DIGESTIBLE ENERGY AND CARBOHYDRATES IN THE NUTRITION OF TILAPIA

(*Oreochromis niloticus* Linn.)

Joseph Anderson, B.Sc.

A thesis submitted to the University of Aston in Birmingham

for the Degree of Doctor of Philosophy

June, 1985

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SUMMARY

The apparent digestibilities of a series of tropical feeds were evaluated in feeding trials with tilapia. A standardised method of measuring feed digestibility is reported and microassays are described which enable analysis of energy, nitrogen and chromium in the small faecal samples (c. 150 mg) obtainable from individual fish.

From regressions of the digestibility data on chemical measures of feed composition, several statistical models were produced which can be used to predict the digestible energy (DE) of plant-product feeds to an accuracy of ±1.5 MJ/kg DM. The single most accurate predictor of DE was neutral detergent fibre, although this was only applicable to certain feed types. For a wider range of feeds, DE was more accurately predicted by multiple regression equations which included measures of dietary protein, available carbohydrate and fibre.

Factors affecting feed digestibility were also investigated. With soybean meal, it was shown that DE was significantly greater when this feed was given to tilapia as a component of a complete diet than when it was given on its own. DE was also significantly greater after 15 weeks of feeding than after 1 week. However, the DE of soybean was not affected by its level in a diet over a range from 20% to 60% and there was no interaction between the effects of inclusion level and feeding duration. Soybean protein digestibility was independent of inclusion level and feeding duration.

DE values of carbohydrate-rich feeds were generally higher than corresponding values for other species of farmed fish. In growth trials, the carbohydrates in these plant-product feeds were shown to be effectively utilised to spare dietary protein energy for growth, and it was demonstrated that the maximum tolerable level of dietary carbohydrate was at least 40%.

The energy value of different carbohydrates was related to their level in a diet and the number of saccharide units in the molecule. Thus, at low dietary inclusion (10%), glucose spared more protein energy in diets than sucrose, dextrin or starch. At higher levels (40%), the situation reversed and dextrin spared more protein than the lower molecular weight carbohydrate. At all inclusion levels, dextrin was a more available source of energy than starch.

Increasing levels of dietary fibre depressed DE, food utilisation, carcass fat and growth, and it is recommended that fibre levels should not exceed 10% in practical diets for tilapia.

KEY WORDS: Tilapia, Nutrition, Digestibility, Carbohydrates, Fibre.

PREFACE

Background and development of research project

The stimulus and funding for this research were provided by the Tropical Development and Research Institute of the Overseas Development Administration. In 1981, the Institute recognised a need for research on the nutrition of tilapia against a background of increasing activity in the culture of this fish for food in less developed countries. At this time, there were practically no published data on the availability of energy in feeds for tilapia making it difficult to formulate and compound these feeds into well balanced diets. The initial requirement for the present research was, therefore, to investigate the availability of energy in a range of tropical feeds in order to develop a method for predicting this availability (as digestible energy - DE) from the chemical composition of a feed. The value of such a method for predicting DE would be that it could reduce the need for laborious *in vivo* feeding trials and provide feed manufacturers with a rapid means of evaluating local feeds for inclusion in least-cost diets.

The most obvious way of predicting DE from the chemical composition of a feed was to quantify the relationship between the two measures by regression analysis. If a fixed relationship could be identified, it would then be possible to predict one measure from the other. However, the first problem to be encountered was that there were no published DE values for tilapia. The only way to obtain these values was, therefore, by experiment with live animals. The reliability of these experimentally determined DE values were of fundamental importance because the accuracy of any predictive model depends on the reliability of the data base from which predictions are made. For this reason, it

was necessary to precede the work on determination and prediction of DE with a critical examination of the methods currently used in digestibility trials with fish. This work comprised the first part of this research and concluded with recommendations for a simple, accurate method of measuring DE with tilapia under standard conditions (Chapters 3 and 4).

The work on determination and prediction of DE was then able to proceed and was extended to include the determination and prediction of protein digestibility. This work comprised the second part of the research program and enabled a series of conclusions to be made concerning the general principles of digestion in tilapia (Chapters 5 and 6).

At this point the initial research requirements were satisfied, but it was of further interest to extend the work from the availability of food energy and protein to an investigation of the utilisation of these for growth. This work comprised the third part of the project. The first approach here was to examine the relationship between the digestibility of practical feeds and growth in tilapia (Chapter 7). The aim of this was to provide useful information on the relative productive value of different classes of feed and on the utilisation of carbohydrate energy for growth. The second approach was then to examine the utilisation of specific carbohydrates as major non-protein sources of energy and to observe the effect of indigestible fibre on the utilisation of other dietary components (Chapter 8).

The three parts of the project are reported in this thesis sequentially. Throughout the text, the specific aims of each part are developed further, and in the introductory literature review, the overall aim of the project is placed in a broader context of fish

nutrition and diet development.

Most of the energy values used in this thesis are in megajoules (MJ). However, to maintain continuity in the Introduction and Discussion, literature values have been left as kilocalories (kcal) when they have been reported as such. These quantities can be interconverted thus:

1000 kcals = 4184 kJ = 4.184 MJ

Explanation of terminology

The term digestibility is used throughout the text. Strictly, this is a measure of the percentage of food that is not egested and which is therefore assumed to be absorbed. Digestibility must not be confused with digestion efficiency or absorption efficiency, since the digestion of food and the absorption of digestion products are two distinct processes. Digestibility describes the sum effect of digestion and absorption. The term digestibility is, therefore, misleading but its use has become established in the literature and for convenience it is also used throughout this thesis.

The terms digestible energy (DE) and metabolisable energy (ME) are used extensively. These may be defined as follows (Brett and Groves, 1979):

DE = energy remaining from food less faeces (MJ/kg DM)

ME = energy available from food less total exogenous excretions (faecal + nonfaecal) (MJ/kg DM)

The terms 'feed' and 'diet' also require some explanation. A feed (or, 'feedstuff') either refers to a particular crop of plants and their products (e.g. rice grain and rice bran, wheat grain and wheat

middlings) or a concentrated form of animal tissue (e.g. fishmeal, meat and bone meal, poultry by-products). These plant-product and animal-product meals are the raw materials of the feed manufacturing industry; they are treated as discrete commodities and are identified by an international coding system (see Appendix 3). A diet on the other hand is a carefully formulated preparation composed of several feeds and a variety of supplemental nutrients such as vitamins, minerals, preservatives, binders, essential oils and pigments. Thus, 'feed digestibility' and 'diet digestibility' are two distinct measures and in this thesis, their meanings are not interchangeable.

ACKNOWLEDGEMENTS

The funding for this research was provided by the Tropical Development Research Institute (TDRI) and I am indebted to Mr Brian Capper of the animal feeds section for his helpful advice and constant encouragement. The experimental work was conducted in the Fish Culture Unit of the Department of Biological Sciences at the University of Aston, where I was kindly assisted by many members of staff and fellow research students. In particular, I am most grateful to Dr Andrew Jackson for his guidance during the first six months of this project, Professor Alan Matty for supervising the remainder of the project and Dr Niall Bromage for his thorough and constructive review of the manuscript.

The advice and assistance of technical staff at Aston and TDRI were invaluable. For answering numerous inquiries and helping with analytical techniques I am especially grateful to Steve Howitt, John Huddleston, Dennis Reeves and Tony Watson at Aston and Pam Carter and Victor Medlock at TDRI. In addition, my thanks go to Hazel Penzig for her patience and understanding whilst typing this thesis.

Finally, I would like to express my warmest thanks to Mum, Dad, Susan and the rest of my family for their endless support and encouragement.

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LIST OF ABBREVIATIONS

The following is a list of the abbreviations used frequently throughout this thesis. All these abbreviations are fully defined in each chapter when they are first used, but they are also listed here for quick reference. The symbols for SI units, chemical elements and statistical terms follow scientific convention and are not listed.

AAS	Atomic absorption spectrophotometry
ADF	Acid detergent fibre
ANOVAR	Analysis of variance
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ARC	Agricultural Research Council (presently Agricultural and Food Research Council - United Kingdom)
Analar	Analytical grade reagent
CF	Crude fibre
CHO	Available carbohydrate
CHO E	Available carbohydrate energy
CMC	Carboxymethylcellulose
CP	Crude protein
Δh	Temperature change (in calorimetry)
DCP	Digestible crude protein
DE	Digestible energy
DM	Dry-matter
DMD	Dry-matter digestibility
DPE	Digestible protein energy
EDTA	Ethylenediaminetetra-acetic acid
EE	Ether extract (= crude fat)

FCE	Food conversion efficiency
FCR	Food conversion ratio
GE	Gross energy
GPT	Gut passage time
HCELL	Hemicellulose
MAFF	Ministry of Agriculture Fisheries and Food (United Kingdom)
ME	Metabolisable energy
NDF	Neutral detergent fibre
NER	Net energy retention
NFE	Nitrogen-free extract
NPR	Net protein retention
NRC	National Research Council (of the United States National Academy of Sciences)
P/DE	Protein to digestible energy ratio
PE/DE	Protein energy to digestible energy ratio
PER	Protein efficiency ratio
RSD	Residual standard deviation
SD	Standard deviation
SEM	Standard error of mean
SGR	Specific growth rate
bp	Boiling point
ppm	parts per million
w/w	weight per unit weight

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Section 1.1 Introduction

Most of the world's production of farmed warmwater fish is accounted for by only a few families: carps (Cyprinidae), milkfish (Clupeidae), mullets (Mugilidae) and tilapias (Cichlidae). Of these, tilapias are the most widely cultured and are found in over one hundred countries (Balarin and Hatton, 1979). There are about one hundred in the group collectively referred to as tilapia and whilst there is currently a taxonomic debate on generic groupings, many research workers now accept the classification of Trewavas (1982 a, b), who proposes three genera on reproductive and feeding criteria, viz., Tilapia, Sarotherodon and Oreochromis (see Appendix 1). Approximately ten species are important in aquaculture and until recently, most of these were produced in extensive or semi-extensive systems.

All of the food in extensive systems originates from the natural productivity of the pond and although fertilisers are often used to encourage the production of food organisms, fish yields are relatively low and are typically about 5 t/ha/yr. An alternative is to farm fish intensively, but the development of this has been frustrated for many years by the precocious breeding of tilapia. This leads to over-crowding in ponds, excessive competition for food and the production of small fish. However, developments in the commercial production of all-male fry (by hybridisation and/or hormone treatment) has stimulated a renewed interest in intensive farming.

In an intensive system, all of the food is provided by pelleted

feeds, the contribution of natural productivity is negligible and fish stocking density high. At present, intensive tilapia culture is at an experimental stage. However, research has shown that there is a fourfold improvement in yield when ponds are managed intensively and in tanks and cages, yields of 250 t/ha/yr (50 times that of an extensive pond) have been reported (Balarin and Haller, 1982). With this potential as a stimulus, the commercial farming of monosex populations of tilapia in tanks, cages and raceways has begun and the success of such farms in Israel and Kenya has initiated a revival of research effort into improved husbandry techniques (see Balarin and Haller, 1982 for a review of current research and techniques in intensive tilapia culture). However, the development of intensive farming depends not only on improvements in farming technology, but on the availability of compounded feeds.

Pelleted rations are already produced on a commercial scale for salmonids, catfish and carp. For salmonids in particular, enough is known of their nutrient requirements (NRC, 1973, 1981), to enable formulation of practical rations from locally available feedstuffs throughout the world. Much less is known of the nutrient requirements of warmwater fishes (NRC, 1977, 1983). In particular, very little information exists on the nutrition of tilapias and diets are often formulated using values for the nutrient requirements of other fish species and energy terms published for poultry and swine. Recent research in tilapia nutrition has concentrated on the quantitative protein and amino acid requirements of tilapia (Davis and Stickney, 1978; Mazid et al, 1978; Jauncey, 1982; Jackson and Capper, 1982) and on the value of dietary lipids and fatty acid requirements (Viola and Amidan, 1980; Winfree and Stickney, 1981; Viola and Ariela, 1982;

Stickney and McGeatchin, 1983; Takeuchi et al, 1983 a,b). However, there is no published information as yet on the mineral and vitamin requirements or the need for energy and use of carbohydrates in diets for tilapia. Many of the feedstuffs available in tropical countries where tilapia are farmed contain high levels of carbohydrates (e.g. cereals and their by-products and certain root crops) and in feed manufacture, energy is usually considered to be the most important parameter.

For any commercial livestock, if a ration has sufficient levels of essential amino acids, essential fatty acids, crude protein, minerals and vitamins, the most important factor limiting growth is dietary energy. For this reason and because energy supplying nutrients are those present in food in the greatest quantity, these are given first consideration when computing least-cost formulations. If a diet is formulated to satisfy other nutrient requirements first, and then it is found that energy level is not balanced to the animals needs for any given production level, a complete revision of the diet will be necessary (McDonald et al, 1981). Clearly, the development of cheap balanced rations for tilapia from locally available feed ingredients will benefit from an assessment of the role of energy in tilapia nutrition. Two aspects must be considered:

1. the availability of energy in natural feeds

and 2. the utilisation of dietary energy by tilapia.

Section 1.2 Availability of energy in feeds for fish

1.2.1. Energy budgets and feeding tables

The gross energy values of the major nutrient classes are generally

accepted to be: protein, 5.65 kcal/g (23.6 MJ/kg); fat, 9.45 kcal/g (39.5 MJ/kg) and carbohydrate, 4.0 kcal/g (16.7 MJ/kg). However, the net energy of a food (i.e. the amount available for maintenance, activity and growth) depends on the amount lost in faeces and on its heat increment or, "specific dynamic action". These losses form part of an energy budget, the components of which are well established in animal nutrition (see Maynard and Loosli, 1979). The first significant study of physiological energetics in fish was on carp (Ivlev, 1939), but it was not until the review by Winberg (1956), that it became possible to assign values to the components of an energy budget for fish. In a more recent review, Brett and Groves (1979) constructed a summary energy budget for carnivorous fish using values from the literature and this is shown in Fig.1.1.

The net energy value of food is most affected by the amount of energy that is excreted and early estimates put this at about 20% (Winberg, 1956). However, Winberg (1956) overlooked the losses associated with non-faecal excretion and when this is taken into account, the excretion term is closer to 27% (Brett and Groves, 1979). Effectively, Winberg (1956) was reporting digestible energy (DE), whilst Brett and Groves (1979) calculated metabolisable energy (ME). These two energy terms can be defined as follows:

DE = gross energy - faecal energy

ME = gross energy - faecal energy - energy in non-faecal excretions.

In animal feeding tables, energy requirements most commonly refer to net energy. The most useful measure of food energy value would thus seem to be net energy also, since requirement and supply could then be directly equated. However, most tables report feed energy as DE or ME mainly because these are easier to measure than net energy. In

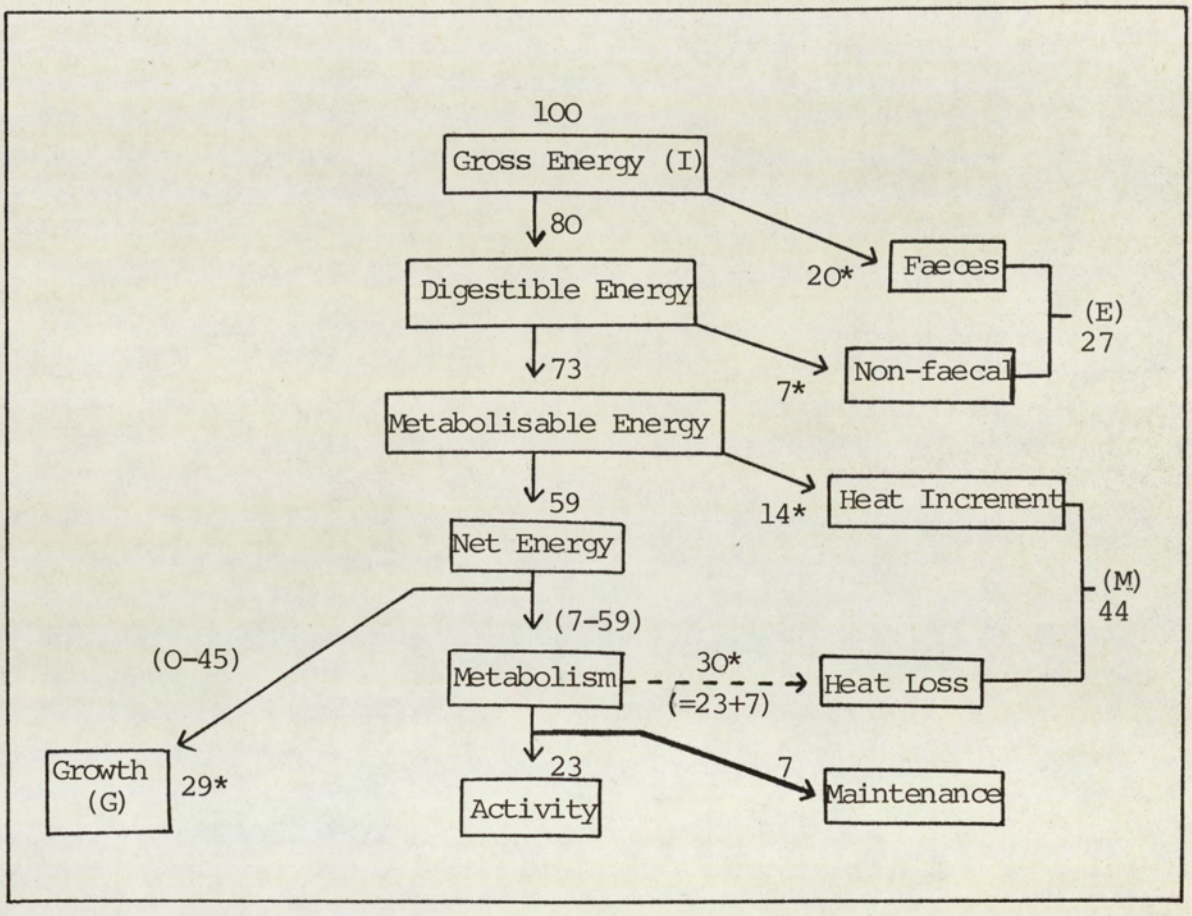


Figure 1.1. Summary energy budget for carnivorous fish

100 calories is ingested (I) and contributes to excretion (E), maintenance (M) and growth (G). Figures marked with an asterix total to 100. The values in brackets indicate the extent to which net energy can be distributed between metabolism and growth. A very active fish will use all of the net energy for metabolism, leaving none for growth, whilst a resting, non-feeding fish will require only enough for maintenance. From Brett and Groves (1979).

addition, net energy is a reflection of the efficiency with which ME is utilised and this varies widely for functions such as maintenance, growth and lactation. This variation is particularly marked with ruminants and if net energy was used to describe energy availability, individual values would be needed for each of the different types of production. For this reason, food energy is reported as ME in ruminant feeding tables and for different production levels, efficiency coefficients are used to convert ME to net energy (MAFF, 1975; ARC, 1980). With monogastric animals, less elaborate energy systems are used and, with pigs for example, the UK Agricultural and Food Research Council states the energy concentration of feedstuffs in terms of DE only (McDonald et al, 1981).

With fish, DE would also seem to be the most convenient measure of energy availability, largely because of the difficulty of determining ME at zero nitrogen balance. ME determination with fish requires collection from the water of both kidney and gill excretions. Metabolism chambers have been constructed which are capable of doing this and ME values published for a limited number of trout feeds (Smith, 1971, 1976, Smith et al, 1980). However, the procedure is time-consuming and fish are stressed as a result of being force-fed and confined in narrow tubes. With tilapia, attempts to determine ME with Smith's chambers have failed due to the excessive stress response (H. Barrash, pers. comm., 1983). Physiological stress affects the nitrogen balance of fish (Smith, 1971) and this in turn affects ME. ME is also affected by level of feeding and several authors have suggested that ME should be abandoned and DE used preferentially as a measure of the availability of food energy in nutrition and production studies with fish (Cho et al, 1982; Jobling, 1983).

In view of this and because of the lack of any data on feeding energetics in tilapia, it is important to measure the DE of food before further refining the energy budget to include ME. This is the approach taken in the present research.

1.2.2 Digestibility and the energy value of dietary nutrients

Early digestibility studies were with trout and the following coefficients summarised by Phillips and Brockway (1959): protein, 90% (Tunison et al, 1942); fat, 85% (McCay and Tunison, 1935) and carbohydrate, 40% (Phillips et al, 1948). Applying these coefficients to gross energy values and correcting for the non-faecal energy losses of protein, Phillips and Brockway (1959) calculated the ME of protein, fat and carbohydrate as respectively, 3.90 kcal/g, 8.0 Kcal/g and 1.6 kcal/g. For carbohydrate and fats, energy losses are confined to the faeces and DE is usually taken to be equivalent to ME (Hilton and Slinger, 1981). For several years, these energy values were taken to be constants despite warnings by Phillips and Brockway (1959) and in the literature they are still widely used when energy levels are calculated in diets for fish. However, these values are not constant and the factors affecting the availability of energy in proteins carbohydrates and fats shall now be considered in turn.

With protein, it is generally accepted that the Phillips and Brockway (1959) value is an underestimate because of the ammoneotelic pattern of nitrogen excretion in freshwater teleosts. Energy losses associated with the excretion of ammonia are substantially lower than those incurred in the biosynthesis and excretion of urea (Forster and Goldstein, 1969). Phillips and Brockway (1959) corrected the gross energy of protein to ME by applying a non-faecal energy term more

appropriate to ureotelic animals than fish, hence the underestimate. In an attempt to correct this, Brett and Groves (1979) calculated the ME of absorbed protein by only subtracting from protein gross energy, a quantity equal to the heat of combustion of ammonia (5.94 kcal/g = 0.95 kcal/g protein). By then applying a digestibility coefficient of 0.9 (90%) they arrived at a theoretical value for dietary protein of 4.23 kcal/g.

Experimentally determined ME values have similarly suffered from inaccurate estimates of the energy contained in non-faecal excretions. Smith (1967) estimated that the branchial excretions of trout contain about 85% ammonia and 15% urea. In metabolism chamber studies, Smith (1971) reported that lyophilised samples of water which contained gill excretions would not combust in a bomb calorimeter and so in a later study calculated energy as the weighted average of the heat of combustion of ammonia and urea. This estimate (4.99 kcal/gN) was used to calculate the ME of casein/gelatin diets fed to trout as 4.5 kcal/g (Smith et al 1980). In their calculation, Smith et al (1980) used a value for the heat of combustion of ammonia of 68.9 kcal/mole. This is lower than the figure of 82.3 kcal/mole used by Brett and Groves (1979) and to an extent, this explains why their ME value is lower than that reported by Smith et al (1980). Because of the inconsistencies of assessing the non-faecal energy term, there are three values for protein ME currently in use throughout the literature, viz. 3.9, 4.2 and 4.5 kcal/g.

The situation is further confused by the fact that ME varies according to the amount of amino acids retained for protein synthesis and the amount deaminated and excreted as ammonia and urea. Thus, ME is underestimated when fish are in negative nitrogen balance and overestimated when they are in positive balance. Because nitrogen

balance is affected by level of feeding, Jobling (1983) suggests that ME is of limited value and that its use in fish nutrition studies should be discontinued. However, the problem of controlling for nitrogen balance is well known in terrestrial livestock nutrition and often, ME is corrected to zero balance by applying a correction factor calculated for each species from known energy losses (or gains) in the metabolism of protein (McDonald et al, 1981). With fish, ME is rarely if ever corrected to zero nitrogen balance at present and values are understandably variable. Thus, until there is standardisation of methodology in metabolism studies with fish, it is perhaps better to restrict attention to the digestibility of nutrients when formulating diets.

The energy value of a nutrient depends more on the amount that is absorbed (i.e. its digestibility) than the amount that is subsequently metabolised. Because of this, digestibility is considered to be the single most important factor determining the availability of energy from dietary nutrients. In general, animal product feeds are more efficiently digested by fish than those of plant origin. Kitamikado et al (1964a) observed that raw meat (fish, beef liver) had protein digestibilities of 91 - 97%, whilst the value of soybean meal was 70%. The variability in digestibility between feeds is large and in a recent study of 55 feeds for rainbow trout, protein digestibilities were reported to range from 32 - 91% (Smith et al, 1980). Clearly, Phillips and Brockway's widely accepted value of 90% for protein digestibility is of limited applicability.

With carbohydrates, digestibility depends largely on the size and complexity of the molecule, and with fats, on the level of hydrogenation (saturation). Monosaccharides such as glucose are readily absorbed

across the gut and have digestibilities of 92 - 99%, whilst a complex polysaccharide such as raw starch has a much lower digestibility of 38% (Phillips et al, 1948). Using these coefficients, the range of DE for carbohydrate can be calculated as 1.6 - 4.0 kcal/g, so a generalised value for this class of nutrient, as for proteins, has little meaning. Fat digestibility is also variable since low melting point, unsaturated fats are more efficiently absorbed than saturated fats (McCay and Tunison, 1935; NRC, 1977; Austreng et al, 1979; Takeuchi et al, 1979).

The relative proportions of carbohydrates, protein and fat in a diet affects digestibility, because there are interactive effects. Thus, Kitamikado et al (1964b) showed that the protein digestibility of diets fed to trout depends on the level of potato starch present in the diet. This has been supported by other workers and it is now generally accepted that the starch content of a diet is negatively correlated with diet digestibility (Neuhaus and Halver, 1969; Spannhof and Kuhne, 1977). The level of a nutrient in a diet can also affect its own digestibility. Thus, in diets fed to trout, the digestibility of starch and dextrin decreased with increasing level of dietary inclusion (Singh and Nose, 1967) and with the channel catfish Ictalurus punctatus, dietary fat level was found to affect fat digestibility (Andrews et al, 1978).

Feed processing and level of feeding are two other factors that are thought to affect digestibility. For example, starch is more available in extrusion processed (floating) pellets than in the same diet when steam pelleted (Hilton et al, 1981). This is because more heat is generated when expanded pellets are manufactured by extrusion and the starch is cooked to a certain extent. Cooked starch is partially

hydrolysed and as such is more digestible than raw starch : 80% versus 38% (Nose, 1967). The effect of feeding level is not as clear, although there is some evidence that digestibility is reduced at high intake levels (Windell et al, 1978). Within limited ranges factors such as body size and water temperature have been shown not to affect digestibility (Windell et al, 1978).

In summary, the energy values of proteins, fats and carbohydrates depend on their gross energy, digestibility and the species characteristics of metabolism. The digestibility of a nutrient is in turn determined by its molecular complexity, dietary inclusion level, interaction with other nutrients, feed processing and, possibly, level of feeding. These factors are often ignored when feedstuff DE's are estimated from literature values for gross energy and digestibility of component nutrients. However, it must be recognised that literature values are not constants since they are often determined under non-standard conditions. In addition, values for one cultured species do not necessarily apply to another. Despite this, researchers often use digestibility values published for animals that are not even in the same taxonomic class as the species under study. When accurate DE values are required for individual species, they are best obtained by in vivo digestibility trials in which the energy content of whole feed and faeces are obtained by direct analysis.

In fish nutrition, it is a reflection of the current status of published data that most of the literature cited in this section has been for trout. However, whilst salmonids are mostly carnivorous, tilapias are predominantly herbivorous (Bowen, 1982) and since digestive physiology is closely linked to feeding habit, digestibility values for salmonids are unlikely to apply to tilapias. In support of this

premiss, the following section examines some of the characteristics of digestion in herbivorous fish in general and tilapias in particular.

1.2.3 Digestion of food by tilapias

From a series of studies in which carnivorous fish were fed well above maintenance, Brett and Groves (1979) computed a summary energy budget thus:

$$100\ I = (44 \pm 7)\ M + (29 \pm 6)\ G + (27 \pm 3)\ E.$$

where, I = rate of ingestion, M = metabolic rate, G = growth rate and E = excretion rate.

The equation for herbivorous fish was

$$100\ I = 37\ M + 20\ G + 43\ E.$$

Clearly with herbivorous fish more energy is lost in the faeces and less is available for growth. This is largely due to high levels of cellulose and ash in the diet of plant feeding fish. The cell walls of plants contain cellulose and are resistant to the alimentary canal secretions of fish (Van Dyke and Sutton, 1977; Stickney and Shumway, 1974). Some fish have a gut microflora which exhibit low levels of cellulase activity (Lindsay and Harris, 1980), but despite this, the digestibility of cellulose by fish has been shown to be approximately zero (Smith, 1971; Hilton et al, 1983). In contrast, plant cell contents (which include soluble carbohydrates, organic acids, lipids, proteins and starch) are highly digestible. The dry-matter digestibility of plant material would therefore appear to depend on the amount of refractile material present and the ability of fish to rupture plant cell walls. There is some evidence for this in the literature. With the exclusively herbivorous grass carp Ctenopharyngodon idella, the dry-matter digestibility of aquatic

macrophytes is 60-70% (Hickling, 1966; Van Dyke and Sutton, 1977). However, when the same species is fed on a plant such as lettuce (Lactuca sativa) with a higher proportion of structural carbohydrates, the digestibility is lower (= 20%, Fischer, 1970). To facilitate access to plant cell contents, herbivorous fish such as tilipias have a variety of anatomical adaptations including specialised teeth and pharyngeal mills. However, tilapias appear to be unique amongst herbivorous fish in that certain types of algae are digested in the gut, without prior treatment in the buccal cavity (Bowen, 1982).

Wild tilapias consume bacteria, blue-green algae, green algae, diatoms, macrophytes and a variety of detrital material. In common with other herbivorous species, the teeth responsible for treating plant material are found on the pharyngeal bone and vary in coarseness depending on diet. The teeth and pharyngeal mill triturates aquatic plants, so increasing the surface area to volume ratio of the food and exposes a greater proportion of the plant material to digestive secretions in the stomach. This treatment improves the efficiency of digestion, but a large proportion of the organic matter in higher plants still remains indigested. For Tilapia zillii, the dry-matter digestibility of an aquatic macrophyte (Najas guadalupensis) was reported to be only 29% and unbroken cells were found in the faeces (Buddington, 1979). This indicates that the cells of higher plants are resistant to intestinal digestion if they are not previously ruptured in the buccal cavity of tilapias. In contrast, blue-green algae are susceptible to intestinal digestion by tilapias (Fish, 1960; Moriarty, 1973). The cell walls of Myxophyceae contain pectic substances, and the presence of a gastric secretion in tilapia capable of digesting pectin led early workers to suggest that this secretion was responsible

for the digestion of blue-green algae (Fish, 1960). However, supporting evidence was scant and it is now generally accepted that the digestion of blue-green algae relies on acid conditions in the stomach per se and not on enzyme hydrolysis.

Gastric pH is as low as 1.25 in tilapia which are digesting food (Moriarty, 1973; Caulton, 1976) and can fall to 1.0 (Payne, 1978). This is lower than the gastric pH of most other fish species (Barrington, 1957) and has been shown to cause direct lysis of the blue-green algae cell wall (Moriarty, 1973). Subsequent research has shown that the same mechanism allows Sarotherodon mossambicus to digest detrital bacteria (Bowen, 1976). Most other vertebrates lack enzymes capable of attacking the cell wall of prokaryotes, so acid lysis is seen to be an adaptation which enables tilapias to exploit a protein rich source for which there is no other vertebrate competition in the wild (Bowen, 1982).

The efficiency with which eukaryotic cells are digested also appears to be improved by gastric acid in tilapia (Caulton, 1976; Bowen, 1976). For example, the cell wall of diatoms are denatured in acid conditions and these plankton are, as a result, reasonably well digested by tilapia. However, lignin and cellulose are resistant to acid lysis and so macrophytes are, predictably, less well digested than phytoplankton. Thus, even with macrophagous tilapias, a substantial amount of the digestible organic matter in their natural diet is provided by epiphytic algae, bacteria and detritus associated with the macrophytes (Bowen, 1980a).

Lysis in the stomach exposes the cell contents of algae, bacteria and diatoms. Gastric acid then decomposes chlorophyll, up to 20% of the carbohydrate and much of the mineral content of the diet (Bowen,

1982). Subsequent digestion of the cell contents occurs in the intestine. Trypsin, chymotrypsin, amylase and esterase activity have been detected in the intestine (Fish, 1960; Nagase, 1964; Moriarty, 1973), which in tilapia is 6 - 8 times the length of the body (Caulton 1976; Pauly, 1976). Amino acids are absorbed in the first quarter of the intestine, whilst the absorption of total organic matter and carbohydrate is not complete until the food has passed through the first half of the intestine. In contrast, the amino acids associated with microbial protein and detritus are absorbed along the entire length of the gut (Bowen, 1980b, 1981).

The efficiency with which the various cytoplasmic nutrients are absorbed depends on their type and source. Thus, algal protein and lipids have greater digestibility coefficients than energy and total organic matter even when cell walls have been ruptured prior to feeding (Kirilenko et al, 1975). This indicates that the digestibility of plant material does not depend solely on the efficiency with which cell walls are ruptured. As discussed in the previous section, the molecular structure of nutrients has a large effect on feed digestibility and because of this, it is often possible to predict the DE of a feed from a knowledge of its chemical composition.

1.2.4. Prediction of DE

Digestibility trials with fish are time-consuming and require experimental facilities often unavailable to a feed manufacturer or farmer. Even when digestibility trials are used to evaluate feeds for fish, many of the techniques used may be criticised on methodological criteria as reviewed in Chapters 3 and 4. In the less developed countries where tilapia are farmed, a wide variety of feeds exist (Gohl,

1981) for which there is no information on digestibility. A simple laboratory method which estimates the DE of feeds without recourse to digestibility trials will therefore be of considerable practical use in the manufacture of rations for tilapia.

A common technique in terrestrial livestock nutrition is to establish an empirical relationship between DE and the proximate composition of a series of forages. Regression analysis is the most useful means of quantifying the relationship because DE can then be predicted as the dependent variable in a model where chemical feed measures are the independent variables. Despite the potential of these predictive models, they often have limited applicability because the digestibility of the feeds used to calculate the regression are often not constant from one crop to another. The digestibility of a single crop or its products is influenced by date of harvesting, maturity and the effects of climate, soil and fertilisation (Van Soest, 1976). However, there is often no alternative to predicting DE in this way when immediate information is required on the availability of energy in novel feeds. Used within the limits set by the samples of known digestibility, regression equations can provide a rapid - if approximate - measure of DE, which is useful in first formulations of complete rations.

Early studies with sheep, cattle and pigs concentrated on predicting digestibility from regression equations in which a single composition factor was used such as crude fibre, crude protein or lignin (Blaxter and Mitchell, 1948; Forbes, 1950; Forbes and Garrigus, 1950 a, b; Mitchell, 1942; Schneider et al, 1946, 1947, 1951). With pigs, fibre (measured as crude fibre, cellulose or modified acid detergent fibre) was found to be the best single predictor of ME or DE (Drennan and Maguire, 1970; Morgan et al, 1975 a, b). However, the

fibre/DE relationship was only valid for cereals. For other feeds, there is greater variation in fat and protein and a more complex relationship to DE. Using multiple regression analysis, the best estimate of DE for a wider range of feeds was an equation which included crude protein, acid ether extract and nitrogen free extract (NFE) (Morgan et al, 1975b).

For poultry feeds, a similar approach was taken (Carpenter and Clegg, 1956) but ME prediction was improved by replacing NFE in the equation with 'starch + sugars'. NFE includes carbohydrates such as starches, soluble sugars and part of the hemicellulose fraction of plant cell walls (Bolton, 1954). It is suggested that the equation including 'starch + sugars' is a more accurate measure of ME for poultry because they are less able to digest (and hence obtain energy from) hemicellulose than pigs. Such species differences in the digestion of food means that predictive equations cannot be applied between animal groups. It is therefore necessary to calculate predictive equations specifically for fish.

From considerations in Section 1.2.3, it is probable that fibre is the most important feed parameter affecting DE in fish. Fibre can be measured by several chemical assays and each describes a different fraction of the feed (Van Soest, 1976). Neutral detergent fibre (NDF) is a measure of plant cell wall and, with ruminants, it is the most important characteristic determining feed value. However, this fraction is digested to varying extents by ruminants and so there is poor correlation with feed digestibility. In contrast, fish are unable to digest plant cell walls to any extent and so the NDF/digestibility relationship may be stronger in fish than in ruminants. The literature on fish nutrition provides evidence of at least a qualitative

relationship between DE and fibre. Increasing the level of fibre in diets reduces feed digestibility and growth rates in several species of fish (Buhler and Halver, 1961; Leary and Lovell, 1975; Bromley and Adkins, 1984).

However a predictive model which relies only on fibre would not be applicable to animal-product meals or non-cereal feedstuffs and so it is equally important to measure the influence of non-fibre chemical measures on DE. As with pigs (Morgan et al 1975 a,b) a more robust predictive model might be obtained for tilapia by regressing DE not only on fibre but on a variety of chemical measures such as protein, fat and available carbohydrate. One of the aims of the research reported in this thesis is to test this hypothesis.

DE values describe the amount of energy absorbed, but give no description of the form in which this energy is provided. Carbohydrates are the cheapest and most abundant energy source in feeds of plant origin but they provide less energy per gram than protein or fat and are metabolised poorly by fish (Cowey and Sargent, 1979). Thus, any study on the availability of energy in natural feedstuffs for fish must include an assessment of the role of carbohydrates in fish nutrition.

Section 1.3 Utilisation of carbohydrates by fish

1.3.1 Protein sparing

A problem in the development of complete artificial diets is the high protein requirement of many species of fish since this component contributes a high proportion of feed costs. Jackson et al, (1982) demonstrated the possibility of substituting expensive fishmeal in diets for tilapia with lower cost plant protein sources such as meals of

groundnut, soybean, sunflower seed, rapeseed and cottonseed. An alternative approach is to "spare" protein in the diet with less expensive ingredients such as lipids and carbohydrates (Halver, 1972) but there is a limit to the amount of non-protein energy that can be tolerated and this depends largely on the species of fish.

With channel catfish, it has been estimated that 0.23g dextrin/100g will spare 0.05g protein (Stickney and Lovell, 1977), but when included at levels greater than 15%, this carbohydrate reduced weight gains. Similarly, long term feeding trials with carnivorous salmonids have shown that high carbohydrate levels depress growth rate, elevate liver glycogen levels, impair liver function and cause mortality (Phillips et al, 1948; Austreng et al, 1977; Refstie and Austreng, 1981, Dixon and Hilton, 1981). Phillips et al, (1948) recommended a maximum level of 12% for carbohydrate in trout diets whilst Buhler and Halver (1961) suggest that 20% is the maximum level for Chinook salmon. More recently, it was reported that the optimum level of digestible carbohydrate in diets for trout is 14% which compares well with Phillips' previously accepted value (Hilton and Atkinson, 1982). The maximum tolerable level of carbohydrate was set at 25%, but it has been demonstrated that both maximum and optimum levels are by no means constant and depend on the overall balance of fat, protein and gross energy in a diet (Hilton et al, 1982).

To assess the possibility of improving carbohydrate utilisation in salmonids through selective breeding, various levels of carbohydrate have been fed to different strains of rainbow trout (Edwards et al, 1977; Refstie and Austreng, 1981). It was demonstrated that there was no interaction between sib group and carbohydrate level in these studies and the conclusion was that diets should contain no more than 17 - 25%

carbohydrate regardless of genetic strain.

There could be greater potential for using carbohydrates in diets for tilapias, since herbivorous fish are superior to carnivorous species in their ability to digest, absorb and metabolise this nutrient (Cowey and Sargent, 1979; Shimeno et al, 1979; Furuichi and Yone, 1980, 1981, 1982 a,b). At present commercial fish feeds produced in the UK contain between 17% to 30% "carbohydrate" for trout and between 22% to 35% for carp (Ewos-BakerLtd. West Lothian, UK). However, carbohydrate levels as high as 45% have been fed to carp in experimental diets with no suppression of growth (Ufodike and Matty, 1983). No similar research results have been published for tilapia but information on maximum carbohydrate levels in diets for this group has obvious commercial significance.

