ACTIVITY [1] IN SOME TISSUES OF RAINBOW TROUT FROM EXPERIMENT 5

TISSUE	Week 0	Week 2			Week 4			Week 6			$\pm$ SEM
		0%	15%	30%	0%	15%	30%	0%	15%	30%	
Dorsel muscle	0.023^{c}_{c}	0.027^{cb}_{b}	0.032^{d}_{c}	0.044^{e}_{b}	0.003^{a}_{a}	0.001^{a}_{a}	0.006^{ab}_{a}	0.002^{a}_{a}	0.004^{a}_{a}	0.011^{c}_{a}	0.002
Liver	0.049^{cd}_{d}	0.127^{d}_{c}	0.141^{e}_{e}	0.214^{f}_{c}	0.012^{a}_{b}	0.029^{b}_{c}	0.027^{b}_{c}	0.015^{a}_{b}	0.018^{a}_{b}	0.018^{a}_{a}	0.003
Stomach	0.017^{b}_{a}	0.012^{b}_{a}	0.024^{d}_{b}	0.016^{c}_{a}	0.038^{e}_{d}	0.006^{a}_{a}	0.007^{a}_{a}	0.009^{ab}_{a}	0.004^{a}_{a}	0.014^{bc}_{a}	0.003
Intestine	0.070^{e}_{e}	0.033^{c}_{b}	0.044^{d}_{d}	0.036^{c}_{b}	0.020^{ab}_{c}	0.018^{ab}_{b}	0.016^{a}_{b}	0.022^{a}_{b}	0.021^{ab}_{b}	0.041^{d}_{b}	0.001
Blood	0.0001^{a}_{a}	ND [2]	0.0001^{a}_{a}	ND	ND	ND	ND	ND	ND	ND	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

Figures in the same column having the same subscript are not significantly different ($p > 0.05$).

[1] Activity figures are in U mole of p-nitrophenol liberated/minute/gram moist tissue at $37^{o}C$

[2] Not Detectable

to produce extremely drastic effects on the performance of the fish (6.4). The overriding influence of the digestible carbohydrate level over that of dietary fibre on carbohydrate digestibility is further shown by the high protein digestibility which ranges between 85% and 89.7% (Fig. 22).

The good health of all fish tend to suggest that the inclusion of upto 30% dextrin had no deleterious consequences on the fish, but this did not improve growth over that achieved with 15% dextrin. The growth retardation discovered by earlier workers when dextrin or some other refined carbohydrate sources were fed to Rainbow trout at over 20% and in some cases over 10% dietary inclusion (Phillips, et al., 1948; Furuichi & Yone, 1971: Austreng et al., 1977) could probably be due to the structure and available sites for enzymatic breakdown in dextrin. However, other investigations on Salmonoids have shown that up to 50% of polysaccharides (starch, dextrins) or 20% of monosaccharides (glucose) can be incorporated into their diets without any deleterious effect on the growth (Buhler & Halver, 1961; Luquet, 1971).

From the results on carcass and liver composition (Tables 37 & 38), it is observed that no persistent rise in muscle glycogen/glucose due to dietary dextrin level occurs, except in the fourth week when the fish on 30% dextrin diet show a significant ($p<0.05$) rise. The fish on the higher dextrin diets had higher liver fat, but no correlation between the dietary dextrin level and liver fat level was evident (Table 38) contrary to the earlier observations of Austreng et al., (1971).

The control diet had the lowest protein digestibilities

(Fig. 21 & 22) probably due to the presence of a high level of cellulose as was suggested by Takeuchi, et al., (1979) when working on Carp, or probably due to the absence of digestible carbohydrate, since the diet containing 15% cellulose (and 15% dextrin) resulted in no significant difference ($p > 0.05$) in digestibility of protein and carbohydrate when compared to the group of fish receiving α-cellulose - free diet.

The results on the activities of α-amylase and α-glucosidase (Tables 39 & 40) show that there is a higher concentration of these enzymes in the liver of Rainbow trout than in the other tissues investigated. This finding with α-amylase is not similar to that of Nagayama and Saito (1978) who obtained the highest α-amylase activity in the intestine of Rainbow trout, the activity in the liver of their fish only ranking second. Nagayama and Saito (1978) however, rightly observed that the high amylase activity in their fish was probably due to the diet. The results obtained from this work show that in the sixth week, the enzyme activity seemed to drop slightly when compared with the value obtained at the end of the fourth week. An assymptote in carbohydrase activity therefore seems to have been achieved in the fourth week when the fish weighed approximately 85 grams. As Kitamikado and Tachino (1960) observed, carbohydrase activity in Rainbow trout is correlated to the fish size, a maximum activity being achieved at about 100 grams body weight.

α-glucocidase in fish tissue hydrolyses the α-D-glucoside including maltose which is liberated from starch or glycogen by the α-amylase action. The α-glucosidase activity in the Rainbow trout in this research was found to be highest in the liver, very low in the muscle and almost absent in the blood. The highest activity indicated by 0.214 U mole of p-nitrophenol liberated per gram moist tissue per minute at 37°C was in the second week in the liver of fish fed 30% dietary dextrin, the activity in the liver of fish fed 15% dextrin ranked next. Nagayama and Saito (1978) obtained the highest α-glucosidase activity for trout in the liver, a value of 0.021 U mole of p-nitrophenol per gram moist tissue per minute at 37°C being recorded by them.

From the findings in this investigation, it is concluded that an inclusion of 15% dietary dextrin improved growth, food conversion, nutrient utilization and digestibilities in Rainbow trout. α-amylase and α-glucosidase hydrolysing enzymes also tend to show maximum activity at 15% dextrin inclusion. These enzymes are present in some tissues of Rainbow trout, and their activity is probably correlated to fish age and size.

An investigation into the dextrin utilization and its effects on carbohydrase activity in Mirror Carp would be necessary for comparison purposes and to help throw more light on the method of carbohydrate utilization in this fish.

CHAPTER 8

CHAPTER 8

EXPERIMENT SIX

SOME ASPECTS OF CARBOHYDRATE METABOLISM IN MIRROR CARP FED DEXTRIN IN ISONITROGENOUS DIETS

8.1. INTRODUCTION

α-amylase and α-glucosidase are known to be present in the tissues of fish, (Kitamikado & Tachino, 1960; Shimeno et al., 1978). Higher carbohydrase activities occur in carp than in trout, and carbohydrase activity has been shown to be partly dependent on the level of dietary carbohydrate (Shimeno, et al., 1978; Nagayama & Saito, 1978). Like in Rainbow trout, not much work has been done on carbohydrase activities in the tissues of Mirror Carp, and there is no available literature on work done to correlate the growth performance, Food Utilization and digestibility of feedstuffs of Mirror carp with the dietary carbohydrate and carbohydrase levels.

This work was therefore undertaken to measure the mentioned criteria and thus, to complement vital knowledge on the carbohydrate nutrition of Mirror carp.

8.2. MATERIALS AND METHODS

8.2.1. EXPERIMENTAL ANIMALS AND SYSTEM

As a result of shortage of supply of fish of the right size

from the Aston Fish Culture Unit, only 90 fish were used for stocking the experimental tanks at a stocking density of 30 fish per tank at the start of this experiment, when fish had an average weight of 30.77 g $\pm$ 1.52 g and a mean length of 10.85 g $\pm$ 0.12g. The fish were stocked in the experimental tanks of "System 6" and fed 3% of their body weight thrice daily for one week on commercial (Edward Baker, Sudbury, Essex), diet, during which time they were allowed to acclimatize.

8.2.2. PREPARATION OF EXPERIMENTAL DIETS

Soluble dextrin from the same stock as the one used for the Rainbow trout experiment (7.2.2) was employed in making three isonitrogenous diets. The three diets, DC-1, DC-2, and DC-3 contained 0%, 22.5% and 45% dextrin (Table 41). Diets were prepared as described in Chapter 2.3, analysed (2.7), (Table 42) and then frozen. Aliquots were weighed out and utilized as required.

8.2.3. FEEDING

The fish were fed 3% of their body-weight thrice daily at 9.00 a.m., 2.00 p.m., and 5.00 p.m., except on the weighing days. At each meal-time, feeding was extended over a period of at least 20 minutes to ensure that the fish in each tank consumed all the food pellets dispensed into the tank.

All fish showed good appetite throughout the six weeks experimental feeding period.

TABLE 41 COMPOSITION OF DIETS FOR EXPERIMENT 6 (g/100 g diet)

INGREDIENTS	DIETS DESIGNATIONS		
	DC - 1	DC - 2	DC - 3
Dextrin	–	22.50	45.00
White fish meal	30.00	30.00	30.00
Casein	10.00	10.00	10.00
α-cellulose	45.00	22.50	–
Mineral mix [1]	4.00	4.00	4.00
Vitamin mix [2]	2.00	2.00	2.00
Cod-liver oil	3.00	3.00	3.00
Corn oil	5.00	5.00	5.00
Chromic oxide	0.50	0.50	0.50
Carboxy-methyl cellulose (Binder)	0.50	0.50	0.50
TOTALS	100.00	100.00	100.00

[1] Composition given on Table 7

[2] Composition given on Table 8

TABLE 42 PROXIMATE COMPOSITION OF EXPERIMENT 6 TEST DIETS FROM BIOCHEMICAL ASSAY (% weight)

COMPONENTS (% weight)	DIETS		
	DC - 1	DC - 2	DC - 3
Moisture	4.54	5.48	6.16
Protein	32.78	30.81	33.87
Fat	9.98	9.70	10.70
Carbohydrate [1]	1.80	21.80	37.30
Ash	4.34	6.75	6.90
Subtotals	53.44	74.54	94.93
Fibre [2]	46.56	25.46	5.07
Cr_2O_3 [3]	0.49	0.49	0.49
Energy [4]	16.85	18.98	20.04

[1] Hydrolysable carbohydrate

[2] Computed as difference between subtotal and 100

[3] Presented on dry weight basis

[4] Kilojoules/gram

8.2.4. WEIGHING AND SAMPLING

Just before the start of the experiment, five fish were randomly picked, anaesthetised (2.4), and analysed (2.5; 2.7; and 2.8). All 30 fish left in each tank were then weighed. Thereafter, fish were individually weighed fortnightly through the six weeks experimentation period. During each weighings after the first, fish were stripped for faeces, and six fish were randomly picked per tank for complete biochemical composition and enzyme activities assay. For uniformity of procedure, weighing and sampling was usually done between 8.00 a.m. and 9.00 a.m.

8.2.5.1. STANDARD CURVES

The standard curves (Figs. 23 & 24) plotted as detailed in Chapter 2.8.3., and the standard curve equations derived as described in Chapter 7.2.5.1, were used to express the α-amylase and α-glucosidase activities in some tissues of Mirror carp.

8.2.5.2. α-AMYLASE AND α-GLUCOSIDASE ACTIVITIES

The α-amylase and α-glucosidase activities were estimated in the dorsal muscle, liver, fore-gut, hind-gut and blood of Mirror carp, as detailed in Chapters 2.8.1. and 2.8.2., using known weights of tissues. Using the standard curve equations (Figs. 23 & 24) the α-amylase and α-glucosidase activities were then computed.

8.2.6. ANALYSES OF EXPERIMENTAL DATA AND STATISTICAL METHODS

These were performed as detailed in Chapter 2.10.

8.3. RESULTS

8.3.1. GROWTH PERFORMANCE

No significant difference ($p > 0.05$) exists between the initial weights of the Mirror carp. (Table 43). By the sixth-week however, there is a large difference between the mean weights of the fish from the different treatment groups (Table 43). As shown in Fig. 25, the growth response of the fish on 45% dietary dextrin is much greater than those of the fish on 0% and 22.5% dietary dextrin. Progressive increase in dietary dextrin level progressively improves Specific Growth Rate (SGR), and percentage daily weight gain (Table 43).

8.3.2. FOOD CONVERSION

The Food Conversion Ratio (FCR) shows a stepwise improvement (decrease) with increase in dietary dextrin level. The best FCR of 1.32 was obtained with 45% dextrin in the diet. The FCR's obtained with 0% and 22.5% dietary dextrin were rather poor (Table 43).

8.3.3. PROTEIN UTILIZATION

The Protein Efficiency Ratio (PER) improved by showing an increase with increase in the level of dietary dextrin. The PER

at 45% dietary dextrin is very good while the PER's at 0% and 22.5% dietary dextrin are rather poor, values for them being below one.

The Apparent Net Protein Utilization (NPU) increases with increase in dietary dextrin level. The lowest Apparent NPU of 13.70% was obtained with the control fish. The best apparent NPU was 35.92%. This is obtained in fish fed 45% dietary dextrin. Protein sparing action is thus at a maximum at this level of dietary carbohydrate.