Two factors determine the efficiency with which carbohydrates are utilised for growth, viz., the ability to digest and absorb carbohydrate molecules and the characteristics of carbohydrate metabolism.

1.3.2. Carbohydrate digestion

The most important carbohydrase in fish is α - amylase. This attacks α - 1,4 glucosidic bonds in large glucose polymers such as starch. The hydrolysis products are then further digested by α - glucosidase which splits the α - 1,6 glucosidic bonds. Disaccharides are hydrolysed by β - glucosidase, β - galactosidase or by specific enzymes such as maltase, but their activity relative to α - amylase is low (Nagayama and Saito, 1979).

Carbohydrase activity is significantly different between herbivorous and carnivorous groups of fish (Vonk, 1941; Fish, 1960; Nagayama and Saito, 1968; Shimeno et al, 1979). For example,

amyloclastic activity in the pancreas of carp was reported to be a thousand times greater than in the carnivorous dogfish Squalus sp. or pike, Esox sp. (Vonk, 1941). Similarly, amylase activity in the gut lumen of the carnivorous yellowtail, Seriola quinqueradiatia was eighty times lower than in carp and as a consequence, starch digestibility was considerably poorer (Shimeno et al, 1979). Enzyme activity can also be affected by diet and with carp and yellowtail, carbohydrase activity was found to be positively correlated with carbohydrate level (NRC, 1977; Shimeno et al, 1979). The type of carbohydrate also affects carbohydrase activity. Thus, soluble starch was found to increase amylase activity in trout intestine whilst raw starch adsorbed the enzyme and reduced its activity (Spannhof and Plantikow 1983).

The tilapia Sarotherodon mossambicus exhibits high levels of amylase activity throughout its alimentary canal including the buccal cavity. The related, but more carnivorous perch, Perca fluviatilis exhibits less amylase and more protease activity which is consistent with a diet containing high levels of protein (Fish, 1960). As with carp, high carbohydrate diets stimulate amylase activity in tilapia (Nagase, 1964). Trypsin activity was positively correlated with dietary protein level, but similar responses were not found for lipase. Enzyme adaptation to diet in this way has significance for the use of carbohydrates in commercially produced compounded rations.

Carbohydrate hydrolysis products are known to be absorbed at different rates across the gut of monogastric animals. Thus, at equal concentrations, galactose, glucose, fructose, mannose, xylose and arabinose are absorbed at different rates in decreasing order of magnitude (McDonald et al, 1981). This pattern also appears to hold with fish. Erman (1969) showed that the rate of absorption in yearling

carp depends on the concentration of various carbohydrates in the gut and on their molecular structure. Glucose, galactose and uronic acid were more rapidly assimilated than mannose and xylose. However, the value of carbohydrates as energy yielding nutrients depends not only on the ability of fishes to absorb them, but also on the efficiency with which they are metabolised.

1.3.3 Carbohydrate metabolism

Glucose is catabolised by two biochemical routes, i.e. the Embden-Meyerhof and pentose phosphate pathways. The role of these in fish nutrition has been extensively reviewed (Hochachka, 1969; Tarr, 1972; Cowey and Sargent, 1972; Walton and Cowey, 1982) and so the discussion here is restricted to points of divergence in the catabolism of glucose by herbivorous and carnivorous fishes.

Glucose is metabolised more rapidly by mammals than by fishes. To an extent, this may arise from differences in the activity of glycolytic enzymes. Phosphorylation during glycolysis is a preliminary step in both anaerobic and aerobic catabolism of glucose, so the activity of enzymes such as glucokinase could dictate the efficiency with which this sugar is metabolised. In support of this reasoning Cowey and Sargent (1979) demonstrated that hepatic hexokinase activity is indeed ten times higher and the rate of glucose oxidation faster in the omnivorous rat than in the carnivorous plaice, Pleuronectes sp.. Amino acids appear to be preferentially catabolised for energy by fish. Thus, carp oxidise glutamate (which on deamination enters the tricarboxylic acid cycle) more rapidly than glucose (Nagai and Ikeda,1972).

Glucose tolerance in fish is generally poor and is considered to be analagous to that of a diabetic mammal (Palmer and Ryman, 1972;

Furuichi and Yone, 1981; Shimeno et al, 1979). However, there are differences in glucose tolerance between carnivorous and herbivorous fish. After oral administration of glucose, blood sugar was found to be higher and recovery to pre-adminstration level slower with carnivorous yellowtail than with carp (Shimeno et al, 1979). This was confirmed by Furuichi and Yone (1981) who showed that glucose tolerance was higher in carp than the semi-carnivorous red sea bream, Chrysophrys major, which in turn was more tolerant than the fully carnivorous yellowtail. In a previous paper, these authors showed that the maximum level of dextrin in diets for these species followed the same order with optimum level being greatest for the herbivorous species (Furuichi and Yone, 1980)

The relative activity of enzymes in muscle and liver also corresponds with the dietary habit of fishes. Thus hepatic hexokinase activity and presumably the ability to phosphorylate glucose is less in the carnivorous trout than in the herbivorous carp (Nagayama et al, 1972). Further, the gluconeogenic enzymes, glucose - 6 - phosphatase and fructose - 1,6 - diphosphatase were more active and the glycolytic enzyme phosphofructokinase less active in yellowtail than carp (Shimeno et al,1979).

In fasting mammals, liver glycogen is rapidly mobilised to maintain blood sugar levels. In comparison, the mechanism of glycogenolysis is rather inefficient in fish. Many species when deprived of food will use tissue fat (Savitz, 1971) or even protein (Inui and Yokote, 1974) in preference to glycogen. As a result, when fish have not eaten for several weeks, liver glycogen remains constant or even increases (Chang and Idler, 1960; Nagai and Ikeda, 1971; Larsson and Lewander, 1973; Hayashi and Ooshira, 1975). However, the ability to use glycogen as a

short term energy source differs from one species to another (Hilton, 1982). The tilapia Sarothoredon mossambicus exhibits a rapid decrease in liver glycogen when deprived of food (Swallow and Fleming, 1969), indicating that this species may be better adapted to metabolising carbohydrate substrates for energy. The same results have also been reported for Tilapia rendalli (Caulton and Bursell, 1977). Despite these possible exceptions, gluconeogenesis appears to have a more important role than glycogenolysis in maintaining blood sugar in fish and non-essential amino acids appear to be the most important substrate for the synthesis of new sugar (Cowey and Sargent, 1979).

The higher activity of gluconeogenic enzymes and the lower activity of glycolytic enzymes in carnivorous fish would indicate that they are inferior to herbivorous species in their ability to catabolise carbohydrates and they preferentially mobilise protein substrates for energy. Furuichi and Yone (1982a) suggest that species differences in the maximum tolerable level of dietary carbohydrate is a direct result of differences in the activity of glycolytic and gluconeogenic enzymes and that their activity is regulated by post-prandial secretion of insulin. The same authors later showed that the efficiency of carbohydrate metabolism also depends on absorption rate (Furuichi and Yone, 1982b). Blood insulin levels peak 2 hours after feeding and any carbohydrate assimilated before the hormone has stimulated maximum enzyme activity will be poorly catabolised. Thus, glucose which is rapidly absorbed produced poorer growth and food conversion in carp than the more slowly absorbed α - starch. With trout, Abel et al (1979) similarly found that starch, more than sucrose, stimulated glucokinase activity. Perhaps this is also mediated by differences in the rate of carbohydrate absorption and post-prandial secretion of insulin.

It is usually only an assumption that enzyme activity is related to the efficiency with which a substrate is metabolised and causal relationships are often assumed, not proved. To measure gluconeogenesis directly, Cowey et al (1977) measured the conversion of radioactive alanine to glucose in trout and confirmed that the rate of conversion was related to diet composition. Radioactive glucose was formed rapidly in fish fed on high protein diets, but the rate of this gluconeogenesis decreased when dietary protein was substituted by carbohydrate.

This is the biochemical basis of protein sparing in diets for fish. By providing carbohydrate as an energy source in the diet, a smaller proportion of dietary protein is catabolised and a larger amount is available for protein synthesis (i.e. growth). The efficiency of protein retention thus depends on the level of non-protein energy in the diet. For this reason, the relationship between protein and energy must be considered when developing diets for tilapia, or any other group of fish.

1.3.4. The Protein/Energy relationship

DE values are often quoted for diets with no indication of the level of protein fed. However, the latter is a particularly important measure, since growth responses may be due equally to changing protein or DE levels in experimental diets; often an increase in DE is made at the expense of protein. Even in isonitrogenous diets, as DE is increased, the ratio of protein: energy will fall. Hence, protein/DE (P/DE) ratios are a more accurate measure of diet quality than either quantity on its own and they are often expressed as mg protein/kcal DE.

P/DE ratios are correlated with a variety of responses. As the

ratio decreases, lipid deposition increases in the fish carcass and the protein efficiency ratio (g liveweight gain/g protein fed) increases (Page and Andrews, 1973; Lee and Putnam, 1973). High P/DE ratios result in large livers and high levels of liver sugar in rainbow trout (Lee and Putnam, 1973). Condition (the relationship of weight to length) is inversely related to P/DE ratio (Winfree and Stickney, 1981). In many cases, growth rate has been shown to change with the P/DE ratio and for each species, there is an optimum value at which growth rate is maximised. The optimum P/DE for growth is about 90 for carp and catfish, whilst for the carnivorous yellowtail and brook trout it is between 125 - 150 (Winfree and Stickney, 1981). Eels and grass carp occupy intermediate positions, but the optimum for mammalian rations is considerably lower at about 70 mg/kcal. Comparison between studies on fish is often confounded due to the various methods authors have used to determine DE and because fish at different stages of development are used in the various studies. This is an important source of error because it is now established that the optimum P/DE ratio changes as fish grow (Winfree and Stickney, 1981). Juvenile fish need high protein levels, but as they grow, this requirement falls. Sarotherodon aureus fry grew well on a diet with a P/DE ratio of 123 (56% protein, 4.6 kcal DE/g), but this became growth limiting before the fish reached 5g and the optimum value for 7.5g fish fell to 108 (34% protein, 3.2 kcal DE/g, Winfree and Stickney, 1981).

Lipid is often used to spare protein in diets for fish and its inclusion reduces the P/DE of a diet. With tilapia, the P/DE of diets was reduced from 110 to 83 by including fat at 12% of the ration, and whilst there was a slight reduction in growth, the efficiency of protein retention increased substantially (Jauncey and Ross, 1982). It is a

common finding that protein retention improves when dietary protein is decreased in an otherwise isocalorific diet. However, this is only applicable to diets already adequate in ME and similar trends are not found with sub-maintenance rations (Garling and Wilson, 1976). Because of this, it is important to report levels of protein and energy in a ration; P/DE ratios on their own are not sufficient.

Recent publications have provided a better picture of protein/energy relationships in feeds for tilapia. Jauncey (1982) obtained maximum growth in 1.8 - 8.5g S. mossambicus with a diet containing 116.6 mg protein/kcal ME (at a dietary protein level of 40%), whilst for Tilapia zillii, the value was 103, at 35% protein (Mazid et al, 1979). These values compare well with the data of Winfree and Stickney (1981), but in all cases if larger tilapia had been used, it is likely that the optimum P/DE would have been lower. For catfish, a much lower figure of 88 is reported (Garling and Wilson, 1976), but these fish were larger than any of the tilapia in the studies cited above (105 - 1000g versus 0.016 - 9.0g). To compare optimum P/DE in diets for different fish, it is necessary to control for fish weight in feeding experiments.

Comparing the existing data for tilapias, the optimum P/DE for the growth of juveniles of comparable size increases in the order S. mossambicus > S. aureus > T. zillii. This may reflect species differences or simply be due to the fact that different protein sources, energy levels and environmental conditions were used in the studies from which these data were taken. For Oreochromis niloticus there are no published P/DE values, but a certain amount of information on optimum protein and energy levels appeared in a newsletter published by the International Centre for Aquaculture,Auburn University, Alabama, USA

(Vol 3, No.3, 1980). Using these data, the optimum P/DE value for growth can be calculated as 120 in a 36% protein diet and 109 in a 26% diet. A P/DE range of 125 - 111 appears to be optimum for O.niloticus, but no information was given regarding the size of experimental fish.

For wild S.mossambicus, the P/DE for maximum growth is reported as 25 mg/kJ (= 105 mg/kcal) whilst the maintenance ration is 4 mg/kJ (= 17 mg/kcal), (Bowen, 1982). Again, fish size was not indicated, but it may be assumed that the author was referring to adult populations. Earlier, this author showed that wild tilapia select detritus to optimise P/DE (Bowen, 1979). Juveniles, which require higher P/DE intake than adults, feed in shallow lake margins where the protein content of food is higher. In contrast, adults feed in deeper water where the quality of food (measured in terms of P/DE) is lower.

Clearly, P/DE ratios are a valuable measure of quality in fish diets and for any given value, certain predictions can be made concerning the growth response of a species. However, Cowey and Sargent (1979) suggest it is more important to consider the proportion of total dietary energy provided by protein, since this acts as both a nutrient and an energy source in fish. Using data for rainbow trout from Lee and Putnam (1973), they calculated a relationship between protein retention and the protein energy/DE ratio of the diets (i.e. PE/DE). Within the limits of these data, it was possible to predict protein retention from the proportion of protein energy in the diet. This approach should also be considered when diets are developed for tilapia because the efficiency of protein retention is, perhaps, the most important measure of the productive value of a feed and any means of predicting protein retention from dietary analysis clearly deserves further attention.

Section 1.4 Summary of review and research aims

Summary

When a complete diet is formulated for tilapia it is necessary to consider the supply of energy from the natural feeds which will make up the diet. The foregoing review identified two important areas for study; firstly, the availability and secondly, the utilisation of food energy. With reference to first, the availability of energy, the review can be summarised as follows:

The amount of energy that is derived from a food depends on its gross energy and the ability of the animal to digest the food. The percentage of the gross energy that is absorbed is called the digestible energy (DE). This energy is then partitioned within the animal and utilised for different functions such as maintenance, activity and growth. The amount used for these different functions can be described in an energy budget and from this, it is clear that only a portion of a food's gross energy is available for growth. To the feed manufacturer, it is valuable to know this amount (the 'net energy'), so that diets can be made to satisfy the animal's energy requirements. However, the review described some of the problems with the concept and measurement of net energy and, with fish, showed why it is more reliable to measure the available energy as digestible energy.

The DE of a feed is governed by the digestibilities of its protein, fat and carbohydrate components. Early work showed differences between the DE of protein fat and carbohydrate, but indicated that within each of these classes of nutrients, DE was more or less constant. This then suggested that the overall DE of a feed could be calculated from simple measurement of the proportion of protein, fat and carbohydrate

present. This method of calculating the DE of feeds is still widely reported in the literature on fish nutrition. However, more recent literature has shown that the DE's of specific proteins, fats and carbohydrates are not constant as originally assumed, because they are affected by factors such as their molecular structure, their dietary inclusion level and the feeding rate. It was concluded that the only reliable way of estimating the DE of whole feeds is to conduct in vivo digestibility trials in which the energy contained in faeces is measured and subtracted from the gross energy of the feed.

In comparison to the number of digestibility trials conducted with terrestrial livestock, very few have been carried out with fish. Of those that have been reported for fish, most were with rainbow trout. This is a carnivorous species, whilst tilapia is herbivorous, so DE values for trout cannot be expected to apply to tilapia. In support of this, the review included a section on the digestion of food by herbivorous fish in general and tilapia in particular. It was shown that there are considerable differences between tilapia and carnivorous species in their ability to digest plant material and this reinforced the need for digestibility trials with tilapia.

In any feed evaluation program, it is impossible to measure the DE of all existing feeds. One way around this is to predict DE from the chemical composition of a feed. In this way, the feed need only be subjected to simple chemical analysis to obtain an estimate of DE. The techniques used for predicting DE in this way were reviewed for terrestrial livestock and it was suggested that similar techniques could be applied to tilapia. In particular, it was considered necessary to evaluate the level of dietary fibre as a predictor of DE. This method would, of course, only apply to fibrous plant feeds; for other feeds a

different approach would be necessary and it was suggested that other dietary components (e.g., protein, fat, digestible carbohydrate) should be evaluated as predictors of DE, preferably in a multifactorial approach.

At this point in the review, the emphasis shifted from an account of energy availability to an account of energy utilisation. Energy can be derived from protein, fat and carbohydrate in most feeds and all three sources can be effectively utilised for growth by fish. However, the main emphasis was given to carbohydrates because this nutrient class is the most abundant source of energy in many plant-product feeds and until now has been neglected in studies of tilapia nutrition.

Naturally occurring carbohydrates were shown to have value in commercial diets because they can spare protein energy and thus reduce diet costs. However, it was shown to be essential to determine the maximum amount of carbohydrate that can be tolerated in a diet, because excessive amounts can suppress growth, or even kill fish. The maximum level that can be tolerated was shown to be higher in herbivorous fish than in carnivorous species, probably because the former are better adapted to digesting and metabolising carbohydrates. This should be investigated with tilapia. It was also evident that the amount of carbohydrate utilised for energy depends on the amount of energy supplied by protein and vice-versa. Thus, any study of the utilisation of plant feeds and their component carbohydrates must give attention to protein-energy relationships and preferably the digestible protein: digestible energy ratio.

Aims

The preliminary aim was to develop techniques for conducting

digestibility trials with tilapia. The method had to be simple, applicable to all types of feed and above all reliable, because much of the subsequent research was to depend on the accuracy of these digestibility values. Only when this method was developed and some of the factors affecting digestibility were investigated would it be valid to proceed with the rest of the work.

The main aim was to determine the digestibilities of a range of feeds containing different levels of protein, fat, carbohydrate and fibre and to evaluate these dietary components as predictors of digestibility. In doing so, it was hoped to provide a means of estimating the digestibility of any feed without recourse to feeding trials. The two digestibility measures of most interest here were digestible energy and digestible protein.

The final aim was to investigate the relationship between DE and growth, and to examine the role of carbohydrate energy in tilapia nutrition. There were two specific interests here. The first was to determine how the available carbohydrate energy of natural feeds affects growth, carcass composition and the retention of protein and energy. The second was to establish how the energy value of carbohydrates depend on their molecular structure and level of inclusion in a diet.

CHAPTER 2

GENERAL MATERIALS AND METHODS

This Chapter describes the materials and methods common to all experiments. Procedures and materials specific to individual experiments are described in the relevant chapters.

Section 2.1 Fish stock and husbandry

The species of tilapia studied was Oreochromis (Oreochromis) niloticus (Linn). The classification of Trewavas (1982a) is adopted in naming this species, but for an alternative classification see Appendix 1. Stocks of fry were obtained from the Institute of Aquaculture, University of Stirling. These were quarantined for three weeks after arrival and then either used directly in feeding trials or grown-on to produce larger fish (50-200g) for digestibility experiments.

During quarantine, the fish were held in 150 l aquaria. Each aquarium was supplied with airstones and maintained at 28 C. To compensate for evaporation and to remove nitrogen in solution, tap water was directed into each aquarium and overflowed to drain at an exchange range of 1 l min^{-1}. Fry were graded daily according to size to prevent the faster growing fish cannibalising the smaller ones and fed ad libitum five times daily on trout pellets (BP Nutrition UK Ltd.: fry 00 - 03). Faeces were siphoned from the aquaria daily. Four and two days before transfer to the main holding and experimental facility, food was withheld and each quarantine aquarium was flushed with a 2 ppm solution of malachite-green to kill any ectoparasites and fungi. The experimental system to which quarantined tilapia were then transferred

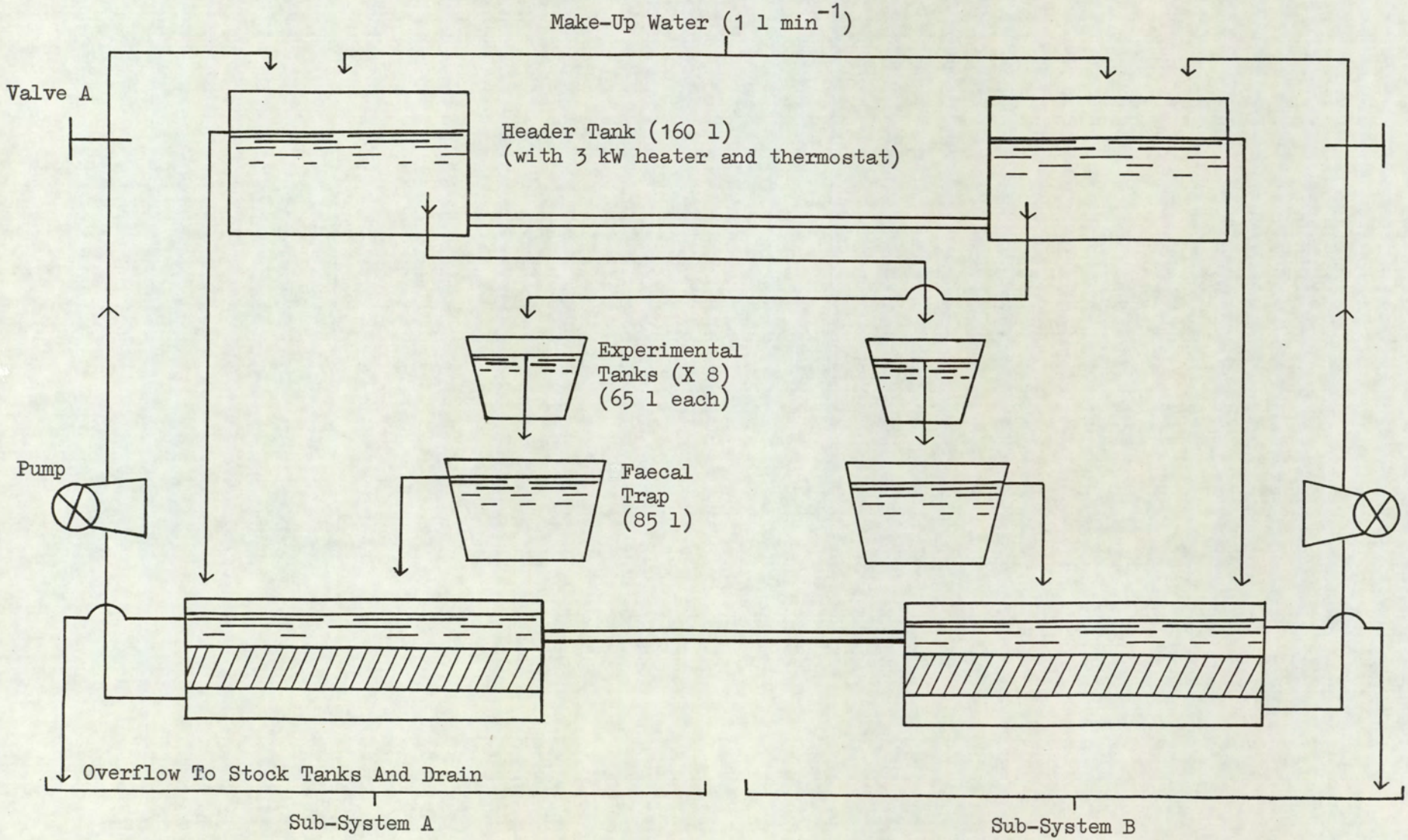
was first treated to kill any pathogens that may have been present. Sodium hydroxide crystals were placed in the system and the caustic solution circulated through the experimental tanks for 24 h. This was then flushed out of the system and the tanks scrubbed with an iodophor detergent (FAM 30-Evans Vanodine Ltd. UK). The system was again flushed with fresh water, fry were transported from quarantine, and as they grew, they were fed ad libitum three times daily on progressively larger pellets (Ewos-Baker Ltd., UK, Omega Nos. 4 and 6) until the day before a feeding trial, when food was withheld.

The sex of tilapia was determined by inspection of the genital papilla as recommended by Maar et al (1966). However, this was only possible in fish larger than 30 g. Smaller fish had to be killed and dissected to display the gonads which were visibly differentiated only in fish larger than 10 g. Fish smaller than 10 g could not be sexed.

Section 2.2 The system of tanks used to maintain fish during feeding trials.

All experiments were conducted with fish held in opaque, circular tanks fitted with lids. The tanks formed part of a recirculating water system and as such they shared a common water reservoir. In this way environmental variables were constant in each tank and diet was the only obvious factor to vary. Figure 2.1 shows the basic design of the system. Essentially, it is a combination of two systems, each with its own header tank, 8 experimental tanks, solids trap, biological filter and water pump. The advantage of recirculating systems are that they require very little throughput of water (max. 1% total volume per hour). Further, it is possible to heat the water without excessive

FIGURE 2.1 THE SYSTEM OF TANKS USED TO MAINTAIN FISH DURING FEEDING TRIALS



energy loss and this is an essential requirement, since tanks were maintained at 26 - 28 C throughout the course of this project. The general principles of closed recirculation systems in aquaculture are described by Spotte (1970). More specific details of the type of system in use at the Fish Culture Unit, Aston University and the operation of biological filters can be found in Roberts (1976) and Jauncey (1979).

The experimental system constructed for the present research contained 16 tanks which were identical in size and colour. Warm, filtered, aerated water was directed into each 65 l tank at a rate of 2 l min^{-1} and left at the same rate through a central standpipe. Faeces were carried in the effluent water through a common drain to one of two solids (faecal) traps. The faecal traps each had a working capacity of 85 l and were cleaned once per week during each experiment. Water passed from the traps to one of two submerged, down-flow biological filters, where it was drawn through gravel beds by the action of a Beresford PV52 pump (James Beresford and Sons, Ltd., Birmingham, UK). Filtered water was then pumped vertically upwards 3 m and sprayed into the header tanks, each of 160 l capacity.

Each header tank contained a 3 kW immersion heater (Bunting Titanium Ltd., West Bromwich, UK), under the thermostatic control of a mercury contact thermostat (Gallenkamp and Co. Ltd., UK) and electronic relay device (EC 980, Gallenkamp and Co. Ltd., UK). Heated water then passed from each header tank through a submerged standpipe to a pressure-equalising ring main which supplied the experimental tanks. The vertical distance between each header tank standpipe and its corresponding ring main (i.e. the water 'head') was 2 m. The gravity feed of water to the ring main generated pressure in excess of that

required to provide each experimental tank with the maximum rate of water exchange needed in a feeding trial. Valves between the header tanks and ring mains (Valve A Fig. 2.1) and individual valves at each tank enabled fine control of water flow rate.

Water was pumped to the header tanks at a faster rate than was necessary to supply the experimental tanks. Excess water from the header tank left via an overflow pipe and was directed back to the filter by gravity feed. The vigorous spraying action of water entering the header tank and the venturi effect caused by water leaving via the overflow pipe were sufficient to maintain oxygen tensions at 6.0 mg/l, or higher.

The total working volume of the system was approximately 2200 l. Additional 'make-up' water was dripped into each header tank at a rate of approximately 0.5 l min^{-1}. Excess water left the system at the filter overflow and was directed to drain via four 150 l holding or 'stock' tanks. The overall exchange rate (1 l min^{-1}) was sufficient to remove nitrogen in solution and compensate for evaporation. Water quality was monitored twice weekly throughout each experiment and was always found to lie within the range indicated in Table 2.1. To prevent excessive collection of suspended solids in the system, filter gravel beds were agitated once a month by digging and the displaced sediment pumped to drain.

The 16 tank system was originally two 8 tank systems (sub-systems). The sub-systems were combined to provide a larger number of tanks with a common water reservoir and this enabled a larger number of dietary treatments to be applied in any one feeding trial. Each trial represents considerable investment of time and experimental resources. To prevent the collapse of an experiment due to the

TABLE 2.1 Water quality data

Parameter	Range	Method of determination
Temperature	As preset $\pm$ 0.5 C	0 - 50 C thermometer
Dissolved Oxygen	6.0 - 8.0 mg/l	Titrimetric, using Winklers reaction (APHA, 1975).
pH	6.8 - 7.5	Electrical pH meter (Pye Unicam, Cambridge, model 791 mk.2).
Total Ammonia	$<$ 0.1 mg/l.	Colorimetrically, with Nesslers reagent (APHA, 1975).
Nitrate	0 - 5 mg/l.	Phillips Electrode.
Hardness	19.5 - 21.0 mg Ca_2CO_3/l	Titration with EDTA (APHA, 1975).

mechanical failure of system components, several safety devices were incorporated into its design. The essential feature of the modified system was that water from the header tank in one system was directed to the experimental tanks of the other and vice-versa (Fig. 2.1). However, if the sub-systems were connected in this way only, and a pump failed (an event which is twice as likely when two pumps are operating independently), both systems would drain as a result of overflow at the filter connected to the redundant pump. To prevent this, the header tanks of each system were connected at their bases with a 4 cm diameter pipe, as were the filters. In this way, water heads were equalised in both filters and both header tanks and so the failure of one pump would have been compensated by the continued operation of the other.

The shared water reservoir was heated by the action of two independent 3 kW heaters, one in each header tank. These heaters were switched on and off at intervals determined by the thermostatic devices. If one heater failed in the modified system, the other remained on twice longer than usual and so kept the water at the required temperature. Conversely, if a thermostatic relay failed (the most common fault), its heater remained on constantly. In this circumstance, the other functioning thermostat detected the rise in water temperature and switched off the heater it was connected to. In simulation tests, the power supplied by a single 3 kW heater was not sufficient to raise the 2200 l water reservoir by more than 4 - 5 C above the preset temperature within 24 h. Water temperature was monitored at least once every 24 h and remedial action taken if there was any change in the preset value.

In addition to the system used for feeding trials, two other heated recirculating water systems were used in the course of this research.

These have the same design as the sub-systems already described. The main holding system consisted of two circular tanks (800 l and 220 l respectively) and these were used to maintain 50 - 300g tilapia for digestibility trials. Individuals were occasionally removed from this system and placed in a gravel bedded 330 l glass aquarium fitted with two Eheim 2021 power filters (Eheim Ltd., GDR). This aquarium was used for spawning tilapia and it was stocked at a ratio of 3 females: 1 male as recommended by Balarin and Haller (1982). Fry from the Aston broodstock supplemented stocks of fry from Stirling University and they were maintained in the second auxillary holding system which consisted of eight 65 l tanks.

Section 2.3 Diet preparation and analysis.

2.3.1. Diet preparation

A variety of ingredients were used to prepare diets as listed in Tables 2.2, 2.3 and 2.4. Where necessary, the ingredients were processed in a laboratory hammer mill to ensure that all dietary components would pass through a 1 mm mesh. This aided mixing and ensured diet homogeneity. For each feeding trial, a mix was formulated which on its own provided to excess all the essential amino acids, essential fatty acids, minerals and vitamins necessary for growth. Test ingredients were then added to this premix and their level adjusted where necessary, with polypropylene powder. This plastic was in the form of microscopic spheres and for unit volume, these present the smallest surface area for the adsorption of nutrients or interference with digestion.

Diet formulations are described for each feeding trial in

TABLE 2.2 Dietary ingredients

Ingredient	Source	Specification
Glucose (α D (+) Form)*	Sigma Chemical Co.Ltd	Grade III
Sucrose*	"	Grade II
Dextrin*	"	Type III 80% water soluble
Starch*	"	75% Amylopectin, 25% amylose
α -Cellulose	"	99.5% pure
Carboxymethylcellulose	"	Na Salt high viscosity
Maize oil	Boots Co.Ltd.	
Cod liver oil	"	
Mineral/vitamin premix	See tables 2.3 and 2.4	
Chromic oxide (Cr_2O_3)	B.D.H. Chemicals Ltd.	Fine powder
Polypropylene (Grade G X 543M)	I.C.I Ltd. (UK).	Microscopic spheres max.diameter 0.1mm
Fishmeal	Ewos-Baker Ltd.	Full analysis in Chapter 5
Wheat middlings	BP Nutrition (UK) Ltd.,	"
Wheat bran	"	"
Wheat grain	"	"
Ground maize	"	"
Soybean	"	"
Cassava	"	"
Rice bran	"	"
Sorghum	"	"
Copra	"	"
Rapeseed	"	"
Sunflower	"	"
Palm kernel meal	"	"
Meat and bone meal	"	"
Poultry by-product meal	"	"
Groundnut	Mars Foods Ltd. (UK).	Hand picked and sorted. Expelled by TDRI.

*Derived from maize.

The scientific names of plant products feeds, the fraction of the plant used and international feed numbers are given in appendix 3.

TABLE 2.3 Formulation of the mineral premix

Test diet H440 Western Fish Nutrition Laboratory

(NRC, 1973: Premix No.5 and Salt No. 2).

	(g/100g)
Aluminium chloride	0.015
Potassium iodide	0.015
Cuprous chloride	0.010
Manganese sulphate	0.080
Cobalt chloride	0.100
Zinc sulphate	0.300
Calcium biophosphate	13.580
Calcium lactate	32.780
Ferric citrate	2.970
Magnesium sulphate	13.200
Potassium phosphate	23.980
Sodium biphosphate	8.700
Sodium chloride	4.350
	100.00

TABLE 2.4 Formulation of the vitamin premix

As used in experimental diets for tilapia (Jackson et al 1982). When included at 1g/100g dry diet, the vitamin mix provides.

	(mg)
Thiamin hydrochloride	5
Riboflavine	5
Calcium pantothenate	10
Nicotinic acid	20
Biotin	0.6
Pyridoxine hydrochloride	4
Folic acid	1.5
Cyanocobalamin	0.01
Inositol	200
Ascorbic acid	100
Choline chloride	400
Menadione	4
Alpha-tocopherol acetate	40
Para-amino benzoic acid	5
Vitamin A acetate	200 IU

All ingredients mixed with α -cellulose.

The mineral premix (Table 2.3) and the above vitamin premix were mixed together to produce a mineral/vitamin premix at a ratio of 2g minerals : 1g vitamins.

subsequent chapters, but pellets were always prepared by the same procedure. Each diet was formulated to required levels of protein, fats, minerals and vitamins. The amount of diet needed for the feeding trial was then calculated and the requisite amount of each ingredient weighed to the nearest 0.01 g and placed in the bowl of a Hobart A200 food mixer (Hobart Ltd., London, UK). The dry diet was then mixed for 5 min at low speed, after which the supplemental fats were added and mixing continued for a further 5 min. Water was then added in measured amounts until a paste had been formed of the required consistency. This was then extruded through the mincer attachment of the food mixer, using a 2, 3 or 5 mm die depending on the size of pellet required. The strings of diet were then laid on trays and either frozen at - 20 C when semi-moist diets were required, or dried for 14 h in a forced-air drying cabinet at 40 C when dry pellets were required. Both types of diet were subsequently broken in a food liquidiser (Kenwood Ltd., UK), passed through a series of sieves (1.0, 2.0, 2.8 and 4.0 mm) and stored in plastic bags at - 20 C.

2.3.2. Diet analysis

Prior to analysis, samples of each diet were ground with a pestle and mortar to pass a 1 mm sieve. All analyses except crude fibre were performed on an "as received" basis and the results subsequently adjusted to a dry-matter basis by determining the moisture content of each diet. Each analysis was duplicated, and when the error between duplicates was greater than 5%, a third analysis was performed. The methods used were as detailed in AOAC (1970), MAFF (1973) and Harris (1970).

Crude protein (CP)

Measured as nitrogen (x 6.25) and determined by one of the following methods :

1. Micro-Kjeldahl, with a Markham still (Markham, 1942; Broadstreet, 1965). Sample size: 40 - 100 mg.

2. Semi-automatic (Tecator) Kjeldahl.
   Sample size: 0.5 - 20 g.

3. Modification of the salicylate-dichloroisocyanurate reaction. Sample size: 30 - 40 mg. (Crooke and Simpson, 1971, see Chapter 3).

Crude fat

Determined as Soxhlet petroleum ether extract (EE) bp 40 - 60 C. (MAFF,1973).

Energy

Determined by one of the following :

1. Combustion in an adiabatic bomb calorimeter (Gallenkamp and Co. Ltd. UK). Sample size: 0.5 - 1.0 g.

2. Combustion in a micro-bomb calorimeter (Model AHI2/EF, Newham Electronics Ltd., London). This is a ballistic calorimeter, with electronic ignition and is a development of the apparatus described by Phillipson (1964). Sample size: 10 - 30 mg. Samples were pelleted in a bench press and weighed

to the nearest 0.01 mg.

3. Chemical oxidation with dichromate. Sample size 20 - 100 mg. Samples weighed to the nearest 0.01 mg. (O'Shea and Maguire, 1962, see Chapter 3).

For 1 and 2, the instruments were calibrated with benzoic acid (British Chemical Standards No. 190K). For chemical oxidation, a correction equation was calculated as described in Chapter 3.

Fibre

Three fibre assays were used: crude fibre (CF), acid detergent fibre (ADF) and neutral detergent fibre (NDF).

CF is the insoluble material (mainly cellulose) that remains after treating feed samples with dilute sulphuric acid and sodium hydroxide under specified conditions (Harris, 1970). It is the official legal analysis of fibre in many countries, but it does not represent any definite chemical fraction. Moreover, CF only measures about 10 - 50% of the lignin and 15 - 25% of the hemicellulose in a feed sample and so underestimates the amount of indigestible material present (Van Soest, 1976). Efforts to improve the assay have led to over a hundred modifications of the original CF determination, including the addition of oxidising agents, use of special solvents and different concentrations of acid and alkali (Pomeranz and Meloam, 1978). One of these modifications was ADF and this is now considered to be a more accurate measure of indigestible material in feeds than CF (Van Soest, 1963). ADF is determined by refluxing with an acid detergent (cetyl

trimethylammonium bromide in sulphuric acid) and unlike CF, it measures indigestible residues such as lignin, cutin, tannin-protein complexes (leather), heat damaged protein, plant silica and soil minerals (Van Soest, 1976). However, ADF does not measure hemicellulose and this is an important constituent of plant cell walls. To include hemicellulose with the other residues measured by ADF, feed samples must be refluxed with a neutral detergent as recommended by Van Soest and Wine (1967). The residue (NDF), more than any other feed assay, measures the total material in a feed that is resistant to vertebrate digestive secretions. However, despite the obvious value of NDF as a measure of feed quality, most feed tables still report fibre as CF, or occasionally, ADF.

In this thesis, NDF replaces the traditional CF and ADF assays as a measure of true fibre. However, CF and ADF were also determined so that the regression equations calculated in the present research can be used in conjuction with existing feed tables. All three assays (CF, ADF and NDF) were determined according to the procedures outlined in Harris (1970) and hemicellulose was estimated as NDF minus ADF.

Nitrogen free extractives (NFE)

$$NFE = 100 - (\%Ash + \%CF + \% EE + \% CP)$$

NFE is a component of the Weende proximate analysis system for feeds. It is supposed to represent soluble carbohydrate, but actually includes hemicellulose, lignin and acid insoluble ash (Harris 1970). Despite this, it is still widely reported and, to enable comparison with the results of other workers, it is also calculated in this thesis.

Alternative values can be obtained for NFE by replacing CF in the above equation with ADF or NDF. Where NFE is calculated using CF it is referred to as NFE1, with ADF it is NFE2 and with NDF, it is NFE3.

Thus : NFE1 = As above

NFE2 = 100 - (% Ash + % ADF + % EE + % CP)

NFE3 = 100 - (% Ash + % NDF + % EE + % CP)

In addition to estimating 'carbohydrate' in this way, the method described below was used to provide a more direct measure of available carbohydrate.

Available carbohydrate (CHO)

Available carbohydrate was measured as glucose plus starch by a modification of the method recommended by Bolton (1960). Starch granules in the sample are disrupted with hot alcohol to remove any lipoprotein coats. The starch is then gelatinised in an autoclave and incubated overnight with amyloglucosidase (from Rhizopus mold) which produces quantitative yields of glucose from soluble starch (manufacturers guarantee, Sigma Chemical Co. Ltd., UK). The glucose is then determined in a glucose analyser. The exact procedure was as follows :-

1 - 2 g of sample was weighed into a 100 ml conical flask. 85% ethanol was added to wet the sample and the flask then placed in a water bath until the alcohol had evaporated. The flask was covered with foil and autoclaved at 120 C for 1 h. When cool, 2.5 ml of 2M acetate buffer (pH 4.5) was added and mixed by swirling. 1 ml of an aqueous solution of amyloglucosidase (concentration, 1.0 mg/ml; activity 5000 -

10000 IU/g) was then added, the sides of the flask rinsed with a little distilled water and the flask incubated in a reciprocating water bath overnight at 37 C. Proteins were precipitated by adding 5 ml of Carrez I (5% m/v $ZnSO_4$, $7H_2O$ aqueous solution) and then 5 ml of Carrez II (3.67% potassium ferricyanide, aqueous solution). The suspension was filtered through Whatman No.1 paper into a 100 ml volumetric flask and made up to volume with distilled water. Glucose was determined on a glucose analyser (Model 27, Yellow Springs Instruments Ltd., Ohio, USA). In this instrument, membrane bound glucose oxidase converts glucose to hydrogen peroxide and this is determined electrometrically.

Chromic oxide

Determined by two methods:

1. Wet acid digestion (Furukawa and Tsukahara, 1966).
2. Atomic absorption spectrophotometry (AAS) as described in chapter 3.

Moisture and Ash

Moisture: oven drying at 105 C, to constant weight

Ash : incineration in a muffle furnace at 450 C for 12 h.

Section 2.4 Experimental procedures

2.4.1. Growth measurement : some considerations

The growth of fish in intensive systems is extremely variable because of genetic variation and the establishment of a feeding hierachy (Anglesea, 1982). In intensive farms, the problem is not so acute

because tilapia are stocked at densities at which intra group dominance is inhibited (Balarin and Haller, 1982), but in experimental tanks, the variation in growth between individuals is large even within the same experimental group on the same dietary treatment. As experimental fish grow, the variance and the weight of each group gradually increases so that at the end of a feeding trial, the variance of the mean final weight may be so large that it reduces or even masks the statistical significance of any differences between experimental groups. The sensitivity of analysis of variance of mean final weights can be improved by increasing the number of fish in an experimental group but this is always limited by the number of fish or tanks that are available. A better method of measuring growth is to calculate the growth rate of each group.

Growth rate is an increase in weight per unit time and can be calculated for each group either on the basis of the mean weight increase of all fish, or by measuring the weight increase of individuals. The disadvantage of using the mean weight is that it provides only one value for the growth rate of a group ($n = 1$) and so different groups cannot be compared statistically. By weighing fish individually, a mean and variance can be calculated for the growth rate of different experimental groups and these can then be compared statistically. However, this requires that individuals are either kept in separate tanks (which is impractical), or that they are marked to enable identification when fed as a group. Marking not only increases the number of observations of growth in a feeding trial, but it also enables the identification of any deaths. This is important because when a fish dies, the mean weight of the group alters. If fish are marked, the dead fish's growth rate can be excluded from the calculation

of the mean. However, if they are not marked, there is no alternative but to use the mean final weight of the remaining fish to calculate growth rate. This then introduces variation to the data that is due not to the effect of diet, but to the death of the fish. Marking is also necessary in digestibility trials since it permits faeces to be collected from the same fish on successive days.

2.4.2. Anaesthesia and marking

Before marking, fish were anaesthetised with either benzocaine (ethyl p-aminobenzoate, Sigma Chemical Co. Ltd. UK), or 2 - phenoxyethanol (Sigma Chemical Co. Ltd., UK). When benzocaine was used, it was first dissolved in a small amount of acetone before mixing with water in a bucket. Benzocaine was used at a concentration of 0.05 g/l and 2 -phenoxyethanol at 0.05 ml/l. Fish were placed in the anaesthetic solution until they lost equilibrium (usually 2 min) after which they were marked and then returned to well-aerated water. This water condition was necessary because fish anaesthetics and handling are known to cause physiological stress (Smit et al 1977; 1979a, b, c) and during recovery, the requirement for oxygen by tilapia increases (Ross and Ross, 1983). Hence, if anaesthetised fish are returned to water at low oxygen tensions, there is a greater risk of mortality. After anaesthesia in the present research, the water flow rate to experimental tanks was doubled to increase oxygen levels and typically, all fish recovered within 5 min. Fish would accept food immediately, but as a precaution, a 24 h recovery period was imposed before the start of a feeding trial.

To mark fish, a 15 mm x 5 mm numbered tag (Charles Neal Ltd., Finchley, London) was attached to a plastic thread which was inserted

through the dorsal muscle at a point midway along the dorsal fin. The thread was driven through the fish with a tagging gun as used in the clothing industry (Kimbal Systems Ltd., Leics., UK). The needle of this gun was dipped in absolute alcohol before tagging to prevent infection. The tags did not appear to affect the behaviour of the fish in any way and were still in place after two years. However, fish weighing less than 25 g could not be marked in this way because of the size of the tag.

In the feeding trial with pure carbohydrates (chapter 8), fry of between 2.0 - 12.0 g were used and an alternative method of marking was attempted as described by Mighell (1969). Copper rods of 1 mm diameter, with plastic handles were dipped in liquid nitrogen and pressed against the side of an anaesthetised fish for about 1 s. This removed scales, killed the epidermis and left a dark mark which normally persisted for about 7 d. A variety of brand signs were attempted, but because of the small size of the fish, the skin surface available for alternative signs was limited. These signs had to be renewed every 14 d and this caused considerable stress, noticeable by the cessation of feeding. Post-branding stress of this type has also been noticed in catfish weighing less than 27 g but not in larger individuals (Joyce and El-Ibiary, 1977). Because of the susceptibility of small fish to stress, the variable effects of stress and because of the practical difficulties of re-branding at 14 d intervals, tilapia fry were left unmarked.

2.4.3. Weighing and feeding

Before the start of a feeding trial, the fork length of each fish was measured to the nearest 0.1 cm, they were weighed to the nearest

0.01 g, marked when appropriate and assigned to one of the experimental tanks. Fish of similar size were selected from stock to ensure that the mean initial weight and variance of experimental groups did not differ significantly ($p < 0.05$). This was always tested by one-way analysis of variance before the start of a feeding trial, because the effects of dietary treatments can only be compared when all individuals originate from the same population. In addition, the sex ratio of each group was balanced to eliminate any sex-related growth differences.

Before diets were offered, one of the groups was selected at random, all individuals killed and the carcasses dried to constant weight at 105 C. These were then analysed chemically and the results compared with a similar analysis of carcasses from the end of the trial. This enabled calculation of the net protein and energy retentions and food conversion efficiency as described in Section 2.4.5.

The water flow rate to all tanks was balanced, they shared a common water supply and they were equally illuminated at constant photoperiod (15 L : 9 D). Water temperature was kept at 26 - 28 C throughout all experiments. Each day, the food ration for each group was weighed into a plastic container and then offered in three equal portions at 0900, 1300 and 1800 h. The amount given was calculated as a fixed percentage of dry diet to total group live weight. When semi-moist diets were used, the exact moisture content of each diet was determined and the feeding rate adjusted accordingly to ensure that all groups received the same proportion of diet on a dry matter basis. Typically, meals were consumed within 30 s, so there was little problem with nutrient leaching from the pellets or loss of food from the tanks.

Fish were weighed individually every 7 d. To reduce handling stress, some authors prefer to anaesthetise fish whilst weighing.

However, anaesthesia is itself stressful as already discussed (Section 2.4.2) and it does not appear to reduce handling stress in tilapia (Ross and Ross, 1983). For this reason, tilapia were not anaesthetised when they were weighed in the present research. After netting from the tank, water was allowed to drain from the fish for approximately 30 s before individuals were weighed on a digital readout top-pan balance with automatic tare control. The feeding rate was adjusted by measuring the total group weight every 7 d and as fish grew, they were offered progressively larger pellets.

2.4.4. Post mortem analyses

Carcass analysis

At the end of a trial, all fish were killed by a heavy blow across the head. The length of each carcass was then recorded, they were weighed, dissected when necessary and dried to constant weight at 105 C for moisture determination. Dried carcasses from each group were pooled, ground to an homogenous powder with a pestle and mortar and stored in air tight jars for subsequent proximate analysis. Protein was determined by macro-kjeldahl, fat by extraction with petroleum ether, energy with an adiabatic bomb calorimeter and ash in a muffle furnace (see Section 2.3.2).

Liver glycogen

Whole livers were dissected from all individuals in an experimental group, frozen in liquid nitrogen and stored in individual LP3 tubes at - 20 C for subsequent glycogen analysis by the method of Lo et al (1970).

2.4.5. Feed trial data analyses

Specific growth rate (SGR)

The growth of wild fish undergoes a series of 'stanzas' (Ricker, 1979). Within each growth stanza, there is a period of rapid weight increase followed by a plateau period during which there is no growth. A curve of weight against time describes an 'S' shape and the decelerating stage at the top of the S reflects environmental conditions (usually seasonal) and the onset of sexual maturity. However, in an intensive culture system, there is a constant supply of food and environmental conditions are (usually) optimum for growth. The growth of farmed fish is therefore confined to one stanza and closely approximates an exponential curve. This can be expressed mathematically (after Brown, 1957):

$$W_F = W_I \times e^{g(T-t)}$$

where

W_F = Final weight

W_I = Initial weight

e = Base of natural Logarithms (2.718)

(T-t) = Time interval in days

g = Instantaneous or 'specific' growth rate

Rearranging to obtain g

$$g = \frac{\text{Loge } W_F - \text{Loge } W_I}{(T-t)} \quad \text{And SGR} = g \times 100$$

This is the growth rate formula used in the present research and it measures the percentage weight increase per day (%/day).

Food conversion efficiency

The simplest measure of food conversion is :

food conversion ratio (FCR)

$$(FCR) = \frac{\text{Dry food offered (g)}}{\text{Live weight gain (g)}}$$

or the inverse of this

food conversion efficiency (FCE)

$$(FCE) = \frac{\text{Live weight gain (g)}}{\text{Dry food offered (g)}} \times 100\%$$

These are 'apparent' measures, since it is usually only an assumption that all of the offered food is eaten. Both formulae assume that the weight gain of a fish is due to an increase in body tissue. However, in some cases a portion of the weight increase is due to an increase in the proportion of body water. The most accurate way of measuring food conversion is therefore, to measure the dry matter weight gain of fish. In the present research, this was essential, since there were significant differences ($p < 0.05$) between the mean moisture content of different groups at the end of a feeding trial.

The dry matter FCE was calculated from :

$$FCE(DM) = \frac{D_F - D_I}{Do} \times 100$$

Where D_I = Total dry matter contained in experimental group at start of trial

D_F = Total dry matter contained in group at end of trial.

D_o = Total dry matter contained in food offered

This provides a more accurate index of the efficiency with which different diets are converted to body tissue, but unfortunately, it was not possible to compare these data statistically in the present research. When a diet is offered to a group, the weight of individual fish can be measured if they are marked, but it is not possible to measure the amount of food eaten by each fish. For this reason, there can only be one value of FCE for a group contained in a single tank, as based on the total weight gain of all fish and the total food offered. Thus, n = 1 and as a consequence parametric statistics are not possible. This restriction also applies to the other measures of food utilisation described below (i.e., PER, NPR, NER).

Protein efficiency ratio (PER).

$$\text{measured as } \frac{\text{Liveweight gain (g)}}{\text{Protein in food offered (g)}}$$

PER is a crude measure of protein utilisation. It takes no account of the variations in body fat and water levels that accompany growth. However, because PER is so widely reported in the literature, it is retained in conventional form throughout this thesis to allow comparison with the results of other workers.

Apparent net protein retention (NPR)

In the same way that dry-matter FCE is an improved measure of gross food conversion, NPR is a more accurate measure of the productive value of protein unconfounded with variations in body fat or water. NPR estimates the percentage of ingested protein retained.

$$NPR = \frac{P_F - P_I}{Po} \times 100\%$$

where

P_I = Total protein contained in group at start of trial

P_F = Total protein contained in group at end of trial

P_o = Total protein in food offered.

This measures the 'apparent' NPR because no correction was made in the present research for endogenous nitrogen losses.

Apparent net energy retention (NER)

$$NER = \frac{E_F - E_I}{Eo} \times 100\%$$

where E_I = Total energy (MJ/kg) contained in group at start of trial.

E_F = Total energy contained in group at end of trial.

E_o = Total energy in food offered.

Again, this is an 'apparent' measure because endogenous energy losses were not measured.

Condition factor (K)

In fish, condition is usually measured as a length/weight ratio. It is most accurately measured by the formula:

$K = 100\ W/L^b$ (Le Cren, 1951).

where,

W = Weight (g), L = Length (cm)

b = mean population exponent derived from a length/weight analysis such as $W = aL^b$, where a is a constant.

Thus, condition measures the deviation of the weight of an individual from the average weight to length of the population. It is used as a rapid, convenient estimate of the physiological state of a fish in terms of the food reserves it has available for metabolism (Caulton and Bursell, 1977). In wild populations, K is selected for by environmental pressures. Under culture conditions the same selective pressures do not apply and there is greater variation in condition. In the present research, a detailed analysis of the length/weight relationship was not possible because of the limited number of fish and so it was approximated as 3.

Thus: $K = 100 \times W/L^3$.

Statistical analyses

A variety of parametric statistical tests were used. One-way (Single Factor) experimental designs were analysed with the appropriate analysis of variance (ANOVAR) and differences between means tested by the multiple comparison technique of Duncan (1955). The more complex, factorial, design in Chapter 4 was analysed with a two-way split-plot ANOVAR and differences between marginal means tested with Tukey's Honestly Significant Difference test (Kirk, 1968). Single factor and multiple regressions were calculated as outlined in Snedecor and Cochrane (1972). Multiple regressions were computed with a statistical package (MINITAB, Penn. State University, USA) on a Harris 800

computer. Some of the ANOVAR were computed with library programs on a Hewlett Packard 2000 computer. All other statistical analyses were performed on a hand calculator.

CHAPTER 3

THE METHODOLOGY OF DIGESTIBILITY DETERMINATION

Section 3.1 Introduction

Many of the techniques currently utilised in digestibility trials with fish are adaptations of methods used for terrestrial livestock. However, the separation and quantitative recovery of waste products from water presents several problems which are unique. As a result, most techniques employed in fish digestibility studies can be criticised on methodological grounds and these criticisms are reviewed below.

The earliest methods employed quantitative recovery of all faeces and excretions by draining aquaria and filtering the water (Migita *et al*, 1937, Tunison *et al*, 1942). This is a laborious technique, appropriate for evaluating only a limited number of feeds. In addition, faeces may be contaminated by gill and kidney excretions thereby reducing the accuracy of digestibility estimates. An improvement was the development of fish metabolism chambers (Post *et al*, 1965; Smith *et al*, 1980). Gill excretions are collected in solution at one end of the chamber and at the other, faeces are separated from urine by catheterising the fish's ureter. Fish are force-fed for several days, during which time all of the excreta and egesta are collected in the chamber. Water at the rear of the chamber contains faeces in suspension and these can be concentrated in a rotary evaporator for quantitative measurement (Smith *et al*, 1980). However, these metabolism studies are still laborious and can only be applied to a limited number of feeds at any one time. An alternative is the use of

a dietary indicator which obviates the need for quantitative collection of all faeces.

The indicator is a substance in the diet that is not absorbed by the animal under study. The assumption is that the absolute quantity of indicator in the feed remains the same as it passes through the gut, whilst the ratio of nutrients to indicator changes as nutrients are absorbed. Thus, only a small portion of the total faecal output has to be collected and analysed for the indicator and feed component under study. Indicators have been used in terrestrial livestock digestibility studies since the late 19th century (Hyden, 1960). Chandler et al (1964) have suggested that an indicator should have the following characteristics. It should:

1. Have no influence on the digestive process
2. Have no palatability effects
3. Not be absorbed

and 4. Move through the gut at the same rate as the nutrients.

A large range of indicators have been used in the literature; some are indigestible substances naturally occuring in the feed material (internal indicators), whilst others (external indicators) are mixed with the diets before feeding. Some of the internal indicators that have been used include lignin (McDonald et al, 1981); crude, acid and neutral detergent fibre (Tacon et al, 1983); cellulose (Buddington, 1979); hydrolysis resistant ash (Bowen, 1981); silicon dioxide (Barnicoat, 1945) calcium, iron and zinc (Lied et al, 1982). External indicators have included carmine, dysprosium, barium sulphate and soot (Maynard and Loosli, 1979); ferric oxide (Barnicoat, 1945); polyethylene and cerium-144 (Knapka et al, 1967); magnesium ferrite (Barrash et al, 1983); titanium oxide (Lied et al, 1982) and celite (Schmitz et al,

1983). However, of all reference substances the one used most extensively in both terrestrial and aquatic livestock digestibility studies is the external indicator chromic oxide (Cr_2O_3).

Several authors have questioned the value of chromic oxide as an indicator. The most serious objection arising from studies of cattle, pigs and poultry is that these animals exhibit diurnal variations in chromic oxide egestion (Kane et al, 1952; Elam et al, 1959; Moore, 1957; Haenlein et al, 1966). However, with rats and humans, this pattern has not been found and digestibility values obtained with the chromic oxide method agree closely with those determined by the traditional method of total faecal collection (Schurch et al, 1950; Irwine and Crampton, 1951). With fish, the daily egestion pattern of chromic oxide has not been reported, but this indicator has been used in digestibility studies with fish for at least 25 years. Nose (1960) and Inaba et al (1962) first used chromic oxide to measure protein digestibility with goldfish and rainbow trout. A few years later, Furukawa and Tsukahara (1966) developed a simple chemical assay for chromic oxide and the indirect digestibility trial became firmly established in fish nutrition research. However, the validity of using chromic oxide has recently been questioned again. Bowen (1978) reported marked variation in the digestibility of a single diet fed to Sarotherodon mossambicus and suggested that these tilapia selectively rejected dietary chromic oxide. How this could be achieved by fish was not clear, but the same phenomenon has been reported for prawns which retained large chromic oxide particles in the proventriculus (Forster and Gabbot, 1971). With prawns this problem was overcome by grinding the indicator, before mixing with the diet, to reduce the particle diameter to 5 µm or less. Bowen (1978) did not state the nature of the chromic oxide used

in his tilapia study and further, he employed a higher inclusion level (6% by weight) than is usual (0.5 - 1.0%). Foltz (1979) points out that chromic oxide should only be used at less than 1% of the diet and he offers a variety of other criticisms of Bowen's (1978) results. In response, Bowen (1979) later concurred that the chromic oxide technique is probably accurate and valid when applied to diets that are fed as dry pellets, but maintains that this technique is not applicable to certain naturally occurring diets containing living bacteria and algae. Another possible source of error is the high specific gravity of chromic oxide (= 5.21). De Silva and Owoyemi (1983) have shown that the gut passage time (GPT) of dietary components can be related to their density. If the GPT of chromic oxide is similarly affected by its density, then it may move through the gut at a rate unequal to the other dietary components, so reducing the accuracy of digestibility determination. However, in the absence of any direct evidence to the contrary, chromic oxide must still be considered a suitable indicator for inclusion in dry, pelleted diets with the provision that it is homogeneously distributed throughout the pellet at a level no greater than 1%. Numerous authors have reported the suitability of chromic oxide for fish digestibility studies (Nose, 1960; Inaba et al, 1963; Nehring, 1963; Smith and Lovell, 1973; Pappas et al, 1973; Cho et al, 1974, 1976; Lall and Bishop, 1976; Austreng, 1978; Windell et al, 1978 a, b; Jobling, 1981; Jauncey, 1982; Lied et al, 1982) and because of this support, chromic oxide was also chosen as the indicator in the present study.

The acceptance of indicator methods has considerably reduced the effort involved with digestibility studies. However, the techniques used for collecting faeces differ considerably between laboratories, so complicating comparison of results. A common procedure is to allow

faeces to accumulate in aquaria and then to collect by siphoning or netting. However, this introduces inaccuracy due to bacterial action (Smith and Thorpe, 1976) and leaching of nitrogen and other soluble organic material (Smith and Lovell, 1973; Stickney and Lovell, 1977; Windell et al, 1978b). Leaching losses can be considerable and have the effect of overestimating digestibility. Beamish (1972) reported that 18% of the faecal protein nitrogen in bass faeces was leached during immersion, whilst Smith et al, (1980) put this figure at 50% for rainbow trout faeces left immersed in a metabolism chamber for 24 h. Inaccuracies due to leaching can be overcome by evaporating the water in which faeces have been immersed and then lyophilising the concentrate. Protein digestibilities based on this technique are about 10 percentage points lower than values obtained by analysing leached faeces (Windell et al, 1978a; Smith et al, 1980). However, evaporative techniques are time consuming and unless gill and kidney excretions are separated from the water, faeces collected in this way will be contaminated with non-faecal nitrogen.

Leaching of nutrients from intact faeces is, evidently, not the only problem. When faeces are left in a culture tank, water is continually agitated by the movement of fish and Blackburn (1968) has shown that up to 17% of bass faeces can appear as suspended solids after several hours immersion.

The longer faeces are left immersed, the greater the potential for error. Windell et al, (1978b) have shown that most of the leaching occurs within the first hour of defaecation, but in many digestibility studies, the faecal immersion period is much longer. Ideally, to prevent error, faeces should be collected the instant they leave fish. Clearly, this is impossible, so several authors have resorted to

manual removal of faeces from the posterior portion of the intestine. These techniques include manual stripping (Austreng, 1978; Windell et al, 1978 b; and suction through an anal catheter (Stickney and Lovell, 1977). However, these techniques are also subject to error because digestion and absorption are not fully completed in fish until food material reaches the rectum and consequently, material that has been removed by dissection, stripping or suction may not be true faeces. In addition, stripped faeces may be contaminated with body mucus, urine or sexual products. Austreng (1978) showed that digestibility coefficients are highest when faeces are sampled from the most posterior part of the rectum in rainbow trout, indicating that material anterior to this is only partially digested. Windell et al, (1978 b) found no absorption of nutrients in the lower 2.5 cm of the intestine in 200 g rainbow trout. However, in routine digestibility trials, these authors had difficulty controlling the amount of faeces stripped; often faecal strings were collected that were much longer than 2.5 cm and these will have contained undigested material. Dissecting faeces from the intestine allows greater control. For catfish, Stickney and Lovell (1977) showed that reasonably accurate estimates of digestibility can be obtained by sampling from the intestine posterior to the ileocaecal valve, whilst for rainbow trout, Austreng (1978) states that faecal stripping must be limited to the area between the ventral fin and anus. With other fish species, a corresponding area of the gut must be defined before intestinal dissection, or stripping, can be used to provide reliable digestibility values.

Intestinal dissection is wasteful of fish, whilst suction and stripping are stressful; all three methods are time consuming and can only be applied to relatively large fish. For these reasons, automated

techniques have been developed to improve the accuracy of collecting faeces from aquarium water. This method can be applied to groups of small fish and is useful for the routine evaluation of large numbers of feeds. Faeces leave the aquaria continually, in the effluent water and can be collected in a settling column (Cho et al, 1975) or on rotating screens (Choubert et al, 1979). The advantage of this approach is that fish can be maintained under standard culture conditions, but the disadvantage is that leaching or breakup of faeces can still occur in the effluent water and settling column. To recover dissolved nitrogen, Ogino et al (1973) combined a settling column with an ion exchange column. Water leaving the settling column passed over a strongly basic resin where cationic substances were retained and subsequently eluted with hydrochloric acid. This apparatus is suitable for nitrogen balance studies, but because it does not permit separation of non-faecal nitrogen and nitrogen leached from the faeces, there is still error in the determination of protein digestibility.

Perhaps the best apparatus yet described is that of Choubert et al (1982). Faeces are collected on a succession of flat screens which move along a horizontal plane beneath an effluent pipe. The screens deposit faeces on a dry container, where they are immediately frozen. Choubert et al (1982), estimate that the faeces are only exposed to water for 6 - 15 s and so their apparatus would appear to provide digestibility estimates which could not be questioned. However, the apparatus can only be used with one aquarium (and therefore one feed) at a time and for the routine evaluation of large numbers of feeds, this method could be prohibitively expensive.

To summarise, the methods used in digestibility trials depend on the anatomical features and size of fish, the number of feeds to be

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evaluated and available facilities. Because faeces cannot be collected the instant they are released by fish, all methods of faecal collection currently employed are subject to error. At best, the method chosen for collecting faeces can only aim to standardise errors, reduce them to a minimum and measure their magnitude. These requirements have been applied to the present digestibility trials with tilapia and are discussed in the following sections.

Section 3.2 The collection and analysis of faeces

The major problem in the present trials was collection of a sufficiently large sample of faeces for analysis. Two approaches were taken to overcome this difficulty, namely:

1. increasing the amount of sample collected and
2. reducing the amount required for analysis.

3.2.1 Collection of faeces

3.2.1.1 Manual collection from fish

Methods

Initial attempts to manually strip faeces were unsuccessful. The intestine in tilapias is highly coiled along its entire length and the uncoiled portion immediately anterior to the anus that is available for stripping is very short. Even with 200 g fish it was usually impossible to obtain any faeces by stripping. The alternative was to kill fish and remove the gut contents by dissection.

Ten fish in the size range 8 - 60 g were fed to satiation 3 times daily (0900, 1300 and 1700 hr) on a high fibre diet (Table 3.1) for 7 d. Ross and Jauncey (1981) estimated that the vent arrival time for

TABLE 3.1 Formulation and analysis of the diet used to determine weight of faeces obtainable by intestinal dissection

Formulation* (g/100g)	Fishmeal	45.6
	Maize oil	7.0
	Cod liver oil	4.0
	Mineral/vitamin mix	3.4
	α-cellulose	40.0
Analysis	Moisture (%)	28.5
	Crude Protein (% DM)	35.7
	Ether Extract (% DM)	15.7
	Ash (% DM)	6.1
	Fibre (by calculation % DM)	40.0

* Ingredient specifications are given in Table 2.2

O. niloticus is 6.8 h at 26 C. Thus, on the eighth day of feedings, fish were killed with a sharp blow to the head, 7 h after the morning feed to ensure the presence of faeces in the posterior intestine. The entire intestine was removed, teased, straightened and measured, then the contents of the most posterior third were removed. This material was dried to constant weight in a forced air oven at 105 C, cooled in a desiccator and weighed to the nearest 0.1 mg.

Results

Intestinal dissection yielded negligible amounts of faeces from tilapia less than 30 g in weight (Table 3.2). Individuals larger than 30 g provided measurable amounts of faeces and there was a tendency for the amount to increase with fish size over the range studied. However, on average, it was only possible to obtain about 30 mg of dry faeces from the most posterior third of the gut in fish averaging 40 g. This amount of faeces is insufficient for chemical analysis. Even when pooled from nine fish, the total faecal mass was only 300 mg dry-matter and this is still insufficient for a single determination of energy in a standard bomb calorimeter.

A high fibre diet was chosen to provide relatively large amounts of faeces. However, when low fibre feeds were evaluated (e.g. grains, protein concentrates), less faeces were obtained. The conclusion was that intestinal dissection will not yield sufficient faeces for digestibility trials with tilapia unless sufficient numbers of fish larger than 50 g were available. Even if these fish were available, there would be theoretical objections to this method of collecting faeces because nutrient absorption occurs along the entire length of the gut in tilapia (Bowen, 1982). There is, therefore, no clearly defined

TABLE 3.2 Weight of faeces obtainable by intestinal dissection

Fish weight (g)	Fresh wt faeces (mg)	Dry wt faeces (mg)	% Dry matter
30.09	60.4	16.5	27.34
32.84	46.1	12.6	27.33
34.58	128.4	33.7	26.31
34.92	172.1	46.2	26.85
47.78	156.5	45.8	29.27
48.04	121.9	35.5	29.12
48.32	139.6	34.6	27.79
52.31	149.3	37.9	25.36
57.94	135.5	34.1	25.17
$\bar{X}$ 42.98 ± 9.95	123.31 ± 42.6	32.99 ± 11.51	26.84 ± 1.62

$\bar{X}$ = mean ± standard deviation

rectal area from which it would be valid to collect material for analysis. With tilapia, gut contents only become faeces when they are expelled from the body.

Because of these practical and theoretical difficulties, it was considered preferable to collect faeces from water.

3.2.1.2 Collection of faeces from water

The simplest method was to collect individual strings of faeces from the bottom of experimental tanks, with a siphon tube. This was the method used in all the digestibility experiments in this thesis, except that reported in Chapter 4 where special collecting chambers were constructed (see below). Each experimental tank was self cleaning (Section 2.2) and so, after a meal, uneaten food particles and faeces were automatically removed in the effluent water. One hour after offering food, the water flow to each tank was stopped and faeces uncontaminated with food accumulated at the bottom of the tank. These were collected at regular intervals by siphoning through a fine gauze. Faeces were then transferred to a clean, labelled petri dish, dried to constant weight at 105°C, ground with a pestle and mortar and stored in airtight tubes over a desiccant. The amount of faeces collected depended on the size and number of fish per tank and could be increased by collecting on successive days. The number of days over which faeces were collected was the same for all tanks.

Siphoning was time-consuming, but because faeces could be collected within 5 min of egestion, it was considered to be the most accurate method of collection with the available facilities. The main disadvantage was that, regardless of the number of fish per tank, or the number of faecal samples collected, the material from each tank only

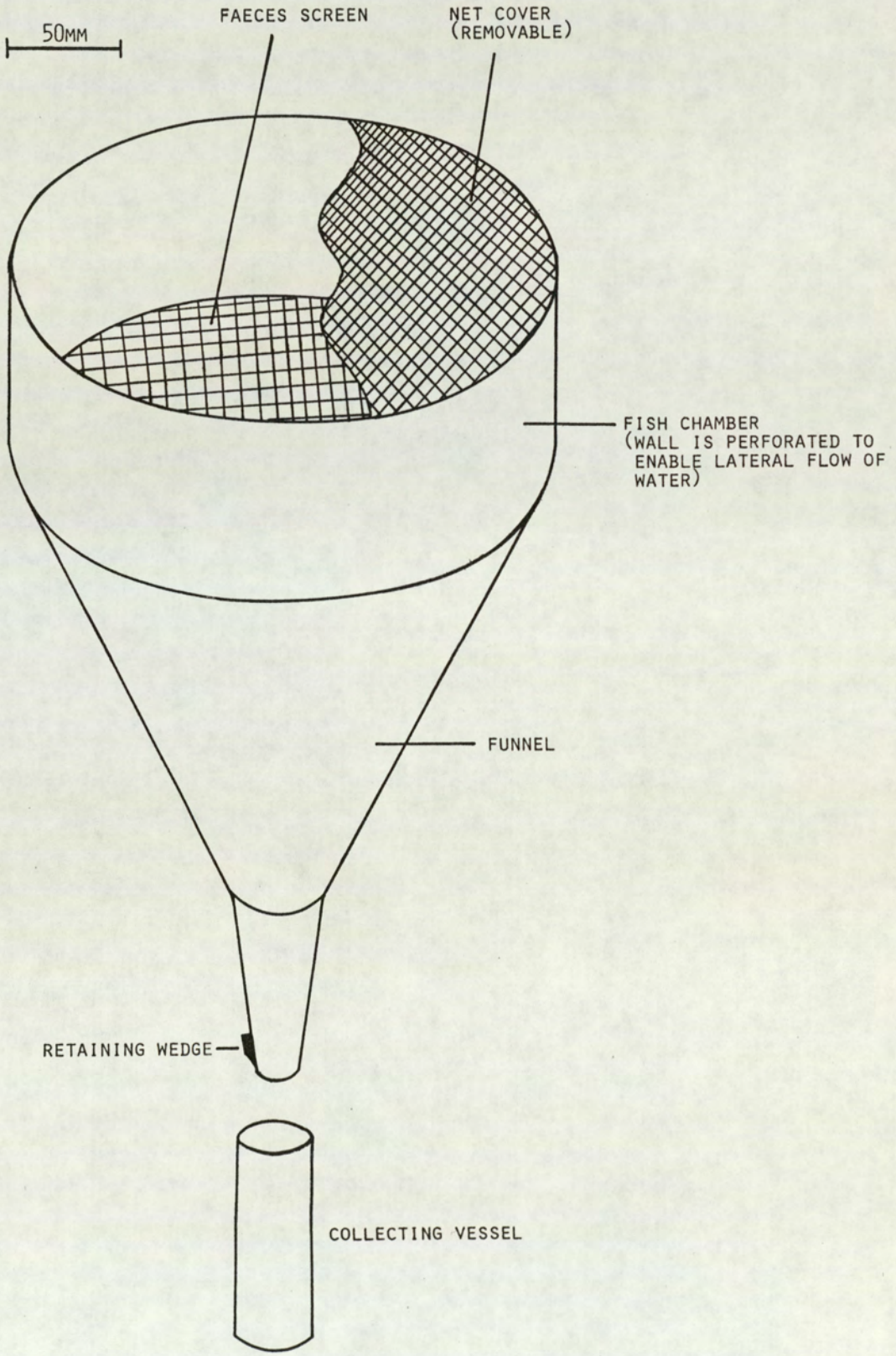
represented a single sample in statistical analysis, i.e. n = 1. Where the experimental design included replicate treatments (i.e. Chapter 4), the number of tanks required exceeded the number that were available. To overcome this limitation, faecal collection chambers were constructed to isolate individual fish (Figure 3.1).

Each chamber was immersed in an experimental tank and fish were placed in the upper part of the apparatus. Faeces passed through a 10 mm mesh screen and settled in a glass bottle at the apex of the funnel. This collecting bottle was removed at 5 min intervals, the contents passed through a fine gauze and the retained faeces treated in the same way as siphoned faeces. Each experimental tank was large enough to contain 3 of these chambers, so tripling the number of treatments possible with existing facilities. A total of 48 chambers were constructed, thereby providing sufficient replicates for the experiment reported in Chapter 4. The chambers were constructed by welding together a non-corrosive, non-toxic plastic funnel and colander. They are simple and inexpensive to assemble, but greatly increase the power of an experimental design.

The only difficulty was that tilapia would not accept food when placed in the chambers, even when starved for 7 d. To overcome this, fish on each dietary treatment were marked (Section 2.4.2), placed together in an experimental tank for group feeding and, 1 h after a meal, transferred individually to the chambers for collection of faeces. An average of 160 mg DM faeces could be collected from a single 100 - 150 g tilapia fed at 3% body weight, over 3 successive days of collecting. This amount was sufficient for duplicate determinations of chromium, protein (nitrogen x 6.25) and energy using the methods described in the section below.

FIGURE 3.1 FAECAL COLLECTION APPARATUS

(NOTE: WHEN IN USE, ENTIRE APPARATUS IS SUBMERGED IN AN AQUARIUM)



3.2.2 Analysis of faeces

Many of the existing techniques for analysing energy, protein and chromic oxide were unsuitable for the small samples available. Standard techniques were, therefore, adapted to enable duplicate determinations of all the above faecal components in a sample not exceeding 150 mg. In the present section, all analyses were in triplicate.

3.2.2.1 Measurement of faecal energy

Methods

Two methods were compared, namely microbomb calorimetry and chemical oxidation. To enable comparison, ten feeds were first analysed for energy in an adiabatic macrobomb calorimeter (Section 2.3.2) to provide reference values. This instrument requires a minimum sample of 0.5 g and so was unsuitable for faecal analysis even though it is highly accurate. Prior to analysis, all feeds were ground with a mortar and pestle, to a powder that would pass through a 1 mm mesh. Subsamples of each feed were dried to constant weight at 105 C for moisture determination. Energy was determined in samples 'as received' and later adjusted to a dry-matter (DM) basis.

Micro-bomb calorimetry

This instrument is described in Section 2.3.2. Because it is a ballistic apparatus, energy determinations with this machine are subject to greater error than with the adiabatic machine. To reduce this error, the peak heat value of each sample was corrected for ambient heat loss. Tangents were drawn to the Δh and post Δh portions of the temperature curve. The point at which these tangents meet is the

corrected peak heat value and this estimates the theoretical heat rise in a perfectly insulated apparatus. Corrected peaks were calculated for the benzoic acid standard and all individual samples, thereby reducing the effect of daily variations in operating conditions such as temperature of the laboratory. Values obtained by micro-bomb calorimetry are compared with the reference values in Table 3.4

Chemical oxidation

The wet combustion procedure was, in principle, the same as that described by O'Shea and Maguire (1962). However the concentrations and quantity of reagents used in the present oxidation were adjusted to levels more appropriate to the analysis of micro-samples. In addition, a heating stage was included to ensure complete oxidation of high fibre samples. the method was as follows:

Reagents: 1.5 N Potassium dichromate

0.3 N Sodium thiosulphate

20% Potassium iodide solution

Sulphuric acid (concentrated)

All reagents were 'Analar' grade, prepared on the day of use.

Oxidation procedure

20 - 50 mg of sample were placed in a 250 ml conical pyrex flask, which had been previously calibrated to 100 ml. Exactly 8 ml of potassium dichromate were transferred to the flask from an analytical pipette, followed by 16 ml of concentrated sulphuric acid from a calibrated repipette dispenser. The resulting mixture was heated over a bunsen for 5 min and then left to cool for 1.5 h. Duplicate blanks

were run with each set of samples analysed. The oxidised solution in each flask was then made up to 100 ml with distilled water and left to cool. 20 ml of the potassium iodide solution were then added from a measuring cylinder after which flasks were placed in a light-proof cabinet for 25 min. 50 ml of distilled water were then added to each flask and the liberated iodine titrated with 0.3 N sodium thiosulphate. The end point was reached when the solution changed from a dark red colour to emerald green. This point was very easily recognised and so a starch indicator was not necessary.

Rationale and calculation

Potassium dichromate is reduced in quantitative amounts as it is used to oxidise food molecules. Unreduced dichromate reacts with iodide, converting this to iodine, which is then estimated by titration with thiosulphate. Thus, to calculate the quantity of 1.5 N dichromate used to oxidise a known weight of material, the sample titre is subtracted from the blank titre and the resulting figure divided by 5 to adjust for normality. This figure for 'ml of dichromate used' is then converted to energy units by applying an oxidation coefficient (C) which was, initially, obtained from O'Shea and Maguire (1962). The oxidation coefficient of a feed relates the quantity of dichromate needed for its oxidation, to its energy content as determined by direct calorimetry. Thus, C is measured in units of ml $K_2Cr_2O_7$/kJ. If x is the amount of dichromate used to oxidise an unknown sample, then x/c is its energy value. Energy values for the ten reference feeds were calculated using C values from O'Shea and Maguire (1962) and these appear in Table 3.4 under 'equation A'.

Correction factor for incomplete oxidation of protein

Oxidation coefficients (C), in the present context, are derived from correction equations to compensate for the incomplete oxidation of protein by dichromate. As sample protein levels increase, so C decreases (O'Shea and Maguire, 1962). Thus, C values are empirically determined quantities and only apply to specified reaction conditions. The present oxidation procedure differs from that reported by O'Shea and Maguire (1962) in that these authors did not apply a heating stage. In addition, different reference feeds were used. Thus, C values derived from the data of O'Shea and Maguire (1962) are not entirely applicable to the present oxidation conditions. This explains why energy values obtained with O'Shea and Maguires equation (A) are low when compared with adiabatic calorimeter values (Table 3.4). To remedy this, a correction equation (B) was derived from the present data (Table 3.3).

Results

The published correction equation compares with that determined in the present experiment, thus:

$$C\ (ml\ K_2Cr_2O_7/kcal) = 23.39 - 0.069\ CP + 0.000226\ CP^2$$

.... Equation A, O'Shea and Maguire (1962)

$$C\ (ml\ K_2Cr_2O_7/kcal) = 22.30 - 0.0821\ CP$$

.... Equation B, present data

where CP = crude protein.