8.3.4. PATHOLOGY

The blood glucose level of all the fish appear good. No significant differenee ($p > 0.05$) in blood glucose level is found

in the fish on 22.5% and 45% dietary dextrin, and the fish for the initial assay. (Table 44). The highest Hepato-somatic Index (HSI) occurs in the fish on 45% dietary dextrin, while the control fish which showed the poorest growth had the lowest H.S.I. A significant difference ($p < 0.05$) is observed in the faecal moisture content of all the fish. The increase thus obtained in faecal moisture content is correlated with the increase in dietary dextrin level. There was however, no evidence of diarrhoea in any of the groups, and no mortality was recorded (Table 44).

8.3.5. CARCASS AND LIVER COMPOSITION

The composition of the carcass and the liver of the fish from proximate biochemical analysis are represented in Table 45 and 46 respectively.

From the results, it is observed that there was no significant difference ($p > 0.05$) between the moisture contents of the carcass, but the muscle glucose/glycogen content increased with increase in dietary dextrin level. The fish fed on the diet containing 45% dextrin showed higher carcass fat content than the rest from the fourth week. No significant difference ($p > 0.05$) existed between the carcass fat in the fish fed 0% and 22.5% dietary dextrin. Generally, the protein content of the tissues of the fish fed 22.5% dietary dextrin is significantly ($p < 0.05$) higher than those of the other two groups. The inclusion of dextrin as well as the level of such inclusion did not have any significant ($p > 0.05$) effect on the ash content of the carcass.

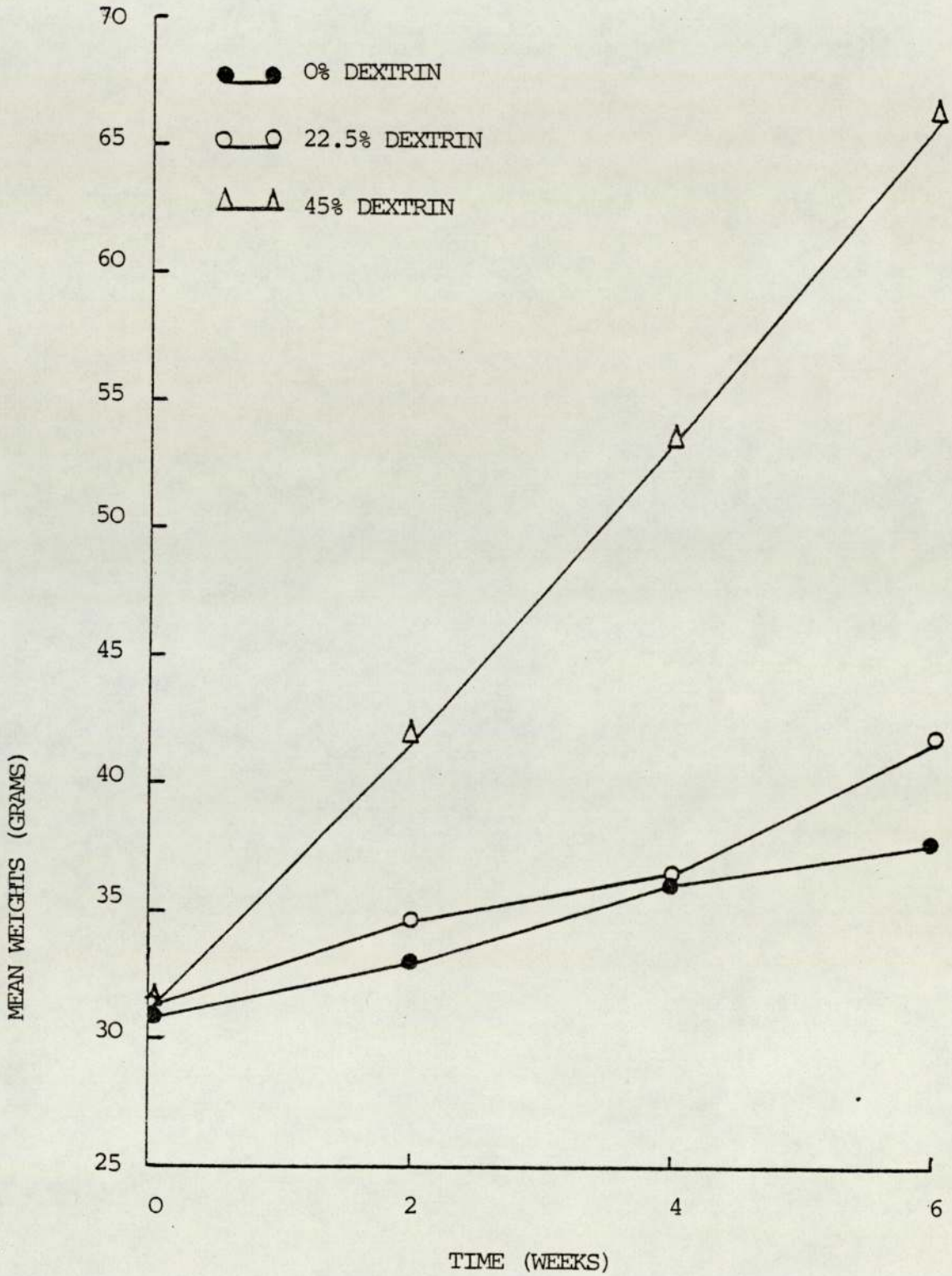


FIG. 25 Effect of different levels of dietary dextrin on weight gain of Mirror Carp

TABLE 4 3 GROWTH AND FOOD UTILIZATION OF MIRROR CARP FED FOR 6 WEEKS ON DIFFERENT LEVELS OF DIETARY DEXTRIN

MEAN VALUES	DIETS			
	DC-1	DC - 2	DC - 3	± SEM
Initial weight (g)	30.82 [a]	31.50 [a]	31.79 [a]	1.159
Final weight (g)	37.79 [a]	42.12 [a]	66.48 [b]	5.770
Weight gain (%)	21.00 [a]	30.66 [b]	83.58 [c]	0.631
S.G.R. [1] (%)	0.49 [a]	0.69 [b]	1.76 [c]	0.048
F.C.R. [2]	5.15 [c]	3.78 [b]	1.32 [a]	0.096
P.E.R. [3]	0.63 [a]	0.93 [b]	2.27 [c]	0.045
Apparent N.P.U. [4] (%)	13.70 [a]	21.36 [b]	35.92 [c]	0.714

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Specific growth rate

[2] Food Conversion Ratio

[3] Protein Efficiency Ratio

[4] Net protein Utilization

TABLE 44 PERFORMANCE OF MIRROR CARP FROM EXPERIMENT 6

	INITIAL	FINAL			
		DC - 1	DC - 2	DC - 3	± SEM
Blood glucose (mg%)	66.51 ^{b}	60.95 ^{a}	67.21 ^{b}	67.00 ^{b}	1.549
HSI [1]	1.33 ^{c}	0.98 ^{a}	1.11 ^{b}	2.01 ^{d}	0.052
% Moisture in faeces	–	77.11 ^{a}	84.99 ^{b}	90.12 ^{c}	1.051
Gross mortalities		0	0	0	

Figures in the same row haging the same superscript are not significantly different ($p > 0.05$)

[1] Hepato-somatic Index

TABLE 45 CHANGE IN CARCASS COMPOSITION OF MIRROR CARP FROM EXPERIMENT 6

(% weight)

	Week 0	Week 2			Week 4			Week 6			
DIETARY DEXTRIN		0%	22.5%	45%	0%	22.5%	45%	0%	22.5%	45%	± SEM
Moisture	77.21^{a}	76.51^{a}	77.07^{a}	76.31^{a}	77.32^{a}	77.32^{a}	76.25^{a}	77.57^{a}	77.07^{a}	75.02^{a}	1.895
Carbo-hydrate [1]	0.49^{c}	0.26^{a}	0.53^{c}	0.67^{d}	0.27^{a}	0.30^{a}	0.66^{d}	0.36^{ab}	0.43^{bc}	0.64^{d}	0.051
Fat	6.95^{c}	5.22^{ab}	4.77^{a}	5.55^{ab}	4.42^{a}	4.84^{a}	6.38^{bc}	4.64^{a}	4.45^{a}	7.55^{c}	0.199
Protein	12.24^{a}	14.01^{b}	15.63^{c}	13.81^{b}	15.29^{c}	14.47^{bc}	13.59^{b}	14.14^{b}	14.70^{bc}	13.93^{b}	0.512
Ash	2.57^{a}	3.13^{a}	3.19^{a}	2.72^{a}	3.31^{a}	3.16^{a}	2.70^{a}	3.09^{a}	2.74^{a}	2.25^{a}	0.605
TOTALS	99.46	99.13	101.19	99.06	100.61	100.09	99.58	99.80	99.39	99.39	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Total glucose and glycogen

TABLE 46 CHANGE IN LIVER COMPOSITION OF MIRROR CARP FROM EXPERIMENT 6

(% weight)

	Week 0	Week 2			Week 4			Week 6			
DIETARY DEXTRIN		0 %	22.5%	45%	0%	22.5%	45%	0%	22.5%	45%	± SEM
Moisture	78.59 ^{a}	81.59 ^{a}	78.22 ^{a}	76.58 ^{a}	81.50 ^{a}	78.28 ^{a}	77.45 ^{a}	80.00 ^{a}	79.46 ^{a}	76.41 ^{a}	3.641
Carbohydrate [1]	3.73 ^{cd}	1.83 ^{a}	4.53 ^{d}	7.86 ^{f}	1.16 ^{a}	3.51 ^{cd}	6.28 ^{e}	2.11 ^{ab}	2.88 ^{bc}	6.17 ^{e}	0.083
Fat	4.56 ^{e}	4.00 ^{cd}	3.86 ^{cd}	3.57 ^{bc}	4.12 ^{de}	3.48 ^{ab}	3.01 ^{a}	4.00 ^{cd}	4.04 ^{cd}	3.73 ^{b}	0.245
Protein	11.14 ^{a}	12.21 ^{b}	13.01 ^{c}	10.99 ^{a}	12.11 ^{b}	12.95 ^{c}	12.00 ^{b}	12.25 ^{b}	12.81 ^{c}	12.00 ^{b}	0.359
Ash	1.78 ^{e}	1.25 ^{c}	1.03 ^{a}	1.11 ^{a}	1.41 ^{d}	1.20 ^{c}	1.18 ^{bc}	1.23 ^{c}	1.09 ^{a}	1.12 ^{ab}	0.041
TOTALS	99.80	100.88	100.65	100.11	100.30	99.42	99.92	99.59	100.28	99.43	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

[1] Total glucose and glycogen

In the liver, dietary dextrin did not produce any significant ($p > 0.05$) change in the moisture content. However, there is a correlation between the increase in dietary dextrin level and increase in liver glucose and glycogen level. Liver fat deposition was highest in the fish fed 0% dextrin (control group). As with the carcass, the highest liver protein deposition occurs in the fish fed 22.5% dietary dextrin. Throughout the six weeks of the experiment, the liver ash content of the Mirror carp on 0% dextrin was higher than those of the fish receiving the dextrin diets. During each fortnightly measurement, however, the liver ash content of the fish receiving the dextrin diets were not significantly different ($p > 0.05$).