TABLE 3.3 Oxidation coefficients and dry-matter protein content of ten feeds

Feed	Oxidation coefficient (C) (ml $K_2Cr_2O_7$/kJ DM)*	Crude protein % (CP)
Basal diet (Chapter 5)	4.04	48.03
Wheat bran	5.07	15.38
Soybean	4.30	44.24
Fishmeal	3.96	70.27
Groundnut	4.46	50.48
Copra	5.18	20.20
Maize	5.04	8.95
Rapeseed	4.92	35.45
Cassava	5.13	2.75
Rice bran	5.04	12.54

$$C \text{ (ml } K_2Cr_2O_7\text{/kJ DM)} = 5.32 - 0.0196 \text{ CP}$$

$$(r = 0.91,\ p < 0.001)$$

* kJ determined by adiabatic calorimetry.

All values are means of triplicate determinations

(The specifications of these feeds, their scientific names and international feed numbers appear in Appendix 3).

TABLE 3.4 Energy values (MJ/kg DM) of ten feeds as determined by bomb calorimetry and chemical oxidation

Feed	Bomb calorimetry		Chemical oxidation	
	Adiabatic Macro-bomb	Ballistic Micro-bomb	Equation A (O'Shea and Maguire, 1962)	Equation B (present values)
Basal diet (Chapter 5)	21.81	23.10	17.84	20.92
Wheat bran	16.23	16.84	15.38	16.36
Soybean	17.76	19.28	15.38	17.13
Fishmeal	17.74	16.15	14.96	17.79
Groundnut	20.02	22.75	18.23	20.57
Copra	16.74	15.09	16.43	17.59
Maize	16.53	17.09	15.30	16.17
Rapeseed	17.46	18.62	16.93	18.54
Cassava	15.05	15.61	13.92	14.64
Rice bran	14.28	12.69	13.35	14.15

All values are means of triplicate determinations

C values computed with equation A are, on average, 5% higher than those computed with equation B. For all reference feeds, chemically determined energy values agreed more closely with directly determined values (from the adiabatic calorimeter) when the present correction equation was used. O'Shea and Maguire's (1962) equation provided much less accurate values (Table 3.4). In most cases, the error between adiabatic calorimeter and chemical oxidation values was less then 5% when the new equation was used. O'Shea and Maguire (1962) point out that a correction equation should also be calculated when the fat content of samples exceed 10%. This was not necessary in the present research.

The agreement between microbomb and macroadiabatic calorimeter values was much poorer and the conclusion is that chemical oxidation is a more accurate estimator of energy than microbomb calorimetry provided that a correction equation is applied.

3.2.2.2 Measurement of faecal chromium and nitrogen

Digestion procedure

Existing methods for chromic oxide and protein determination require two separate digestions. Protein is measured in a kjeldahl digest (Harris, 1979) and chromium in a nitric and perchloric acid digest (Furukawa and Tsukahara, 1966). However, because of the limited amount of material available in the present study, chromium and nitrogen were analysed in the same acid digest. The digestion procedure was as follows:

Approximately 40 mg of dry sample were weighed into a 30 ml kjeldahl digestion tube. 5 ml of concentrated sulphuric acid (Analar) were added and the tubes heated for 5 min on a thermostatically

regulated heating rack in a fume cupboard. Catalyst was then added in the form of two kjeldahl tablets, each containing 7.5 mg selenium and 1.5 g potassium sulphate (Thomson and Capper Ltd., Cheshire, UK). This raised the temperature of the reaction to 300 C. Heating was continued for 1.5 h, after which the tubes were cooled for 15 min. Distilled water was then added to the digest and the resulting solution quantitatively transferred to a 500 ml volumetric flask, which was then filled to the calibration mark with more distilled water and the contents thoroughly mixed. A 60 ml aliquot was then stored in a plastic sample bottle at 4 C, for subsequent analysis. A blank and a chromium standard were determined with each set of samples analysed. The chromium standard (2 ppm Cr) was prepared by placing 1 ml of a 1 mg ml^{-1} solution of chromic nitrate in a clean kjeldahl tube and then subjecting this to the same digestion procedure as the samples.

Digested samples, standards and blanks were then analysed for chromium and nitrogen using the following procedures.

Determination of chromium

Methods

Chromium (Cr) was determined in a Perkin Elmer 303 atomic absorption spectrophotometer (AAS), with the detection wavelength set at 357.9 nm. For all sample determinations, the oxidant/fuel mixture was air/acetylene (air/C_2H_2). However, standard curves for aqueous Cr were also determined in a hotter, nitrous oxide/acetylene flame (N_2O/C_2H_2) for comparative purposes. Standard curves for aqueous chromium in both flame conditions are given in Figure 3.2. For routine determinations, the machine was set to give readings in mg Cr l^{-1} (ppm Cr) using a top standard of 2.0 Mg Cr l^{-1}. Cr III (chromic nitrate) and Cr VI

(Potassium dichromate) gave identical standard curves. For each reading, the integration period was 3 s and for all samples, 6 readings were recorded. The mean of these 6 values were calculated and taken as the sample value. For each batch of seventeen digested samples, a blank and a standard were determined. Within a set of 200 samples or more, these blanks and standards served as useful internal reference values to check for drift in the calibration line of the AAS or for variation between digestion batches.

The sensitivity of chromium detection can be affected by treatment with acid and catalyst (Lied et al, 1982). This was investigated as follows. Firstly, aqueous chromium standards were subjected to the digestion procedure described above and analysed on the AAS. This acid/catalyst treated chromium curve appears on Figure 3.2. Secondly, calibration curves were determined for ground, dried diets containing approximately 0.5% chromic oxide with standard additions of aqueous chromic nitrate. The purpose of this second test was to assess if organic material interfered with chromium detection. One of these diet calibration lines is shown in Figure 3.2.

The recovery of chromium was assessed by preparing a cellulose standard. Cellulose powder (Whatman Standard Grade) and ground chromic oxide were dried at 105 C for 24 h. The dried materials were then weighed to required levels and mixed in a ball mill for 24 h. Percentage recovery was determined with samples ranging from 10 mg to 120 mg in an air/C_2H_2 flame.

Finally, the AAS method of chromium detection was compared with optical spectrophotometry. A diet containing chromium was dried, ground and samples digested with nitric and perchloric acids as described by Furukawa and Tsukahara (1966). Sample size ranged from

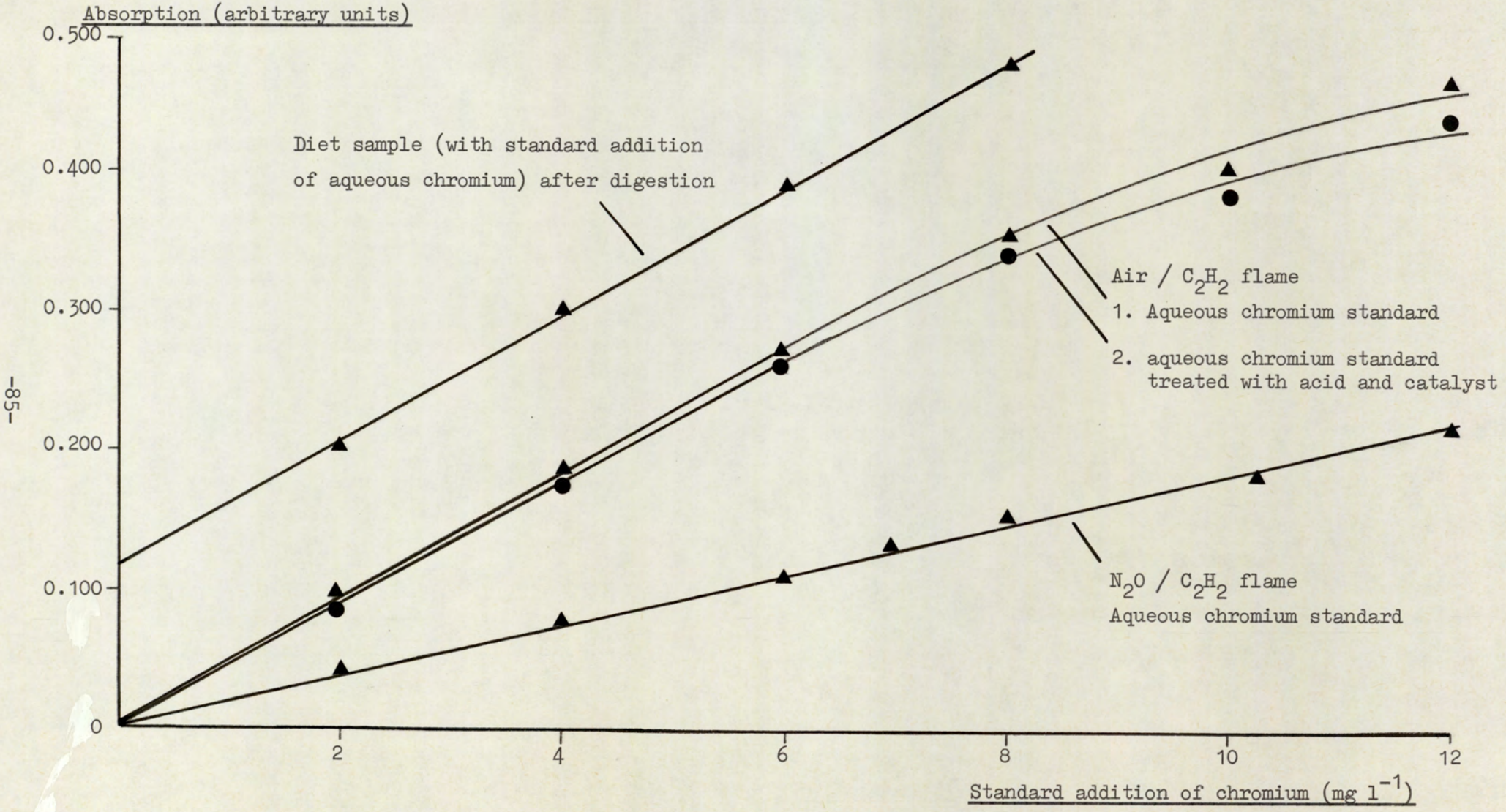
5 - 140 mg. The digests were quantitatively diluted to a final volume of 100 ml with distilled water and an aliquot analysed by AAS using the conditions described above. A second aliquot was taken and the optical density read on an ultra violet spectrophotometer (model CE 272, Cecil Instruments Ltd., UK) at 350 nm. Optical density was then converted to % chromic oxide with the equation given by Furukawa and Tsukahara (1966). The different estimates obtained by the two detection methods are given in Figure 3.4

Results

The N_2O/C_2H_2 flame provided more stable readings than the air/C_2H_2 flame and, unlike the latter, the former gave a standard curve which was linear over the range studied (0 - 12 ppm Cr; Fig. 3.2). However, the maximum Cr concentration in the present diluted digests was less than 2.0 ppm. The air/acetylene flame gave a linear standard curve over this range and the greater sensitivity of this flame argued in favour of its use. In this thesis, all subsequent measurements of Cr were, therefore, made with the air/C_2H_2 flame.

When aqueous Cr standards were treated with acid and catalyst, the absorption suppression was minimal (Fig. 3.2.). Lied et al (1982), reported a much greater suppression. However, the relatively large interference reported by these authors was possibly due to their use of more concentrated digests. Lied et al (1982) only diluted the 5 ml acid digest to 75 ml with distilled water, so their final samples contained nearly 7 times more acid and catalyst than the diluted digests in the present study. When such concentrated digests were tested in the present work the AAS gave inconsistent readings. The first samples in a batch of these concentrated samples gave stable readings.

FIGURE 3.2 CALIBRATION CURVES FOR THE DETECTION OF CHROMIUM BY ATOMIC ABSORPTION SPECTROPHOTOMETRY



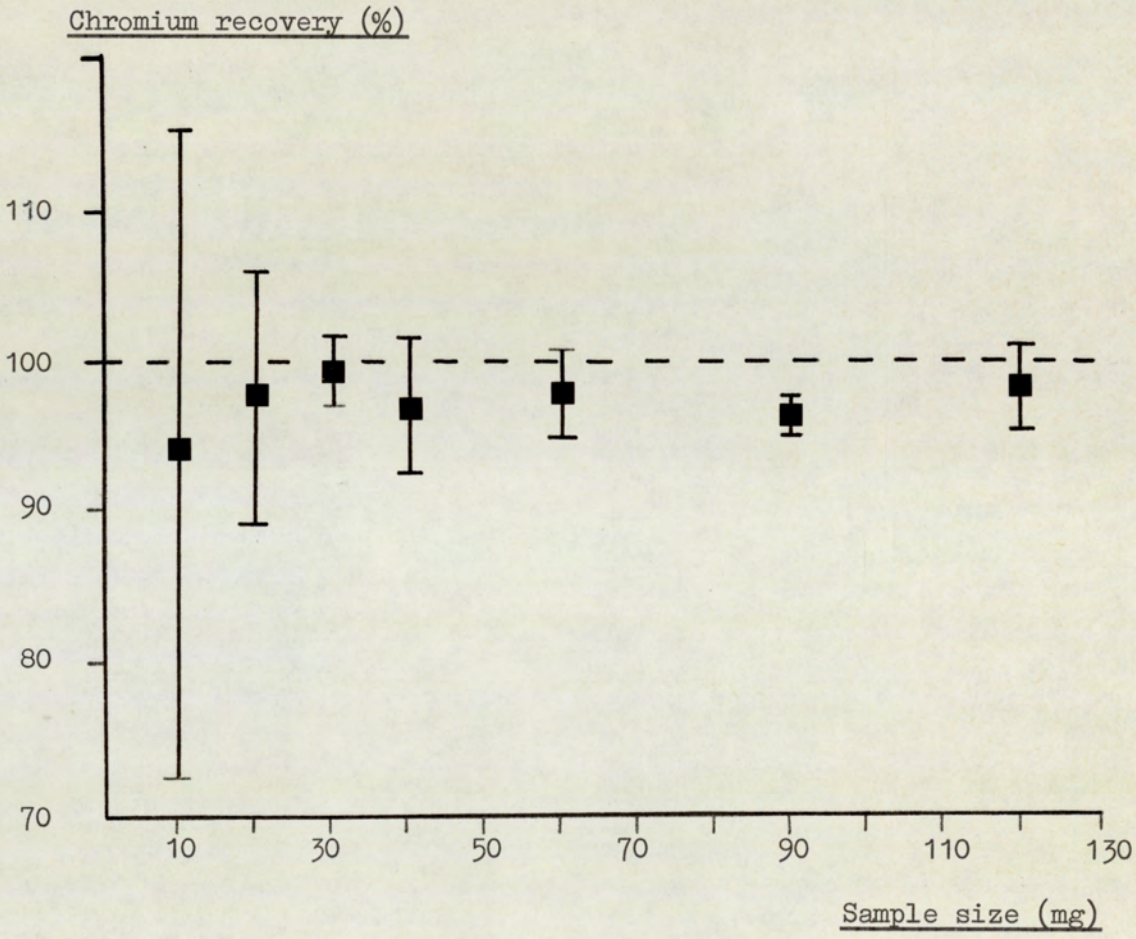
However, after about 20 samples, replicate readings on a single sample were unacceptably erratic even when the integration period was set to 10 s. Cleaning the AAS burner rectified this temporarily and the conclusion was that the high levels of potassium salt in the catalyst tablets interfered with the flame. Diluting digests to 500 ml ameliorated this problem and reduced the interference of acid and catalyst with chromium detection.

Figure 3.2 also shows the absorption curve for a diet to which chromium was added. The slope of this curve is identical to that for an aqueous chromium standard, showing that there was no interference with chromium detection in the presence of organic material.

The recovery of chromium was over 95% in samples weighing more than 20 mg (Fig. 3.3). Recoveries fell to less than 95% in smaller samples and the reproducibility of the assay decreased drastically (as indicated by the size of the confidence limits). For these reasons, it was decided that routine determinations of chromium in faeces should be conducted on samples no smaller than 30 mg.

When samples were digested with nitric and perchloric acids as detailed by Furukawa and Tsukahara (1966), the chromic oxide recovery using AAS was not dependent on sample size. Samples as small as 5 mg gave similar chromic oxide estimates to 140 mg samples (Fig. 3.4). However, when Cr was detected in the nitric/perchloric acid digests by optical spectrophotometry as recommended by Furukawa and Tsukahara (1966), the chromic oxide estimates clearly depended on sample size. In small samples, chromic oxide appeared to be overestimated (Fig. 3.4). An absolute measure of percentage recovery is not possible from the data given in Fig. 3.4, because the original chromium content of the diet sample is not known with sufficient accuracy. However, it is

FIGURE 3.3 EFFECT OF SAMPLE SIZE ON THE RECOVERY OF CHROMIUM IN A SULPHURIC ACID DIGEST, USING ATOMIC ABSORPTION SPECTROPHOTOMETRY



Samples were taken from a cellulose powder standard containing 0.819 % Cr_2O_3 (= 0.560 % Cr), on a dry-matter basis (see text). Data points are each the mean of four determinations with 95 % confidence limits.

still apparent from this data that AAS is a more sensitive means of detecting Cr in small samples than optical spectrophotometry, even when the present sulphuric acid digestion is replaced with the conventional nitric/perchloric acid digestion. The sulphuric acid digestion was used throughout the rest of the work reported in this thesis because it is simpler (and safer) than the nitric/perchloric digestion and because the recovery of chromium by the latter procedure is reported to be poor (Lied et al, 1982).

Determination of nitrogen

Methods

Nitrogen was determined in the same sulphuric acid digest as chromium. Digested samples were analysed for nitrogen in a technicon autoanalyser (model II, Technicon Instruments Corp., Tarrytown, NY, USA) using a modification of the technique described by Crooke and Simpson (1971). The procedure was as follows:

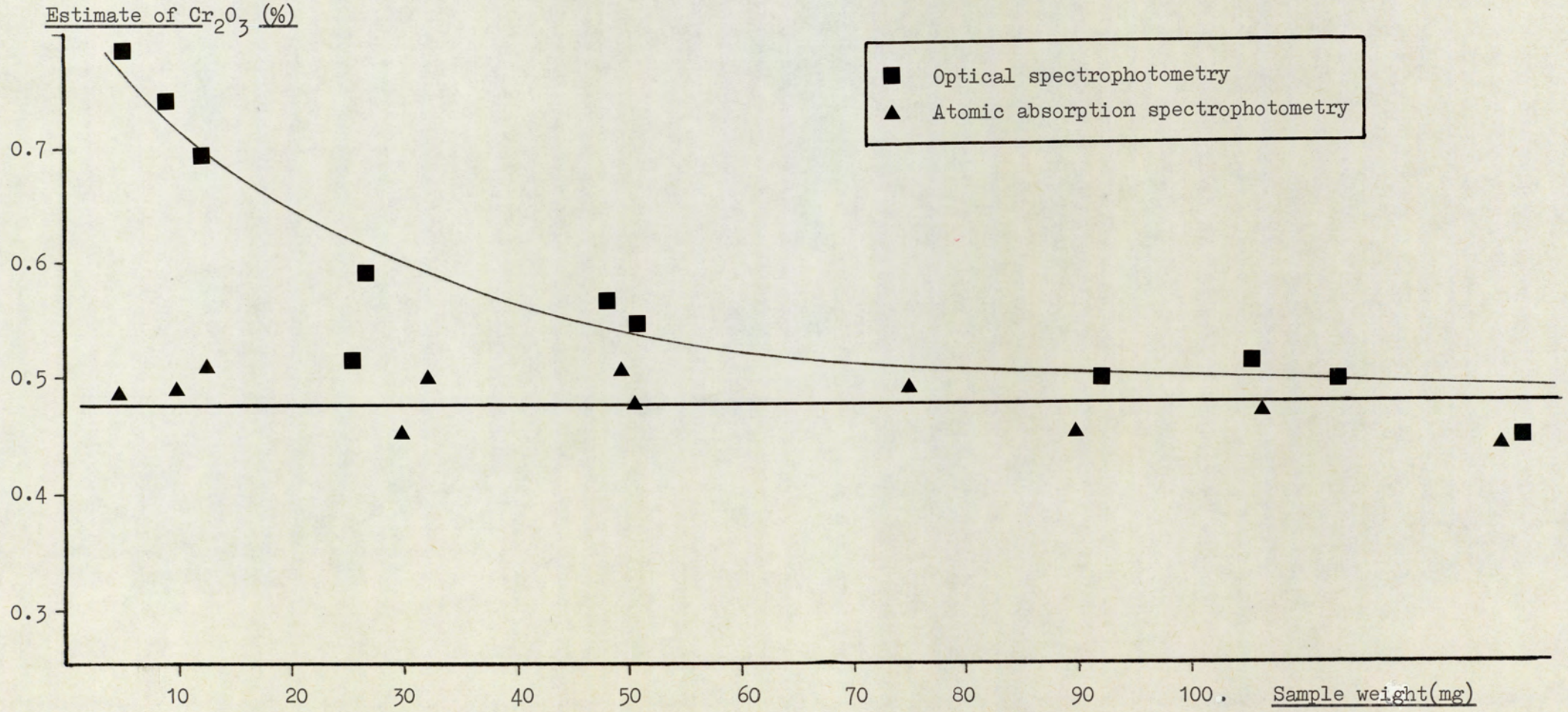
Reagents

Salicylate: 85 g sodium salicylate and 0.6 g sodium nitroprusside in 100 ml distilled, deionised water.

Cyanurate: 24 g sodium hydroxide and 5 g sodium dichloroisocyanurate in 1000 ml distilled, deionised water (stored at 4 C).

Diluent: 2 kjeltabs (total 3 g K_2SO_4, 15 mg Se - Thomson and Capper Ltd., Cheshire, UK)in 5 ml concentrated sulphuric acid. Heat to 300 C for 1 h. Dilute to 500 ml with distilled, deionised water.

FIGURE 3.4 ESTIMATION OF CHROMIUM IN NITRIC / PERCHLORIC ACID DIGESTION OF A DIET USING ATOMIC ABSORPTION AND OPTICAL SPECTROPHOTOMETRY



Nitrogen

Standard: 0.943 g Ammonium Sulphate in 1000 ml diluent solution. This provided a stock solution of 200 mg N l^{-1}. By adding various amounts of diluent to aliquots of this stock solution, standards were prepared to give the following concentration range: 3, 6, 9, 12, 15, 18 mg N l^{-1} (= ppm N).

Autoanalyser operation

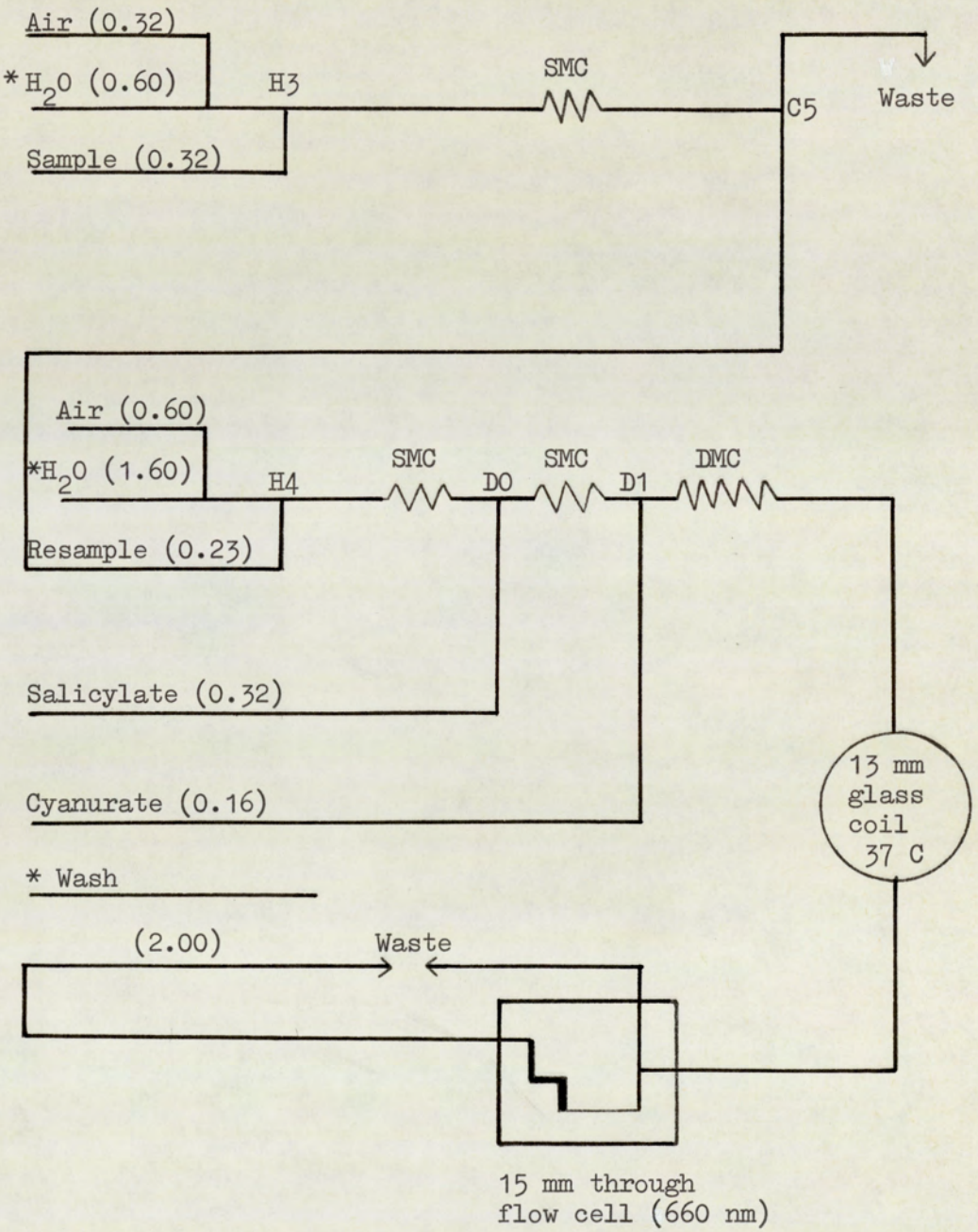
The form of the manifold was similar to that described by Crooke and Simpson (1971), but the first sample dilution step was decreased to bring the present samples into the working range of the instrument. The manifold used in the present study is represented diagrammatically by Figure 3.5. This employs a two stage dilution (1:2.9 and 1:8.0; overall 1:23.2), which is 5.6 times less than the manifold described by Crooke and Simpson (1971)*.

Samples were aspirated at a rate of 30 h^{-1} using a 1:2 cam to ensure complete separation of samples. A wetting agent, Brij-35 (polyoxyethylene lauryl ether), was included at 2 - 3 drops per litre in the sampler wash solution and in the same quantity in the water used for diluting the samples. After the sample was diluted and mixed with reagents, it passed into a delay coil at 37 C and then on to a 15 mm flow cell where Transmittance was read at 660 nm. Transmittance curves

* Note: Crooke and Simpson (1971) appear to have calculated their dilution factor incorrectly as 1:109. Sample dilution is correctly calculated as sample flow rate/total (= water + sample) flow rate, and using Crooke and Simpson's (1971) values, this is 1:132.

FIGURE 3.5 DIAGRAM OF AUTOANALYSER MANIFOLD USED TO DETERMINE NITROGEN IN SULPHURIC ACID DIGESTS

Flow rate (tube size) is given in parenthesis (ml min^{-1})



* Brij-35 included at 2 - 3 drops per 1000 ml

SMC = single mixing coil, DMC = double mixing coil, C5, D0, D1, H3 and H4 are standard glass fittings.

Note : pulse suppressor not necessary.

were discrete and smooth; a pulse suppressor was not necessary.

The ammonium sulphate standards were run at the beginning and end of each batch of samples and a mean standard curve for the batch computed by regressing nitrogen concentration on Log_{10} Transmittance.

To assess the accuracy and reproducibility of the nitrogen assay, the following tests were performed:

1. Nitrogen recovery. An aqueous urea solution was prepared to give 5 mg N ml^{-1}. Aliquots were digested with sulphuric acid and catalyst as described earlier. The volume of aliquots was chosen to give a final concentration of 1, 2, 4 or 9 mg N l^{-1} when the digest was diluted to 500 ml with distilled water. These digested urea samples were then analysed for nitrogen on the autoanalyser and percentage recovery calculated. The results appear in Table 3.5.

2. Reproducibility. Nitrogen was determined in 10 separate samples of the same experimental diet (diet 3, Chapter 4). The results are given in Table 3.6.

Results

The autoanalyser was accurate to $\pm$ 0.1 mg N l^{-1}. Table 3.5 shows that the recovery of nitrogen from a urea standard was, in most cases, complete and was always greater than 97%. This indicates that there is no apparent loss of nitrogen in the digestion procedure. The reproducibility of the assay was high (Table 3.6). The coefficient of variation for replication of 10 samples of the same diet was 2.07%. This compares well with the level of accuracy expected from conventional

TABLE 3.5 Recovery of nitrogen by autoanalyser analysis

Urea Standard (mg N l^{-1})	Autoanalyser Estimate mg N l^{-1}	% Recovery
1	1.0	100
2	2.0	100
4	3.9	97.5
9	9.0	100

Triplicate aliquots of each urea standard were digested with sulphuric acid and selenium catalyst before analysis. Values are means of triplicate measures

TABLE 3.6 Reproducibility of autoanalyser protein assay

Sample weight (mg)*	mg N l^{-1}	% Protein**
43.80	5.3	37.8
35.93	4.3	37.4
48.92	6.0	38.4
34.94	4.4	39.4
43.11	5.3	38.4
43.55	5.2	37.4
37.90	4.6	37.9
44.82	5.6	39.0
43.81	5.5	39.2
38.70	4.7	37.2

Variation between these replicate measures of protein:

Range = 37.2 - 39.4%

Mean = 38.2%. Standard deviation = 0.79

Coefficient of variation = 2.07%

* All samples taken from diet 3, Chapter 4

** % protein = $(\frac{\text{mg N } l^{-1}}{2} \times 6.25) \div$ sample wt (mg)

digestion and distillation with a Markham still (Markham, 1942).

Section 3.3 Effect on digestibility of immersing faeces for different periods

3.3.1 Introduction

The effect on digestibility of leaching and breakup of faeces in water was reviewed in Section 3.1. Specialised equipment designed to reduce this effect was also described in the same Section, but expense prohibited the use of such equipment in the present research. Intestinal dissection was not appropriate for tilapia (Section 3.2.1) and so in the digestibility trials reported throughout this thesis, faeces were collected from water after egestion by fish. The experiment reported in this Section examines the type of error associated with this method of collecting faeces.

3.2.2 Methods

Six groups of 13 fish were maintained in the recirculating water system (Fig. 2.1). All groups were of similar mean weight (approx. 45 g). Each group was kept in a separate tank and they were all fed on the same diet (diet 3, Chapter 4) for 3 weeks. The feeding rate was 2.5% of total tank liveweight day^{-1} and this amount was offered in 3 equal meals at 0900, 1300 and 1700 hr. On days 22 to 26 inclusive, faeces were collected from each tank for 2 h by siphoning as described in Section 3.2.1.2. On day 22, faeces were collected every 5 min; on day 23, every 60 min; on day 24, every 15 min; on day 25, every 10 min; and on day 26, every 30 min. By allocating these immersion periods to different days in a random order any time-dependent effects of feeding

were reduced. Faeces were also collected at the same time each day to prevent possible interference with daily digestion cycles, should these exist. This experimental plan provided 5 faecal immersion periods each with 6 replicates.

All diet and faecal samples were analysed in duplicate for nitrogen, energy and chromium by the methods described in Section 3.2.2. All values were adjusted to a dry-matter basis and digestibility calculated for each sample using the following formulae.

Dry-matter digestibility (DMD %).

$$\left[1 - \left(\frac{\%\ \text{Cr in feed}}{\%\ \text{Cr in faeces}}\right)\right] \times 100$$

Digestible energy (DE %).

$$\left[1 - \left(\frac{\%\ \text{Cr in feed}}{\%\ \text{Cr in faeces}} \times \frac{\text{Energy in faeces (MJ/kg DM)}}{\text{Energy in feed (MJ/kg DM)}}\right)\right] \times 100$$

This provides a percentage coefficient. To obtain DE in units of MJ/kg DM, the energy content of the diet is multiplied by the percentage coefficient then divided by 100.

Digestible crude protein (DCP %)

$$\left[1 - \left(\frac{\%\ \text{Cr in feed}}{\%\ \text{Cr in faeces}} \times \frac{\text{Nitrogen in faeces (\%)}}{\text{Nitrogen in feed (\%)}}\right)\right] \times 100$$

These formulae provide 'apparent' estimates of digestibility because faeces also contain endogenous material from the fish, such as sloughed intestinal epithelial cells, mucus, catabolised digestive

enzymes and bacteria. This leads to an underestimation of the proportion of food actually absorbed by the fish. For example, Nose (1967) estimated that metabolic faecal nitrogen (i.e. endogenous nitrogen from the fish) could amount to 5 - 17 % of total faecal nitrogen. Nevertheless, apparent digestibilities do represent the net value of a feed and so are a more realistic and practical measure than true digestibility.

The apparent digestibility coefficients calculated from data in the present experiment are summarised in Tables 3.7, 3.8 and 3.9. The data in each Table was subjected to a one-way analysis of variance and differences between means tested with a multiple range test (Duncan, 1955).

Section 3.3.3 Results

The analyses of variances for data in Tables 3.7, 3.8 and 3.9 show that the length of the faecal immersion period had a significant effect on estimates of apparent digestibility. There was a progressive decline in estimates of DE, DCP and DMD as faeces were left immersed for longer periods. For DMD, there was a significant reduction after 10 min and a further significant reduction after 30 min. This trend was the same for DE, but not for DCP. Estimates of protein digestibility only showed a significant decline after 30 min, but anomalously, the reduction after 60 min was non-significant (probably because of the large standard deviation of this mean).

These results conflict with those reported by Windell et al (1978a) who showed that digestibility estimates increase with faecal immersion period. Of course, both Windell's and the present results only reflect errors in the determination of apparent digestibility. The assumption

TABLE 3.7 Effect of faecal immersion period on estimates of apparent digestible energy (DE)

Tank	Mean Fish wt (g) ± S.D.	DE (%) Immersion period				
		5 min	10 min	15 min	30 min	60 min
1	43.46 ± 17.13	78.34	78.62	76.90	73.95	76.18
2	46.16 ± 15.14	80.24	77.19	77.33	74.55	78.29
3	45.61 ± 14.13	79.46	77.27	76.05	75.56	74.48
4	45.48 ± 22.20	80.11	76.56	75.85	74.92	73.87
5	46.60 ± 24.32	80.92	76.60	77.64	75.39	75.58
6	43.44 ± 17.59	80.80	76.89	76.61	74.09	75.69
mean*		79.98^{a}	77.19^{b}	76.73^{bc}	74.74^{d}	75.68^{cd}
S.D.		0.96	0.76	0.70	0.66	1.54
coefficient of variation		1.2%	1.0%	0.9%	0.9%	2.0%

* Means with a common superscript are not significantly different (i.e. $p > 0.05$).

Analysis of variance summary

Source of variation	Degrees of freedom	Sums of squares	Mean square	F
Between immersion periods	4	94.31	23.58	
Within immersion periods	25	23.95	0.96	24.61 ($p < 0.01$)
Total	29	118.26		

Standard Error of Mean (SEM) = 0.40.

TABLE 3.8 Effect of faecal immersion period on estimates of apparent digestible crude protein (DCP)

Tank	Mean Fish wt (g) ± S.D.	DCP (%) Immersion period 5 min	10 min	15 min	30 min	60 min
1	43.46 ± 17.13	92.84	93.01	93.85	92.26	90.95
2	46.16 ± 15.14	93.13	90.51	94.30	90.37	92.28
3	45.61 ± 14.13	94.00	91.96	93.52	90.95	86.53
4	45.48 ± 22.20	92.45	93.57	92.10	89.76	92.26
5	46.60 ± 24.32	93.45	93.22	92.23	89.80	93.45
6	43.44 ± 17.59	92.72	93.42	92.29	90.73	92.58
mean*		93.10^{a}	92.61^{a}	93.05^{a}	90.64^{b}	91.34^{ab}
S.D.		0.56	1.18	0.96	0.93	2.49
coefficient of variation		0.6%	1.3%	1.0%	1.0%	2.7%

* means with a common superscript are not significantly different (i.e. $p > 0.05$).

Analysis of variance summary

Source of variation	Degrees of freedom	Sums of squares	Mean square	F
Between immersion periods	4	29.05	7.26	
Within immersion periods	25	48.38	1.94	3.75 ($p < 0.05$)
Total	29	77.42		

Standard error of mean (SEM) = 0.57

TABLE 3.9 Effect of faecal immersion period on estimates of apparent dry matter digestibility (DMD)

Tank	Mean Fish wt (g) ± S.D.	DMD (%) Immersion period 5 min	10 min	15 min	30 min	60 min
1	43.46 ± 17.13	64.49	63.89	61.40	59.95	56.49
2	46.16 ± 15.14	68.22	64.66	65.42	61.23	59.83
3	45.61 ± 14.13	67.57	64.25	63.82	62.31	57.24
4	45.48 ± 22.20	67.23	63.64	62.88	54.89	59.63
5	46.60 ± 24.32	68.73	62.76	65.35	58.26	62.65
6	43.44 ± 17.59	67.91	65.80	63.47	58.90	61.80
Mean*		67.36^{a}	64.17^{b}	63.72^{b}	59.26^{c}	59.61^{c}
SD		1.50	1.02	1.53	2.60	2.43
Coefficient of variation		2.2%	1.6%	2.4%	4.4%	4.1%

* Means with a common superscript are not significantly different (i.e. $p > 0.05$)

Analysis of variance summary

Source of variation	Degrees of freedom	Sums of squares	Mean square	F
Between immersion periods	4	277.49	69.37	
Within immersion periods	25	91.50	3.66	18.95 ($p < 0.01$)
Total	29	368.99		

Standard error of mean (SEM) = 0.78

of these experiments is that the diet is equally digestible by all groups at all times. It is only the effect of leaching of organic material, bacterial action or physical breakup of faeces that causes the change in estimates of digestibility when faeces are left immersed. An increase in digestibility with faecal immersion can be explained by postulating that organic material is leached from faeces whilst immersed. The present results cannot be explained by the same postulate. The two most obvious explanations for the decrease in digestibility with immersion are either that the faeces gained organic material whilst immersed, or that chromium was lost. The former is unlikely because the only possible gain of material was from bacterial growth on the faeces and this was unlikely over the short period studied. The most reasonable explanation, therefore, is that chromium was lost from immersed faeces.

Inspection of the data for faecal composition showed that there were no significant differences in the energy or nitrogen content of faeces between the 5 min and 60 min immersion periods. By contrast, there was a significant reduction in faecal chromium with immersion period (Table 3.10). As far as is known, this effect has not been previously reported in the literature and there is no obvious explanation. One possibility is that faeces broke up on immersion and the denser chromium particles were lost from the faeces. By collecting over shorter periods, the amount of chromium lost was reduced and the estimate of digestibility higher. However, it must be pointed out that faecal breakup was not visually apparent in the present experiment and the reason for loss of chromium requires further investigation.

One way of estimating the error due to loss of chromium is to plot a curve of apparent digestibility against faecal immersion period,

TABLE 3.10 Effect of faecal immersion period on estimates of faecal chromium

	% Chromium in faeces				
	Immersion period				
Tank No.	5 min	10 min	15 min	30 min	60 min
1	1.039	1.022	0.956	0.921	0.848
2	1.161	1.044	1.067	0.952	0.921
3	1.110	1.035	1.020	0.979	0.863
4	1.126	1.015	0.994	0.818	0.914
5	1.180	0.991	1.065	0.884	0.988
6	1.150	1.079	1.010	0.898	0.966
Mean*	1.128^{a}	1.031^{b}	1.019^{b}	0.909^{c}	0.917^{c}
SD	0.050	0.030	0.043	0.056	0.055
Coefficient of variation	4.4%	2.9%	4.2%	6.2%	6.0%

* Means with a common superscript are not significantly different (i.e. $p > 0.05$)

Analysis of variance summary

Source of variation	Degrees of freedom	Sums of squares	Mean square	F
Between immersion periods	4	0.1974	0.0493	
Within immersion periods	25	0.0571	0.0023	21.60 ($p < 0.01$)
Total	29	0.2545		

Standard error of mean (SEM) = 0.02

assume that the relationship is linear and extrapolate digestibility to time zero. This provides an approximate theoretical estimate of digestibility if faeces had been collected the instant they were released. However, the assumption of linearity is invalid with the present results because the reduction in digestibility is more rapid over the first 30 min of faecal immersion than subsequently (Tables 3.7, 3.8 and 3.9). If it can be assumed that reduction in digestibility is linear over the first 10 min of immersion, an estimate of the error can be obtained by calculating the reduction in digestibility between the 5 min and 10 min immersion periods. For DE, the estimate for the 10 min period is 96.5% of the estimate for the 5 min period; for DMD, this estimate is 95.3% and for DCP it is 99.5%. It is possible that the reduction in digestibility estimates between time zero and 5 min of faecal immersion are of a similar magnitude.