No significant difference was observed ($p > 0.05$) in the digestibility of dietary dextrin at 22.5% and 45% dietary inclusions. The carbohydrate digestibilities at these levels range between 94% and 97% which is very high (Fig. 26). In the control fish (fed 0% dietary dextrin), carbohydrate digestibility of the

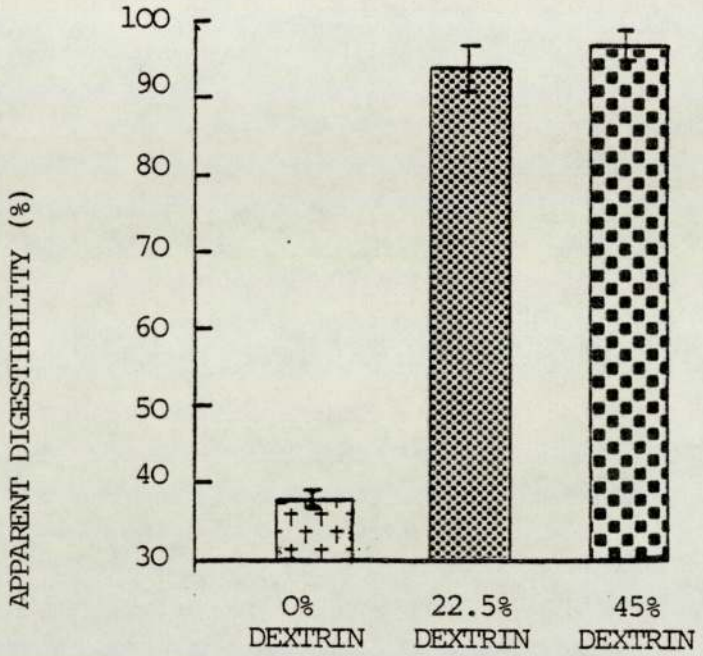


FIG. 26 Apparent digestibility of dietary carbohydrate in Mirror Carp from Experiment 6

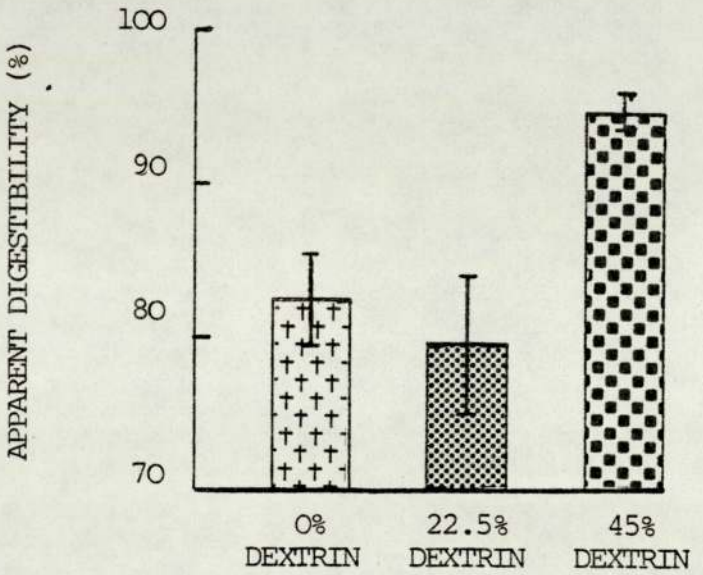


FIG. 27 Apparent digestibility of dietary protein in Mirror Carp from Experiment 6

trace (1.80% from Table 42) dietary carbohydrate is 38%.

The effect of dietary fibre on gut transit time and carbohydrate digestibility is probably minimal since no significant difference ($p > 0.05$) is obtained in the protein digestibilities of fish fed 0% and 22.5% dietary dextrin. The protein digestibility at 45% dietary dextrin is significantly ($p > 0.05$) higher and is approximately 94.5% (Fig. 27).

8.3.7. CARBOHYDRASE ACTIVITY

8.3.7.1. α-AMYLASE

The results of estimation of α-amylase activity in the dorsal muscle, liver, fore-gut, hind-gut and blood are shown in Table 47. From these results, the highest α-amylase activity occurs in the liver of Mirror carp. There is some correlation between dietary dextrin level and the enzyme activities, (increase in dietary dextrin level resulting in higher α-amylase activity.) The highest liver α-amylase activity of 443.374 U moles of maltose liberated/minute/gram moist tissue at 37°C was obtained in the second week from fish on 45% dextrin diet. This value fell to 270.042 U moles of maltose liberated/ minute/gram moist tissue at 37°C in the fourth week, and remained at around this level till the sixth week. A simi lar trend in fluctuations of α-amylase activity in the other tissues and at other dietary dextrin levels was also observed. α-amylase activity in the blood was not detectable.

TABLE 47 α-AMYLASE ACTIVITY [1] IN SOME TISSUES OF MIRROR CARP FROM EXPERIMENT 6

TISSUE	Week 0	Week 2			Week 4			Week 6			± SEM
		0%	22.5%	45%	0%	22.5%	45%	0%	22.5%	45%	
Dorsal muscle	28.369^{c}_{c}	27.055^{c}_{c}	18.247^{b}_{a}	30.741^{c}_{b}	$9,870^{a}_{a}$	19.442^{b}_{b}	30.002^{c}_{a}	7.530^{a}_{a}	8.825^{a}_{a}	24.882^{bc}_{b}	± 4.0
Liver	170.635^{d}_{d}	112.849^{b}_{d}	136.539^{c}_{c}	443.374^{g}_{c}	82.290^{a}_{c}	211.751^{e}_{c}	270.042^{f}_{c}	62.083^{a}_{b}	173.420^{d}_{d}	262.969^{f}_{d}	± 14.5
Foregut	0.550^{a}_{a}	0.890^{a}_{a}	16.952^{cd}_{a}	19.768^{d}_{a}	15.736^{c}_{b}	15.374^{c}_{a}	37.443^{e}_{a}	5.460^{b}_{a}	14.793^{c}_{b}	14.613^{c}_{a}	± 1.5
Hindgut	16.954^{b}_{b}	8.941^{a}_{b}	22.949^{c}_{b}	21.653^{c}_{a}	14.051^{b}_{b}	12.909^{b}_{a}	51.796^{e}_{b}	7.868^{a}_{a}	25.403^{c}_{c}	40.979^{d}_{c}	± 1.1
Blood	ND [1]	ND	ND	ND	ND	ND	ND	ND	ND	ND	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

Figures in the same column having the same subscript are not significantly different ($p > 0.05$)

[1] Activity figures are in U moles of maltose liberated/minute/gram moist tissue at 37°C

[2] Not Detectable

TABLE 48 α-GLUCOSIDASE ACTIVITY [1] IN SOME TISSUES OF MIRROR CARP FROM EXPERIMENT 6

TISSUE	Week 0	Week 2			Week 4			Week 6			± SEM
		0%	22.5%	45%	0%	22.5%	45%	0%	22.5%	45%	
Dorsel muscle	0.017^{c}_{a}	0.012^{b}_{a}	0.021^{e}_{a}	0.024^{f}_{a}	0.012^{b}_{a}	0.009^{a}_{a}	0.020^{de}_{a}	0.018^{cd}_{a}	0.020^{d}_{a}	0.014^{b}_{a}	0.005
Liver	0.110^{g}_{c}	0.168^{h}_{d}	0.073^{e}_{b}	0.041^{b}_{b}	0.052^{c}_{b}	0.063^{de}_{b}	0.056^{cd}_{b}	0.087^{f}_{c}	0.095^{f}_{c}	0.029^{a}_{b}	0.010
Foregut	0.092^{d}_{b}	0.058^{bc}_{b}	0.096^{d}_{b}	0.088^{d}_{c}	0.062^{c}_{c}	0.064^{c}_{b}	0.050^{b}_{b}	0.053^{c}_{b}	0.065^{c}_{b}	0.039^{a}_{b}	0.004
Hindgut	0.174^{d}_{d}	0.123^{bc}_{c}	0.178^{de}_{c}	0.186^{e}_{d}	0.108^{a}_{d}	0.128^{bc}_{c}	0.121^{bc}_{c}	0.129^{bc}_{d}	0.117^{ab}_{d}	0.107^{a}_{c}	0.015
Blood	ND[2]	ND	ND	ND	ND	ND	ND	ND	ND	ND	

Figures in the same row having the same superscript are not significantly different ($p > 0.05$)

Figures in the same column having the same subscript are not significantly different ($p > 0.05$)

[1] Activity figures are in U mole of p-nitrophenol liberated/minute/gram moist tissue at 37°C

[2] Not detectable

8.3.7.2. α-GLUCOSIDASE

The results of α-glucosidase activity is given in Table 48 as U mole of p-nitrophenol liberated/minute/gram of moist tissue at 37°C.

The α-glucosidase activity in the hind-gut of Mirror carp is significantly higher than those in the other tissues ($p < 0.05$), for most of the fish in most of the measurements. In the second week, the α-glucosidase activity in the liver of the fish on the control diet was higher than the value obtained for the hind-gut. In the sixth week, the difference between the α-glucosidase activity in the liver and hind-gut of the fish on the control diet is not significant ($p > 0.05$). In most of the measurements, the extent of enzyme activity in the tissues of the fish receiving the three different levels of dietary dextrin ranked in the order 45% ≥ 22.5% > 0%, and the extent of activity in the different tissues decreased in the order hind-gut > fore-gut > liver > dorsal muscle > blood. No detectable activity was infact, obtained in the blood.

8.4. DISCUSSION AND CONCLUSIONS

The results obtained from this investigation tend to suggest that Mirror carp are capable of utilizing upto 45% dextrin in diet. When added to the diet of Mirror carp, 45% dextrin improved growth, Food Efficiency, Protein Efficiency and Apparent Protein Utilization. The growth performance of the control fish

whose diet was devoid of dextrin was inferior. These observations are opposed to the findings of Furuichi and Yone (1980) but are in agreement with those of Buhler and Halver (1961) when they worked on Chinook salmon and concluded that the fish could tolerate upto 48% of dietary carbohydrate provided that the feed is nutritively adequate and balanced. The diet of Furuichi and Yone (1980) does not seem to be nutritionally balanced. Casein, which is a purified/synthetic protein was their major protein source. Casein is low in certain essential amino acids and some other nutritional factors which would be present in white fish meal. Furthermore, the levels of amino acid they added to the diets containing 0% to 40% dextrin varied from 3.6% to 1.2%. The insufficient supply of essential amino acids to their carp on 40% dextrin diet, could therefore, have affected the carbohydrate utilization in their fish.

The health of all the fish in this investigation appeared good. There was no evidence of persistent hyperglycaemia in fish fed the high (45%) dextrin diet. Rather, the control fish showed a significant ($p < 0.05$) hypoglycaemia when compared to the fish used for the initial blood assay (Table 44). The significantly ($p < 0.05$) higher value of 2.01 Hepato-somatic Index for fish on 45% dextrin is indicative of better growth. This value is not too high as to suggest hepatomegally (Heidinger & Crawford 1977).

From the tissue proximate analyses, (Table 45 & Table 46), the dietary dextrin level had no effect on the moisture content of the carcass and liver of the experimental fish. There was however, a positive correlation between muscle and liver glucose/glycogen contents, and dietary dextrin levels. Some fat deposition over what is obtained with the control and 22.5% diets is obtained in the fish on the 45% dextrin diet.

The highest carcass protein deposition in the second and sixth weeks was obtained in the fish fed 22.5% dextrin diets, while the liver analysis showed that this group had the highest protein deposition all through the experimental period. Though the ash content of the liver tends to have a negative correlation with the level of dietary dextrin, the highest liver ash being obtained in the control fish, there is no significant difference ($p > 0.05$) between the carcass ash content of all the fish. These results tend to suggest that the inclusion of upto 45% dextrin to the diet of Mirror carp produces no harmful effects. However, the 22.5% dietary dextrin seems to be better tolerated, though it does not produce the best growth.

As confirmed in the diegestibility studies (Figs. 26, & 27) the addition of dextrin to the diet of Mirror carp significantly ($p < 0.05$) improves nutrient digestibility. Takeuchi et al., (1979) reported of decrease in digestibility of energy with increase in cellulose content of their diets. The diet containing

22.5% dextrin had the same (22.5%) quantity of ∝-cellulose. Regardless of the fact that the 45% dextrin diet had no ∝-cellulose, the fish on this diet manifested a carbohydrate digestibility that was not significantly different ($p>0.05$) from that of the fish on 22.5% dextrin diet.

The increase in ∝-amylase and ∝-glucosidase activities in the tissues of Mirror carp with increase in the level of digestible carbohydrate as dextrin further shows that the ability of Mirror carp to utilize dietary and tissue carbohydrate has a positive correlation with the level of digestible carbohydrate in the diet. The influence of diets on the enzymatic activities of carp, (Kawai & Ikeda, 1972; Nagayama & Saito, 1978), and Tilipia Mossambica (Nagase, 1964) and the action of digestive enzymes in carp (Bondi & Spandorf, 1954) have been documented. Results from this investigation are in agreement with most of the findings of previous work. Mirror carp are stomachless fish. Higher concentrations of ∝-amylase and ∝-glucosidase enzymes were detected in the hind-gut than in the fore-gut. The majority of carbohydrate digestion and absorption must therefore, be occurring in the hind-gut. Similarly, as there is more ∝-amylase enzyme present in the liver than in the muscle, most of the tissue glycogen catabolism would occur in the liver. This is not surprising as a lot of glycogen is stored in the liver. The low ∝-glucosidase enzyme activity in the muscle is perhaps

indicative of low supply of energy for muscular activities from carbohydrate sources. Nevertheless, an appreciable supply of energy from carbohydrate source appears to be present.

It is concluded that dextrin upto 45% when incorporated into a nutritionally balanced carp diet containing upto approximately 32% good quality protein like fish meal, improves growth, feed and protein utilization, protein and carbohydrate digestibilities, protein sparing and carbohydrase (α-glucosidase and α-amylase) activities.