Faecal immersion appears to have less effect on estimates of DCP than DE or DMD. This is because so much dietary protein is absorbed by the fish that for unit loss of chromium, the relative decrease in DCP is small when compared with the decrease in DMD or DE. Despite this, faeces should still be collected within 5 min intervals when determining DCP to reduce to a minimum the small error inherent in this determination.

Section 3.4 Discussion and recommendations

The literature review and experimental results in this Chapter show that digestibility trials with fish are subject to considerable error. The methods developed here have aimed to standardise the error and reduce it to a minimum. For convenience, a summary of the

recommended method for digestibility trials with tilapias, appears at the end of this Chapter. This is the method which will be used for all other digestibility trials described later in this thesis.

The faecal collection method was chosen for simplicity and because it enables the collection of many samples in a short period. The only limitation is the availability of aquaria in trials with large numbers of feeds or experimental treatments, but this can be overcome by using the collecting apparatus in Figure 3.1. The methods of analysis were also designed to enable rapid throughput of large numbers of faecal samples. In general, it was possible to perform 45 sulphuric acid digestions per day. When sufficient samples had been digested for batch analysis (approx. 200), these could be analysed on the AAS and autoanalyser within 2 days. For energy determinations, approximately 35 samples could be analysed daily. The value of these rapid assays was that many replicates could be conducted on each sample, thereby increasing the accuracy of determination. The coefficient of variation for 5 replicate determinations of energy in a single sample was 1.1 - 5.2% depending on the type of sample. For nitrogen, this was 2.07% (10 replicates) and for chromium, it was 4.2 - 6.3% (5 replicates). Thus, the level of accuracy was similar for all 3 assays of faecal composition. Another major advantage of the present methods is that all assays can be performed in duplicate on a faecal sample weighing no more than 150 mg. This is particularly valuable when working with small fish.

Using a combination of these assays, digestibility estimates were highly reproducible. When faeces were collected within 5 min of egestion, the coefficient of variation for DE was 1.2%; for DCP, 0.6% and for DMD, 2.2% (Tables 3.7 - 3.9). The only potential source of

error was loss of chromium from immersed faeces. This could not be avoided, but by standardising faecal immersion to 5 min, any error was minimised. As a result, apparent digestibility estimates in this thesis are probably greater than 95% of 'true' apparent values.

Recommended procedure for digestibility determination

Faecal collection: Siphon faeces from tanks containing replicate groups of fish, or collect faeces from individuals in chambers. Collect faeces every 5 min. Total weight of faeces required: c.200 mg dry-matter.

Faecal analysis

Energy: 20 - 50 mg samples. Chemical oxidation with dichromate using a correction equation for incomplete oxidation of protein.

Chromium and nitrogen: 40 mg samples. Digestion with sulphuric acid and selenium catalyst at 300 C. Dilute digests to 500 ml with distilled water and assay for chromium with an air/acetylene flame on an AAS, and for nitrogen with the dichloroisocyanurate reaction on an autoanalyser.

Diet analysis: Either with conventional macro methods (Section 2.3.2) or the present micro methods.

Calculations: Adjust all values to a dry-matter basis and compute digestibility according to the formulae in Section 3.3.2.

Using this procedure, the following levels of accuracy can be expected.

	coefficent of variation for replicate measures
Digestible energy	1.2%
Digestible crude protein	0.6%
Digestible dry-matter	2.2%

CHAPTER 4

THE EFFECTS ON FEED DIGESTIBILITY OF THE DURATION OF A FEEDING TRIAL AND THE LEVEL OF TEST FEED INCLUSION IN A REFERENCE DIET

Section 4.1 Introduction

When the digestibility of a single feed such as groundnut, soybean or rice bran is assessed, it is often necessary to supplement that feed with other nutrients to make a balanced experimental diet. If a feed is deficient in vital nutrients or very high in fibre and is given, on its own, to experimental animals, the process of digestion and absorption can be impaired, thereby affecting the digestibility of that feed. In addition, the voluntary intake (or 'palatability') of plant-product feeds is usually low if the feed is not mixed with dietary supplements such as lipids and animal protein sources, and this can also affect digestibility.

To overcome these problems, the "substitution method" of diet formulation is now widely used in digestibility studies. Here, a basal, or "reference" diet is prepared which has a nutrient profile closely resembling a standard production diet. A known amount of this reference diet is then substituted with the "test feed". Thus:

Experimental diet = Reference diet + Test feed.

By determining the digestibilities of the experimental diet and the reference diet in separate trials, it is then possible to calculate test feed digestibility by difference.

The advantage of this method is that animals can be provided with a nutritionally adequate diet which they will accept under standard husbandry conditions. The main disadvantage however, is that the substitution method relies on the assumption that there is no interaction between the test feed and reference diet. This assumption is not usually tested in routine trials, but it implies that the digestibility of the experimental diet is always the simple sum of the digestibilities of its two components. However, in some cases (particularly with high fibre feeds), there is a possibility that the digestibility of the experimental diet is the product of an interaction between its two components. In other words, within an experimental diet, the test feed can affect the digestibility of the reference diet and vice-versa. If this occurs, the digestibility of a test feed will then depend on its level of inclusion in the experimental diet. Thus, by measuring the digestibility of a single test feed at a variety of substitution levels in the experimental diet, it is possible to test the assumption of non-interaction between dietary components. One of the aims of this chapter is to perform this test with tilapia.

The duration of a feeding trial may also affect digestibility. In the literature, this length of time (which shall be called the "feeding period"), seems to be determined on an arbitrary basis. Thus, some authors force-feed their experimental animals and collect faeces as soon as these are produced (Jobling, 1981). Others permit normal feeding activity and collect faeces after a feeding period of 3 - 5 d (Smith et al, 1971; 1980); 9 d (Smith and Lovell, 1971); 28 - 35 d (Windell et al, 1978; De Silva and Perera, 1984) or as long as 240 d (Kirilenko et al, 1975). However, the digestive physiology of tilapia adapts to different diets with time. For example, Nagase (1964) showed that

amylase activity in S. mossambicus was greater on high-carbohydrate diets than on carbohydrate-free diets, after a feeding period of 6 weeks. This type of enzyme adaptation may affect the digestibility of a feed, but because this is not instantaneous, digestibility values obtained after a short feeding period may not be accurate. The second aim of this chapter is, therefore, to examine the effect of feeding period duration on digestibility.

In any intensive tilapia culture operation, the greatest feed costs are during the grow-on stage as fish are raised from fingerlings to market size. Thus, whilst it is accepted that the digestion and absorption of feeds by fry are different from those by post-juveniles, it is of most commercial interest to determine the digestibility of feeds for the latter. For this reason, the experiment reported in the present chapter was conducted with tilapia in the middle of the size range usually encountered in the grow-on stage (i.e., 100 - 150 g).

To summarise, the aim of this chapter is to determine if the digestibility of a single feed, such as soybean meal, depends on:

1. Its level of inclusion in a diet.
2. The duration of the feeding trial.

Section 4.2 Methods

4.2.1 Experimental design

Two factors were examined, namely: test feed inclusion level (A) and feeding period (B). The design used was a split-plot partial factorial (Kirk, 1968) with repeated measures on the feeding period factor. The 'plots' were groups of 6 fish and measurements of digestibility on these plots were 'split' (repeated) over two feeding

periods ('sub-plots'). Each group (plot) received only one level of factor A (i.e. one diet), but all levels of factor B (i.e. digestibility scores were obtained from the same individuals after each feeding period). There were four test feed inclusion levels (20%, 40%, 60% and 100%) and two feeding periods (1 week and 15 weeks). Formally, this is known as a 4 x 2 split-plot design with 6 replicates and repeated measures on one factor (SPF 4.2 - Kirk, 1968; pp. 245-318). The present experimental design is illustrated by Table 4.1. Each fish was weighed at the beginning of the experiment and at the end of the 15 week collection period.

4.2.2 Diets

There were 5 diets. The formulation and analysis of these appear in Table 4.2. The reference diet (diet 1) was formulated to provide, to excess, the nutrients required by tilapia for maintenance and growth. The test feed was soybean meal (soya). This feed was substituted in the reference diet at 20, 40 and 60% by weight to produce diets 2, 3 and 4 respectively. Diet 5 was soybean meal on its own, with no nutrient supplements. Chromic oxide was added to each diet, after they had been mixed, at a level of 0.5%. All diets were presented to the fish as dry pellets.

4.2.3 Feeding, and the collection of faeces

Each of the groups were fed on their respective diets at a rate of 1.0% of the total group liveweight per day. This amount was offered each day in 3 equal meals, the first at 0900, the second at 1300 and the last at 1700 h. One week after the first meal, faeces were collected from each fish for 5 d as described below. This feeding was continued

TABLE 4.1 Design of a two-factor experiment to determine the effects on digestibility of feeding period and level of test feed in a reference diet

FACTOR A (soya inclusion level)	FACTOR B (Feeding period)	
	B_1 (1 week)	B_2 (15 weeks)
A_1 (20%)	$S_1 - S_6$	$S_1 - S_6$
A_2 (40%)	$S_7 - S_{12}$	$S_7 - S_{12}$
A_3 (60%)	$S_{13} - S_{18}$	$S_{13} - S_{18}$
A_4 (100%)	$S_{19} - S_{24}$	$S_{19} - S_{24}$
Reference (0%)	$S_{25} - S_{30}$	$S_{25} - S_{30}$

The symbols $S_1 - S_{30}$ represent individual fish. All fish were of similar weight (mean $\pm$ standard deviation = 139.5 $\pm$ 15.2 g). Individuals were randomly allocated to one of 5 tanks, so that each tank contained a group of 6 fish. Four groups received the experimental diets, one diet per group, and the fifth group received a basal (or 'reference') diet. The reference diet was used to calculate the digestibility of soya in each of the four experimental diets ($A_1 - A_4$) at both feeding periods ($B_1 - B_2$) as described in Section 4.2.4.

TABLE 4.2 Formulation and analysis of reference and experimental diets containing different levels of soybean meal

Formulation

Component of experimental diet (g/100g)	DIET 1	2	3	4	5
Reference*	99.5	79.5	59.5	39.5	0
Soybean meal	0	20	40	60	99.5
Cr_2O_3	0.5	0.5	0.5	0.5	0.5

Analysis

Dry matter (%)	92.66	87.47	87.54	90.61	91.27
Crude protein (% DM)	39.25	41.00	45.35	47.06	49.69
Ether extract (% DM)	11.03	9.42	7.03	5.94	1.92
Gross energy (MJ/kg DM)	19.23	19.20	19.39	19.06	18.25
Chromium (% DM)	0.36	0.36	0.37	0.35	0.37

* Formulation of reference diet (g/100g): fishmeal, 50; dextrin, 30; α-cellulose, 6; carboxymethylcellulose, 1; cod liver oil, 3; maize oil, 5; mineral/vitamin premix, 5.

for another 15 weeks, after which faeces were again collected from each fish over a second 5 d period. The two collection periods were, therefore, separated by 15 weeks and these constituted the two levels of "feeding period" (factor B in Section 4.2.1).

Faeces were collected in chambers as described in Section 3.2.1.2 and illustrated in Figure 3.1. One hour after the 0900 h meal, each fish was transferred from its experimental tank to a separate collection chamber. Faeces were then collected by removing the collecting vessel from the immersed chamber at 5 min intervals. The contents of the collecting vessel were passed through a fine mesh and the retained faeces transferred to a dry glass petri dish. In this way, faeces were only immersed for a maximum of 5 min, in accordance with the recommendations in Chapter 3. This procedure was continued for 2 h each morning of the collection period and faeces from all 5 mornings pooled for individual fish. After 2 h in a chamber, fish were returned to their respective experimental tanks. Pooled faeces from each fish were dried to constant weight at 105 C, ground to an homogenous powder and stored over a desiccant for subsequent analyses of chromium, nitrogen and energy, using the methods described in Chapter 3.

4.2.4 Calculation of test feed digestibility

Digestible energy (DE) and digestible crude protein (DCP) coefficients were calculated for all 5 diets using the chromium ratio method described in Section 3.3.2. DE and DCP coefficients were then calculated for the soybean meal component of the experimental diet using the following formula:

$$DTF = \frac{100}{x} \left(DED - \frac{(100 - x)\ DRD}{100}\right)$$

where:

DTF = Digestibility coefficient (%) of test feed (Soya)

DED = Digestibility coefficient (%) of experimental diet

DRD = Digestibility coefficient (%) of reference diet

x = Level of inclusion of test feed in experimental diet (%)

Digestible energy (DE) and digestible crude protein (DCP) values were determined for the reference diet after both feeding periods. These measures were used as DRD values in the above formula to calculate the DE and DCP of the test feed at the corresponding feeding periods. Both measures of reference diet digestibility were the mean of 6 replicates. Digestibility values for the experimental diets (DED) were individual estimates for each fish. As such, there were 6 replicate values for soya digestibility at each of four inclusion levels. These measures were repeated at the two feeding periods, giving a total of 48 scores for soya DE and 48 scores for DCP.

The data were subjected to the split-plot factorial analysis for a design with repeated measures as described by Kirk (1968, pp. 249-251). A posteriori tests of the main effects of soya inclusion level were made with Tukey's Honestly Significant Difference Test as recommended by Kirk (1968, p. 268). This procedure tests specific differences between each feed inclusion level as an average over both levels of factor B (feeding period).

Section 4.3 Results

All diets, including the 100% soya diet, were equally well accepted by the fish and there were no mortalities throughout the course of the

16 week trial. The fat content of the experimental diets decreased and their protein content increased as soya was included at higher levels and this balanced the final gross energy values.

The mean digestible energy (DE) and digestible crude protein (DCP) values for the reference diet and four experimental diets are given, together with their standard deviations in Table 4.3. The DE and DCP of the soya component in each of the four experimental diets were calculated from these data and are also given in Table 4.3. The complete data for soya DE and DCP were subjected to statistical analyses as described in the previous section and the corresponding analysis of variance summaries are given in Tables 4.4 and 4.5 respectively.

The DE of soya was significantly affected by both test-feed level and feeding period (i.e. F-values for both the A and the B factor in Table 4.4 are significant, $p < 0.05$). DE was significantly higher when measured after 15 weeks than after 1 week ($p < 0.01$). In addition, DE decreased significantly as the level of soya was increased ($p < 0.05$). There was no interaction between the feeding period and inclusion level factors (i.e. the F-value for the AB interaction was non-significant, $p > 0.05$, Table 4.4). Thus, the effect of soya level on DE was the same at both feeding periods.

At both feeding periods, the DE of soya decreased with increasing level of inclusion in the experimental diet (Fig. 4.1). However, analysis of the main effects of factor A (test feed inclusion level) revealed that the decrease in soya DE was only significant at the 100% inclusion level. In other words, the DE of soya was not affected by its inclusion level within the range studied. It was only when tilapia were fed on soya alone that there was a significant decrease in DE.

In contrast to these results for DE, DCP was not significantly

TABLE 4.3 Apparent digestibility of experimental diets and the soya component of these diets at 4 levels of inclusion and 2 feeding periods

1. Digestibility of experimental diets

Diet	Digestible energy (%)		Digestibile crude protein (%)	
	1 week	15 weeks	1 week	15 weeks
1 Ref. diet	83.78 (1.53)	83.43 (2.71)	94.02 (0.71)	91.34 (2.08)
2 (20% soya)	82.57 (1.63)	82.73 (1.86)	93.78 (0.91)	91.45 (1.20)
3 (40% soya)	80.24 (0.62)	81.83 (1.67)	94.10 (0.37)	93.88 (0.28)
4 (60% soya)	77.52 (1.12)	80.42 (1.06)	93.96 (0.62)	92.87 (2.87)
5 (100% soya)	69.10 (2.04)	71.56 (1.88)	94.93 (0.63)	94.74 (1.95)

2. Digestibility of soya component of above diets

Soya inclusion level	Digestible energy (%)		Digestible crude protein (%)	
	1 week	15 weeks	1 week	15 weeks
20% (diet 2)	77.73 (8.15)	79.95 (9.28)	92.80 (4.55)	91.90 (6.00)
40% (diet 3)	74.81 (1.69)	78.27 (4.20)	94.04 (0.74)	97.69 (0.69)
60% (diet 4)	73.48 (1.86)	78.57 (1.76)	94.12 (1.03)	94.08 (4.80)
100% (diet 5)	69.10 (2.04)	71.56 (1.88)	94.93 (0.63)	94.74 (1.95)

Values are mean of 6 replicates ± (standard deviation)

TABLE 4.4 Analysis of variance summary for effects of soya level and feeding period on digestible energy of soya

Source of variation	Sums of squares	Degrees of freedom	Mean square	F
BETWEEN SUBJECTS	1182.7	23	-	
A (soya level)	468.5	3	156.2	4.37*
Subjects within groups (mainplot error)	714.2	20	35.7	
WITHIN SUBJECTS	363.8	24	-	
B (feeding period)	113.9	1	113.9	10.37**
A x B	30.3	3	10.1	0.92
B x Subjects within groups (sub plot error)	219.6	20	11.0	
Total	1546.5	47		

* $p < 0.05$ ** $p < 0.01$

ANALYSIS OF MAIN EFFECTS OF FACTOR A (SOYA INCLUSION LEVEL)

A_1 (20%) A_2 (40%) A_3 (60%) A_4 (100%)

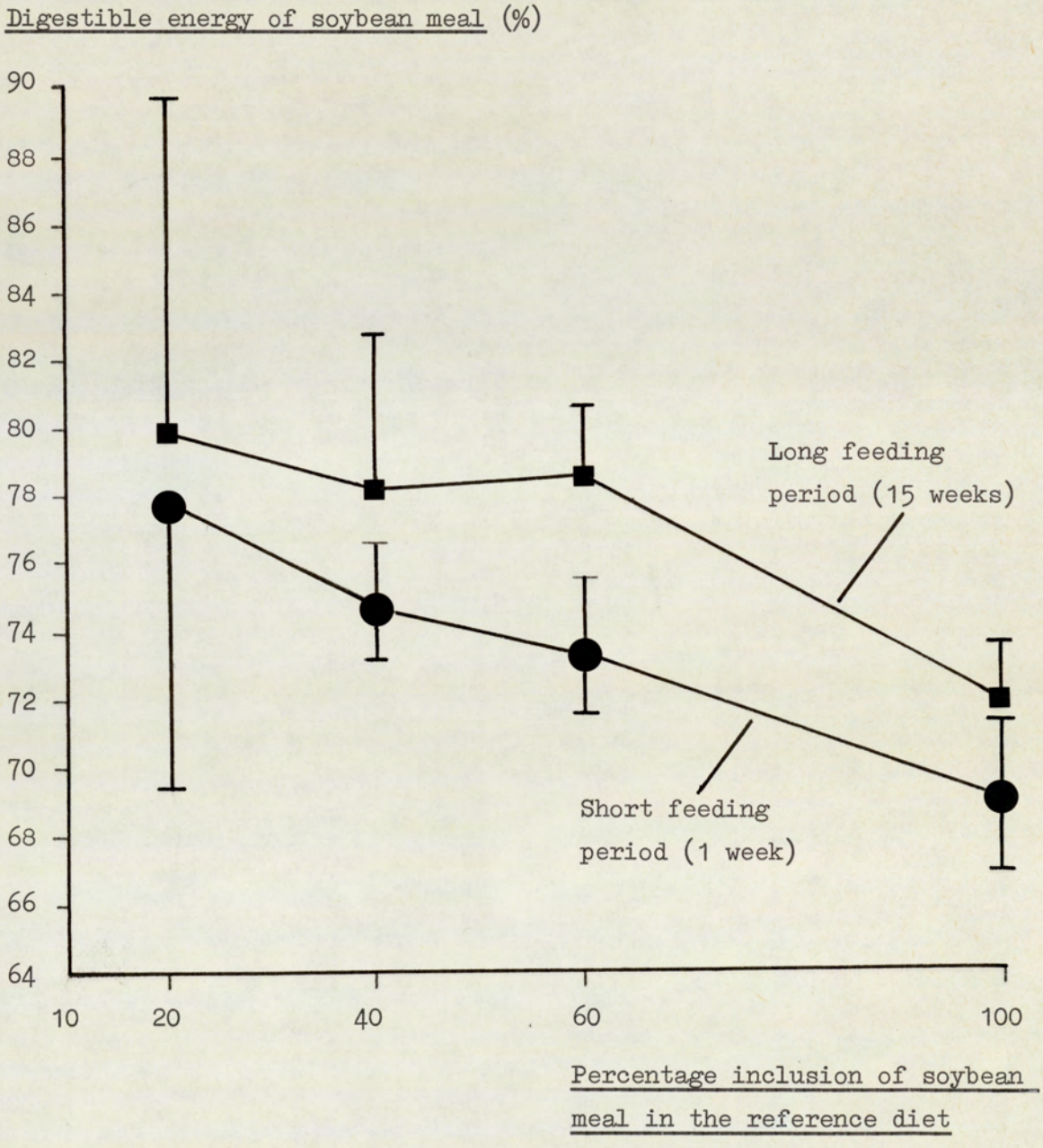
Where levels are underscored, there are no significant differences ($p > 0.05$)

TABLE 4.5 Analysis of variance summary for effects of soya level and feeding period on Digestible crude protein of soya

Source of variation	Sums of squares	Degrees of freedom	Mean square	F
BETWEEN SUBJECTS	406.9	23	-	
A (soya level)	79.1	3	26.4	1.61
Subjects within groups (main plot error)	327.8	20	16.4	
WITHIN SUBJECTS	144.4	24	-	
B (feeding period)	4.7	1	4.7	0.92
A x B	37.6	3	12.5	2.45
B x subjects within groups (sub plot error)	102.1	20	5.1	
Total	551.3	47		

Hence, there are no significant effects on DCP of factor A, factor B or the AB interaction ($p > 0.05$)

FIGURE 4.1 DIGESTIBLE ENERGY OF SOYBEAN MEAL AS A FUNCTION OF INCLUSION LEVEL IN A REFERENCE DIET AND PERIOD OF FEEDING



Data points are each the mean of 6 replicates with 95 % confidence intervals

affected by either soya level or feeding period (Table 4.5). Hence, the digestibility of soya protein was more or less the same regardless of soya inclusion level or the period over which tilapia were fed on the diets.

The standard deviations of DE and DCP values for the experimental diets were uniformly small, as shown in part 1 of Table 4.3. However, the standard deviations of DE and DCP values for the soya component of these experimental diets were noticeably larger and particularly so at the 20% inclusion level (Table 4.3, part 2).

There was very little growth of fish over the 16 week trial (Table 4.6). This was due to the low feeding rate (1.0% total liveweight d^{-1}). Specific growth rates varied from 0.02% d^{-1} for the group fed on the 60% soya diet to 0.06% d^{-1} for the group fed on the reference diet. Clearly, the quantity of feed offered daily was just above the maintenance requirements of these 130 g tilapia.

Section 4.4 Discussion

The DE of soya was clearly affected by both dietary formulation and feeding period. With respect to dietary formulation, the DE of soya was significantly greater when it was given to tilapia as a component of a complete diet, than when it was given on its own. The reason for this is not clear, but it may be that the process of digestion is more efficient in tilapia which are fed on a nutritionally balanced diet. Soybean meal is a high quality protein source and is used widely as a component of many animal diets. However on its own, it is deficient in certain minerals and vitamins (notably, the B-vitamins), it contains sub-optimal levels of methionine and cystine and the solvent extracted

TABLE 4.6 Growth of tilapia throughout the 16 week digestibility trial with soybean based diets

Diet	Initial weight (g)	Final weight (g)	Specific growth rate (% d^{-1})
1 Ref. diet	133.68 (14.6)	143.70 (20.31)	0.06
2 (20% soya)	132.20 (17.26)	136.74 (14.44)	0.03
3 (40% soya)	132.29 (15.76)	138.40 (17.60)	0.04
4 (60% soya)	131.08 (15.30)	133.67 (24.30)	0.02
5 (100% soya)	125.82 (17.1)	129.70 (21.80)	0.03

Values are means of 6 fish $\pm$ (standard deviation)

meal is low in fat. In the present experiment, these deficiencies did not influence voluntary intake (the 100% soya diet was accepted as readily as the 0% (reference) diet), but they clearly affected the energy value of unsupplemented soya. The practical implications of this result are twofold. Firstly, the energy value and hence, the economic value of soya can be increased by the simple expedient of making good its nutrient dificiencies. Secondly, when DE values are published for any feed, they should be reported along with details of the reference diet and the test feed substitution level. Ideally, the digestibility of a novel feed should always be evaluated in a standard reference diet at a standard inclusion level. This approach would then allow more accurate comparison of values between laboratories and provide feed manufacturers with information on the energy value of a feed at a given dietary inclusion level.

The actual level of soya in an experimental diet does not appear to be important as long as the reference diet portion supplies enough nutrients to satisfy the requirements of the test animal. Thus, in the present experiment, there were no significant differences between soya DE's over the substitution range 20-60%*. This shows that there was no significant interaction between soya and the reference diet within this range. However, the reduction in soya DE with increasing substitution level was continuous (Fig. 4.1) and there must be a point at a level greater than 60%, where the soya DE is reduced to a level that is

* This was partly due to the type of statistical analysis used, because the split-plot design sacrifices accuracy on the non-repeated factor (inclusion level) in favour of sensitivity on the other, repeated measures factor (feeding period).

statistically indistinguishable from the 100% level. This level would then indicate the minimum level of nutrient supplementation necessary to cause a significant increase in the DE of soya and this could profitably form the basis of future work.

With respect to feeding period, all estimates of soya DE were significantly greater after 15 weeks than after 1 week ($p < 0.01$), indicating digestive adaptation to diet. This adaptation was clearly independent of fish size, because there was no significant growth throughout the digestibility trial (Table 4.6). It is possible that this increase in DE with time was due to a diet induced increase in the activity of specific digestive enzymes as reported by Nagase (1964). However, it is not known when adaptation occurred because only two feeding periods were studied. It is necessary to determine DE over a greater range of feeding periods to illustrate more closely how digestibility changes with time. At present, the only other data available on the subject are for rainbow trout, in which it was shown that complete adaptation to diet occurs after 3 d (Noue et al, 1980). However, these authors only conducted their digestibility trial for 20 d and so did not demonstrate if there was any adaptation to diet subsequent to this period. The present digestibility trial was over 5 times longer than that of Noue et al (1980) and the results indicate that adaptation to diet may be relatively long-term.

To the fish culturist, the most meaningful period over which to measure digestibility is that which corresponds to the normal growing period under practical husbandry conditions. For intensive tilapia farms, this is typically 3 - 5 months but because it is not practical to run routine digestibility trials over such a long time, much shorter periods are frequently used in feed evaluation programs (Section

4.1). Whilst this is acceptable from a practical viewpoint, the present work shows that it is essential to employ a standard feeding period when measuring the digestibility of a set of feeds. In general this requirement is not satisfied in the literature and this can complicate comparison of DE values between laboratories. In future, when DE values are to be used to formulate practical diets, greater attention should be given to the feeding period utilised by different authors. Ideally, digestibility trials for all fish feeds should be conducted over a standard feeding period.

The foregoing discussion applies only to DE values. DCP, by contrast, was not affected by feeding period or soya level (Table 4.5). One explanation is that the digestion of protein by fish is more efficient than the digestion of other (energy-yielding) nutrients and as evidence for this, DCP values were consistently higher and less variable than DE (Table 4.3, part 2). Where the digestive physiology of an animal is well adapted to the breakdown and absorption of protein, this implies that protein digestibility is relatively unaffected by external factors such as diet formulation and this was observed here. Similarly, where the efficiency of protein digestion is already high, further adaptation is pre-empted and this was also observed here; DCP values were constant over the entire 15 week digestibility trial.

The results here are for soybean meal and they may, of course, not apply to other feeds. In particular, the DE and DCP of high fibre, low protein feeds may be influenced more by reference diet formulation and test feed substitution level and this should be checked in routine digestibility trials.

DE is gaining acceptance as an energy term by manufacturers of fish feed, because unlike ME, it is relatively unaffected by feeding rate as

long as the maintenance requirements are satisfied (Cho et al, 1982). However, this chapter has shown that the DE of feeds can be significantly affected by the methods used in their evaluation. To reduce these effects, DE should be determined at a standard test feed substitution level and over a standard feeding period. The feeding period should be long enough to enable adequate adaptation to diet, but short enough to be practical. The test-feed substitution level should be close to the practical inclusion level in production diets, but otherwise as high as possible. This is because the variation between replicate measurements of test feed digestibility is smaller at high inclusion levels (Table 4.3, part 2). The reason for this is simply that variation between replicate estimates of test-feed digestibility is magnified by proportional calculation from digestibility data for experimental diets. The lower the test-feed inclusion level, the greater the variation between replicates.

To accommodate the above recommendations, all subsequent digestibility trials in this thesis were conducted over a standard period of 4 weeks and at a test-feed substitution level of 60%.

CHAPTER 5

THE DIGESTIBILITY OF A SERIES OF FEEDS

Section 5.1 Introduction

The experiments reported in the two previous chapters examined some of the conditions affecting digestibility determination with tilapia. The conclusion was that digestibility trials must be conducted under standard conditions if digestibility coefficients are to be compared between different feeds. Such standard conditions are applied in the present chapter to a series of experiments in which the dry-matter (DMD), protein (DCP) and energy (DE) digestibilities of seventeen feeds are evaluated.

The evaluation program incorporated two specific aims. The first was to assess the range in digestibility values of feeds for tilapia and to compare these with values for other animals. The second was to provide a data base for the predictive modelling of digestibility on the basis of the chemical composition of feeds. To provide an informative set of data, the feeds selected for evaluation vary widely in their fibre, protein and fat content, although they are all representative of the range of ingredients currently used in tilapia diets.

Section 5.2 Methods

5.2.1 Diets

Seventeen feeds were evaluated as listed in Table 5.3 and Appendix 3. Thirteen were of plant origin, three were animal-product meals and

one was a semi-purified compounded ration which served as a reference diet. Each feed was evaluated by substitution in the reference diet at a level of 60%.

The formulation of the reference diet, the proximate composition and energy values of the feeds and the analysis of experimental diets are given in Tables 5.2, 5.3 and 5.4 respectively. Proximate analyses were performed as described in Section 2.3.2 and energy was determined in an adiabatic bomb calorimeter. Dry pellets (4.0 mm diameter) were prepared as described in Section 2.3.1 (2 kg of each diet) and stored in plastic, airtight containers at normal room temperature.

5.2.2 Fish

Four hundred Oreochromis niloticus fry were grown-on to a weight of approximately 50 g each. Males were separated from females in the growing stock as soon as they could be identified and grown-on in separate tanks. For the digestibility trials, each of fifteen experimental tanks were stocked with 12 males. Throughout the trials all groups had a similar mean weight and standard deviation. The range of group means was from 44.39 $\pm$ 13.36 g (the smallest group) to 49.99 $\pm$ 15.27 g (the largest group). All groups were fed on a commercial diet (Ewos-Baker Ltd., Lothian, UK) for 7 d before the start of a digestibility trial.

5.2.3 Experimental procedure

The reference diet, diet 3 and diet 14 were fed to 6 replicate groups of fish. All other diets were fed to 3 replicate groups according to the design in Table 5.1.

TABLE 5.1 Allocation of tanks and diets in digestibility trials

Tank	DIET CODE			
	Trial 1	Trial 2	Trial 3	Trial 4
1	4	1	7	16
2	4	1	7	16
3	4	1	7	16
4	8	2	9	17
5	8	2	9	17
6	8	2	9	17
7	11	3	10	17
8	11	3	10	17
9	11	3	10	17
10	14	5	12	3
11	14	5	12	3
12	14	5	12	3
13	15	6	13	14
14	15	6	13	14
15	15	6	13	14

To prevent transfer (or "carryover") effects between diets, each digestibility trial was separated by a period of 7 d, during which groups were maintained on a commercial trout diet. In addition, at the end of each trial, fish were randomly relocated between the 15 tanks to eliminate any group dependent effects between trials.

The amount of dry diet given to each group was 2% d^{-1} of the total group liveweight. This was presented in 3 equal portions at 0900, 1300 and 1700 h. Each diet was offered for a standard period of 4 weeks. 1 h after the morning feed on the 28th day of feeding, the water flow to each tank was stopped, faeces were siphoned from tanks, separated from water on a fine nylon guaze and transferred to a labelled, glass petri

dish. The delay of 1 h between feeding and collecting the first samples of faeces was sufficient to prevent contamination with excess food since any uneaten pellets were removed from the self-cleaning tanks in the effluent water during this period. To minimise leaching from the faeces, these were siphoned from each tank every 5 min. Collection was continued in this way for 2 h, after which faecal production diminished. Petri dishes were transferred to a drying oven, kept at 105 C for 20 h and then cooled in a desiccator. Dried faeces were subsequently ground with a mortar and pestle, sealed in airtight LP3 tubes (Luckham Ltd., Burgess Hill, UK) and stored over a desiccant until required for analysis.

Typically, 150 - 300 mg of dry-matter faeces were collected from each group within 2 h, depending on diet, and this was sufficient for duplicate analyses of energy, nitrogen and chromium. Faecal energy was determined by oxidation with dichromate, chromium by AAS and nitrogen by the dichloroisocyanurate reaction as described in Chapter 3. Dry-matter (DMD), protein (DCP) and energy (DE) digestibilities were calculated for the reference diet and for all experimental diets using the formulae given in Chapter 3. The digestibilities of individual feeds were then estimated by proportional calculation as described in Chapter 4.

Section 5.3 Results

The proximate analyses of individual feeds compared well with accepted values for these materials (Table 5.3). The exceptions were rice bran, which was lower in fat and higher in fibre than expected and meat and bone meal which was lower in protein and higher in fat and ash

TABLE 5.2 Formulation of the reference diet

	g/100 g	g/100 g in final ration
Fishmeal	75.00	30.00
Corn oil	11.25	4.50
Cod liver oil	5.00	2.00
Mineral/vitamin premix	7.50	3.00
Chromic oxide	1.25	0.50

When the reference diet was fed on its own to fish, the formulation was adjusted to provide the same concentration of chromium as the final experimental diets thus:

	g/100 g
Fishmeal	75.00
Corn oil	11.25
Cod liver oil	5.00
Mineral/vitamin premix	7.50
Chromic oxide	0.50
Carboxymethylcellulose	0.75

Proximate analysis appears in Table 5.4

TABLE 5.3 Proximate analysis of Feeds

Feed	Dry* matter (%)	Gross Energy* (MJ/kg DM)	As % of dry matter				
			CP	CF	ASH	EE	NFE1
1 Soybean	90.47	19.63	48.84	7.62	6.60	2.12	34.82
2 Ground maize	88.90	18.59	10.11	1.52	1.42	4.21	82.74
3 Wheat middlings	89.21	18.81	17.48	7.15	4.63	4.07	66.67
4 Wheat bran	87.75	18.49	17.57	9.99	6.41	2.72	63.31
5 Cassava	89.72	16.77	3.08	4.27	6.06	0.96	85.63
6 Sunflower	90.59	19.57	31.07	28.33	6.44	2.23	31.93
7 Rice bran	89.11	16.02	14.14	20.29	18.97	0.55	46.05
8 Sorghum	85.62	17.21	12.04	1.75	1.82	3.31	81.08
9 Wheat grain	86.21	18.07	11.52	2.15	1.66	1.67	85.15
10 Copra	88.84	18.84	22.82	14.83	6.10	7.83	48.37
11 Groundnut	93.09	21.51	54.44	3.72	4.65	10.29	26.90
12 Rapeseed	89.24	19.56	39.95	13.36	8.24	2.30	36.15
13 Palm kernel	89.29	20.40	19.25	17.74	4.19	12.27	46.55
14 Meat and bone	95.81	14.07	44.88	ND	43.11	13.17	0
15 Poultry by-product	86.26	18.83	71.26	ND	21.49	6.47	0
16 Fishmeal	92.54	19.17	75.63	ND	18.86	7.46	0

* Mean of triplicate analysis. All other measures are mean of duplicates.
ND = Not Detected.
International feed numbers for each of the above feeds are given in Appendix 3.

CP = Crude protein
CF = Crude fibre
EE = Ether extract
NFE 1 = Nitrogen-free extract: 100 - (% CP + % EE + % Ash + % CF)

TABLE 5.4 Proximate analysis of experimental diets incorporating a series of plant and animal-product feeds

Diet	Dry matter (%)	Gross Energy (MJ/kg DM)	As % of dry matter		
			CP	EE	Chromium*
1 (Soybean)	93.55	20.40	41.11	11.38	0.33
2 (Ground maize)	91.71	20.76	30.48	11.99	0.35
3 (Wheat middlings)	95.38	20.31	29.56	11.22	0.34
4 (Wheat bran)	95.58	20.22	28.94	11.00	0.35
5 (Cassava)	96.56	19.02	22.05	10.32	0.34
6 (Sunflower)	95.09	20.68	34.97	10.38	0.32
7 (Rice bran)	95.65	19.15	28.18	10.10	0.30
8 (Sorghum)	91.27	20.75	31.05	12.41	0.33
9 (Wheat grain)	95.56	20.30	27.65	10.66	0.32
10 (Copra)	94.56	20.51	31.82	13.90	0.31
11 (Groundnut)	91.49	22.30	45.01	13.69	0.31
12 (Rapeseed)	95.14	20.93	45.64	11.15	0.32
13 (Palm kernel)	95.36	21.36	29.40	16.08	0.32
14 (Meat and bone)	97.24	17.38	44.97	16.74	0.32
15 (Poultry)	96.72	20.32	55.97	13.32	0.35
16 (Fishmeal)	97.01	20.66	61.37	14.29	0.35
17 (REFERENCE)	98.54	22.13	47.19	24.91	0.33

All analyses in triplicate.
* = Cr. To estimate Cr_2O_3, divide these values by 0.684

CP = crude protein
EE = ether extract

than usually reported. Proximate analyses of experimental diets (Table 5.4) revealed, as expected from their formulation, a wide range of protein (22 - 61% DM) and fat (10 - 25% DM). This is, of course, a reflection of the variation in composition between individual test feeds in the different diets. The chromium content of diets was constant; the average dry-matter level was 0.33% (= 0.48% Cr_2O_3) and this is acceptably close to the formulated level of 0.50% Cr_2O_3.

The stability of most pellets was high. The exceptions were diets containing animal-product meals and palm kernel meal. A binder could have been added to improve stability in these cases, but this option was rejected because it would have caused unacceptable variability in diet formulation. Even with no binder, the diets which formed unstable pellets were sufficiently water stable for there to have been no significant leaching in the short time these diets were exposed to water before being ingested by the fish (max. exposure time 1 min).

Faeces production appeared to be stimulated after fish consumed a meal. Noticeable amounts of faeces appeared in tanks about 30 min after fish had eaten, and the maximum production rate usually occurred about 2 h later. The faeces from most diets appeared as discrete, intact "strings"; a property which enabled them to be siphoned as individual units. The exception was faeces produced from the palm kernel meal diet. Often, faeces from this diet broke up in the water within the 5 min collecting period. More faeces were produced from diets containing high levels of fibre than those with low levels or no fibre. Thus, up to 3 times more dry matter faeces were produced by fish which had eaten the brans, sunflower or copra than fish which had eaten animal product meals, maize, wheat grain, sorghum or cassava.