CHAPTER 9

CHAPTER 9

GENERAL DISCUSSION AND CONCLUSIONS

The rate of price inflation which has led to the high cost of protein foods including fish has contributed to some human protein deficiences particularly in developing countries. This has led to the need for cheap feed-stuffs for fish, which would result in cheaper fish products. In general, attempts to incorporate carbohydrate sources into fish diets have proved relatively unsuccessful, as the fish portrayed intolerance to high dietary levels. Nevertheless, the few sucessful attempts to feed carbohydrate - rich deits to fish tend to allude to the idea that different fish species are able to tolerate different carbohydrates to different extents. This research was therefore conducted to establish the extent to which Rainbow trout (Salmo gairdneri) and Mirror carp (Cyprinus carpio) could utilise various dietary carbohydrate sources.

During this investigation a dietary inclusion level of 20% cassava or rice was found to significantly increase ($p < 0.05$) growth, food efficiency, protein utilisation and protein sparing action in Rainbow trout (Salmo gairdneri). On the other hand when corn and potato starch were fed to Rainbow trout results showed that the fish could tolerate up to 30% corn or potato starch. The fish however, generally grew better on the 20% than on the 30% cassava or rice diets. The higher Specific Growth Rate (SGR) with cassava and rice diets (1.34% and 1.36% at 20% and 30% cassava respectively) (Table II), when compared with the best S.G.R. obtained with corn and potato starch (0.94% with 20% corn) (Table 23) probably suggests that Rainbow trout are better adapted to utilise cassava than corn or potato. The difference in genetic strains and initial weight of the fish used in the two experiments probably had some influence on their abilities to utilise the

different carbohydrates. No degenerative or neoplastic damage was observed within the livers of fish fed high dietary carbohydrate levels. However, although it has been suggested that the carbohydrate type, feeding period, water quality and other environmental factors have a great deal of influence on liver pathology of fish (Wood & Yasutake, 1955; Struthems, et al., 1975; Ghito, 1976; Pierce, et al., 1980), the short duration of the feeding trials may in part have explained the absence of any liver damage in the very high carbohydrate trout experimental diets. The digestability of dietary cassava in Rainbow trout was found to be significantly ($p < 0.05$) higher than that of dietary rice. The digestibility of potato starch is relatively poor (about 15% for 10% dietary potato), when compared to 45% for 10% dietary corn. This probably contributed to insufficient energy being obtained from the diets, and hence to the observed increase in digestabiltiy with increase in dietary carbohydrate level, since the fish attempted to compensate. Previous researchers (McLaren, et al., 1946, 1947; Benedetto, et al., 1975) and some recent studies (Pieper & Pfeffer, 1980 a & b), have shown that the digestion and absorption of different carbohydrates by Rainbow trout depends on the carbohydrate type and level. The use of casein alone as the dietary protein source in such nutritional studies produces adverse effects on the fish (Lee & Wales, 1973).

Some of the previous work in which the unavailability of carbohydrates to Rainbow trout was reported have been conducted using diets consisting either wholly or partially of semi-purified or poor quality protein. Such protein sources have been shown to cause nutritional disorders. The use of some refined carbohydrate

sources have been shown to lead to growth retardation (Inada, et al., 1963; Hastings, 1968; Austreng, et al., 1977). However, the dietary inclusion of up to 50% of starch and/or dextrin, or 20% glucose has been shown to be well tolerated by salmonoids, and the differences in tolerance levels has, in the main, been attributed to the intestinal carbohydrate digesting ability of the animals (Buhler and Halver, 1961; Luquet, 1971). The use of protein sources cheaper than fish meal, and carbohydrates to substitute or spare as much as possible of protein has been suggested (Jauncey, 1979). When dextrin was used as the dietary digestible carbohydrate source in the trout diet, poorer growth was obtained with a 30% dextrin diet than with a 15% dextrin diet. The differences in growth and food utilisation of the fish on the dextrin diet when compared to those on the control (0% dextrin) diet was significant ($p < 0.05$).

The ability of Rainbow trout to metabolise limited quantities of carbohydrate (Stimeno, et al., 1978; Walton & Cowey, 1979) was portrayed in this research by the lower levels of α-amylase and α-glucosidase enzymes in Rainbow trout (Nagayama & Saito, 1968, 1979). It was also found that these enzymes were unevenly distributed in the tissues of Rainbow trout. The level of digestible carbohydrate in the diet, in addition to the carbohydrate type was found to have an influence on the ability of the fish to metabolize carbohydrate as has also been previously shown (Hers & DeWulf, 1967; Cowey et al., 1977; Shimeno et al., 1978; Nagayama & Saito, 1978).

Mirror carp are better adapted than Rainbow trout to metabolise higher dietary and tissue carbohydrates (Johnston, 1977; Shimeno, et al., 1977). This is not surprising because in their natural environments, Mirror carp are omnivorous while Rainbow trout are mainly carnivorous. Hence, though the former

is a warm water fish, the latter requires more dietary energy particularly in the form of protein. It is for this reason that higher dietary levels of carbohydrate were employed in the Mirror carp diets. Accordingly, 15%, 30% and 45% cassava, rice, corn or potato starch or a control containing 45% α-cellulose were fed to Mirror carp in isonitrogerous diets. For the studies with dextrin, 0%, 22.5% and 45% of this carbohydrate source was incorporated in the carp diets.

Results indicate that the Mirror carp fed 45% cassava or rice showed better growth and feed utilisation than the fish fed lower quantities of cassava or rice. No significant difference ($p > 0.05$) was observed in the carcass protein deposition. With the cassava or rice diets, a maximum Specific Growth Rate (SGR) of 2.57% was obtained at 45% inclusion of rice in diet. With the corn and potato diets however, the maximum SGR obtained was 1.19% which was achieved at 45% inclusion of corn. The two stocks of Mirror carp used in Experiments 2 and 4 were from different brood stocks, spawned at different times and were of different mean sizes at the start of each of the two sets of experiments. This could have contributed to the differences obtained in the SGR of the control groups for the two experiments. The discovery by Shimeno et al., (1978), that there were higher activities of glycolytic and pentose phosphate cycle enzymes in the liver of fish receiving carbohydrate diets and the knowledge that long term (over 23 days) fasting of salmonoids evoked the activities of some gluconeogenic enzymes (Lin et al., 1977) suggests that the possible source of the tissue glucose/glycogen content of the control fish is from gluconeogenesis. This must have also led to the observations of Wittenberger and Nitca (1966), and Murat (1976a)

of high glycogen content level maintenance in tissues of carp starved for several months.

The digestibility observations in this study persistently show very poor carbohydrate digestibility in the fish fed the control diet, which contained only trace quantities ($< 3\%$) of hydrolysable carbohydrate. This suggests that incorporation of at least a "threshold" level of digestible carbohydrate in the diets of Rainbow trout and Mirror carp may be necessary to initiate the secretion of sufficient quantities of digestive enzymes. Low protein digestibility has been reported in fish fed high fibre-containing diets (Kitamikado, et al., 1964; Takeuchi, et al., 1979). This could explain the increasing protein digestibility observed with the increasing levels of starch and reduced α-cellulose content of the diets. It is likely during the present investigation, that the high fibre content of some of the diets have had some effect on the hydrolysable carbohydrate digestibility in the Rainbow trout and Mirror carp. However, examination of the rectal content of the fish in this research showed no evidence of diarrhoea or inconsistency in the rectal contents of the fish receiving the high fibre diets. The pattern of variation in enzyme activities with dietary digestible carbohydrate (Chapters 7 & 8) tend to confirm that the digestibility of carbohydrate by trout and carp is probably to a large extent dependent on carbohydrate type, and on whether a significant quantity of the digestible carbohydrate is present.

The value of carbohydrate to fish would appear to be basically for the supply of metabolic energy. Thus protein is best used for growth, leaving the energy demand to be met by

dietary lipid and carbohydrate. Metabolic energy produced as heat is usually regarded as being a waste to the fish (Cowey & Sargent, 1979). With an adequate supply of dietary carbohydrate, the process of gluconeogenesis (evidenced by the presence of tissue carbohydrate in the control fish whose trace quantities of dietary digestible carbohydrate were very poorly digested), would be minimised. Hence, dietary protein would be spared.

The energy derived from dietary protein, fat and carbohydrate by fish is below that obtained from heat of combustion as determined; by bomb calorimetry. This is not only because the energy yielding materials in the diet are not completely digested and absorbed, but also because protein nitrogen is not fully oxidised in the tissue during tissue catabolism. Unfortunately, a review of energy contents of food compounds ascribed by different authors varies (Cowey & Sargent, 1979), This is probably mainly because the quantity of energy derived from different food compounds by different animals varies with species, and with the dietary composition. However, the most reliable practical digestible energy values for protein, fat and carbohydrate in fish diets (Cowey & Sargent, 1979) are 4.5 K cal/g for protein (Lee & Putnam, 1973), 9 K cal/g for fat (Page & Andrews, 1973), and 2.7 K cal/g for carbohydrate (Page & Andrews, 1973). When the standard values for heat of combustion of fat, protein and starch (9.4, 5.6 and 4.2 K cal/g respectively) are used to compute the energy contents of the test diets used in this research, it is found that the control diets often contained the least energy. The proportion of this energy that is actually digested and absorbed by the fish would vary with the digestibilities of the different

diets, and are presented below in K cals/100 g of diet (fat being assumed to be 95% digested):

Rainbow trout (% dietary carbohydrate)	Cassava	Rice	Corn	Potato
0	299	299	329	329
10	344	344	344	330
20	370	354	370	345
30	379	371	402	384
Mirror carp				
(% dietary carbohydrate)				
0	222	222	164	164
15	301	313	294	275
30	343	362	323	313
45	408	415	378	377

Several workers in recent years have reported that fish, when fed a variety of diets regulate their food consumption so as to maintain a relatively constant evergy intake, that is, fish appear to eat to satisfy their energy needs (Cowey et al., 1972; Lee & Putnam, 1973; Page & Andrews, 1973; Cho et al., 1976). Takeuchi et al., (1979a) while working on carp observed that an increase in dietary lipid from 5% to 15% which led to an increase of digestible energy from 320 to 460 K cal/100 g diet did not lead to better growth or protein utilisation. However, diets containing less than 310 K cal/100 g (15 K Joules/g) of digestible energy resulted in poor growth, PER and NPU (Takeuchi et al., 1979a). At the feeding regimes used in this research, less than enough dietary energy in the form of protein and fat was supplied to the experimental fish. If the observations of Takeuchi et al., (1979a) are correct, then maximum energy must be derived from the carbohydrates by the experimental fish employed in this research. In general, results from this research indicate that Mirror carp and Rainbow trout can best utilise approximately 45% and 20% respectively of the various carbohydrates tested.

From these results, it is concluded that digestible dietary carbohydrate is important in the diets of Rainbow trout and Mirror carp, if maximum use is to be made of the dietary protein. It has also been shown that trout and carp could utilise some crude carbohydrate sources better than they could utilise dextrin. The acceptability of the different carbohydrates vary, and probably depend on the nature and number and type of glucosidic linkages in the macromolecules. The incorporation of cassava, rice and corn, and to a lesser extent potato and dextrin, over a"threshold" level greatly enhance

growth in Rainbow trout and Mirror carp. Nutrient digestibilities and the general health of the fish receiving the crude carbohydrate diets appear to be good. α-amylase and α-glucosidase enzyme activities in these two fish species tend to have a positive correlation with the level of dietary digestible carbohydrate, until an optimum enzyme level is achieved.

It is recommended that further studies be undertaken to determine the minimum or critical level of high quality protein (such as white fish meal) that could be used with high levels of well accepted carbohydrate sources such as rice, cassava, and corn for the formulation of Rainbow trout (Salmo gairdneri) and Mirror carp (Cyprinus carpio) diets, to enhance growth, without causing any deleterious consequences.

The exact role of dietary fibre, the energy balance in intermediary metabolism and the occurrence of the enzymes involved in gluconeogenic activities in these two fish species also need further investigation.

APPENDIX I BRAND SIGNS USED IN MARKING FISH

BRAND SIGNS	|	−	⊥	⊤	∨	∧	>	<	+	×
CORRESPONDING SERIAL NUMBERS	1	2	3	4	5	6	7	8	9	10

REFERENCES

REFERENCES

ABEL, V.H., PIEPER, A., & PFEFFER, E. (1979), Untersuchugen an wachsenden Regenbogenforellen (Salmo gairdneri, R.) über die intermediare Anpassung and Protein order Kohlenhydrate als Energietrager im Futter. Zitschrift fur Tierphysiologie, Tierernahrung and Futtermittelkunde, 41 : 325-334.

AKINRELE, I. A. (1964). Fermentation of cassava, J. Sci., Food and Agric., 15: 586-594.

ANDREWS, J.W., PAGE, J.W., & MURRAY, M.W. (1977) Supplementation of a semi-purified casein diet for catfish with free amino acids and gelation. J. Nutr., 107: 1153-1156.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC) (1975). Official methods of Analysis of the AOAC, (Hortwitz, W. Ed.) 12th Ed. Publ. 1. by AOAC, Washington, D.C.

AUSTRENG, E. (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aqua culture. 13. 265-272.

AUSTRENG, E. (1979). Fat and protein in diets for salmonoid fishes. VI. Digestibility and feed utilization by Rainbow trout (Salmo gairdneri, R.) fed diets containing different levels of fat. Scientific Report of the Agric Univ. Norway, 58 : 2-12.

AUSTRENG, E., RISA, S., EDWARDS, D. J. & HVIDSTEN, H., (1977) Carbohydrate in Rainbow trout diets. II. Influence of carbohydrate level on chemical composition and feed utilization of fish from different families. Aquaculture 11 : 39-50.

BAGENAL, T.B. (1971). Eggs and early life history. Part 1. Fecundity. In, Methods for Assessment of Fish Production in Fresh Waters, 2nd Ed. (W. E. Ricker Ed.), pp 166-179. Oxford IBP Handbook No. 3, Blackwell Scientific Publ.

BARRINGTON, E.J.W. (1962). Digestive enzymes. In, Advances in Comparative Physiology and Biochemistry (O. Lowenstein, Ed.), Vol. 1 : pp 1-67. New York Academic Press.

BECKETT, J.S. (1971). Interim report of herring tagging in the gulf of St. Lawrence, 1970. Int. Comm. North-west Atl. Fish Res. Doc. 71/95. Ser. No. 2565.

BENDER, A.E. & MILLER, D.S. (1953). A new brief method of estimating net protein value. Biochem. J. 53 : vii

BENEDETTO, F.E., FARMANFARMAIAN, DIBENEDE, F.E. & FARMANFA, A. (1975). Intestinal absorption of naturally occurring sugars in Rainbow trout (Salmo gairdneri) under free swimming conditions. Comp. Biochem. and Physiol. A. p 555.

BERGOT, F. (1979). Carbohydrate in Rainbow trout diets: Effects of the level and source of carbohydrate and the number of meals on the growth and body composition. Aquaculture, 18: 157-167.

BERNFELD, P. (1955). Amylase α and β In, Methods in Enzymology (S. Colowick & N.O. Koplan, Ed.), I : 149-158. New York, Academic Press.

BLACK, E.C., ROBERTSON, A.C., & PARKER, R.R. (1961). Some aspects of carbohydrate metabolism in fish. In, Carbohydrate Metabolism in some heterothermic animals, (A.W. Martin, Ed.,) pp. 89-124. University of Washington Press.

BONDI, A., & SPANDORF, (1954). The action of the digestive enzymes of the carp. Br. J. Nutr., 8 : 240-246.

BOURNE, E. J. (1951). Some recent developments in the academic fields of starch chemistry. Chem. & Ind. (London), 1047-1052.

BOWEN, S.H. (1979). Chromic oxide in food assimulation studies (comments). Trans. Am. Fish Soc., 108: 650-652.

BRETT, J.R. & SHELBOURN, J.E. (1975). Growth rate of young sockeye salmon (Oncorhynchus nerka) in relation to fish size and ration level. J. Fish. Res. Bd. Can., 32: 2103-2110.

BROCK, J. A. (1977). Freeze and laser marking of channel catfish. Prog. Fish-Cult., 39: 138.

BROWN, M.E. (1946). The growth of brown trout (Salmo trutta Linn.) II. Growth of two-year old trout at a constant temperature of 11.5°C. J. Exp. Biol., 22: 145-155.

BROWN, M.E., (1957), Metabolism. In, The Physiology of Fishes, Vol. I, Academic Press, N.Y.

BUHLER, D.R., & HALVER, J.E. (1961). Nutrition of salmonoid fishes. IX. Carbohydrate requirements of chinook salmon. J. Nutr., 74 : 307-318.

CHAO, L.P. & BIRKBECK, T.H. (1978). Storage of marine fish erythrocytes in liquid nitrogen, J. Fish. Biol. 13: 483-488.

CHO, C.Y., SLINGER, S.J. & BAYLEY, H.S (1976). Influence of level and type of dietary protein and level of feeding on feed utilization by Rainbow trout. J. Nutr., 106: 1547-1556.

CLAYSON, D.B., (1975). Nutrition and experimental carcinogensis: A review. Cancer Res., 35 : 3292-3300.

COLLARD, P. (1963). A species of Corynebacterium isolated from fermenting cassava roots. J. Applied Bacteriol. 26: 115-116.

CORDIER, D. & CORDIER, M. (1957). Phosphorolyse du glycogene du muscle strie chez les poissons marins. Compt rend. Soc. Biol. (Paris), 151: 1909-1911.

COULSON, R.A. & HERNANDEZ, T. (1979) Increase in metabolic rate of the alligator fed proteins or amino acids. J.Nutr., 109, 538-550.

COWEY,C.B., HIGUERA, D.L. ADRON, J.W., (1977). The effect of dietary composition and of insulin on gluconeogenesis in Rainbow trout (Salmo gairdneri). Br. J. Nut., 38: 385.

COWEY, C.B. KNOX, D., WALTON, M.J., & ADRON, J.W. (1977). The regulation of gluconeogenesis by diet and insulin in Rainbow trout (Salmo gairdneri). Br. J. Nutr., 38: 463-470.

COWEY, C.B., POPE, J.A., ADRON, J.W. & BLAIR, A. (1972). Studies on the nutrition of marine flatfish. The protein requirement of plaice (pleuronectes platessa). Brit. J. Nut., 28: 447-456.

COWEY, C.B. & SARGENT, J.R. (1972). Fish Nutrition. A-V Mar. Biol., 10: pp 383-492.

COWEY, C.B. & SARGENT, J.R. (1979). Nutrition. In Fish Physiology, VIII (W.S. Hoar, D.J. Randall & J.R. Brett, Ed). 11-17. Academic Press Inc.

CREACH, Y. & SERFATY, A. (1974). Le jeune et la realimentation chez la carpe. J. Physiol. (Paris), 68: 245-260.

CULLING, C.F.A. (1963). Handbook of histopenthological techniques (including muscle technique). London Butterworths.

DICKIE, L.M. (1963). Estimation of mortality rates of Gulf of St. Lawrence cod from results of a tagging experiment. Int. Comm. Northwest Atl. Fish. Spec. Publ. 4: 71-80

DUNCAN, D.B. (1955). Multiple range and multiple F-test. Biometrics, 11: 1-42.

DYKE, J.M.V. & SUTTON, D. L. (1977). Digestion of duckweed (Lemna sp.) by the grass carp (ctenopharyngodon idella). J. Fish. Biol., 11: 273-278.

EDWARDS, D.J., AUSTRENG, E., RISA, S. & GJEGREM, T. (1977). Carbohydrate in Rainbow trout diets. 1. Growth of Fish of different families fed diets containing different proportions of carbohydrate. Aquaculture, 11: 31-38.

ELLIOTT, J. M. (1975a). The growth of brown trout (Salmo trutt a L.) fed on maximum rations. J. Animal Ecol., 44; 805-821.

ELLIOTT, J. M. (1975b). The growth of brown trout (Salmo trutta L.) fed on reduced rations. J. Animal Ecol., 44: 823-842.

ERMAN, YE, Z. (1969). Intestinal absorption of various sugars in yearling carp. J. Ichtyology, 10: 534-537.

FERRIRA, J. T., SMITH, G. L., SCHOONBEE, H. J., & HOLZAPFEL, C.W., (1979). Comparison of anaesthetic potency of Benzocaine Hydrochloride and MS-222 in two freshwater fish species. Prog. Fish Cult., 41: 161-163.

FISHMAN, W.H. & BERNFELD, P. (1955). Glucuronidases. In, Methods in Enzymology, (S. Colowick & N.O. Kaplan, Ed.), 1 : 262-269. New York Academic Press.

FOLTZ, J.W. (1979). Chromic oxide in Food Assimulation Studies (Comments). Trans. Am. Fish. Soc., 108: 650-652.

FURUICHI, M. & YONE, Y. (1971a). Studies on nutrition of red sea bream - IV. Nutriative value of dietary carbohydrate. Report of Fishery Research Laboratory Kyushu University, No. 1, 1971 75-81.

FURUICHI, M. & YONE, Y. (1971 b). Studies on nutrition of red sea bream -IV. A study of carbohydrate utilization by glucose and insulin - tolerance tests. Report of Fishery Research Laboratory Kyushu University, No. 1, 1971: 91;100

FURUICHI, M., & YONE, Y. (1980). Effect of dietary-dextrin levels on the growth and feed efficiency, the chemical composition of liver and dorsal muscle, and the absorption of dietary protein and dextrin in fishes. Bull. Jap. Soc. Scient. Fish., 46 : 225-229.

FURUKAWA, A., (1976). Diet in yellow-tail culture. In Proceedings of the Fish International Conference on Aquaculture Nutrition, (K.S. Price (Jr), W.N. Shaw & K. S. Danberg, Eds.) pp. 85-104 Coll. Mar. Stud. Univ. Del. Newark.

FURUKAWA, A & TSUKAHARA, H. (1966). On the acid digestion method for determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jap. Soc. Scient. Fish., 32: 502-506.

GAS, N., (1972). Alterations structurales du muscle blanc de carpe au cours du jeune prolonge. C.R. Acad. Sci., 275 : 1403-1406.

GERKING, S.D., (1971). Influence of rate of feeding and body weight on protein metabolism of bluegill sunfish. Physiol. Zool., 44: 9-19.

GERKING, S.D., (1972). Revised food consumption estimate of bluegill sunfish population in Wyland Lake, Indiana, U.S.A. J. Fish. Biol., 4 : 301-308.

GHITTINO, P (1976). Nutritional factors in trout hepatoma. Prog. Exp. Tumor. Res., 20: 317-338.

GUNNES, K., & REFSTIE, T., (1980). Cold-branding and fin-clipping for marking of salmonoids. Aquaculture, 19: 295-299.

HALVER, J.E., (1967). Cristalline aflatoxin and other vectors for trout hepatoma. Trout Hepatoma Res.Conf. Papers. USDHEWUSDI Res. Rep., 70: 78-102.

HALVER, J. E. (1976). Nutritional Research with Salmonoids. In, Proceedings of the First International Conference on Aquaculture Nutrition, (K.S. Price (Jr.), W. N. Shaw & K. S. Danberg, Eds.), pp 25-37. Coll. Mar. Stud. Univ. Del. Newark.

HALVER, J. E. & MITCHELL, I. A. (1967). Trout hepatoma research conference papers. USDHEW-USDI Res. Rep., 70: 199.

HAMADA, A, IDA, T., TSDA, T., & KARIYA, T. (1975). Studies on the growth structure of fishes. I. Maximum growth of carp. Bull. Jap. Soc. Scient. Fish., 41: 147-154.

HASTINGS, W. H. (1967), Progress in sport fisheries, 1966: feed formulation, physical quality of pelleted feed; digestibility. Fish Wildl. Serv. (U.S) Res. Publ., 39: 137-141.

HASTINGS, W.H. (1968) Fish food processing. New developments in Carp and trout nutrition. EIFAC/TG 23-42.

HASTINGS, W.H. (1969). Nutritional Scores. In Fish in Research, (O.W. Neuhaus & J. C. Halver Eds). pp 263-292. Academic Press, New York.

HASTINGS, W.H. & DICKIE, L.M. (1972). Feed formulation and evaluation. In, Fish Nutrition, (J.E. Halver, Ed.), pp 327-374. Academic Press, New York.

HASWELL, M.S. & RANDALL, D.J. (1978). The pattern of carbon dioxide excretion in the Rainbow trout, Salmo gairdneri. J. Exp. Biol., 72: 17-24.

HEIDINGER, R.C., & CRAWFORD, S.D., (1977). Effect of temperature and feeding rate on the liver-somatic index of the large mouth bass, (Micropterus salmoides) J. Fish. Res. Bd. Can., 34: 633-638.

HELMREICH, E. (1969). Control of synthesis and breakdown of glycogen starch and cellulose. In, Comprehensive Biochemistry, 17, Carbohydrate Metabolism, (M. Florkin & E.H. Stotz, Ed.), 17: 17-92. Elsevier Pub. Co. Amsterdam, Lond. N.Y.

HELMREICH, E., & CORI, C.F. (1969). In, Advances in Enzyme Regulation (Weber, Ed.) : p. 91. Pergamon, Oxford.

HENDRICKS, J.D., HUNTER, L.J. & WALES, J.H. (1976). Postmortem bile damage to Rainbow trout (Salmo gairdneri) livers. J. Fish. Res. Bd. Can., 33: 2613-2616.