Mean chromium values in the faeces ranged from 0.39% DM for the

palm kernel diet to 1.13% DM for the groundnut diet. Protein (N x 6.25) in the faeces ranged from 5.7% for the sunflower diet to 22.7% for the poultry by-product meal, whilst faecal energy ranged from 6.1 MJ/kg DM for the fishmeal diet to 15.6 MJ/kg DM for the sunflower meal.

Digestibility values for experimental diets and test feeds appear in Tables 5.5 to 5.8. Dry matter digestibilities (DMD) for the test feeds covered a wide range, from 16.0% for meat and bone meal to 79.5% for groundnut meal. DCP followed a similar trend; this was lowest for meat and bone meal (56.9%) and highest for groundnut meal (93.4%). DE was lowest for palm kernel meal (8.2% of GE, 1.7 MJ/kg DM) and highest for fishmeal (84.5%, 16.2 MJ/kg DM). Of the cereals and cereal by-products evaluated, rice bran had the lowest DE (31%, 5.0 MJ/kg DM) and wheat grain the highest (73.6%, 13.3 MJ/kg DM). For the oilseed cakes and meals, sunflower had the lowest energy value (3.6 MJ/kg DM) and groundnut the highest (17.9 MJ/kg DM). Thus, the range in DE is greater within the oilseeds than in the cereals evaluated. Of the animal product meals, fishmeal had the highest and meat and bone meal the lowest digestibility. Only one root was evaluated (cassava) and this was well digested (DMD = 74.0%; DE = 80.0%, 13.4 MJ/kg DM). Even though cassava contains only a small amount of protein (3.1% DM), this fraction of the feed was also well absorbed (DCP = 75.8%).

For most diets, the variation between replicate digestibility trials was low (Tables 5.5 and 5.6). The standard deviation of digestibility coefficients are, in general, of the same order as published values for trout and pigs (Table 5.5; Smith et al, 1980; Morgan et al, 1975a). For the highly digestible feeds (soya, cassava, groundnut), the agreement between replicates is better than usually

TABLE 5.5 Apparent digestibility of experimental diets

percentage coefficients

Diet	Digestible energy DE (%)	Digestible crude protein DCP (%)	Dry-matter digestibility DMD (%)
1 (Soybean)	79.2 ± 0.8	91.0 ± 0.5	64.0 ± 1.2
2 (Ground maize)	77.5 ± 1.2	87.6 ± 1.5	64.5 ± 1.1
3 (Wheat middlings)*	68.4 ± 1.8	86.8 ± 0.5	55.7 ± 2.1
4 (Wheat bran)	54.9 ± 1.7	81.1 ± 1.4	43.3 ± 0.7
5 (Cassava)	83.2 ± 0.7	81.2 ± 0.1	69.4 ± 1.0
6 (Sunflower)	46.3 ± 1.6	88.5 ± 0.8	28.4 ± 3.2
7 (Rice bran)	56.0 ± 2.9	79.9 ± 1.9	35.9 ± 2.9
8 (Sorghum)	73.4 ± 2.1	87.8 ± 1.7	66.0 ± 3.0
9 (Wheat grain)	79.4 ± 2.8	88.5 ± 2.4	71.1 ± 2.4
10 (Copra)	56.0 ± 4.2	86.7 ± 3.6	34.4 ± 7.8
11 (Groundnut)	85.2 ± 1.8	91.7 ± 1.7	72.6 ± 1.9
12 (Rapeseed)	70.9 ± 2.9	81.4 ± 1.7	44.7 ± 5.1
13 (Palm kernel)	40.3 ± 1.7	80.4 ± 0.7	18.4 ± 3.7
14 (Meat and bone)*	75.0 ± 1.5	71.3 ± 4.1	34.5 ± 9.2
15 (Poultry)	67.1 ± 3.4	74.9 ± 1.4	38.3 ± 4.6
16 (Fishmeal)	85.9 ± 3.2	89.0 ± 1.8	52.5 ± 2.3
REFERENCE*	88.0 ± 1.7	89.3 ± 1.7	62.4 ± 2.2

mean values ± standard deviation.

* n = 6.

TABLE 5.6 Apparent digestibility of experimental diets

Digestible nutrient levels

Diet	Digestible energy (DE) (MJ/kg DM)	Digestible crude protein (DCP)[1] (g/Kg DM)	Dry-matter digestibility[2] (DMD) (g/Kg DM)
1 (Soybean)	16.2 ± 0.2	374.1 ± 2.1	640.3 ± 12.1
2 (Ground maize)	16.1 ± 0.3	267.0 ± 4.6	644.6 ± 11.0
3 (Wheat middlings)*	13.9 ± 0.4	256.5 ± 1.5	556.6 ± 20.8
4 (Wheat bran)	11.1 ± 0.4	234.7 ± 3.9	433.0 ± 7.0
5 (Cassava)	15.8 ± 0.1	179.0 ± 0.2	694.0 ± 9.9
6 (Sunflower)	9.6 ± 0.3	309.4 ± 2.9	283.9 ± 32.0
7 (Rice bran)	10.7 ± 0.6	225.2 ± 5.2	359.0 ± 28.5
8 (Sorghum)	15.2 ± 0.4	269.4 ± 5.2	659.7 ± 29.5
9 (Wheat grain)	16.1 ± 0.6	244.7 ± 6.7	710.6 ± 23.8
10 (Copra)	11.5 ± 0.9	275.8 ± 11.4	343.7 ± 78.3
11 (Groundnut)	19.0 ± 0.4	412.9 ± 7.7	726.3 ± 18.5
12 (Rapeseed)	14.9 ± 0.6	371.6 ± 7.7	447.0 ± 50.9
13 (Palm kernel)	8.6 ± 0.4	236.4 ± 2.1	183.7 ± 37.2
14 (Meat and bone)*	13.0 ± 0.3	320.4 ± 18.8	345.3 ± 92.0
15 (Poultry)	13.7 ± 0.7	418.7 ± 7.9	382.8 ± 45.5
16 (Fishmeal)	17.8 ± 0.7	545.9 ± 10 9	525.3 ± 22.9
REFERENCE*	19.5 ± 0.4	421.6 ± 8.2	623.8 ± 22.0

Mean values ± standard deviation.
*n = 6

1 = g digestible protein kg^{-1} dry diet
2 = g digestible dry-matter kg^{-1} diet

TABLE 5.7 Apparent digestibility of individual feeds

Percentage coefficients

Feeds	Digestible energy DE (%)	Digestible crude protein DCP (%)	Dry-matter Digestibility DMD (%)
Soybean	73.3 ± 1.4	92.1 ± 0.8	65.1 ± 2.0
Maize	70.5 ± 2.0	86.5 ± 2.5	65.9 ± 1.8
Wheat middlings	55.4 ± 3.1	85.1 ± 0 9	51.2 ± 3.5
Wheat bran	32.9 ± 2.8	75.6 ± 2.3	30.6 ± 1.2
Cassava	80.0 ± 1.2	75.8 ± 0.1	74.1 ± 1.7
Sunflower	18.5 ± 2.7	87.9 ± 1.4	8.8 ± 0.1
Rice bran	31.4 ± 3.3	73.7 ± 3.1	18.3 ± 4.8
Sorghum	72.0 ± 3.4	85.0 ± 2.8	68.4 ± 4.9
Wheat grain	73.6 ± 4.6	88.0 ± 4.0	76.9 ± 4.0
Copra	34.7 ± 7.0	84.9 ± 6.0	15.7 ± 13.1
Groundnut	83.3 ± 2.9	93.4 ± 2.9	79.5 ± 3.1
Rapeseed	54.2 ± 6.9	76.2 ± 2.8	32.9 ± 8.5
Palm kernel	8.2 ± 2.7	74.5 ± 1.2	-
Meat and bone	64.7 ± 5.4	56.9 ± 10.8	16.0 ± 8.3
Poultry by-product	53.2 ± 5.7	65.2 ± 2.4	22.2 ± 6.2
Fishmeal	84.5 ± 5.4	88.7 ± 3.0	46.0 ± 3.8

Mean values ± standard deviation.

TABLE 5.8 Apparent digestibility of individual feeds digestible nutrient levels

Feeds	Digestible energy (DE) (MJ/kg DM)	Digestible crude protein (DCP) (g/kg DM)	Dry-matter digestibility (DMD) (g/kg DM)
Soybean	14.4 ± 0.3	449.9 ± 4.0	651.4 ± 20.2
Maize	13.1 ± 0.4	87.4 ± 2.5	658.6 ± 18.3
Wheat middlings	10.4 ± 0.6	148.7 ± 1.5	511.9 ± 34.7
Wheat bran	6.1 ± 0.5	132.9 ± 4.0	305.8 ± 11.7
Cassava	13.4 ± 0.2	23.3 ± 0.1	740.9 ± 16.5
Sunflower	3.6 ± 0.5	273.2 ± 4.4	88.4 ± 1.2
Rice bran	5.0 ± 0.5	104.2 ± 4.4	182.5 ± 47.5
Sorghum	12.4 ± 0.6	102.4 ± 3.4	683.6 ± 49.3
Wheat grain	13.3 ± 0.8	101.2 ± 4.6	768.6 ± 39.6
Copra	6.5 ± 1.3	194.2 ± 13.6	156.9 ± 130.5
Groundnut	17.9 ± 0.6	508.2 ± 15.5	794.7 ± 30.8
Rapeseed	10.6 ± 1.4	304.2 ± 11.3	329.2 ± 84.9
Palm kernel	1.7 ± 0.6	143.4 ± 2.3	-
Meat and bone	9.1 ± 0.4	255.2 ± 48.6	160.2 ± 83.3
Poultry by-product	10.0 ± 1.1	464.8 ± 16.7	222.4 ± 62.1
Fishmeal	16.2 ± 1.0	671.0 ± 22.4	460.0 ± 38.2

Mean values ± standard deviation

1 = g digestible protein kg^{-1} feed.

2 = g digestible dry-matter kg^{-1} feed.

expected in digestibility studies. For the more indigestible (high fibre) and animal-product meal diets, the reproducibility of the digestibility assay is slightly poorer, but agreement between replicates is still within acceptable limits. The DMD of the reference diet was 62.4% with a standard deviation of 2.2%. Eight of the diets had higher and eight had lower standard deviations than this and so there was no indication that the reference diet assay was more or less reproducible than assays for the other diets. When 6 replicates were used instead of 3, the standard deviation of digestibility coefficients was not noticeably smaller.

The only feed for which the digestibility value was suspect was palm kernel meal. The digestibility of the experimental diet was so low that digestibility values for this meal were less than zero when estimated by proportional calculation. Possible reasons for this result are considered in the next section.

Section 5.4 Discussion

The digestibility of all feeds was determined by the substitution method. It has already been shown that this method provides more meaningful figures than direct determination in which digestibility is evaluated in single feeds with no nutrient supplements (Chapter 4). To evaluate feeds using the substitution method, there are two alternative types of formulation. The first is to formulate diets to constant nutrient levels by adjusting the proportions of nutrients in a supplement. The second is to formulate a standard reference diet which on its own provides all essential nutrients to excess, and to substitute the test feed in this at a constant level.

Both options were considered but the first was rejected for the following reasons. To formulate diets to set nutrient levels, some authors group feeds into classes on the basis of similarity of proximate composition (e.g. Morgan et al, 1975 a). For each class, a reference diet is then formulated which, when mixed with feeds within a class, produces diets that are more or less isonitrogenous and isoenergetic. This was attempted in the present work. Three feed classes were considered: "energy feeds" (wheat, maize, cassava, sorghum), "high fibre feeds" (wheat bran, rice bran, sunflower, palm kernel) and "protein concentrates" (meat and bone, poultry by-product, fishmeal). For each of these three classes, a reference diet was formulated which, when mixed with the test feeds, produced experimental diets to given nutrient specifications. Using linear programming techniques, many alternative formulations were attempted, but the "best average" reference diet for each class still produced diets which varied widely in nutrient levels. This was due to the fact that even within a feed class, the proximate composition of the feed varied widely. The only way to formulate all diets to set nutrient levels would have been to prepare a reference diet (or supplement) for each of the 16 test feeds. This option was rejected because it would require the evaluation of 16 reference diets in addition to 16 test feed/reference diet mixtures, and because of the unquantified effects of the different reference diets on the digestibility of each test feed.

The constant level substitution method used in this research has the advantage that digestibility is not confounded with test feed level between diets. This is important, because it is not known if the digestibility of the feeds in this study change with inclusion level. Chapter 4 showed that for soybean meal, there is no change, but for high

fibre feeds this may not be the case (Chapter 8). All digestibility values in this chapter are for feeds at 60% of the same reference diet and this provides an acceptable basis for comparisons between feeds. The 60% level was used because digestibility is measured more accurately at high inclusion levels and because, with soybean at least, digestibility does not change with inclusion level up to this limit (Chapter 4).

As indicated in Section 5.3, the digestibility of palm kernel meal is unusually low. One explanation for this could be interaction between palm kernel meal and the reference diet. The high proportion of fibre in the experimental diet containing palm kernel meal may have depressed the digestibility of the reference diet component of the experimental diet. Such synergistic interaction between the feed and reference diet could, in this case, be mediated by a reduction in gut passage time (GPT). If fibre reduces GPT (as discussed in Chapter 8), the digestibility of the reference diet would be less when it is mixed with palm kernel meal than when it is fed to fish on its own. If so, the assumption that there is no interaction between test feed and reference diet is invalid. However, the other high fibre feeds evaluated (sunflower, rice bran, copra) did not affect the digestibility of their respective experimental diets in the same way as palm kernel meal and it is unlikely that fibre level per se accounts for the spurious result with this feed. It is more likely that the exceptionally low digestibility of palm kernel meal is an artefact; the result of poorly bound faecal strings and subsequent loss of material on siphoning.

For the other feeds, the substitution method was taken to be a valid procedure and faeces were collected intact. All experimental

diets contained sufficient levels of protein, fat, minerals and vitamins for growth (Tables 5.2, 5.4) and the only source of fibre was from the test feeds. The difference in digestibility between the reference diet and the experimental diet can, therefore, be directly attributed to the test feed. The reproducibility of the assay was good, as discussed in the previous section. When 6 replicates were used, the standard deviations of mean digestibility values were similar to that when 3 replicates were used and so there was no obvious increase in accuracy by repeating the assay more than 3 times.

Within the series of feeds evaluated, protein digestibility (DCP) varied from 56.9% to 93.4%. Digestible energy (DE) and dry-matter digestibility (DMD) had even wider ranges and the two appeared to be related. This relationship is explained by the fact that the energy value of a feed is directly related to the amount of organic matter that is absorbed across the gut. DE ranged from 18.5% to 84.5% of gross energy (not including the low value for palm kernel meal), whilst DMD ranged from 8.8% to 79.5%. Clearly, the digestibility of feeds for tilapia is so variable that it is difficult to make generalisations concerning the amount of energy or protein available in different classes of feed. The only useful qualitative observation is the higher the fibre content of a feed, the lower its digestibility. This trend is examined more closely in the next chapter and more complex relationships between digestibility and feed composition are investigated.

The range of DE was greater in the oilseeds than in the cereals. This was probably because the range of protein and fat (the major source of dietary energy for fish) was greater in the oilseeds evaluated than in the cereals. The use of carbohydrate as an energy source by tilapia

is examined in Chapter 8, but it is of interest here to note the high digestibility of cassava. This feed has a nitrogen-free extract value of over 85%, yet up to 80% of the gross energy of cassava was absorbed by tilapia. Other high carbohydrate feeds (maize, wheat grain, sorghum) also had high digestible energy values, and whilst this observation on its own gives no indication of the value of carbohydrates as energy sources in tilapia nutrition, it does at least show that this class of nutrients can be well digested and absorbed.

Of the three animal-product meals evaluated, fishmeal had the highest digestibility. DE and DCP values for this feed were superior to those for meat and bone and poultry by-product meals. Fishmeal is, traditionally, one of the highest quality sources of protein in compounded diets for a wide variety of livestock, both terrestrial and aquatic. Not only does fishmeal have a good amino acid profile (none of the essential amino acids for fish are lacking), but for an animal-product meal it is relatively low in ash. The lower digestibility of meat and bone and poultry by-product meals can be ascribed to their higher ash content; for the former this was 43.1% and for the latter, 21.5%. Most of this ash represents the mineral components of bones and has no energetic value. For meat and bone meal, this means that nearly half of the material has no energetic value. In addition, only small amounts of the ash fraction are absorbed, and this explains the low DMD of meat and bone meal (= 16%). The high fibre plant feeds (sunflower, rice bran and copra) had similar DMD values to meat and bone meal but the DE's of these roughages were always lower (Table 5.7). Ash and fibre are equally indigestible feed fractions and so the only obvious explanation for the greater availability of energy in meat and bone meal is the relatively high amount of fat in this feed. Fats are more

digestible than carbohydrates in fish (NRC, 1977), and this explains why the DE's of the high carbohydrate feeds, copra, sunflower and rice bran were lower than the high-fat meat and bone meal.

It is common for meat and bone meal to have ash values of about 30% (NRC, 1977; Gohl, 1981). Higher values indicate that a high proportion of bones have been included with the animal carcasses in the preparation of the meal. The meat and bone meal sample used in this study was clearly of low quality. It had a lower protein content, a higher level of fat and over 50% more ash than other samples of feed described as meat and bone meal. Such variation between samples of the same feed must be considered when reporting digestibility values. Variation in the levels of protein, carbohydrate, fat and indigestible feed fractions between different batches of the same feed affect digestibility and so values in the literature should be treated as estimates rather than absolutes. This proviso also applies to the present research. The digestibility values for tilapia should be considered as representative of the different feeds and not as definitive values. The data in Tables 5.7 and 5.8 are considered accurate for the feeds evaluated, but before these values are used in diet formulation, the proximate composition of new feed samples should be compared with that of the relevant feed in Table 5.3.

The composition of most of the feeds evaluated in this research compare well with accepted values for these materials (Table 5.3; Gohl, 1981; NRC, 1977). Any slight variation between the values in Table 5.3 and other published data reflect differences in feed processing conditions and with the plant-product meals, the additional effects of strain variation, soil, climate and fertilisation (Van Soest, 1976). The only notable deviations from accepted values are meat and bone meal

as already discussed and rice bran. The rice bran sample had a fibre content of over 20% and only 0.6% fat. Although not stated by the feed supplier, it is evident from this that the sample was solvent extracted. Gohl (1981) states that pure rice bran should have a crude fibre value of 10 - 15%. Clearly, the bran in this study was adulterated with husks and is of poor quality, intermediate between bran and "rice mill feed". The digestibility of this sample is, therefore, possibly lower than would be expected for a more typical sample of rice bran with a lower fibre content. For all other feeds, the digestibility values in Tables 5.7 and 5.8 may be taken as representative.

As far as is known, the values in Tables 5.7 and 5.8 are the only published data at present on the digestibility of these feeds for tilapia. In contrast, there is a very extensive literature on the digestibility of feeds for terrestrial livestock and a modest amount of data for salmonid fish (NRC, 1973; 1981). The only significant collection of digestibility data for warmwater fish appears in the National Research Council (USA) publications "The Nutrient Requirements of Warmwater Fishes" (NRC, 1977, 1983). However, even in these summary volumes, there is only a limited amount of digestibility data and most are for channel catfish, Ictalurus punctatus. The only energy terms in these publications are metabolisable energy (ME) values for poultry; the suggestion being that these figures can be used to balance dietary energy levels for warmwater fish. However, if the present data for tilapia are compared with the values for poultry notable differences are apparent (Tables 5.9 and 5.10).

Poultry ME's are lower than the present DE values for tilapia with respect to the high-protein feeds meat and bone meal, fishmeal, soya and

TABLE 5.9 Energy values (MJ/kg DM) of feeds for fish, pigs and poultry

FEED	TILAPIA(DE)	CATFISH(DE)	TROUT(DE)			PIGS(DE)			POULTRY(ME)
	A	B	C	D	E	B	F	G	G
Soybean	14.4	10.8	13.6	13.5	13.9	16.2	17.7	-	11.3
Maize	13.1	4.6	-	6.6	-	16.8	16.8	-	15.6
Wheat middlings	10.4	-	7.5	7.6	7.6	-	13.5	-	10.6
Wheat bran	6.1	10.4	-	-	-	11.8	9.0	-	5.2
Cassava	13.4	-	-	-	-	-	-	-	14.7
Sunflower	3.6	-	-	-	-	-	-	-	7.4
Rice bran	5.0	-	-	-	-	-	-	12.6	10.2
Sorghum	12.4	-	-	-	-	-	-	15.1	15.9
Wheat grain	13.3	10.7	-	-	-	15.4	16.4	-	14.3
Copra	6.5	-	-	-	-	-	-	13.4	6.9
Groundnut	17.9	-	-	-	-	-	17.9	-	13.7
Rapeseed	10.6	-	12.5	8.1	-	-	-	14.8	10.6
Palm kernel	1.7	-	-	-	-	-	-	9.4	8.8
Meat and bone	9.1	14.5	-	15.0	14.2	8.8	8.5	-	7.8
Poultry by-product	10.0	-	-	13.9	15.6	-	-	-	12.8
Fishmeal	16.2	16.3	19.7	18.8	19.2	13.5	19.4	-	13.6

A Present values
B NRC (1977)
C Smith et al (1980)
D Cho et al 1982
E Smith (1976)
F Morgan et al (1975a)
G Gohl (1981)

TABLE 5.10 Protein digestibility (%) of feeds for several fish species

FEED	TILAPIA[1]	CARP[2]	CATFISH[2]	TROUT[3]	PLAICE[2]	RED SEA BREAM[2]
Soybean	92.1	81-96	72-84	75-85	68.0	78.0
Maize	86.5	66.0	60.0			
Wheat middlings	85.1			89.1		
Wheat bran	75.6					
Cassava	75.8					
Sunflower	87.9					
Rice bran	73.7		71.0			
Sorghum	85.0		41.0			
Wheat grain	88.0	84.0	82.0			
Copra	84.9					
Groundnut	93.4	85.0				
Rapeseed	76.2			76.4		
Palm kernel meal	74.5					
Meat and bone meal	56.9		75.0	70.3		
Poultry by-product meal	65.2			74.7		
Fishmeal	88.7	95.0	80.0	86.7	86.0	61-87

1 Present values

2 NRC (1977)

3 NRC (1981)

groundnut. Thus, poultry ME underestimates the available energy in protein concentrates for tilapia. In contrast, the ME values are higher than the present DE values for rice bran, sunflower, sorghum and maize, and similar for the meals derived from wheat, copra and rapeseed. Thus, poultry ME values tend to overestimate the value of plant-product meals, especially those with high levels of fibre. These differences probably reflect differences in digestive physiology between fish and chicken. As discussed in Chapter 1, fish, more than most other classes of livestock, rely on protein as an energy source and are, in general, less capable of digesting carbohydrates. An additional factor may be that DE has been compared with ME in Table 5.9. In low protein feeds, the difference between ME and DE is usually small (Morgan et al, 1975a). However, in high protein feeds, as more protein is catabolised, a greater proportion of non-faecal nitrogen is excreted and the difference between ME and DE increases. This is one of the arguments for using ME instead of DE as an energy term when evaluating feeds, but as discussed in Chapter 1, the current consensus in fish nutrition is that DE is more reliable than ME (Cho et al, 1982; Hilton and Slinger, 1981; Jobling, 1983). In view of this, and because marked differences have been shown between poultry ME and tilapia DE, the use of poultry ME to balance energy in diets for tilapia should be discontinued.

The change in diet formulation caused by using the present DE values instead of poultry ME, is illustrated by the pair of hypothetical least-cost rations in Appendix 2.

Energy terms for poultry are always given as ME because the dietary waste egested by birds is a mixture of faeces and urine. Energy terms for ruminants are also given as ME because the amount of information

available on feeding metabolism for this group enables this refinement (MAFF, 1975; ARC, 1980). In contrast, energy terms for pigs and other monogastrics are currently reported as DE for a variety of practical reasons (McDonald et al, 1981). It is more meaningful, therefore, to compare the present energy terms for tilapia with corresponding values for pigs, rather than poultry or ruminants. Table 5.9 shows that plant-product meals have a lower DE in tilapia than in pigs which reflects the less efficient digestion of carbohydrates by fish. The exception is groundnut, which has the same energy value for both pigs and tilapia. Unlike the other plant-product meals, groundnut is relatively low in carbohydrate and most of its energy is provided by protein and fat. In this respect, groundnut resembles an animal-product meal more closely than any of the plant product meals in this study. The DE's of animal-product meals are higher in tilapia (and the other fish species in Table 5.9) than in pigs. This is probably due to the importance of animal proteins as energy sources in fish nutrition and the higher digestibility of lipids by fish than terrestrial livestock (Stickney and Lovell, 1977).

Of the three groups of fish in Table 5.9, trout are mainly carnivorous, catfish are omnivorous, and tilapia are predominantly herbivorous. The relative digestibilities of plant and animal-product meals in these groups reflect the dietary habits of these fish. Thus, high carbohydrate feeds are relatively well digested by tilapia whilst the high protein animal-product meals have a higher digestibility in trout (Tables 5.9, 5.10). There is not enough data on catfish DE for a comprehensive comparison of results, but from the figures available, it appears that the digestibility of plant-product meals is higher in tilapia than catfish. Catfish are reported to digest the protein in

plant products as well as, or better than, terrestrial monogastrics, but not as well as ruminants (NRC, 1977). DE and DCP coefficients for plant products are higher for tilapia than catfish (Tables 5.9 and 5.10), indicating that tilapia are even better adapted to a diet of plants than catfish. The DE of meat and bone meal is higher in catfish (and trout) than in tilapia, but this could be due to the low quality of the sample used in this study as discussed earlier. Similarly, fishmeal has higher DE in trout than catfish or tilapia, presumably because the digestive physiology of trout is more adapted to a carnivorous diet of animal proteins.

In summary, the relative efficiency with which tilapia digests and absorbs feed material appears to be related to the herbivorous habit of this group. When compared with pigs and poultry, the energy value to tilapia of high-carbohydrate plant product meals is low, but in comparison with other, more carnivorous fish species the energy value of these meals is high. The more efficient use of plant materials as energy sources by tilapia when compared with trout is probably due to a combination of physiological and morphological adaptations to an herbivorous diet by tilapia (Sections 1.2.3 and 1.3.2). The consequence is that more of the energy in plant material is available for sparing protein in diets for tilapia than for more carnivorous fish.

The productive value of the plant-product meals evaluated in this chapter is assessed more directly in the series of growth trials reported in Chapter 7 and the protein sparing value of different carbohydrates is examined in Chapter 8. However, it is appropriate at this stage to examine in more detail, the relationship between the chemical composition of feeds and their digestibility in tilapia.

CHAPTER 6

PREDICTION OF THE DIGESTIBILITY OF FEEDS FROM THEIR CHEMICAL ANALYSIS

Section 6.1 Introduction

The time and resources necessary for the digestibility trials in Chapter 5 make the direct estimation of digestible energy (DE) and protein digestibility (DCP) a difficult procedure to carry out routinely. The same difficulty has been reported for other classes of livestock and the need for alternative, rapid assays to estimate DE or metabolisable energy (ME) is well recognised (Schneider *et al*, 1951; Sibbald *et al*, 1963).

Most attempts to estimate DE without recourse to *in vivo* digestibility trials have relied on computing regression equations to quantify the relationship between the digestibility of feeds and their proximate composition (Section 1.2.4). The components of proximate analysis (protein, fat, carbohydrate and fibre) are the independent variables in these equations and DE is predicted as the dependent variable (Drennan and Maguire, 1970; Morgan *et al*, 1975b). The exact form of the equation is determined by the requirements of the investigator. Some authors choose to examine the predictive power of one chemical measure at a time (Gallup and Briggs, 1948; Forbes, 1950), whereas others regress DE on mathematical functions combining measures of protein, fat and carbohydrate (Carpenter and Clegg, 1956). Some authors go further and use multiple regression analysis to provide information on the relative contribution to DE of several feed components at once (Schneider *et al*, 1951; Morgan *et al*, 1975b).

Regardless of the type of predictive regression equation produced, if their correlation coefficients are large enough, all can be used, with equal effect, to estimate digestibility from simple chemical tests of feed composition. In this respect, predictive equations have two clear advantages over balance trials. Firstly, they enable rapid checks on the energy value of feeds for quality control or legislative purposes; checks for which it is not practical to run balance trials. Secondly, the equations provide rapid estimates of DE when novel feeds are evaluated for inclusion in compounded diets.

As far as is known, regression techniques have not previously been used to predict the digestibility of feeds for fish. The aim of this chapter is, therefore, to establish the value of this approach for estimating digestible energy (DE), digestible crude protein (DCP), and dry-matter digestibility (DMD) in feeds for tilapia.

Section 6.2 Methods

Initial regressions were based on the feed composition data in Table 5.3 and the digestibility values in Tables 5.7 and 5.8. DE, DCP and DMD were each treated as the y variable ("dependent variable", "predictand", "response") in separate equations. When DCP and DMD were the predictands, percentage digestibility coefficients were used and when DE was the predictand, digestibility was expressed as MJ/kg DM (Table 5.8). Equations which predict DE in units of energy rather than as percentage coefficients are considered to be more useful for feed formulation.

The independent variables (predictors) were analytical measures of feed composition. Protein was measured as crude protein (CP) and fat

as ether extract (EE). Initially, carbohydrate was measured as nitrogen-free extract (NFE) and fibre as crude fibre (CF). However, because of the inadequacy of NFE and CF as analytical measures, feed samples were subjected to further chemical analysis as described in Section 6.2.1.

6.2.1 Chemical analyses

Alternative measures of fibre were obtained as acid detergent fibre (ADF), neutral detergent fibre (NDF) and hemicellulose (HCELL) using the analytical methods described in Section 2.3.2. Carbohydrate was measured directly as "available carbohydrate" (CHO) and indirectly as nitrogen-free extract in three different forms (NFE1, NFE2, NFE3). The methods for determining CHO and NFE 1 - 3 are described in Section 2.3.2.

The carbohydrate and fibre assays were only applied to the plant-product feeds because animal-product meals contain only trace amounts of these components.

6.2.2 Regression analyses

Digestibility and feed composition data were available for 16 feeds and one basal diet. For each of these feeds, there were 12 composition measures (predictors) as listed in Tables 5.3 and 6.1. DE, DCP and DMD were regressed against these predictors in a number of ways to produce 3 different types of equation.

Type 1 Single factor regressions: individual x variables.

In these equations the predictand (DE, DCP or DMP) was regressed against each of the predictors in separate regression equations.

Type 2 Single factor regressions on functions of x-variables

The single predictor in these equations was an arithmetic function combining measures of the energy-yielding feed fractions protein, fat and carbohydrate. The forms of these functions appear in Table 6.8.

DE was the only predictand used in these regressions because DCP and DMD do not vary as a function of protein, carbohydrate and fat in the same way that the energy value of a feed does.

Type 3 Multiple regressions

In these regressions, protein, fat, carbohydrate and fibre were included as independent variables in the same equation. The choice of predictors for multiple regression was determined from consideration of the single factor regressions. Only those predictors with a noticeable effect on digestibility in type 1 equations were considered. In addition, there were practical limitations to the choice of predictors. When there are k predictors, the number of regression equations that can be computed for any one dependent variable is $2^k - 1$. In the present research, this is $2^{12} - 1 = 4095$. For 3 dependent variables this increases to 12285. Not only is it impractical to compute such a large number of equations, but for theoretical reasons, a large number of these equations would be invalid. For example, the three measures of fibre (CF, ADF, NDF) could not be included in the same equation, because these measure a similar feed fraction and are significantly correlated with one another (Table 6.2). The same restriction applies to the three measures of nitrogen-free extract (NFE 1, 2, 3) and available carbohydrate (CHO) (Table 6.3). A multiple regression equation in which independent variables

are highly correlated is said to suffer from multicolinearity and is invalid (Edwards, 1979). Multicolinearity is also a potential problem when too many predictors are included in one equation. For example, NFE is calculated by difference from other chemical measures and if all these are included together with NFE in the same regression equation, the sum approaches 100% and the predictors cannot be considered to be acting independently of one another. In the present research, independent variables were checked for multicolinearity in each regression.

The predictors were assigned as follows: x_1 = crude protein (CP), x_2 = ether extract (EE), x_3 = carbohydrate (NFE 1, 2, 3 or CHO) and x_4 = fibre (CF, ADF, NDF or hemicellulose). From the entire set of independent variables (Tables 5.3 and 6.1), a series of sub-sets were selected to avoid multicolinearity (Table 6.10). All multiple regressions were run on a Harris 800 computer using a statistical package (MINITAB, Penn. State Univ. USA). The program applied a combined "step-up, step-down" procedure (Ryan et al, 1982). Each subset was analysed using this procedure to arrive at the equation which gave the best prediction of the dependent variable, as judged by the smallness of the residual standard deviation (RSD).

The step-up, step-down procedure operates in the following manner. First, all independent variables within a subset are entered into a file. A single factor regression is then calculated for the first variable and the F-statistic for this variable is stored. The next step is to add a second variable to the existing single factor model and to store the F-statistic for this new variable. This procedure continues through all the x-variables, and these are eliminated from the model if their F-statistic falls below a specified

level. In the present research, this elimination level was F = 4 (t = 2). After this elimination process (step-down), the program attempts to add variables (step-up). The F-statistic is calculated for each x variable not already in the model and the variable with the largest F-statistic is added provided that the value of F is greater than 4. When no variables can be added or eliminated from the model, the program stops. In this way, all combinations of predictors in a subset are tested and only those variables with relatively large partial regression coefficients are included in the regression equation.

Regression equations were only considered suitable for predicting DE when their RSD was less than 2.0 MJ/kg DM.

For each of the 3 types of regression analysis, feeds were grouped into 2 categories; namely, 1) plant-product feeds (n = 13) and 2) plant and animal product feeds together (n = 17). In category 1, all of the predictors were used in the 3 types of regression. For category 2, carbohydrate and fibre were not used as predictors because these feed components are not present in animal-product meals. Zero values for carbohydrate and fibre reduce the correlation between the dependent variable and these predictors and so reduce the value of these equations. An example of this is given in Section 6.3.4.

Section 6.3 Results

6.3.1 Feed analyses

The range of values for fibre and carbohydrate in the plant-product feeds is high as shown in Table 6.1. Available carbohydrate (CHO = "glucose + starch") ranges from 2% DM (sunflower, copra) to over 60% DM (cereals and cassava). Available carbohydrate values for the

TABLE 6.1 Further chemical analysis of feeds

	As percentage of dry-matter						
	ADF[1]	NDF[2]	HCELL[3]	NFE1[4]	NFE2[5]	NFE3[6]	CHO[7]
Soybean	10.23	15.43	5.20	34.82	32.21	27.01	5.53
Ground maize	2.26	14.69	12.43	82.74	82.00	69.57	64.57
Wheat middlings	9.54	36.84	27.30	66.67	64.28	36.98	30.71
Wheat bran	13.57	51.90	38.33	63.31	59.73	21.40	16.30
Cassava	8.69	9.38	0.69	85.63	81.21	80.52	65.65
Sunflower	34.58	44.63	10.05	31.93	25.68	15.63	2.14
Rice bran	35.69	64.72	29.03	46.05	30.65	1.62	8.52
Sorghum	6.73	13.87	7.14	81.08	76.10	68.96	63.19
Wheat grain	2.77	19.79	17.02	85.15	82.38	65.36	60.32
Copra	27.31	54.70	27.39	48.37	35.89	8.50	2.25
Groundnut	5.92	6.64	0.72	26.90	24.70	23.98	6.02
Rapeseed	20.36	29.24	8.88	36.15	29.15	20.27	3.81
Palm kernel meal	35.71	63.98	28.27	46.55	28.58	0.31	2.69

Scientific names and international feed numbers for the above feeds appear in Appendix 3

1 Acid detergent fibre
2 Neutral detergent fibre
3 Hemicellulose
4 NFE1 = 100 - (% CP + % EE + % ASH + % CF)
5 NFE2 = 100 - (% CP + % EE + % ASH + % ADF)
6 NFE3 = 100 - (% CP + % EE + % ASH + % NDF)
7 Available carbohydrate

cereals, cereal by-products and cassava are in good agreement with literature values, but the present estimates for oilseeds are generally lower than usually reported (e.g., Bolton, 1960). Fibre, (as NDF), ranges from less than 7% DM (groundnut) to 65% DM (rice bran).

For all feeds, the proportion of indigestible material recovered by the three fibre assays increased in the order NDF > ADF > CF (compare Tables 6.1 and 5.3). This difference is largely due to the greater amount of hemicellulose in the fibrous feeds (Table 6.1). Correlation between the different measures of fibre are given in Table 6.2 and it is clear that CF is more closely correlated with ADF ($r = 0.95$) than NDF ($r = 0.77$). As discussed in Section 2.3.2 neither CF or ADF measures hemicellulose and this is possibly why these two measures are more closely related to one another than to NDF. There is no significant correlation between hemicellulose (HCELL) and CF or between HCELL and ADF. Thus, within the samples analysed, the level of hemicellulose is independent of the level of CF (mainly cellulose) or ADF (cellulose + other indigestible residues). Hemicellulose is, however, significantly correlated with NDF as may be expected, since this assay is designed to recover hemicellulose.

As the proportion of indigestible material recovered by the 3 fibre assays increase in the order NDF > ADF > CF, so the estimates of "digestible carbohydrate" decrease in the order NFE3 < NFE2 < NFE1 (Table 6.1). All 3 indirect estimates of digestible carbohydrate correlate highly with the direct chemical measure of available carbohydrate (Table 6.3). However, intercept values for these regressions decrease in the order NFE3 < NFE2 < NFE1 showing that CHO is more closely estimated by NFE3 than any of the other indirect measures. NFE1 and NFE2 include hemicellulose and so overestimate

TABLE 6.2 Correlation between measures of dietary fibre

Fibre Assay

	CF	ADF	NDF	HCELL
CF	-	r = 0.95 ($p < 0.001$)	r = 0.77 ($p < 0.01$)	r = 0.34 (NS)
ADF	-	-	r = 0.85 ($p < 0.01$)	r = 0.42 (NS)
NDF	-	-	-	r = 0.84 ($p < 0.001$).

r = Correlation coefficient
NS = Not significant
CF = Crude fibre
ADF = Acid detergent fibre
NDF = Neutral detergent fibre
HCELL = Hemicellulose

TABLE 6.3 Regressions of NFE1, NFE2 and NFE3 on available carbohydrate (CHO).

NFE1 = 37.5 + 0.75 CHO ($r = 0.93$, $p < 0.001$).

NFE2 = 28.7 + 0.84 CHO ($r = 0.95$, $p < 0.001$).

NFE3 = 9.1 + 0.97 CHO ($r = 0.96$, $p < 0.001$).

NFE 1 - 3 = Different forms of nitrogen-free extract

digestible carbohydrate, particularly in high fibre feeds. CHO and NFE3 are, therefore, clearly superior to NFE1 and NFE2 as estimates of the amount of carbohydrate available for digestion and absorption by fish. There are, however, noticeable differences between NFE3 and CHO (particularly within the oilseeds) and possible reasons for this are discussed in Section 6.4.

6.3.2 Single factor regressions on individual chemical measures

Tables 6.4, 6.5 and 6.6 list the single factor regressions of DE, DCP and DMD on individual feed composition measures for the plant-product feeds. These regression equations are numbered and referred to throughout the text in parentheses.