HENNEBERG & STOHMANN (1865) in LLOYD., et al., The nutritients and their proximate analysis. In, Fundamentals of Nutrition,

(L.E. Lloyd, B.E. McDonald & E. W. Crampton, Eds. 1978). pp 14-17. W.H. Freeman & Co.

HERS, G.H., & DeWULF, H. (1967). The stimulation of glycogen synthesis and of glycogen synthetase in the liver by the administration of glucose. Eur. J. Biochem. 2 : 50-56.

HESTRIN, S., FEINGOLD, D.S., & SCHRAMM, M., (1955). Hexoside hydrolases. In, Methods in Enzymology (S. Colowick & N.O. Kaplan, Eds.), 1: 311-257. Academic Press, New York.

HODGE, J.E., & DAVIS, H.A., (1952). Selected methods for determining reducing sugars. USDA Bur. Agric. Ind. Chem. A.I.C., pp. 333.

HODGE, J.E. & HOFREITER, B.T. (1962). Determination of reducing sugars and carbohydrates. Methods in Carbohydrate Chemistry, 1 : 380-294.

HOFER, R., (1979a). The adaptation of digestive enzymes to temperature, season and diet in roach, (Rutilus rutilus) and rudd (Scardinius erythrophthalmus); Proteases. J. Fish. Biol., 15: 373-379.

HOFER, R., (1979b). The adaptation of digestive enzymes to temperature, season and diet in roach, (Rutilus rutilus, L.) and rudd, (Scardinius erythrophthalmus). 1. Amylase, J. Fish. Biol., 14: 565-572.

HOKANSON, K.E.F., KLEINER, C.F., & THORNSLUND, T.W., (1977). Effect of constant temperatures and diel temperature fluctuations on the specific growth and mortality rates and yield of juvenile Rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Can., 34: 639-648.

HUDSON, G.J., JOHN, P.M., BAILEY, B.S., & SOUTHGATE, D.A.T. (1976). The automated determination of carbohydrate. Development of a method for available carbohydrates and its application to foodstuffs. J. Sci. Fd. Agric., 27: 681-687.

HUISMAN, E.A., (1976). Food conversion efficiencies at maintenance and production levels for carp. (Cyprinus carpio L.) and Rainbow trout, (Salmo gairdneri R.) Aquaculture, 9: 259-273.

INADA, D., OGINO, C., TAKAMATSU, C., TAKAMATSU, C., UEDA, T. & KARAKAWA, K. (1963). Digestibility of dietary components in Fishes II. Digestibility of dietary protein and starch in Rainbow trout. Bull. Jap. Soc. Scient. Fish., 29: 242-244.

ITAZAWA, Y. & TAKEDA, T. (1979). An estimation of the minimum level of dissolved oxygen in water required for normal life of fish - II. An experiment with carp under carbon dioxide accumulation. Bull. Jap. Soc. Scient. Fish., 45: 323-327.

JAN, L., HSU, Y.L., & WU, J.L. (1977). Nutritive values of leaf protein concentrates for grass carp, (Ctenopharyngodon idellus) B.I. Zool. As., 16: 91-98.

JAUNCEY, K. (1979). Growth and nutrition of carp in heated effluents. PhD Thesis, University of Aston in Birmingham, England, p. 181.

JOBLING, M., & DAVIES, P.S., (1979). Gastric evacuation in plaice, (Pleuronectes platessa L.) : effects of temperature and meal size. J. Fish. Biol., 14: 539-546.

JOHNSTON, I.A., (1977). A comparative study of glycolysis in red and white muscles of the trout (Salmo gairdneri) and Mirror carp (Cyprinus carpio). J. Fish. Biol., 11: 575-588.

JOHNSTON, I.A., DAVISON, W., & GOLDSPINE, G. (1976). Energy metabolism of carps swimming muscle. J. Comp. Physiol., 114: 203-216.

JOYCE, J.A., & El-IBIARY, H.M., (1977). Persistency of hot brands and their effects on growth and survival of fingerling channel cat fish. Prog. Fish Cult. 39: 112-114.

KEADING, L.R., & KAYA, C.M., (1978). Growth and diets of trout from contrasting environments in a geothermally heated stream: The Firehole River of Yellowstone National Park. Trans. Am. Fish. Soc., 107: 432-438.

KAWAI, S., & IKEDA, S. (1972). Studies on digestive enzymes of fish-II. Effect of dietary change on the activities of digestive enzymes in carp intestine. Bull. Jap.Soc. Scient. Fish., 38: 265-270.

KAYA, C.M., (1978). Thermal resistance of Rainbow trout from a permanently heated stream, and of two hatchery strains. Prog. Fish. Cult., 40: 128-142.

KELSO, J.R.M. (1972). Conversion, maintenance and assimulation for Walleye, (Stizostedion vitreum vitreum) as affected by size, diet and temperature. J. Fish. Res. Bd. Can., 29: 1181-1192.

KETIKU, A.O. & ONYENUGA, V.A., (1968). Preliminary report on the carbohydrate constituents of cassava and yam tuber. Nigerian J. Sci., 4: 25-30.

KETOLA, H.G., (1978). Nutritional requirements and feeding of selected coolwater fishes: A Review. Prog. Fish. Cult., 40: 127-132.

KILAMBI, R.V., ADAMS, J.C., BROWN, A.V., & WICKIZER, W.A., (1979). Effects of stocking density and cage size on growth, feed conversion and production of Rainbow trout and channel catfish. Prog. Fish. Cult., 39: 62-66.

KILBORN, L.G. & MACLEOD, J.J.R., (1919). Observations on the glycogen content of certain invertebrates and fishes. Quart. J. Exper. Physiol., 12, : 317-330.

KITAMIKADO, M., MORISHITA, T., & TACHINO, S., (1964). Digestibility of dietary protein in Rainbow trout. 2, Effect of starch and oil contents in diets and size of fish. Bull. Jap. Soc., Scient. Fish., 30: 50-54.

KITAMIKADO, M., & TACHINO, S., (1960). Studies on the digestive enzymes of Rainbow trout - 1. Carbohydrases. Bull. Jap. Soc. Scient. Fish., 26: 679-684.

KLAIN, G.J., (1977). In vivo effects of glucagon on fatty acid synthesis in fasted and refed rats. J. Nutr., 107: 942-948.

KLONTZ, G.W. & SMITH, L.S. (1968). Methods of using fish as a biological research subject. In, Methods of Animal Experimentation III (Ed. Cay, W.L.) 323-385 pp. NY. Academic Press

KONO, H., & NOSE, T., (1971). Relationship between the amount of food taken and growth of fishes. I. Frequency of feeding for maximum daily ration. Bull. Jap. Soc., Scient. Fish., 37: 169-175.

KOOPS, H., TIEWS, K., TIEWS, J., & GROPP, J. (1974). Starke-und fetterverwertung netzkafiggehaltener regenbogenforellen (Salmo gairdneri). Aquaculture, 4: 277-286

KWAIN, W., (1975). Effects of temperature on development and survival of Rainbow trout, (Salmo gairdneri) in acid waters. J. Fish. Res. Bd. Can., 32: 493-497.

LEATHERLAND, J.F., CHO, C.Y., & SLINGER, S.J., (1977). Effects of diet, ambient temperature and holding conditions on plasma thyroxine level in Rainbow trout (Salmo gairdneri). J. Fish Res. Bd. Can., 34: 677-682.

LEE, D.J., & PUTNAM, G.B., (1973). The response of Rainbow trout to varying protein energy ratios in a test diet. J. Nutr., 103 916-922.

LEE, D.J. & SINNHUBER, R. D. (1972). Lipid requirements. In, Fish. Nutrition, (J.E. Halver, Ed.) pp. 145-177. Academic Press, New York.

LEE, D.J., SINNHUBER, R.O., WALES, J.H., & PUTNAM, G.B. (1978) Effect of dietary protein on the response of Rainbow trout (Salmo gairdneri) to aflatoxin B.1. J. Nat. Cancer Inst., 60: 317-320.

LEE, D.J. & WALES, J.H. (1973). Observed liver changes in Rainbow trout (Salmo gairdneri) fed varying levels of a casein-gelatin mixture and herring oil in experimental diets. J. Fish. Res. Bd. Can., 30: 1017-1020.

LEE, R.A., (1969). Bioenergetics and feeding and growth of large-mouth bass in aquaria and ponds. M.S. Thesis Oregon State University, Corvallis, Oregon, U.S.A., p. 63.

LEGER, C., LUQUET, P., & BOUDON, M., (1975). Effects de la suppression des glucides dans l' alimentation de la Truite arc-en-ciel a la temperature de 10°C. II. Evolution des compartments corporels avec reference particulieve aux acides gras des familles W9, W6, and W3. Ann. Hydrobiol., 6: 71-93.

LEGER, C., LUQUET, P., & BOUDON, M., (1976). Effects de la suppression des glucides dans lpalimentation de la Truite arc-en-ciel a la temperature de 16°C. II. Evolution des

compartments corporels avec reference particuliere aux acides gras des familles W9, W6 et W3 et influence d'um changement de temperature. Ann. Hydrobiol., 7: 185-201.

LEWANDER, K. (1976). Metabolic effects of insulin in the European eel, (Anguilla anguilla L.). General and Comparative Endocrinol., 2: 455-467. Academic Press, Inc.

LIED, E., GJERDE, J., & BAAEKKAN, O.R., (1975). Simple and rapid technique for repeated blood sampling in Rainbow trout. (Salmo gairdneri). J. Fish. Res. Bd. Can., 32 : 699-701.

LIN, H., ROSMOS, D.R., TACK, P.I., & LEVEILLE, G.A., (1977). Effects of fasting and feeding various diets on hepatic lipogenic enzyme activities in coho salmon. (Oncorhynchus Kisutch Walboum) J. Nutr., 107: 1477-1483.

LIN, H., ROSMOS, D.R., TACK, P.I. & LEVEILLE, G.A., (1978). Determination of glucose-utilization in coho salmon (Onchorhynchus kisutch walbaum). (6-H-3)-Glucose and (U-C-14)-Glucose. Comp. Biochem. A., 59: 189-191.

LINDSAY, G.J.H., & HARRIS, J.E., (1980). Carboxymethylcellulase activity in the digestive tracts of fish. J. Fish. Biol., 16: 219-233.

LLOYD, L.E., McDONALD, B.E., & CRAMPTON, E.W., (1978) (Ed.)

Fundamentals of nutrition. (W.H. Freeman & Co., San Francisco), pp. 47-50.

LOVELAND, P.M., NIXON, J.E., PAWLOWSK, N.E., & EISELE, T.A., (1978). Aflatoxin -B1 and aflatoxin metabolism in trout (Salmo gairdneri) and the effects of dietary cyclopropene. J. Env. Pathol., 2: 707-718.

LUQUET, P., (1971). Efficacite des proteines en ralation quec leur taux d' incorporation dans l' alimentation de la Truite arc-en-ciel. Ann. Hydrobiol., 2 : 175-186.

LUQUET, P., LEGER, C., & BERGOT, F., (1975). Effects of carbohydrate suppression in diets of Rainbow trout kept at a temperature of 10°C. I. Growth in relation to level of protein ingestion. Ann. Hydrobiol., 6: 61-70.

LUQUET, P., LEGER, C., & BERGOT, F., (1976). Effects de la suppression des glucides dans l'alimentation de la Truite arc-en-ciel a la temperature de 16°C. I. Croissance en fonction du niveau d' ingestion proteique. Ann. Hydrobiol., 7: 179-184.

McCAULEY, R.W., ELLIOTT, J.R., & READ, L.A.A. (1977). Influence of acclimation temperature on preferred temperature in the Rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc., 106: 362-365.

McERLEAN, A.J., & KENNEDY, V.S., (1968). Comparison of some anaesthetic properties of benzocaine and MS-222. Trans. Am. Fish. Soc., 97: 496-498.

McFARLAND, W.N., (1959). A Study of the Effects of Anaesthesia on the Behaviour and Physiology of Fishes. Publ. Inst. Mar. Sci., 6: 23-55.

McLAREN, B.A., HERMAN, E.F., & ELUEHJEM, C.R., (1946). Nutrition of Rainbow trout; studies with purified rations. Arch. Biochem., 10: 433-441.

McLAREN, B.A., HERMAN, C. F., & ELUEHJEM, C.R., (1947). Nutrition of trout, studies with practical diets. Proc. Soc. Exp. Biol. Med., 65: 97-101.

McLEAN, A.E.M., & MAGEE, P.M., (1970). Increased renal carcinogenesis by dimethylnitrosamine in protein defficient rats. Br. J. Exp. Pathol., 51: 587-590.

McLEAY, D.J. (1977). Development of a blood sugar bioassay for rapid measuring of stressful levels of pulpmill effluent to salmonoid fish. J. Fish. Res. Bd. Can., 34: 477-485.