6.3.2.1 Prediction of digestible energy (DE)

All 3 measures of fibre are highly correlated with DE (Table 6.4). The residual standard deviation of equations containing fibre decreases in the order NDF < ADF < CF (4, 5, 6). Thus NDF is better than ADF as a predictor of DE and ADF is in turn, a better predictor than CF. For all measures of fibre, the regression coefficients are negative. These decrease in the order NDF < ADF < CF, whilst regression intercepts increase in the order NDF > ADF > CF. The reduction in scatter about the regression line and the change in slope as NDF replaced ADF and CF is illustrated graphically in Figure 6.1. Essentially, the change in slope shows that there is a smaller decrease in DE for unit change in NDF than for unit change in ADF or CF.

The correlation between DE and NDF is particularly high. The coefficient of determination (r^2) is 87.2%. This measures the proportion of variance in DE that is attributed to its regression on NDF

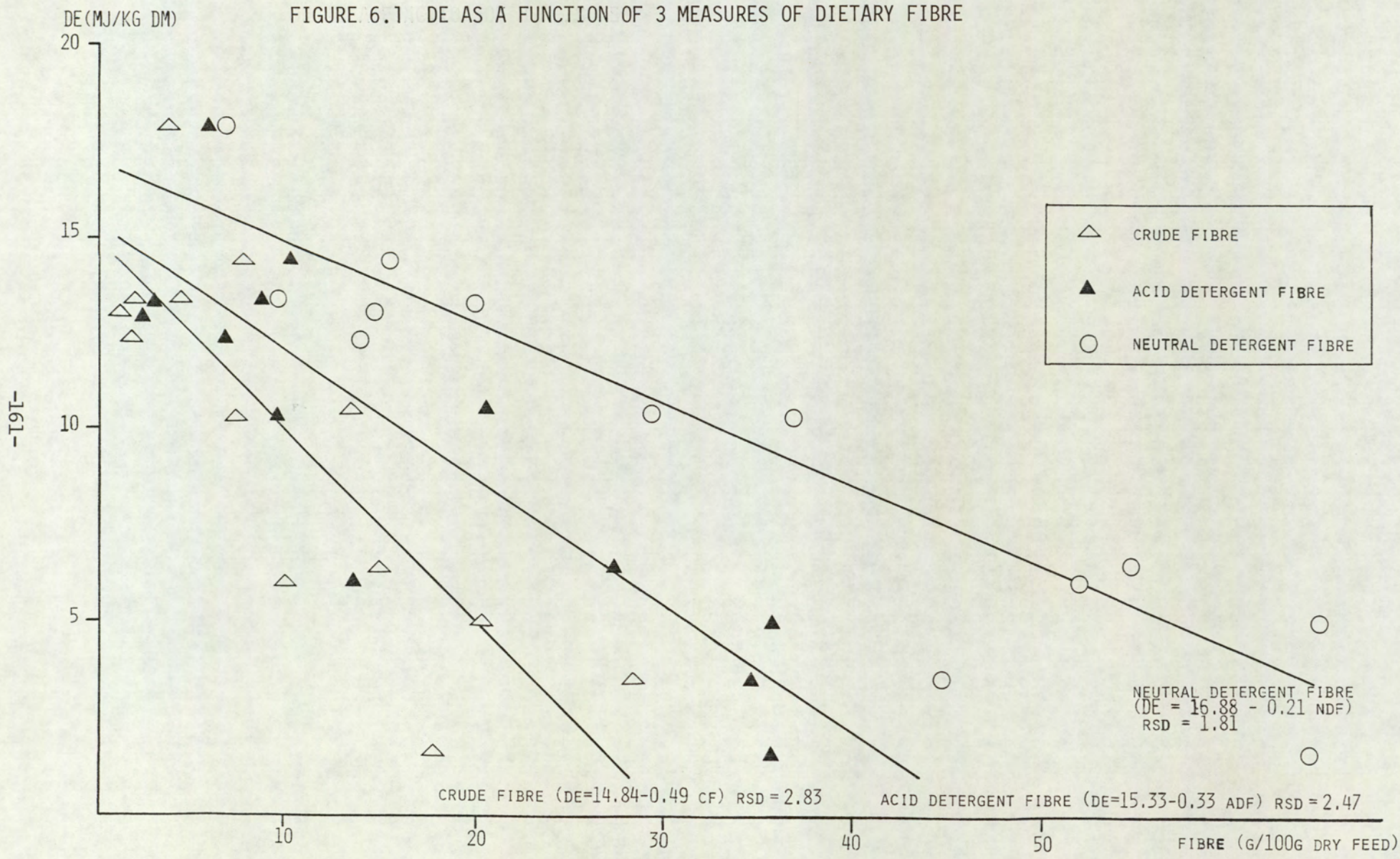
TABLE 6.4 Single factor regressions of digestible energy (DE) (MJ/kg DM) on chemical measures of plant-product feeds (n = 13)

X	C	b (± SE)	r^2 (%)	RSD	P	Equation No.
CP	7.97	0.08 (0.09)	7.1	4.88	NS	1
EE	10.60	-0.17 (0.40)	1.6	5.03	NS	2
ASH	12.37	-0.42 (0.30)	14.8	4.68	NS	3
CF	14.84	-0.49 (0.10)	68.7	2.83	***	4
ADF	15.33	-0.33 (0.06)	76.2	2.47	***	5
NDF	16.88	-0.21 (0.02)	87.2	1.81	***	6
HCELL	14.37	-0.28 (0.08)	49.1	3.62	**	7
NFE1	7.03	0.05 (0.06)	5.2	4.95	NS	8
NFE2	6.10	0.08 (0.06)	14.2	4.69	NS	9
NFE3	6.12	0.11 (0.04)	40.8	3.90	*	10
CHO	7.76	0.08 (0.05)	22.1	4.47	NS	11
GE	4.05	0.31 (0.98)	0.9	5.04	NS	12

x = Independent variable (chemical measure)
c = Intercept (equation constant)
b = Regression coefficient (± standard error of coefficient)
r^2 = Square of correlation coefficient x 100% = 'coefficient of determination'
RSD = Residual standard deviation
P = Significance level. NS = Not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (F-test)

CP = Crude protein
EE = Ether extract
CF = Crude fibre
ADF = Acid detergent fibre
NDF = Neutral detergent fibre
HCELL = Hemicellulose
NFE 1 - 3 = Different forms of nitrogen-free extract (see Section 2.3.2)
CHO = Available carbohydrate
GE = Gross energy

FIGURE 6.1 DE AS A FUNCTION OF 3 MEASURES OF DIETARY FIBRE



and describes the percentage of the total variation in data shared by those x and y variables. The residual standard deviation (RSD) of this equation (6) is lower than any of the other single factor regressions and is considered suitable for predicting DE. It is the only single factor regression with a RSD small enough to enable accurate prediction of DE.

In addition to the 3 measures of fibre mentioned above, hemicellulose is also significantly correlated with DE (7). This relationship is, however, not as strong as for the other 3 measures of fibre indicating that the hemicellulose fraction of fibre on its own is a poor predictor of DE. The only non-fibre chemical measure for which there was significant correlation with DE was the indirect measure of carbohydrate, NFE3 (10). None of the other measures (CP, fat, ash, NFE1, NFE2, CHO or GE) were significantly correlated with DE.

6.3.2.2 Prediction of dry-matter digestibility (DMD)

For the plant-product feeds, all 3 measures of fibre correlate highly with DMD (28, 29, 30). The RSD's of these equations are low enough for all three to be used with equal effect to predict DMD. To a lesser degree, there is also correlation between DMD and the more accurate measures of digestible and available carbohydrate such as NFE2, NFE3 and CHO (33, 34, 35). However, the correlation between these measures and DMD is not high enough to enable the use of carbohydrate to predict DMD to an acceptable degree of accuracy. Hemicellulose is also correlated with DMD, but as for the carbohydrate measures, the RSD of this equation (31) is too high for predictive purposes. None of the other chemical measures (CP, EE, Ash, NFE1 or GE) were significantly

TABLE 6.5 Single factor regressions of digestible crude protein (DCP %) on chemical measures of plant-product feeds (n = 13)

x	c	b (± SE)	r^2(%)	RSD	P	Equation No
CP	77.89	0.22 (0.11)	24.5	6.29	NS	13
EE	81.93	0.25 (0.57)	1.7	7.18	NS	14
ASH	87.43	-0.75 (0.41)	23.4	6.34	NS	15
CF	85.71	-0.27 (0.24)	10.3	6.86	NS	16
ADF	87.16	-0.26 (0.15)	22.3	6.39	NS	17
NDF	89.01	-0.18 (0.08)	31.9	5.97	*	18
HCELL	87.40	-0.27 (0.15)	23.3	6.34	NS	19
NFE1	85.68	-0.05 (0.09)	2.3	7.16	NS	20
NFE2	83.02	0 (0.08)	0	7.24	NS	21
NFE3	81.18	0.05 (0.07)	4.4	7.08	NS	22
CHO	82.59	0.02 (0.08)	0.3	7.23	NS	23
GE	43.75	2.09 (1.25)	20.3	6.46	NS	24

x = Independent variable (chemical measure)
c = Intercept (equation constant)
b = Regression coefficient (± standard error of coefficient)
r^2 = Square of correlation coefficient x 100% = 'coefficient of determination'
RSD = Residual standard deviation
P = Significance level. NS = Not significant, * = $P < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (F-test).

CP = Crude protein
EE = Ether extract
CF = Crude fibre
ADF = Acid detergent fibre
NDF = Neutral detergent fibre
HCELL = Hemicellulose
NFE 1 - 3 = Different forms of nitrogen-free extract (see Section 2.3.2)
CHO = Available carbohydrate
GE = Gross energy

TABLE 6.6 Single factor regressions of dry-matter digestibility (DMD) (%) on chemical measures of plant product feeds (n = 13)

x	c	b (± SE)	r^2 (%)	RSD	P	Equation No.
CP	45.33	0.02 (0.52)	0	28.71	NS	25
EE	51.54	-1.37 (2.23)	3.3	28.24	NS	26
ASH	63.33	-2.96 (1.63)	22.9	25.21	NS	27
CF	76.50	-3.01 (0.42)	82.1	12.14	***	28
ADF	78.77	-2.01 (0.24)	86.9	10.40	***	29
NDF	84.96	-1.20 (0.15)	85.1	11.10	***	30
HCELL	63.28	-1.38 (0.53)	38.4	22.54	*	31
NFE1	12.25	0.59 (0.33)	22.3	25.32	NS	32
NFE2	11.66	0.68 (0.27)	35.9	22.29	*	33
NFE3	19.19	0.79 (0.18)	63.4	17.38	**	34
CHO	28.40	0.68 (0.22)	46.2	21.06	*	35
GE	67.80	-1.18 (5.54)	0.4	28.66	NS	36

x = Independent variable (chemical measure)
c = Intercept (equation constant)
b = Regression coefficient (± standard error of coefficient)
r^2 = Square of correlation coefficient x 100% = 'coefficient of determination'
RSD = Residual standard deviation
P = Significance level. NS = Not significant, * = $P < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (F-test).

CP = Crude protein
EE = Ether extract
CF = Crude fibre
ADF = Acid detergent fibre
NDF = Neutral detergent fibre
HCELL = Hemicellulose
NFE 1 - 3 = Different forms of nitrogen-free extract (see Section 2.3.2)
CHO = Available carbohydrate
GE = Gross energy

correlated with DMD.

6.3.2.3 Prediction of digestible crude protein (DCP)

For the plant-product feeds, DCP is significantly correlated with only one chemical measure; namely NDF (Table 6.5 (18)). However, this correlation is only significant at the 5% level and r^2 is much lower than when NDF is regressed on DE or DMD. Clearly DCP is not as dependent on dietary fibre level as DE or DMD. Only 31. 9% of the variation in DCP between feeds is due to NDF and so this regression is clearly inadequate for predicting DCP. Table 6.5 shows that, within the range of feeds evaluated in this study, none of the chemical measures are suitable for predicting the digestibility of protein using single factor regressions.

When the above regressions for DE, DMD and DCP were extended to include data from all feeds (plant-product and animal-product meals together, n = 17), only the non-fibre/non-carbohydrate measures were used as predictors for the reasons given in Section 6.2. Including data for animal-product meals in the above regressions did not noticeably alter the degree of correlation between DE or DMD and CP, EE, Ash or GE as shown in Table 6.7 (37-40, 45-48); all of these regressions remained non-significant. However, when DCP was regressed against ash or GE, the correlation between these variables was increased by including animal-product meals in the regressions (compare 15 with 43 and 24 with 44). The correlation between DCP and ash is significant at the 5% level when data for all feeds are included in the regression, whilst the same relationship is not significant when only the plant-product data are used. Similarly, DCP and GE are significantly correlated only when data for animal-product meals are used together

TABLE 6.7 Single factor regressions of digestible energy, digestible crude protein and dry-matter digestibility on chemical measures of all feeds (n = 17)

1. Digestible energy (MJ/kg DM)

x	c	b (± SE)	r^2 (%)	RSD	P	Equation No.
CP	8.03	0.09 (0.05)	14.6	4.78	NS	37
EE	9.20	0.25 (0.20)	9.7	4.92	NS	38
ASH	10.44	0.03 (0.12)	0.5	5.16	NS	39
GE	-4.12	0.80 (0.63)	9.5	4.92	NS	40

2. Digestible crude protein (%)

x	c	b (±SE)	r^2 (%)	RSD	P	Equation No.
CP	81.21	0 (0.1)	0	10.28	NS	41
EE	81.30	-0.03 (0.42)	0	10.28	NS	42
ASH	87.03	-0.54 (0.18)	36.7	8.18	*	43
GE	19.50	3.30 (1.0)	41.3	7.88	**	44

3. Dry-matter digestibility (%)

x	c	b (±SE)	r^2 (%)	RSD	P	Equation No.
CP	47.57	-0.12 (0.30)	1.1	26.58	NS	45
EE	44.87	-0.20 (1.08)	0.2	26.69	NS	46
ASH	52.94	-0.85 (0.56)	13.3	24.88	NS	47
GE	-6.06	2.66 (3.38)	4.0	26.19	NS	48

See footnote on previous table for explanation of terms

with data for plant-product feeds. When animal-product meal data are included in the regression, there is larger variation in ash and energy within the data set and this probably accounts for the improvement in correlation. Whatever the mechanism, ash and gross energy appear to have some influence on the digestibility of protein in feeds of animal origin.

6.3.3 Single factor regressions of digestible energy (DE) on functions of chemical measures

When DE was regressed against the various functions of protein, fat and carbohydrate described in Table 6.8, the correlation was significant in all cases. This was only true for the plant-product feeds. Animal-product meals were not included in the regressions because they contain no carbohydrate or fibre and so artificially increase the data variance.

The functions in Table 6.8 can be grouped into 3 sets of four, namely: A-D (49-52), E-H (53-56) and I-L (57-60). Within each group, the only variation between functions is the replacement of one measure of carbohydrate for another. There was a decrease in RSD as NFE2 replaced NFE1 and NFE3 replaced NFE2. Thus, functions containing NFE3 as a measure of carbohydrate predicted DE more accurately than functions containing other forms of nitrogen-free extract (functions C, G and K). When CHO replaced NFE3 (functions D, H, L), there was a slight increase in RSD (52, 56, 60). However, as a component of an energy function, CHO was still a better predictor of DE than NFE1 or NFE2.

The difference between the 3 groups of functions was in the multiplication coefficients assigned to EE and CP. In group E-H, EE was multiplied by 2.25. This is a common procedure in animal nutrition

TABLE 6.8 Single factor regressions of digestible energy (DE) (MJ/kgDM) on functions of chemical measures for plant-product feeds

x (Function)	c	b(± SE)	r^2 (%)	RSD	P	Equation No
A (CP + EE + NFE1)	-16.31	0.31 (0.08)	56.0	3.36	**	49
B (CP + EE + NFE2)	-9.34	0.25 (0.05)	65.6	2.97	***	50
C (CP + EE + NFE3)	-1.35	0.18 (0.03)	81.3	2.19	***	51
D (CP + EE + CHO)	-1.22	0.21 (0.04)	71.8	2.69	***	52
E (CP + 2.25 EE + NFE1)	-11.38	0.24 (0.09)	39.9	3.93	*	53
F (CP + 2.25 EE + NFE2)	-8.81	0.23 (0.06)	57.1	3.32	**	54
G (CP + 2.25 EE + NFE3)	-2.02	0.18 (0.03)	77.3	2.42	***	55
H (CP + 2.25 EE + CHO)	-1.71	0.20 (0.04)	65.9	2.96	***	56
I (1.25 CP + NFE1)	-21.65	0.37 (0.06)	77.6	2.40	***	57
J (1.25 CP + NFE2)	-10.50	0.26 (0.04)	76.1	2.48	***	58
K (1.25 CP + NFE3)	-1.19	0.18 (0.02)	83.5	2.06	***	59
L (1.25 CP + CHO)	-2.07	0.22 (0.03)	81.8	2.16	***	60

x = Independent variable (function of chemical measures)
c = Intercept (equation constant)
b = Regression coefficient (± standard error of coefficient)
r^2 = Square of correlation coefficient x 100% = 'coefficient of determination'
RSD = Residual standard deviation
P = Significance level. NS = Not significant, * = $P < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ (F-test).

CP = Crude protein
EE = Ether extract
CF = Crude fibre
ADF = Acid detergent fibre
NDF = Neutral detergent fibre
HCELL = Hemicellulose
NFE 1 - 3 = Different forms of nitrogen-free extract (see Section 2.3.2)
CHO = Available carbohydrate
GE = Gross energy

studies (Carpenter and Clegg, 1956) and it gives more weight to the energy contribution of the fat component in feeds, (on average, fat provides 2.25 more gross energy per unit weight than carbohydrates). However, in the present study, when EE was multiplied by 2.25, the accuracy of DE prediction invariably decreased (compare 49 with 53, 50 with 54, 51 with 55 and 52 with 56). Thus, fat did not appear to provide an important contribution to the energy value of a function and so in functions I-L, it was eliminated.

Preliminary regressions of DE against functions containing only protein and carbohydrate showed that these functions were better predictors of DE than functions which also contained EE. In fish nutrition, protein is known to be an important energy source (Sections 1.3.3 and 1.3.4) and so in an attempt to further improve the accuracy of DE prediction, protein was multiplied by a variety of coefficients. On the basis that the gross energy of dietary protein is 23.6 MJ/kg and carbohydrate is 16.7 MJ/kg (Section 1.2.1), protein provides 1.41 more gross energy per unit weight than carbohydrate. Regressions were, therefore, calculated for DE on functions in which CP was multiplied by 1.41. However the RSD's of these equations were not noticeably lower than equations with functions in which CP was not adjusted. By experimenting with several multiplication factors, DE was found to be most accurately predicted with a function in which CP was multiplied by 1.25. These equations, (57-60), give the best prediction, (i.e. lowest RSD's) of DE for all functions examined. Within this group, function K was the best predictor of DE (59). The full equation is:

$$DE\ (MJ/kg\ DM) = 0.18(1.25\ CP + NFE3) - 1.19$$

6.3.4 Multiple regressions

Multiple regressions are only reported for the plant-product feeds (Table 6.10). When data for the animal-product meals were included in these regressions, the RSD always increased. This is because the chemical measures with the highest partial regression coefficients were digestible carbohydrate and fibre, and these components are not found in animal-product meals.

The following equations illustrate the extent to which regression statistics of a multiple regression equation changed by including animal-product meal data:

Plant-product feeds only

$$DE\ (MJ/kg\ DM) = 3.47 + 0.25\ CP + 0.13\ CHO - 0.26\ CF$$

$$RSD = 1.55,\ R_1^2 = 89.9\%$$

Plant-product plus animal-product feeds

$$DE\ (MJ/kg\ DM) = 7.17 + 0.13\ CP + 0.09\ CHO - 0.27\ CF$$

$$RSD = 3.26,\ R_1^2 = 57.6\%$$

Most of the multiple regression equations with significant partial regression coefficients and multiple correlation coefficients were those in which DE was the dependent variable (Table 6.10). For DCP and DMD, multiple regressions were not tabulated because so few of these were statistically significant. Instead, the few significant relationships for DCP and DMD are given in full in Sections 6.3.4.2 and 6.3.4.3.

6.3.4.1 Prediction of digestible energy (DE)

A large number of significant regressions were computed from the subsets of independent variables in Table 6.9. The RSD's of many of these regression equations were small enough to enable accurate prediction of DE. However, there were too many equations to list them all and so Table 6.10 only provides regression statistics for the equations which are of most practical use in feed evaluation.

All of the regressions listed have highly significant multiple correlation coefficients. Most of these regressions include a measure of fibre and in all cases, the partial regression coefficient for this feed component is large and negative. By contrast, the partial regression coefficients for protein and carbohydrate, when significant are small and positive. For dietary fat (EE) the partial regression coefficient was never significant (62, 65, 67).

When fibre was included with protein in multiple regressions, the accuracy of DE prediction increased as CF was replaced by ADF and then NDF (compare 61, 63 and 64). Thus, DE is influenced more by NDF than ADF or CF and this is in agreement with the single factor regressions (Table 6.4). NDF alone accounted for so much of the variation in DE between feeds, that the inclusion of other chemical measures in the multiple regression equation was always non-significant (64-68).

The only regression which does not contain fibre and in which all predictors have significant partial regression coefficients is equation 73. In this equation, crude protein and available carbohydrate (CHO) together account for 84% of the between feed variation in DE. Replacing CHO with NFE3 increased the RSD of the multiple regressions. This contrasts with the results of single factor regressions of DE on individual chemical measures (Table 6.4) and energy

TABLE 6.9 Subsets of chemical measures used in multiple regression analysis

The complete data set comprised 12 measures of feed composition. These were grouped into the following subsets for multiple regression analysis with digestible energy, digestible crude protein or dry-matter digestibility.

SUBSET	INDEPENDENT VARIABLES (PREDICTORS)				
1	CP	EE	ASH	CF	
2	CP	EE	ASH	ADF	
3	CP	EE	ASH	NDF	
4	CP	EE	ASH	HCELL	
5	CP	EE	ASH	CF	NFE1
6	CP	EE	ASH	ADF	NFE2
7	CP	EE	ASH	NDF	NFE3
8	CP	EE	ASH	CF	CHO
9	CP	EE	ASH	ADF	CHO
10	CP	EE	ASH	NDF	CHO
11	CP	EE	ASH	CF	GE
12	CP	EE	ASH	ADF	GE
13	CP	EE	ASH	NDF	GE

Within each subject, all possible combinations of variables were examined using step up/step down multiple regression analysis. Multiple regression statistics for the combination giving the lowest RSD in each subset appear in Table 6.10.

CP = Crude protein
EE = Ether extract
CF = Crude fibre
ADF = Acid detergent fibre
NDF = Neutral detergent fibre
HCELL = Hemicellulose
NFE 1 - 3 = Different forms of nitrogen-free extract (see Section 2.3.2)
CHO = Available carbohydrate
GE = Gross energy

TABLE 6.10 Multiple regression of digestible energy (MJ/kg DM) on chemical measures of plant-product feeds (n = 13)

Chemical measures	C	Regression coefficients x_1	x_2	x_3	x_4	RSD	R^2	R_1^2	F	P	Equation No.
CP + CF	12.3	0.13	-	-	-0.52	2.07	84.8	81.7	27.8	***	61
CP + EE + CF	13.1	0.15	-0.31^{ns}	-	-0.52	1.79	89.8	86.4	26.4	***	62
CP + ADF	13.2	0.10	-	-	-0.34	1.98	86.1	83.4	31.1	***	63
CP + NDF	16.0	0.03^{ns}	-	-	-0.21	1.82	88.3	86.0	37.8	***	64
EE + NDF	16.7	-	0.06^{ns}	-	-0.22	1.88	87.5	85.0	34.9	***	65
NFE3 + NDF	19.2	-	-	-0.04^{ns}	-0.25	1.77	88.9	86.6	39.8	***	66
CP + EE + NDF	16.0	0.03^{ns}	0.02^{ns}	-	-0.21	1.91	88.3	84.4	22.7	***	67
CP + NFE3 + NDF	38.4	-0.23^{ns}	-	-0.23^{ns}	-0.48	1.73	90.5	87.3	28.4	***	68
CF + HCELL	16.9	-	-	-	-0.39 - 0.19	1.82	88.3	86.0	37.8	***	69
CP + CF + HCELL	14.8	0.08	-	-	-0.44 - 0.14	1.43	93.5	91.3	43.1	***	70
NFE1 + CF	22.4	-	-	-0.10	-0.66	2.24	82.2	79.6	23.1	***	71

Continued .....

TABLE 6.10 (continued) Multiple regression of digestible energy (MJ/kg DM) on chemical measures of plant-product feeds (n = 13)

NFE2 + ADF	22.1	-	-	0.09	-0.46	1.89	87.3	84.7	34.3	***	72
CP + CHO	-3.5	0.34	-	0.22	-	1.93	86.8	84.2	33.0	***	73
CP + CHO + CF	3.47	0.25	-	0.13	-0.26	1.55	92.4	89.9	36.4	***	74
CP + NFE3 + CF	5.42	0.19	-	0.09	-0.30	1.51	92.7	90.3	38.1	***	75

C = Intercept (equation constant)

x_1 = protein, x_2 = fat, x_3 = carbohydrate, x_4 = fibre

RSD = Residual standard deviation

R^2 = square of multiple correlation coefficient x 100%

R_1^2 = R^2 corrected for degrees of freedom*

F = (Mean square regression/mean square residual)

* When a new independent variable is added to the regression equation, R^2 increases even if this variable is of no value. To correct for this R_1^2 is calculated thus:

$$R_1^2 = 1 - \frac{\text{Sums of squares of residual}/n - k - 1}{\text{Total sums of squares}/n - 1}$$ (where k is the number of x variables)

R_1^2 is thus, an approximately unbiased estimate of the population R^2

*** = $p < 0.001$ (F test)

functions (Table 6.8) in which NFE was superior to CHO as a predictor of DE. However, the difference in RSD between regressions with either CHO or NFE3 is small and more feeds will have to be evaluated before it can be stated conclusively which measure of carbohydrate is a more accurate predictor of DE.

The multiple regressions with lowest RSD's were those including protein, fibre and one of the more accurate measures of carbohydrate (74, 75). In these equations, the chemical feed measures account for about 90% of the variation in DE between feeds. In comparison, NDF on its own accounts for 87% of this variation (6) and the RSD of this single factor equation is only slightly higher than the multiple regression equations 74 and 75. Thus, the multiple regression equations only improve the accuracy of DE prediction slightly when compared with the single factor NDF equation and so the additional work of measuring protein and carbohydrate may, in some circumstances, be unnecessary.

Crude fibre and hemicellulose were not significantly correlated (Table 6.2) and so even though these are both fibre measures, it was valid to include them together as independent variables in multiple regression analysis (69, 70). These equations are not of practical use for predicting DE because of the method by which HCELL is estimated (Section 2.3.2). However, equation 69 is instructive because it shows that CF and HCELL together, are as accurate as NDF at predicting DE (compare 69 and 6). This indicates that DE is predicted more accurately by NDF than CF because the latter assay does not include the indigestible hemicellulose fraction. When CP is included with CF and HCELL, there is a marked reduction in RSD (70). The accuracy with which DE is predicted by this equation is similar to the accuracy of

equation 74, but the latter is of more practical use because CHO is easier to measure than HCELL.

The use of CF, ADF and their respective NFE values as independent variables yields acceptable predictive equations (71 and 72). ADF and NFE2 are superior to CF and NFE1 as predictors. The partial regression coefficient of NFE3 was not significant when included with NDF. In each NFE/fibre combination, NFE produced equations with smaller RSD's than similar equations in which carbohydrate was measured by CHO.

6.3.4.2 Prediction of dry-matter digestibility (DMD)

For plant-product feeds, the only multiple regression with a significant correlation coefficient included CF and HCELL as the predictors:

$$DMD(\%) = 85.1 - 2.61\ CF - 0.78\ HCELL \quad \ldots\ldots \quad (76)$$

$$R_1^2 = 91.5\%,\ p < 0.001$$

$$RSD = 8.02$$

The RSD of this equation is lower than any of the single factor regressions of DMD on fibre or carbohydrate (Table 6.6).

When the DMD regressions were extended to include all feeds (plant and animal-product feeds), the partial regression coefficient of ash became significant.

Thus,

$$DMD(\%) = 77.1 - 2.4\ CF - 1.35\ ASH \quad \ldots\ldots \quad (77)$$

$$R_1^2 = 65.9,\ p < 0.001$$

$$RSD = 15.10$$

$$DMD(\%) = 83.4 - 0.9\ NDF - 1.6\ ASH \quad ..... \quad (78)$$

$$R_1^2 = 67.3,\ p < 0.001$$

$$RSD = 14.80$$

Clearly, DMD is affected by the large amount of ash in animal-product meals, possibly for the same reason that DCP is significantly correlated with ash in the single factor regression with all feeds (43).

6.3.4.3 Prediction of digestible crude protein (DCP)

None of the multiple regressions computed for DCP were significant. Of all the regressions for DCP (single factor, functions, multiple regressions), only the single factor regression with NDF was significant (18). However, this regression was only significant at the 5% level, r^2 was only 31.9% and the conclusion is that DCP cannot be predicted from any of the chemical feed measures used in the present study.

Section 6.4 Discussion

The single most powerful predictor of digestibility in plant-product feeds was fibre. Fibre (measured as crude (CF), acid detergent (ADF) or neutral detergent (NDF)) fibre was negatively correlated with digestibility and within limits, this correlation was strong enough to enable estimates of DE or DMD in feeds from an analysis of dietary fibre alone.

In addition, other feed components also proved to be useful predictors of digestibility. Protein and digestible carbohydrate, in

particular, were positively correlated with DE and multiple regressions which included these energy-supplying nutrients in addition to crude fibre, predict DE more accurately than a single factor regression with crude fibre alone (compare 4 and 74). By contrast, dietary fat has no value for predicting DE, DCP or DMD, within the range of fat levels studied: in single factor (individual variables or functions) and multiple regressions, ether extract (EE) was never significantly correlated with DE. This is possibly due to the small range of dietary fat in the feed samples. For most feeds EE levels were less than 5% DM and only three feeds (all oilseeds) contained higher levels (Table 5.3). Thus, whilst fat is a high-energy nutrient, it is present in such small amounts in the feeds tested that it accounts for a relatively small proportion of dietary energy when compared with protein and carbohydrate.

Compared with DMD or DE, the digestibility of crude protein was more or less independent of dietary composition. For the plant-product feeds, DCP was only significantly correlated with one feed component, namely NDF, but this relationship was too weak to enable prediction of DCP. The conclusion is, therefore, that whilst DMD and DE can be satisfactorily predicted for plant-product feeds from analysis of dietary composition, DCP cannot. The only generalisation that can be made, is that the digestibility of protein in plant-product feeds is higher and less variable than the digestibility of dry-matter or energy. In the plant-product feeds evaluated, DCP ranged from 74 - 93%, whilst DE ranged from 8 - 80% and DMD from 9 - 80% (Table 5.7). Thus, the digestive physiology of tilapia, in common with other fish species, appears to be better adapted to digesting and absorbing protein than other, energy supplying nutrients.

The relative value of fibre and available carbohydrate as predictors of digestibility depends on the assays used to evaluate these feed components. With respect to fibre, DE and DMD are predicted more accurately by NDF than ADF or CF. This is because NDF includes all of the indigestible residues in a feed (mainly, the cell wall), whilst ADF does not include hemicellulose and CF does not include hemicellulose or lignin (Section 2.3.2). Because CF and ADF underestimate the amount of indigestible material, these assays overestimate the amount available for absorption and their correlation with digestibility is, therefore, weakened. The NDF assay, by contrast, recovers a much greater proportion of the indigestible feed fraction, it more accurately estimates the material available for absorption and is, therfore, more closely related to digestibility. With DE, the single factor regressions show this clearly, since the residual standard deviations (RSD) of equations which include a fibre measure decrease in the order NDF < ADF < CF. With DMD, single factor regressions all have similar RSD values showing that scatter about the regression line is similar regardless of the fibre assay. However, the regression intercept for DMD increases in the order NDF > ADF > CF. Thus, when there is no fibre in a feed as measured by NDF, the DMD of this feed is higher than when there is no fibre as measured by CF or ADF, again indicating that these latter underestimate the proportion of indigestible material in a feed.

The accuracy of the fibre assay also determines the accuracy of indirect estimates of digestible carbohydrate (nitrogen-free extract - NFE), for the following reasons. NFE is calculated by deducting the sum of measured ash, protein, fat and fibre from the dry-matter weight of a feed. Any errors in the determination of these fractions are

compounded into error of the NFE estimate. Table 6.1 shows that the feed fraction with the largest error is crude fibre. This is particularly apparent in the high-fibre feeds which contain a large proportion of hemicellulose. These fibrous feeds have NDF values which are up to 4 times higher than CF and so clearly, when nitrogen-free extract is calculated "by difference" with CF, the resulting figure (NFE1) is a gross overestimate.

Nitrogen-free extract is more accurately calculated with NDF (the estimate is then referred to as NFE3) than with CF. This improvement in accuracy is reflected in the accuracy with which DMD is predicted by the different estimates of nitrogen-free extract. Thus, DMD is significantly correlated with NFE3, but not with NFE1. Similarly, in regressions of DE on functions of protein and digestible carbohydrate, and in multiple regressions in which digestible carbohydrate was used as a predictor, NFE3 was always superior to NFE1 as a predictor of DE.

Clearly, neutral detergent fibre is superior to ADF and CF both as a predictor of digestibility and as an estimator of digestible carbohydrate by difference. The neutral detergent fibre assay also has the advantage that it only has one reflux stage and therefore is simpler and faster to perform than the crude fibre assay. It is for these reasons that the CF assay should now be abandoned in fish nutrition studies and replaced with NDF.

As early as the 1930's, workers realised the inadequacy of CF and suggested that the carbohydrate portion of a feed should be partitioned into lignin, cellulose and "other carbohydrates" (Crampton and Maynard, 1938). Neutral detergent fibre, essentially recovers the lignin, cellulose and hemicellulose portion of carbohydrates (in addition to cutin, plant silica and soil minerals) and the "other carbohydrates"

include sugars, oligosaccharides and polysaccharides. However, it is now recognised that not all of these carbohydrates are actually digestible and as a result, a series of direct chemical methods have been developed to measure the carbohydrate actually available for absorption (i.e. the "available carbohydrate", Bolton, 1960; Friedemann et al, 1967; Southgate, 1969; Pomeranz and Meloam, 1978).

The method used in the present study (CHO - Section 2.3.2) measures glucose, starch and any other α-linked glucose polymers that are present in feeds. For the cereal grains (maize, wheat grain, sorghum), this direct measure of "available carbohydrate" was closely approximated by the indirect estimate of "digestible carbohydrate", NFE3. This indicates that most of the digestible carbohydrate in cereal grains are provided by starches and glucose. By contrast, CHO values for the oilseed meals (soybean, sunflower, rapeseed, groundnut) were 4 - 7 times lower than NFE3 (Table 6.1). Clearly, a larger proportion of the digestible carbohydrate in oilseeds consists of non-starch, non-glucose residues.

The literature on the composition of the carbohydrate fraction in oilseeds is far from comprehensive, but some data has been published for soybean meal (Smith and Circle, 1978). This oilseed (and presumably, other leguminous seeds) contains relatively high levels of the trisaccharide raffinose and the tetrasaccharide stachyose (Smith and Circle, 1978). These carbohydrates have no food value unless they have been previously hydrolysed by strong acids (Arora, 1983) and so it is appropriate that they are not recovered in the present (CHO) assay. The CHO assay is a specific measure of the amount of carbohydrate available for digestion and absorption regardless of the type of feed material. By contrast, the indirect measure, NFE3 is only accurate

with cereal grains and so is less useful.

The greater robustness of the CHO assay suggests that it may be superior to NFE3 as a predictor of digestibility. However, the regression data fail to reveal conclusively which carbohydrate measure (NFE3 or CHO) is the better predictor of DE (Section 6.3.4). This may be due to the fact that there is a flaw in the CHO assay: it does not measure sucrose. Later in this thesis (Chapter 8) it is shown that sucrose is a particularly valuable source of carbohydrate energy in diets for tilapia. Because sucrose is not recovered in the CHO assay, the contribution of available carbohydrate to DE is underestimated and the correlation between CHO and DE is weakened. An improvement would be to include a stage for measuring sucrose in the assay for available carbohydrate. For the present, it is sufficient that the accuracy of DE prediction is significantly improved by replacing conventional estimates of nitrogen free extract (NFE1, NFE2) with CHO.

The equations of most practical use for predicting DE are reproduced in full in the summary at the end of this chapter. To illustrate the level of accuracy provided by each of the 3 types of regression, DE was predicted with a single factor equation (6), an equation including a function of chemical measures (59) and a multiple regression equation (74). These predicted DE values are given in Table 6.11 where they are compared against values obtained from the digestibility trials in Chapter 5. DE estimates were also calculated by the conventional method of summating energy values for each of the component nutrients in a feed sample and these values also appear on Table 6.11. The summation values were calculated with ME values for protein, fat and carbohydrate as recommended for tilapia by Jauncey (1982). The carbohydrate contribution to total ME was calculated as

TABLE 6.11 Feed energy values (MJ/kg DM) as determined directly and by a variety of indirect methods

FEED	METHOD OF DETERMINATION						
	Direct bioassay (Chapter 5)	Regression prediction			Summated values*		
		Equation 6	Equation 59	Equation 74	A	B	C
Soybean	14.4	13.6	14.3	14.5	15.6	14.5	11.3
Ground maize	13.1	13.7	13.3	13.7	15.5	13.6	12.9
Wheat middlings	10.4	9.0	9.2	9.9	14.6	10.2	9.3
Wheat bran	6.1	5.8	6.4	7.3	13.6	7.4	6.7
Cassava	13.4	14.9	13.7	11.4	13.4	12.7	10.5
Sunflower	3.6	7.3	8.4	4.2	11.4	9.0	7.0
Rice bran	5.0	3.1	2.2	2.8	9.6	3.1	4.0
Sorghum	12.4	13.9	13.6	14.0	15.4	13.6	12.8
Wheat grain	13.3	12.7	12.8	13.4	15.3	12.4	11.6
Copra	6.5	5.2	5.3	5.7	14.2	8.3	7.4
Groundnut	17.9	15.5	15.0	17.0	17.9	17.5	14.9
Rapeseed	10.6	10.6	11.2	10.6	13.6	11.3	8.9
Palm kernel	1.7	3.2	3.1	4.0	14.8	8.1	8.4

* ME values of dietary components taken as: protein, 18.8 MJ/kg; fat, 35.6 MJ/kg; carbohydrate, 14.6 MJ/Kg (Jauncey, 1982). The energy contribution of the carbohydrate component was measured in 3 ways. In A, this contribution is measured as NFE1, in B as NFE3 and in C as available carbohydrate (CHO).

Equation 6: DE = 16.88 - 0.21 NDF

Equation 59: DE = 0.18(1.25 CP + NFE3) - 1.19

Equation 74: DE = 3.47 + 0.25 CP + 0.13 CHO - 0.26 CF

either NFE1, NFE3 or CHO to illustrate the effect of choice of carbohydrate assay on estimates of dietary energy. It is recognised that these final figures are ME values and cannot, strictly, be compared with DE. However, as pointed out in Section 1.2.2, ME and DE are identical measures for carbohydrate and fat. Thus, it is only in the high protein diets that ME will be noticeably smaller than DE.

The most obvious feature of these summated values is that, for each feed, the values decrease as digestible carbohydrate is estimated first, by NFE1 (A), then NFE3 (B) and, finally CHO (C). When compared with DE values determined by direct bioassay, it is clear that A values are all overestimates, particularly with the high fibre feeds, as expected from the discussion earlier in this Section. When NFE3 is the carbohydrate estimate (B-values) the predicted DE's compare much more closely with directly determined values.