McLEAY, D.J. & BROWN, D.A., (1975). Effects of acute exposure to bleached kraft pulpmill effluent on carbohydrate metabolism of juvenile coho salmon (Oncorhynchus kisutch) during rest and

exercise. J. Fish. Res. Bd. Can., 32: 753-760.

MacLEAN, W.C., (Jr.), KLEIN, G.L., LOPEZDE, R.G., MASSA, E., & GRAHAM, G.G. (1978). Protein quality of conventional and high protein rice and digestibility of glutinous and non-glutinous rice by preschool children. J. Nutr., 108: 1740-1747.

MACPHERSON, N.L., (1932). IX. Studies in the behaviour of the carbohydrate and lactic acid of the muscle of the haddock. (Gadus aeglefinus) after death. Biochem. J., 26: 80-87.

MADHAVAN, T.V., & GOPALAN, C., (1965). Effect of dietary protein on aflatoxin liver injury in weaning rats. Arch. Pathol., 80: 123-126.

MADHAVAN, T.V., & GOPALAN, C., (1968). The effect of dietary protein on carcinogenesis of aflatoxin. Arch-Pathol., 85: 133.

MAIR, G., (1977). Zelluloseverdauung bei omnivoren cypriniden. Hausarbeit Univ. Innsbruck, p. 60.

MALEVSKI, Y., MONTGOMERY, M.W., & SINNHUBER, R.O., (1974). Liver fat and protein metabolism in Rainbow trout (Salmo gairdneri) fed cyclopropenoid fatty acids. J. Fish. Res.Bd. Can., 31: 1093-1100.

MALEY, F., McGARRAHAM, J. F., & DELGIACCO, R., (1966). Galactosamine: A precursor of glycogen glucosamine. Biochem.

Biophys. Res. Communs., 23: 85.

MARKING, L.L. (1967). Toxicity of MS-222 to selected fishes. U.S. Fish . Wildl. Serv. Invest. Fish. Control., 10, (Resour. Publ. 18), 10 pp.

MATTY, A.J. & SMITH, P., (1978). Evaluation of a yeast, a bacterium and an algae as a protein source for Rainbow trout. 1. Effect of protein level on growth, grass conversion efficiency and protein conversion efficiency. Aquaculture, 14: 235-146.

MAYNARD, L.A. & LOOSLI, J.K. (Eds) (1979). Animal Nutrition. 6th Edn. Mc Graw - Hill Book Co.

MENZEL, D.W., (1959). Utilization of algae for growth by the angel fish. J. Cons. Perm. Int., Explor. Mer., 24: 308-313.

MILLER, D.S. & BENDER, A.E. (1955). The determination of the net protein utilization by a shortened method. British J. Nutr. 9 : 382-388

MULLER-FEUGA, A., PETIT, J., & SAEAUT, J.J., (1978). Influence of temperature and wet weight on the oxygen demand of Rainbow trout (Salmo gairdneri) in fresh water. Aquaculture, 14: 355-363.

MURAT, J.C., (1976a). Recherches sur la metabolition des glucides tissulaires chez la carpe. These Doct. Etat, Toulouse.

MURAT, J.C., (1976 b). Studies on glucogenolysis in carp liver. Comp. Biochem. Physiol., 55B, 461-465.

MURAT, J.C., (1976c). Particularites du metabolisme due glycogene chez la carpe, apres injections de glycagon ou d'insuline Ann. d'Endocrinologie (Paris), 37: 523-524.

MURAT, J.C., CASTILLA, C., & PARIS, H., (1978). Inhibition of gluconeogenesis and glucagon-induced hyperglycaemia in carp (Cyprinus carpio L.) (Technical note). Gen. clin. Endocrinol., 34 : 243-246.

MURAT, J.C., & PLISTETSKAYA, E.M., (1977). Effects of glucagon on blood-glucose, liver glycogen level and synthetase activity in lamprey and carp. C.R. Soc. Biol., 171: 1302-1305.

MURAT, J.C., & SERFATY, A., (1974). Simple enzymatic determination of polysaccharide (Glycogen) content of animal tissue. Clin. Chem., 20: 1576-1577.

MURAT, J.C., & SERFATY, A., (1975). Effects de ladrenaline, du glucagon et de lpinsuline sur le metabolisme glucidique de la carpe. C.R. Soc. Biol., 169: 228-232.

MURRAY, M.W., (1977). Effects of dietary lipids, dietary protein and environmental temperatures on growth, feed conversion and body composition of channel catfish. J. Nutr., 107: 272-280.

NAGASE, G., (1964). Contribution to the physiology of digestion in Tilipio mossambico (Peters): digestive enzymes and effect of diets on their activity. Z. Vergl. Phys. 49: 270-284.

NAGAYAMA, F., & SAITO, Y., (1968). Distribution of amylase α- and β-glucosidase, and β-galactusidase in Fish. Bull. Jap.

Soc. Scient. Fish. 34: 944-949.

NAGAYAMA, F., & SAITO, Y., (1978). Physiology of digestion: Distribution of several hydrolytic enzymes in fish. EIFAC/T9. 104-116.

NAGAYMA, F., SAITO, Y., & HAYASHI, M., (1967). Distribution of β-glucuronidase in fish. Bull. Jap. Soc. Scient. Fish., 33: 1132-1138.

NEWBERNE, P.M., & ROGERS, A.E., (1970). Aflatoxin carcinogenesis in rats: Dietary effects. In Purchase, I.F.H. (Ed.). Mycotoxins in Human Health, pp. 195-208. London & Basingstoke, Macmillan Press Limited.

NIEDERHOLZER, R., & HOFER, R., (1979). The adaptation of digestive enzymes to temperature, season and diet in roach Rutilus rutilus L. and rudd scardinus erythrophthalmus L. cellulase. J. Fish. Biol., 15: 411-416.

NIMI, A.J., & BEAMISH, F.W.H., (1974). Bioenergetic and growth of large-mouth bass (Micropterus salmoides) in relation to body weight and temperature. Can. J. Zool., 52: 447-456.

NOMURA, T., & KAWATSU, H., (1977). Variation of hemotocrit valves of Rainbow trout blood samples incubated under different conditions. Bull. Jap. Soc. Scient. Fish., 43: 301-306.

NORDIN, J.H., & HANSEN, R G., (1963). Isolation and characterization of galactose from Hydrolysates of glycogen. J. Biol. Chem., 238: 489.

NOSE, T., (1967). Recent advances in the study of fish digestion. EIFAC (Eur. Inland Fish Advis. Comm.) Tech. Pap. 3: 83-94.

NOSE, T., (1971). Determination of nutritive value of food protein in fish III. Nutritive value of casein, white fish meal and soybean meal in Rainbow trout fingerlings. Bull freshwater Fish. Res. Lab., 21: 85-98.

NUTRITION REVIEWS VOL. 37 (8)/ Aug. 1979. Comparison of lipogenesis from dietary glucose in liver and carcass. The Nat. Res. Council.

OKAFOR, N., (1977). Short communication. Micro-organisms associated with cassava fermentation for gari production. J. Applied. Bacteriol; 42, 279-284.

ONO, T., & NAGAYAMA, F., (1957). Enzymatic studies on the glycolysis in fish muscle. I. Activity of phosphorylase. Bull. Jap. Soc. Scient. Fish., 23, 260-264.

OSBORNE, T.B., MENDEL, L.B., & FERRY, E.L., (1919). A method for expressing numerically the growth promoting value of proteins. J. Biol. Chem. 37: 223-224.

PAGE, J. W., & ANDREWS, J.W., (1973). Interactions of dietary levels of protein and energy on channel catfish (Ictalurus punctatus). J. Nutr., 103: 1339-1346.

PALMER, T.N., & RYMAN, B.E., (1972). Studies of oral glucose intolerance in fish. J. Fish. Biol., 4: 311-319.

PALOHEIMO, J.E., & DICKIE, L.M., (1966). Food and growth of fishes III. Relations amongst food body size and growth efficiency. J. Fish. Res. Bd. Can. 23: 1209-1248.

PANDIAN, T.J., (1967). Intake, digestion, absorption and conversion of food in the fishes, Megalops cyprinoides and Ophiocephalus striotus. Marine Biol. 1: 16-32.

PEAT, S., ROBERTS, P.T. P., & WHELAN, N.J., (1952). The occurrence of fructose in Rabbit-liver glycogen. Biochem. J. 51: XVII.

PFEFFER, E., (1977). Studies on the utilization of dietary energy and protein by Rainbow trout (Salmo gairdneri) fed either by hand or by an automatic self-feeder. Aquaculture, 10: 97-107.

PHILLIPS, A.M., (Jr.) (1969). Nutrition, digestion and energy utilization. In Fish Physiology 1: pp. 391-432. (W. S. Hoar, & D.J. Rendell Ed.) Academic Press, N.Y.

PHILLIPS, A.M., & BROCKWAY, D.R., (1956). The nutrition of trout. II. Protein and Carbohydrate. Prog. Fish Cult. 18: 159-164.

PHILLIPS, A.M., (Jr.) & BROCKWAY, D.R., (1959). Dietary calories

and the production of trout in hatcheries. Prog. Fish. Cult., 21, 3-16.

PHILLIPS, A.M., (Jr.)., LIVINGSTON, D.L., & POSTON, H.A., (1966). Use of Calorie sources by Brook trout. Prog. Fish. Cult., 16: 67-72.

PHILLIPS, A.M., TUNISON, A.V., & BROCKWAY, D.R., (1948). The utilization of carbohydrates by trout. Fish. Res. Bull. (N.Y. Cons.Dept.). No. 11, 44 pp.

PIEPER, A., & PFEFFER, E., (1980a). Studies on the comparative efficiency of utilization of gross energy from some carbohydrates, proteins and fats by Rainbow trout (Salmo gairdneri) Aquaculture, 20:323-332.

PIEPER, A., & PFEFFER, E., (1980b) Studies on the effect of increasing proportions of sucrose or gelatinized maize starch, in diets for Rainbow trout (Salmo gairdneri) on the utilization of dietary energy and protein. Aquaculture. 20: 333-324.

PIERCE, K.V., McCAIN, B., & WELLINGS, S.R. (1980). Histopathology of abnormal liver and other organs of starry flounder Platichthys stellatus (pallas) from estuary of the Duwamisch River Seattle, Washington, USA. J. Fish. Diseases, 3: 81-91.

POSTON, H.A., RIIS, R.O., RUMSEY, G.L., & KETOLA, H.G., (1978) Nutritionally induced cataracts in salmonids fed purified and

practical diets. Marine fish Res., 40: 45-46.

PREJS, A., & BLASZCZYK, M., (1977). Relationship between food and cellulose activity in fresh-water fishes. J. Fish. Biol., 11: 447-452.

RAFFIN, J.P., & LERAY, C., (1979). AMP Deaminase in the gill of trout (Salmo gairdneri R) modalities of an activation by cellular Proteolytic enzymes. Comp. Broc. B., 62: 23-29.

REFSTIE, T., (1980). Genetic and environmental sources of variation in body weight and length of Rainbow trout fingerlings. Aquaculture. 19: 351-357.

REINITZ, G.L., (1976). Comparison of techniques for stabilizing hemoglobins of Rainbow trout (Salmo gairdneri) during frozen storage. Comp. Biochem. Physiol., 55B: 357-358.

REINITZ, G.L., (1977). Tests for association of transferrin and lactate dehydrogenase phenotypes with weight gain in Rainbow trout (Salmo gairdneri). J. Fish. Res. Bd. Can., 34: 2333-2337.

REINITZ, G.L., ORME, L.E., & HITZEL, F. N., (1979). Variations of body composition and growth among strain of Rainbow trout. Trans.Am. Fish. Soc. 108: 204-207.

REINITZ, G.L., ORME, L.E., LEMM, C.A., & HITZEL, F.N., (1978a). Differential performance of four strains of Rainbow trout reared

under standardized conditions. Prog. Fish. Cult., 40: 21-23.

REINITZ, G.L., ORME, L.E., LEMM., C.A., & HITZEL, F.N., (1978b). Full-fat soybean meal in Rainbow trout diets. Reprinted from Feedstuffs, 50; 23-24.

REINITZ, G.L., ORME, L.E., LEMM, C.A., & HITZEL, F.N., (1978c). Influence of varying lipid concentration with two protein concentrations in diets for Rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc., 107: 751-754.

REINITZ, G.E., & RIX, J., (1977). Effect of Tricaine methansulfonate MS-222 on hematocrit values in Rainbow trout (Salmo gairdneri). Comp. Biochem. Physiol., 56c: 115-116.

ROALD, S.O., (1977). Effects of sublethal concentrations of lignosulphonates on growth, intestinal flora and some digestive enzymes of Rainbow trout (Salmo gairdneri). Aquaculture, 12: 327-335.

ROGERS, A.E., & NEWBERNE, P.M., (1971). Diets and aflatoxin B1 toxicity in rats. Toxicol. Appl. Pharmacol; 20: 113-121.

RYCHLY, J., (1980). Nitrogen balance in trout II. Nitrogen excretion and retention after feeding diets with varying protein and carbohydrate levels. Aquaculture, 20: 343-350.

RYCHLY, J. & SPANNHOF, L., (1979). Nitrogenous balance in trout. 1. Digestibility of diets containing varying levels of protein and carbohydrate. Aquaculture, 16: 39-46.

SCARPELLI, D.G., (1976). Drug metabolism and Aflatoxin induced hepatoma in Rainbow trout (Salmo gairdneri). Prog. Exp. Tumor. Res; 20: 339-350.

SCHOETTGER, R.A., & JULIN, A.M., (1967). Efficiency of MS-222 as an anaesthetic on four salmonids. U.S. Fish Wildl. Serv. Invest. Fish Control. 13: (Resour. Publ. 19) 15pp.

SEDGWICK, R.W., (1979). Effect of ration size and feeding frequency on the growth and food conversion of juvenile Penoeus merguiensis De Man. Aquaculture, 16: 279-298.

SHIMENO, S., HOSOKAWA, H., HIRATA, H., & TAKEDA, M. (1977). Comparative studies on Carbohydrate metabolism of yellow-tail and Carp. Bull. Jap. Soc. Scient. Fish. 43: 213-217.

SHIMENO S; HOSOKAWA, H., & TAKEDA, M. (1978). The importance of Carbohydrate in the diet of a carnivorous fish. FAO/EIFAC/78/Symp. E/5. 1-32.

SHIREMAN, J.V; COLLE; & ROTTMANN, R.W. (1977). Intensive Culture of grass Carp. (Ctenophargodon idella) in circular tanks. J. Fish. Biol., 11, 267-272.

SIMON, J., & ROSSELIN, G. (1979). Effect of Intermittent feeding on glucose- insulin relationship in the chicken. J. Nutr., 109: 631-641.

SIMS, G.S., (1978). Rapid estimation of Carbohydrate in Formulated Fishery products - protein by difference. J. Sci. Food, 29: 281-284.

SIN, A.W., (1973). The dietary protein requirements for growth of young carp (Cyprinus carpio). Hong Kong Fisheries Bull. no. 3: 77-82.

SINGH, R.P. & NOSE, T. (1967). Digestibility of carbohydrates in young Rainbow trout. Bull Freshwater Res. Lab. 17: 21-25.

SISK, D.B., & CARLTON, W.W., (1972). Effect of dietary protein concentration on response of miniature swine to aflatoxin. Am. J. Vol. Res. 33: 107-114.

SMIT, G.L., BARHAM, W.T; & SCHOONBEE, H.J., (1977). Some effects of the anaesthetic MS-222 on freshwater Fish. S. Afr. J. Sci., 73: 351-352.

SMIT, G.L; HATTINGH, J., & BURGER, A.P., (1979a) Haematological assessment of the effects of the anaesthetic MS-222 in natural and neutralized form in three freshwater fish species: interspecies difference. J. Fish. Biol., 15: 633-643.

SMIT, G.L., HATTINGH, J., & BURGER, A.P., (1979b). Haematological assessment of the effects of the anaesthetic MS-222 in natural and neutralized forms in three freshwater fish species: intraspecies differences. J. Fish. Biol., 15: 645-653.

SMIT, G.L., HATTINGH, J, & BURGER, A.P., (1979c). Haematological assessment of the effects of the anaesthetic MS-222 in natural and neutralized form in three freshwater fish species: haemoglobin electrophoresis, ATP levels and corpuscular fragility curves. J. Fish Biol., 15: 655-663.

SMITH, B.W., & LOVELL, R.T., (1971). Digestibility of nutrients in semi-purified rations by channel catfish in stainless steel troughs. Proc. 25th Annu. Southeast Assoc. Game Fish Comm. 452-459.

SMITH, B.W., & LOVELL, R.T., (1973). Determination of apparent protein digestibility in feed for channel catfish. Trans. Am. Fish.Soc., 102: 831-835.

SMITH, C.E., (1979). The prevention of liver lipoid degeneration (ceroidosis) and microlytic anaemia in Rainbow trout, (Salmo gairdneri R.) fed rancid diets: a preliminary report. J. Fish. Diseases, 2: 429-437.

SMITH, C.M., (1978). The effect of metabolic state on incorporation of ^{14}C pantothenate into CoA in rat liver and heart. J. Nutr. 108: 863-873.

SMITH, R.R., (1971). A method for measuring digestibility and metabolizable energy of fish feeds. Prog. Fish Cult., 53: 132-134.

SMITH, R.R., (1976). Metabolizable energy of feedstuffs for trout. Feedstuffs, 48: 16-17

SMITH, R.R., RUMSEY, G. L; & SCOTT, M.L., (1978a). Net energy maintenance requirements of salmonids as measured by direct calorimetry: Effect of body size and environmental temperature. J. Nutr., 108: 1017-1024.

SMITH, R.R., RUMSEY, G.L., & SCOTT, M.L., (1978b). Heat increment associated with dietary protein, fat, carbohydrate and complete diets in salmonids: comparative energetic efficiency. J. Nutr., 108: 1025-1032.

SOIVIO, A., NYHOLM, K. & HUHTI, M., (1977). Effects of anaesthesia with MS-222, neutralized MS-222 and benzocaine in the blood constituents of Rainbow trout, (Salmo gairdneri). J. Fish. Biol. 10: 91-101.

SOIVIO, A., & OIKARI, A., (1976). Haematological effects of stress on a teleost, (Esox lucius L.). J. Fish Biol., 8: 397-411.

SPATARU, P., (1978). Food and feeding habits of (Tilapia zillii Gervais) (Cichlidae) in lake Kinneret (Israel). Aquaculture. 14: 327-338.

SPATARU, P. & ZORU, M., (1978). Food and feeding habits of (Tilapia aurea Steindachner) (Cichlidae) in lake Kinneret (Israel). Aquaculture, 13: 67-79.

SPIGARELLI, S.A., & THOMMES, M.M., (1979). Temperature selection and estimated thermal acclimation by Rainbow trout (Salmo gairdneri) in a thermal plume. J. Fish. Res. Bd. Can., 36: 366-376.

STICKNEY, R.R; & SHUMWAY, S.E., (1974). Occurrence of cellulase activity in the stomachs of fishes. J. Fish. Biol., 11: 447-452.

STOTT, W.T., & SINNHUBE, R.O., (1978). Dietary protein levels and aflatoxin - Bl metabolism in Rainbow trout (Salmo gairdneri). J. Env. Pathol. & Toxicol., 2: 379-388.

STOTT, W.T., & SINNHUBER, R.O., (1979). Dietary casein levels and aflatoxin -Bl metabolism in Rainbow trout (Salmo gairdneri). ACS Symp. Series No. 99 Pesticide and Xenobiotic Metab. in Aquatic Organisms. (M.A.W. Khan, J.J. Lech. & J. Mann, Eds.) 390-400. Am. Chem. Soc.

STRUTHERS, B.J., WALES, J.H., LEE, D.J., & SINNHUBER, R.O., (1975). Liver composition and histology of Rainbow trout fed cyclopropenoid fatty acids. Experimental and molecular pathology, 23: 164-170.

SUZUKI, A., & SEKIZAWA, Y., (1979). Residue analyses on 2-amino-

4-phenythiazole a piscine anaesthetic in fishes - IV. GC/ms analysis on Rainbow trout. Bull. Jap. Soc. Scient. Fish., 45: 167-171.

SVOBODOVA, Z., (1976). Vliv charakteru potravy na obsah glykogenu v hepatopankreatu a ve svalovine kapru (Cyprinus carpio L.) Zivocisna vyroba (Praha), 21: 891-900.

SWANN, P.F., & McLEAN, A.E.M., (1971). Cellular injury and carcinogenesis. The effect of a protein-free high-carbohydrate diet on the metabolism of dimethylnitrosamine in the rat. Biochem. J., 124: 283-288.

TACON, A.G.J. (1979). The use of activated sludge - single cell protein (A S C P) derived from the treatment of domestic sewage in trout diets. In, Proc. world Symp. on finfish nutrition and fishfeed Technology, Hamburg, June 1978, Vol. II, Berelin, 1979.

TAKEUCHI, T., WATANABE, T., & OGINO, C., (1979a). Availability of carbohydrate and lipid as dietary energy sources for carp. Bull. Jap. Soc. Scient. Fish., 45: 977-982.

TAKEUCHI, T., WATANABE, T., & OGINO, C., (1979b). Optimum ratio of dietary energy to protein for carp. Bull. Jap. Soc. Scient. Fish., 45: 983-987.

TODD, G.C., SHALKOP, W.T., DOOLEY, K.L., & WISEMAN, H.G.,

(1968). Effect of ration modifications of aflatoxicosis in the rat. Am. J. Vet. Res., 29: 1855-1861.

TYLER, A.V., & DUNN, R.S., (1976). Ration, growth and measures of somatic and organ condition in relation to meal frequency in winter flounder, (Pseudopleuronectes americanus) with Hypothesis regarding population homeostasis. J. Fish. Res. Bd. Can., 33: 63-75.

VAHL, O., (1979). An hypothesis on the control of food intake in fish. Aquaculture, 17: 221-229.

VAN SOEST, P.J., & McQUEEN, R.W., (1973). The Chemistry and estimation of fibre. Proc. Nutr. Soc., 32: 123.

VIOLA, S., (1977). Energy values of feedstuffs for carp. Bamidgeh, 29: 29-30.

WALTON, M.J., & COWEY, C.B., (1979). Gluconeogenesis by isolated hepatocytes from Rainbow trout (Salmo gairdneri). Comp. Bioc. B., 62: 75-79.

WARE, D.M., (1975). Growth, metabolism, and swimming speed of a pelagic fish. J. Fish. Res. Bd. Can., 32: 33-41.

WARNER, M.C., DIEHL, S.A., & TOMB, A.M., (1978). Effects of dilution and temperature of analysis on blood-serum values in Rainbow trout (Salmo gairdneri). J. Fish. Biol. 13: 315-319.

WATANABE, T., (1977). Sparing action of lipids on dietary protein in fish - low protein with high calorie content. TECHNOCRAT, 10: 34-39.

WINDELL, J.T., FOLTZ, J.W., & SAROKON, J.A., (1978a). Effect of fish size temperature and amount fed on nutrient digestibility of a pelleted diet by Rainbow trout (Salmo gairdneri). Trans. Am. Fish. Soc., 107: 613-616.

WINDELL, J.T., FOLTZ, J.W., & SAROKON, J.A., (1978b). Methods of faecal collection and nutrient leaching in digestibility studies. Prog. Fish Cult., 40: 51-55.

WINDELL, J.T., HUBBARD, J.D., & HORAK, D.L., (1972). Rate of gastric evacuation in Rainbow trout fed three pelleted diets. Prog. Fish Cult., 34: 156-159.

WINDELL, J. T., & NORRIS, D.O. (1969). Gastric digestion and evacuation in Rainbow trout. Prog. Fish Cult. 31: 20.

WINDELL, J.T., NORRIS, D.O., KITCHELL J.F., & NORRIS, J.S., (1969), Digestive response of Rainbow trout to pellet diets. J. Fish. Res. Bd. Can., 26: 1801-1812.

WINTERS, G.W., (1977). Estimtes of tag extrusion and initial tagging mortality in Atlantic herring (Glupea harengus harungus), released with abdominally inserted magnetic tags. J. Fish. Res. Bd. Can., 34: 354-359.

WITTENBERGER, C., & VITCA, E., (1966). Variation of the glycogen content in the tissues of the carp, during a work in isolated muscles and during starvation. Stud. Univ. Babes-Bolgai Serv. Biol., 11: 117-123.

WOOD, E.M., & YASUTAKE, W.T., (1955). Histopathology of fish I Techniques and principles. Prog.Fish. Cult., 17: 166-171.

WURTSBAUGH, W.A., & DAVIS, G.E., (1977a). Effects of temperature and ration level on the growth and food conversion efficiency of Rainbow trout (Salmo gairdneri R.) J. Fish. Biol., 11: 87-98.

WURTSBAUGH, W.A., & DAVIS, G.E., (1977 b). Effect of fish size and ration level on the growth and food conversion efficiency of Rainbow trout, (Salmo gairdneri, R.) J. Fish. Biol., 11: 99 - 104.

YU, T. C., & SINNHUBER, R.O. (1979). Effect of dietary W3 and W6 fatty acids on growth and feed conversion efficiency of coho salmon (Oncorhynchus kisutch). Aquaculture, 16: 31-38.