Jobling (1983) criticises Jauncey (1982) for using nutrient ME values determined for different fish species, when calculating diet ME for tilapia. Whilst this criticism may be valid on theoretical grounds, the present results show that Jauncey's (1982) approach can be useful for obtaining rapid estimates of energy values in diets for tilapia, provided that nitrogen-free extract is estimated by difference with NDF. The other provision for using this summative approach is that it should not be applied to high fibre feeds. For example, Table 6.11 shows that the summated energy values (B) considerably overestimate the DE of sunflower and copra. Summated energy values using CHO as the carbohydrate assay are most accurate for the cereals, but less accurate for the oilseeds and high fibre feeds. When there is a wide range of dietary fibre as in the present feeds, one of the predictive regression equations should be used.

The relative accuracy of the 3 predictive equations in Table 6.11 is indicated by their respective residual standard deviations. On this basis, the multiple regression equation (74) is more accurate than either of the single factor regressions (6, 59). All regressions provide reasonable predictions of DE for most feeds. However, the accuracy of prediction appears to be lowest with the fibrous feeds. When NDF is used alone to predict DE (6), the error between predicted and determined values for most feeds is less than 10% and for over half the feeds evaluated, this error is less than 5%. However, deviations are particularly noticeable for the most fibrous feeds (sunflower, rice bran) and groundnut. The groundnut sample contained 54% protein and 24% carbohydrate (NFE3), but these energy supplying nutrients are not given any weight in equation 6 and this possibly explains the underestimation of the prediction. In contrast, the equation which combines chemical measures in a function (59) gives weight to the protein and carbohydrate fractions, so improving the accuracy of prediction for a wider range of feeds. However, there is still considerable inaccuracy when this equation (59) is applied to high fibre feeds. The most robust equation appears to be the multiple regression that includes a measure of fibre as well as protein and carbohydrate. This equation (74) should be applied when there is large variation in fibre levels. For other groups of feeds with lower, less variable levels of fibre, DE can be adequately predicted by equation 59 or by summation (method B, Table 6.11).

The agreement between direct and indirect values for rice bran and palm kernel meal is consistently poor, regardless of the method used to estimate DE. It is possible that rice bran and palm kernel meal do not fit the regressions either because they contain dietary factors

affecting digestibility which are not included in the regression, or because the direct determinations are inaccurate. As discussed in Section 5.4, the latter is a more likely explanation.

Dry-matter digestibility is most accurately predicted for plant-product feeds by the multiple regression which includes crude fibre and hemicellulose (76). However, there is only a small difference between the RSD of this equation and the equation including neutral detergent fibre as the sole predictor of DMD (30). Ease of dietary analysis argues in favour of the latter.

Hemicellulose does not correlate as well as the other fibre measures with DE or DMD (7, 31). It is possible that certain parts of the hemicellulose fraction (e.g. the pentosans) are digested by tilapia, and it would be of interest to check this directly by measuring hemicellulose in the faeces.

All of the regressions referred to so far in this Section have only concerned plant-product feeds. The extension of these regressions to include animal-product meals reduced the accuracy of prediction considerably. An alternative approach would be to compute separate predictive equations for each main class of feed as recommended by Schneider et al (1951) for terrestrial livestock. However, only four animal-product meals were evaluated in the present study and this is insufficient for meaningful statistical analysis. Further work is necessary with tilapias to determine the digestibility of a wider range of animal-product feeds, protein concentrates and silages before comprehensive sets of predictive regressions can be published.

Although the inclusion of animal-product meals reduced the predictive value of the equations, certain interesting trends were revealed. High levels of ash in the animal-product meals significantly

increased the correlation between this fraction and DMD in multiple regressions (77, 78). Similarly, the correlation between DCP and ash was significant for all feeds together, but not for plant-product feeds alone. Clearly, the more ash there is in a food, the less material there is available for absorption. In animal-product meals ash could, therefore, be a useful predictor of digestibility. Further work is needed to establish this, but there is already some evidence in the literature on trout nutrition that dietary ash is negatively correlated with protein digestibility in fishmeals (Nose and Mamiya, 1963). The same authors also found that DCP was positively correlated with dietary protein level. However, Nose and Mamiya (1963) only evaluated seven fishmeals with limited ranges of both ash (14-21%) and protein (65-71%). By extending these relationships to cover a wider range of animal-product meals, it may be possible to predict DCP with protein and ash in the same way that fibre and carbohydrate have been used to predict DE and DMD in the present study with plant-product feeds.

In contrast to the results of Nose and Mamiya (1963), there was no correlation between DCP and dietary protein in the present study. This is possibly because Nose and Mamiya (1963) restricted their study to a small number of animal protein concentrates. In the present research, protein was only found to have a significant effect on DE when included with other nutrients in multiple regressions. For example, the single factor regressions of DE on CP or CHO are not significant (Table 6.4), whilst the multiple regression with CP and CHO as two independent variables together, is highly significant (73). This indicates a clear synergistic response. DE is influenced more by the relative amounts of protein and carbohydrate in a feed than by either component on its own. The importance of protein as an energy source is indicated by the

significant partial regression of this nutrient in most of the multiple regressions (Table 6.10), and the results in Section 6.3.3 suggest that the DE value of protein is 1.25 times higher than carbohydrate.

In conclusion, this chapter has shown that regression analysis can be used to predict the digestibility of feeds without recourse to *in vivo* digestibility trials. However, regression prediction can never be as accurate as a biological assay because the former assumes that all nutrients have the same energy value in all diet formulations and under all feeding conditions. This assumption may not be valid for the reasons given in Section 1.2.2. Nevertheless, summative methods or regression predictions of DE are of considerable practical importance because the data required for an estimate of DE can be obtained by chemical analysis, usually within 24 hours. This is a considerable improvement on the conventional *in vivo* trials which, in the present study, required a total of four weeks per feed.

Section 6.5 Summary of predictive equations

The most useful equations for predicting digestible energy in units of MJ/kg DM are:

$$DE = 16.88 - 0.21\ NDF \qquad (6)$$

$$DE = 0.18(1.25\ CP + NFE3) - 1.19 \qquad (59)$$

$$DE = 12.30 + 0.13\ CP - 0.52\ CF \qquad (61)$$

$$DE = 13.20 + 0.10\ CP - 0.34\ ADF \qquad (63)$$

$$DE = 0.34\ CP + 0.22\ CHO - 3.53 \qquad (73)$$

$$DE = 3.47 + 0.25\ CP + 0.13\ CHO - 0.26\ CF \qquad (74)^{*}$$

$$DE = 5.42 + 0.19\ CP + 0.09\ NFE3 - 0.30\ CF \qquad (75)$$

* Equation 74 should be used in preference to the others if the appropriate chemical measures are available, because it provides the most accurate estimates of DE for a wide range of feeds.

The most useful equation for predicting dry-matter digestibility as a percentage is:

$$DMD(\%) = 84.96 - 1.20\ NDF \qquad (30)$$

CHAPTER 7

THE EFFECTS OF DIETARY DIGESTIBLE ENERGY AND AVAILABLE CARBOHYDRATE ENERGY LEVELS ON GROWTH, FOOD CONVERSION AND CARCASS COMPOSITION

Section 7.1 Introduction

The digestible energy (DE) content of a diet controls the amount of diet that is consumed (Hunt, 1980), because fish, like many other animals regulate food consumption to satisfy their energy requirements (Adolph, 1981). Thus, when fish are allowed free access to food, they maintain energy intake, whatever the DE value of the diet, by adjusting the bulk of food that they eat. A possible corollary of this is that fish which are fed on high energy diets will grow faster than those fed on low energy diets, when the amount of diet given is restricted to a fixed level (such as when following a set of hatchery feeding tables). This response to dietary DE on fixed feeding regimes has been verified with many terrestrial animals such as pigs (Drennan and Maguire, 1970), but it has not yet been investigated with tilapia.

It is usually assumed that dietary DE is directly related to the growth of fish, but this assumption is not always valid. For example, Takeuchi et al (1979) fed carp on diets in which DE levels were adjusted by varying the lipid and carbohydrate contents, but they found no simple connection between DE and growth. Lipids and carbohydrates were, however, found to be good dietary energy sources for carp, and growth was shown to depend more on the level of these nutrients in a diet than on overall DE. Clearly, a limitation of DE as a measure of food energy is that it only describes the total amount of energy absorbed and gives no indication of the contributions of different dietary energy sources

such as fats, carbohydrates and proteins. Thus, in studies of the utilisation of dietary energy, it is not sufficient to simply investigate the effect of DE on growth; attention must also be given to the energy contributions and specific nutrients.

Fats have the highest energy value, but in many processed plant-product feeds, this nutrient is present at relatively low levels when compared with proteins and carbohydrates. Certainly, this was the case with the feeds evaluated in this thesis (Chapter 5) and as a consequence dietary fats made only an insignificant contribution to overall DE (Chapter 6). Proteins can also be used as an energy source by fish (Section 1.2.2), but their most important function in fish diets is the provision of amino acids for the proliferation of body tissue. The third energy supplying nutrient is carbohydrate and in many plant-product feeds, this is the cheapest and most abundant source of DE.

There have been several studies on the ability of fish to utilise dietary carbohydrates for energy, but the level which can be tolerated in fish diets remains a topic of considerable controversy (Section 1.3.1, Hilton and Slinger, 1981). Evidence in the literature suggests that herbivorous fish are superior to carnivorous fish in their ability to digest and metabolise dietary carbohydrates (Sections 1.3.2 and 1.3.3). Consequently, there may be considerable potential for sparing dietary protein energy with carbohydrates in diets for tilapia which are predominantly herbivorous. However, until now, the role of carbohydrates in tilapia nutrition has been neglected and it has only been possible to speculate on the utilisation of this nutrient for dietary energy.

The aim of the work reported in this Chapter was, therefore, to measure the response of tilapia to changing DE levels, and to examine

the utilisation of available carbohydrate as an energy source in practical diets compounded from a series of plant-product feeds. Several responses to dietary carbohydrate energy were examined including growth, carcass composition, liver glycogen, net protein retention and net energy retention. In addition, the growth response to dietary fibre was also examined because this feed component is invariably present in plant-product feeds and, as shown in Chapter 6, it acts to reduce energy availability.

Section 7.2 Methods

7.2.1 Diets

The twelve diets used in this growth trial were those described in Chapter 5 which contained plant-product feeds. The diets containing animal-product feeds were not used because these contained no carbohydrate. The diets all shared a common basal mixture (the "reference diet" of Chapter 5) and differed only in their source of plant material. The diets were numbered and composed as indicated in Table 7.1. The proximate analyses of these diets are summarised in Table 7.2 along with their digestible energy and digestible crude protein values.

The basal mixture was formulated to ensure that all the essential fatty acid, essential amino acid, mineral and vitamin requirements of tilapia were satisfied in the final diet. In this way, the growth response of tilapia could be attributed to the effect of the different feeds in nutritionally adequate diets. The only source of available carbohydrate and fibre in each diet was from the plant-product feeds.

The amount of dietary energy provided by protein and carbohydrate

TABLE 7.1 Formulation of experimental diets incorporating a series of plant-product feeds

Diet	Feed g/100 g		Basal mixture g/100 g*
1	Soybean	60	40
2	Maize	60	40
3	Wheat bran	60	40
4	Cassava	60	40
5	Sunflower	60	40
6	Rice bran	60	40
7	Sorghum	60	40
8	Wheat grain	60	40
9	Copra	60	40
10	Groundnut	60	40
11	Rapeseed	60	40
12	Palm kernel	60	40

* Basal diet composition (g/100 g): fishmeal, 75.00; corn oil, 11.25; cod liver oil, 6.25, mineral/vitamin premix, 7.50

TABLE 7.2 Proximate composition of experimental diets

DIET	Dry matter %	As % of dry matter				Gross energy (MJ/kg DM)	Digestible energy (MJ/kg DM)	Digestible crude protein (g/kg DM)
		CP	EE	CHO*	NDF*			
1. Soybean	92.4	41.1	11.4	3.3	9.3	20.4	16.2	374.1
2. Maize	90.5	30.5	12.0	38.6	8.8	20.8	16.1	267.0
3. Wheat bran	95.3	28.9	11.0	9.8	31.1	20.2	11.1	234.7
4. Cassava	97.2	22.1	10.3	39.4	5.6	19.0	15.8	179.0
5. Sunflower	95.5	35.0	10.4	1.3	26.8	20.7	9.6	309.4
6. Rice bran	95.5	28.2	10.1	5.1	38.8	19.2	10.7	225.2
7. Sorghum	91.2	31.0	12.4	37.9	8.3	20.8	15.2	269.4
8. Wheat grain	96.0	27.7	10.7	36.2	11.9	20.3	16.1	244.7
9. Copra	95.2	31.8	13.9	1.4	32.8	20.5	11.5	275.8
10. Groundnut	90.7	45.0	13.7	3.6	4.0	22.3	19.0	412.9
11. Rapeseed	95.2	45.6	11.2	2.3	17.5	20.9	14.9	371.6
12. Palm kernel	94.7	29.4	16.1	1.6	38.4	21.4	8.6	236.4

All analyses in triplicate
* Calculated from data in Table 6.1
CP = Crude protein
EE = Ether extract
CHO = Available carbohydrate
NDF = Neutral detergent fibre

was estimated as follows. Protein energy was calculated by applying an energy equivalent of 23.6 MJ/kg DM to the digestible crude protein value of each diet. The resulting values are referred to as digestible protein energy (DPE). Carbohydrate energy was calculated by applying an energy equivalent of 16.7 MJ/kg DM to the available carbohydrate value for each diet. These values are referred to as available carbohydrate energy (CHO E). To enable comparison between diets, the DPE and CHO E values of each diet are expressed as a percentage of diet DE.

The 12 diets can be broadly classified on the basis of their protein and carbohydrate contents. Diets containing the protein-rich meals of soybean, sunflower, groundnut and rapeseed all contained more than 35% protein and negligible amounts of carbohydrate. The remaining diets all contained much higher levels of carbohydrate (i.e. available carbohydrate and fibre) and for convenience, these are referred to as the 'carbohydrate diets'. All diets were prepared as dry pellets (4.0 mm in diameter) as described in Section 2.3.1.

7.2.2 Experimental procedure

Each of 12 experimental tanks were stocked with 12 tilapia which had previously been marked with plastic tags as described in Section 2.4.2. The fish were distributed between tanks on the basis of their body weights to ensure that all groups had statistically indistinguishable mean weights and variances. These initial mean weights are given in Table 7.3. The water temperature during this trial was 28 C.

Each group was given a different diet at a rate of 1.5% of the group liveweight per day. This daily amount was presented in 3 equal

portions at 0900, 1300 and 1700 h. The fish were weighed every 7 d, so that as the fish grew, the amount of food given was adjusted to maintain the feeding rate at 1.5%. Diets were offered for a total of 91 consecutive days and on the 92nd day, all fish were killed and their weights recorded. Each carcass was identified by its plastic tag and the individual weight gain for each fish calculated. In this way, there were 12 observations of growth for each diet.

Six fish from each group were dissected immediately after they were killed, and their livers removed. These livers were immediately frozen in liquid nitrogen, placed in separate airtight tubes and stored at -20 C for subsequent glycogen analysis as described in Section 2.4.4. After removing their plastic tags, all carcasses were individually dried to constant weight at 105 C for moisture determination. Dried carcasses from each group were then pooled together and analysed for crude protein, fat, ash and gross energy using the methods described in Section 2.4.4. A group of seven fish was killed at the start of the trial and their carcasses similarly analysed to enable the calculation of food conversion efficiency (FCE), net protein retention (NPR) and net energy retention (NER).

Throughout the trial the faecal traps in the recirculating water system were emptied daily and water quality measured weekly. Dissolved oxygen, nitrite, nitrate, total ammonia, pH and hardness were all stable through the 13 week trial and remained within the limits given in Table 2.1.

Section 7.3 Results

Diets

Diet compositions are summarised in Table 7.2. Protein, available

carbohydrate and fibre levels all varied between diets, but fat levels were constant. The water stability and acceptability of these diets were as reported in Section 5.3, with the exception that the rapeseed diet was accepted less readily than the other diets at the beginning of this feeding trial. For the first 4 d, the rapeseed diet had to be presented as five meals before fish would accept the daily quota. Thereafter, this problem resolved itself and the daily quota of all diets were consumed within 3 meals.

Growth, feed conversion, carcass composition and liver glycogen

Growth values are given in Table 7.3, carcass composition in Table 7.4 and feed conversion values in Table 7.5. Where appropriate, data were subjected to one-way ANOVAR and differences between means tested with the multiple range technique of Duncan (1955).

All diets caused a steady increase in fish weight over the 13 week trial. Mortalities were infrequent and occurred randomly. Specific growth rates were low, because of the low feeding rate, but despite this, there were significant differences in the mean weight gain between groups ($p < 0.05$, Table 7.3). The greatest gains were with the groups fed on high protein, low fibre diets (i.e. groundnut and soya). The smallest gains were in groups fed on the high fibre diets (sunflower, rice bran, copra and palm kernel).

The feed conversion ratios (FCR's) of the diets reflected the pattern observed for growth (Table 7.5). The best feed conversions (lowest FCR's) were for the soya and groundnut diets, whilst the worst conversions (highest FCR's) were for the high fibre diets. These FCR values are, however, only crude measures of conversion efficiency as

TABLE 7.3 Growth of tilapia fed on experimental diets containing plant-product feeds

DIET	Mean initial weight (g)	Mean final weight (g)	Mean weight gain (g)
1. Soybean	51.1[a]	97.5	46.5[ab]
2. Maize	50.8[a]	85.4	34.5[abcd]
3. Wheat bran	51.3[a]	82.8	31.5[bcd]
4. Cassava	50.8[a]	86.5	35.6[abcd]
5. Sunflower	50.2[a]	68.2	18.0[d]
6. Rice bran	50.1[a]	73.5	23.4[d]
7. Sorghum	50.8[a]	94.0	43.2[abc]
8. Wheat grain	49.6[a]	83.2	33.7[abcd]
9. Copra	49.6[a]	73.6	24.0[d]
10. Groundnut	51.1[a]	102.3	51.2[a]
11. Rapeseed	49.5[a]	78.0	28.5[cd]
12. Palm kernel	49.6[a]	74.8	25.3[d]

Each value is the mean of 12 fish
Figures with common superscripts in the same column are not significantly different ($p > 0.05$)

TABLE 7.4 Carcass analysis and liver glycogen of tilapia fed on diets containing plant-product feeds

Diet	Moisture (%)	Crude protein (%)	Ether extract (%)	Ash (%)	Gross energy (MJ/kg DM)	Liver glycogen* (% wet liver)
1. Soybean	77.3^{cd}	14.8	2.7	5.0	18.3	5.5^{cd}
2. Maize	74.2^{bc}	15.3	5.4	5.1	20.9	9.3^{b}
3. Wheat bran	77.1^{cd}	14.2	3.5	5.4	18.5	5.2^{cd}
4. Cassava	70.8^{a}	15.3	9.1	6.2	22.8	12.6^{a}
5. Sunflower	78.9^{d}	13.5	1.2	6.0	16.5	2.0^{e}
6. Rice bran	76.5^{bcd}	14.9	2.9	6.5	18.6	4.6^{cd}
7. Sorghum	75.3^{bc}	13.7	5.7	5.3	21.8	9.6^{b}
8. Wheat grain	73.7^{b}	15.5	5.1	6.2	20.3	11.2^{ab}
9. Copra	77.1^{cd}	14.8	2.6	5.4	18.6	5.4^{cd}
10. Groundnut	75.2^{bc}	15.0	4.9	4.5	21.5	6.3^{c}
11. Rapeseed	78.9^{d}	14.2	1.5	5.3	17.9	3.8^{de}
12. Palm kernel	77.3^{cd}	14.0	3.6	4.8	19.8	5.2^{cd}
Initial group**	80.1	13.3	1.5	4.6	18.4	-

Each value for carcass composition is the average of duplicate determinations on 12 pooled carcasses. Moisture values are each the mean of 12 fish and those with a common superscript are not significantly different ($p > 0.05$)

* Each liver glycogen value represents the mean of 6 fish. Values with a common superscript are not significantly different ($p > 0.05$)

** Group sampled for analysis at the beginning of the trial. Mean weight 49.12 ± 11.31 ($n = 7$)

TABLE 7.5 Food conversion of tilapia fed on diets containing plant-product feeds

DIET	Food conversion ratio (FCR)	Food conversion efficiency (%) (FCE)	Protein efficiency ratio (PER)	Apparent net protein retention (%) (NPR)	Apparent net energy retention (%) (NER)
1. Soybean	1.8	15.7	1.5	24.4	14.1
2. Maize	2.6	15.5	1.4	26.5	17.5
3. Wheat bran	2.3	12.5	1.6	24.3	11.5
4. Cassava	2.2	19.8	2.1	47.0	26.9
5. Sunflower	3.9	6.6	0.8	12.0	3.9
6. Rice bran	3.1	10.5	1.2	23.6	10.4
7. Sorghum	2.0	16.5	1.8	29.4	19.4
8. Wheat grain	2.4	13.3	1.6	28.2	14.7
9. Copra	3.8	10.2	1.1	20.6	9.3
10. Groundnut	1.8	18.6	1.4	24.9	19.6
11. Rapeseed	2.7	9.1	0.9	13.4	7.5
12. Palm kernel	2.9	10.2	1.2	21.3	10.3

Each value was calculated from the pooled data for 12 fish

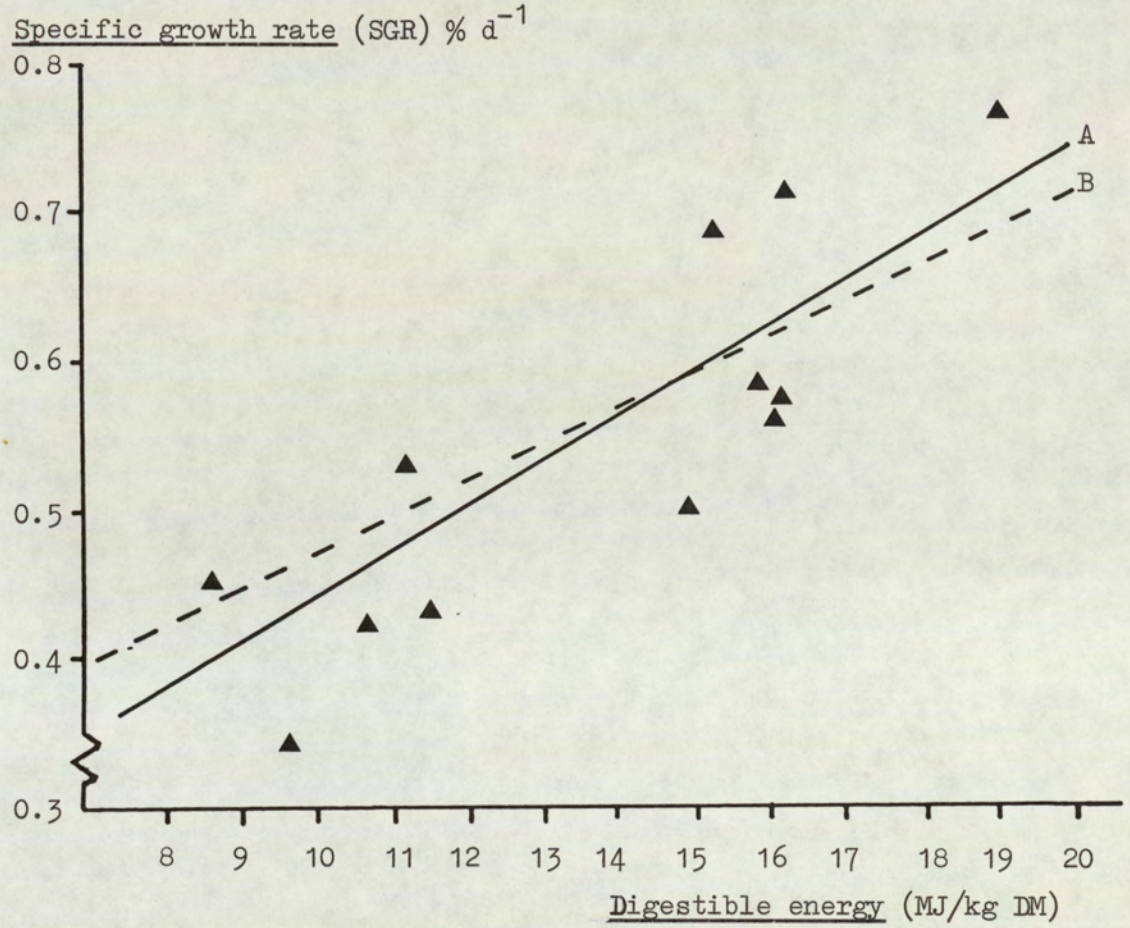
explained in Section 2.4.5. There were significant differences in carcass moisture between groups at the end of the feeding trial ($p < 0.05$, Table 7.4) and because of this, it is more meaningful to compare food conversion efficiencies on a dry-matter basis (FCE). When food conversion efficiencies are compared in this manner, it can be seen that the high carbohydrate diets (cassava, sorghum, maize) are converted at least as efficiently as the high protein diets (Table 7.5).

The carcass composition values in Table 7.4 show that the variations in protein and ash between groups were less than the variation in carcass fat. Carcass fat varied from 1.2% (sunflower diet) to 9.1% liveweight (cassava diet). As carcass fat increased, so the energy content of the carcasses increased (Table 7.4). The differences in liver glycogen between groups were also large and followed a trend similar to that for carcass fat: liver glycogen was lowest in the group fed on the sunflower diet (2.0% of liver wet weight) and highest in the group fed on the cassava diet (12.6% of liver wet weight). These differences were significant at the 5% probability level as indicated in Table 7.4.

The correlations of dietary energy with growth, protein retention, energy retention, carcass fat and liver glycogen

There was positive linear correlation between DE and specific growth rate ($p < 0.001$, Figure 7.1). This relationship applied to the entire range of diets, regardless of their protein, carbohydrate or fibre contents. For the carbohydrate diets, (maize, wheat bran, cassava, rice bran, sorghum, wheat grain, copra and palm kernel), SGR was more closely correlated with the level of carbohydrate energy ($r = 0.96$) than with overall DE ($r = 0.84$), (cf Fig. 7.1 with Fig.

FIGURE 7.1 SPECIFIC GROWTH RATE AS A FUNCTION OF LEVEL OF DIGESTIBLE ENERGY IN DIETS CONTAINING PLANT-PRODUCT FEEDS

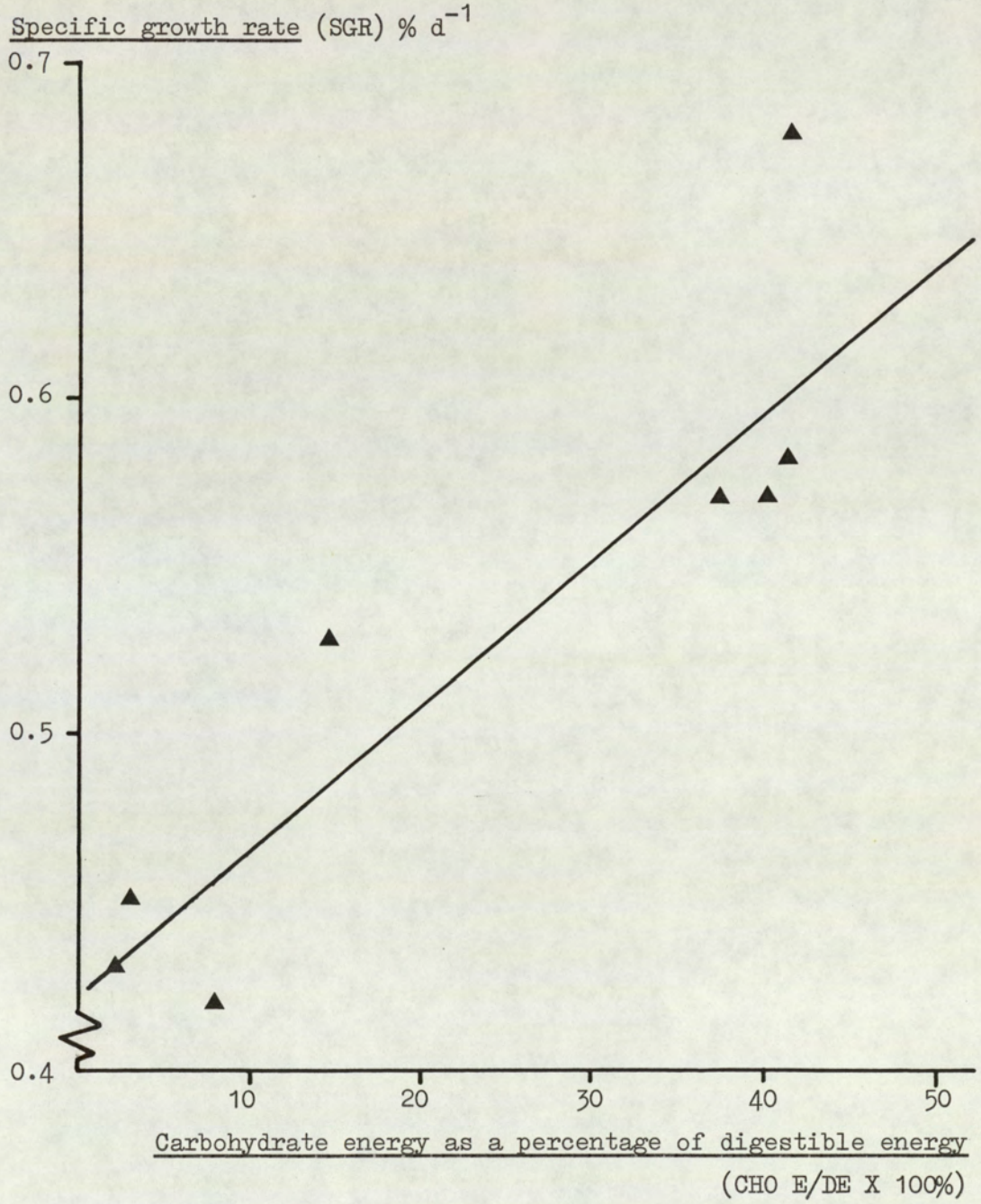


Each data point represents the mean SGR of 12 fish

A. Regression for all diets (n = 12)
SGR = 0.14 + 0.03 DE ($r = 0.84$, $P < 0.001$)
Residual standard deviation = 0.07

B. Regression for 'carbohydrate diets' only (n = 8)
SGR = 0.23 + 0.024 DE ($r = 0.84$, $P < 0.01$)
Residual standard deviation = 0.05

FIGURE 7.2 SPECIFIC GROWTH RATE AS A FUNCTION OF LEVEL OF CARBOHYDRATE ENERGY IN PLANT-PRODUCT FEEDS



Each data point represents the mean SGR of 12 fish

SGR = 0.42 + 0.0045 (CHO E/DE) $r = 0.96$, $P < 0.001$

Residual standard deviation = 0.02

7.2). A corollary of this is that SGR was predicted more accurately by its regression on carbohydrate energy than by its regression on DE. The residual standard deviation (RSD) of the former regression was only 0.02, whilst that of the latter was 0.05 (cf Equation B, Fig. 7.1 with the equation in Fig. 7.2). Dietary carbohydrate energy was also positively correlated with net energy retention (Fig. 7.3) and the levels of carcass fat and liver glycogen (Fig. 7.4) in fish fed on the carbohydrate diets.

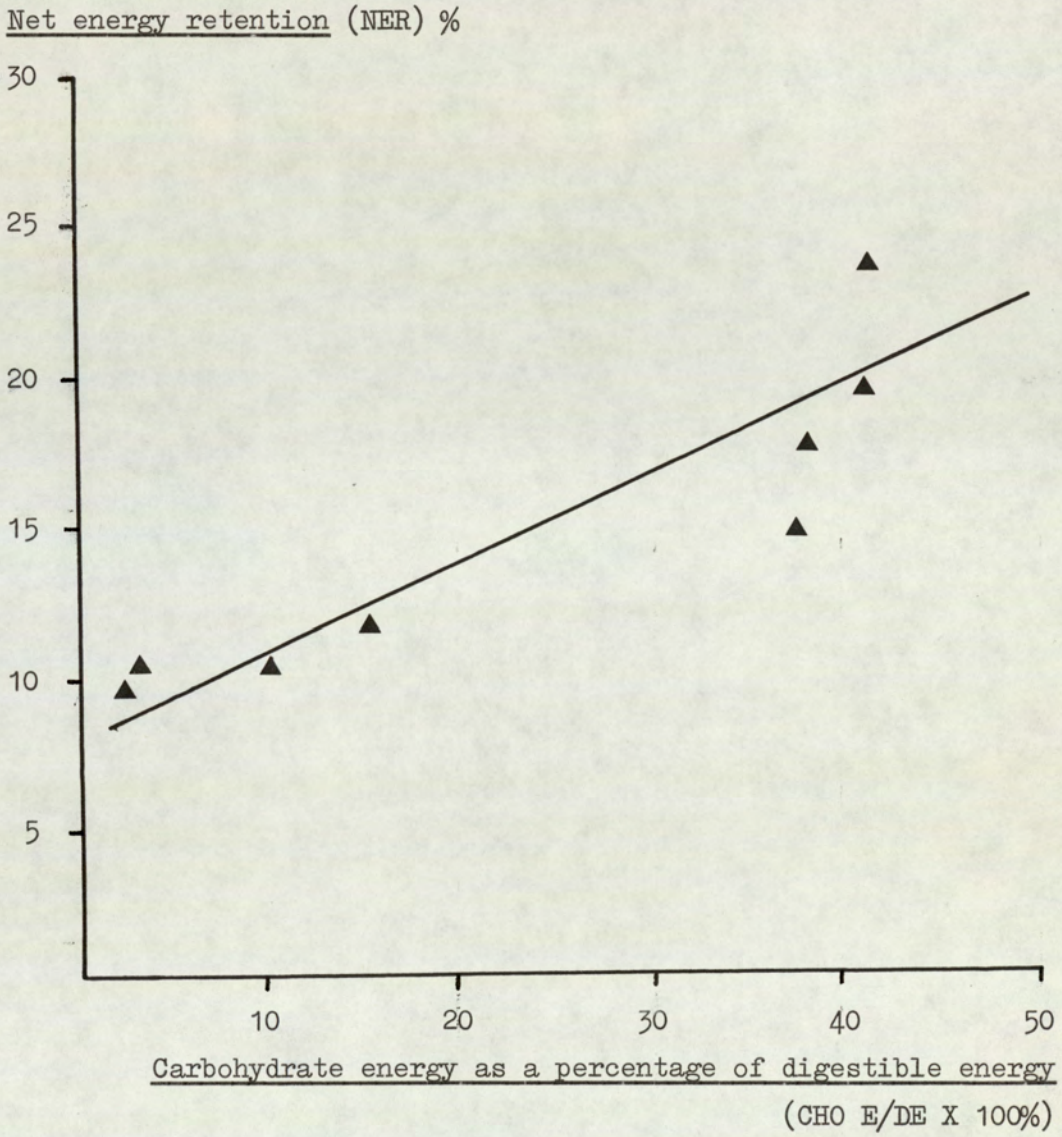
Net protein retention (NPR) was similarly correlated with the level of dietary carbohydrate and this relationship applied to all feeds. As the ratio of available carbohydrate to digestible protein increased, so NPR increased (Fig. 7.5). In other words, an increase in available carbohydrate at the expense of protein in the diets led to an improvement in the efficiency of protein retention. The converse was that NPR decreased as the level of dietary protein increased (Fig. 7.6). Clearly, as a greater proportion of dietary digestible energy is supplied by protein, the efficiency of protein retention decreases.

The level of dietary protein energy also had a clear effect on net energy retention. As more of the overall DE was supplied by protein, less energy was retained by the fish (Fig. 7.6) and, correspondingly, there was a decrease in their levels of carcass fat and liver glycogen (Fig. 7.7).

The correlation between dietary fibre and growth

The total carbohydrate content of the diets consisted of varying amounts of available carbohydrate and fibre (measured as NDF, Table 7.2). As the ratio of NDF to available carbohydrate increased, there

FIGURE 7.3 NET ENERGY RETENTION AS A FUNCTION OF LEVEL OF CARBOHYDRATE ENERGY IN PLANT-PRODUCT FEEDS

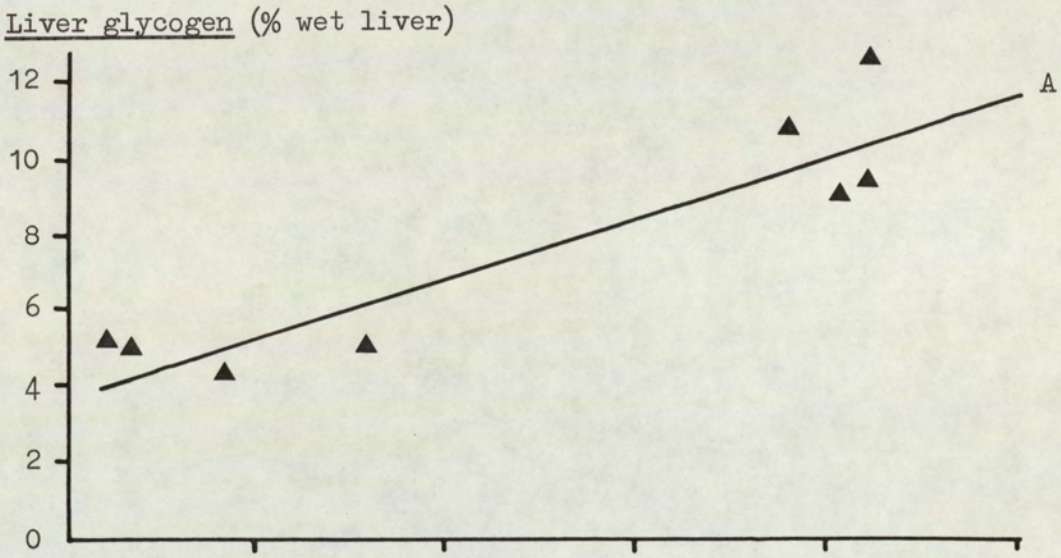


Each data point represents the mean of 12 fish

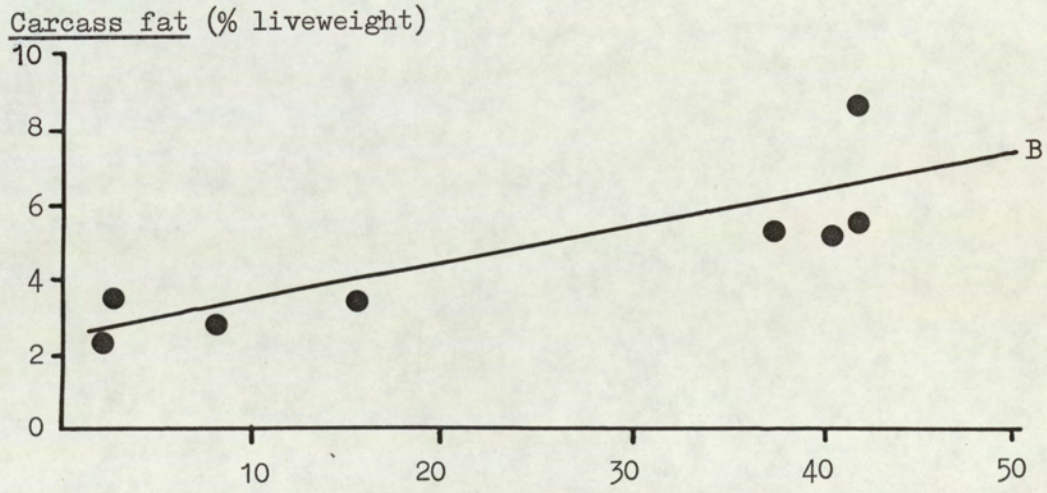
NER = 8.37 + 0.28 (CHO E/DE) $r = 0.85$, $P < 0.01$

Residual standard deviation = 3.46

FIGURE 7.4 LIVER GLYCOGEN AND CARCASS FAT AS FUNCTIONS OF THE LEVEL OF CARBOHYDRATE ENERGY IN PLANT-PRODUCT FEEDS



Each data point represents the mean liver glycogen value of 6 fish



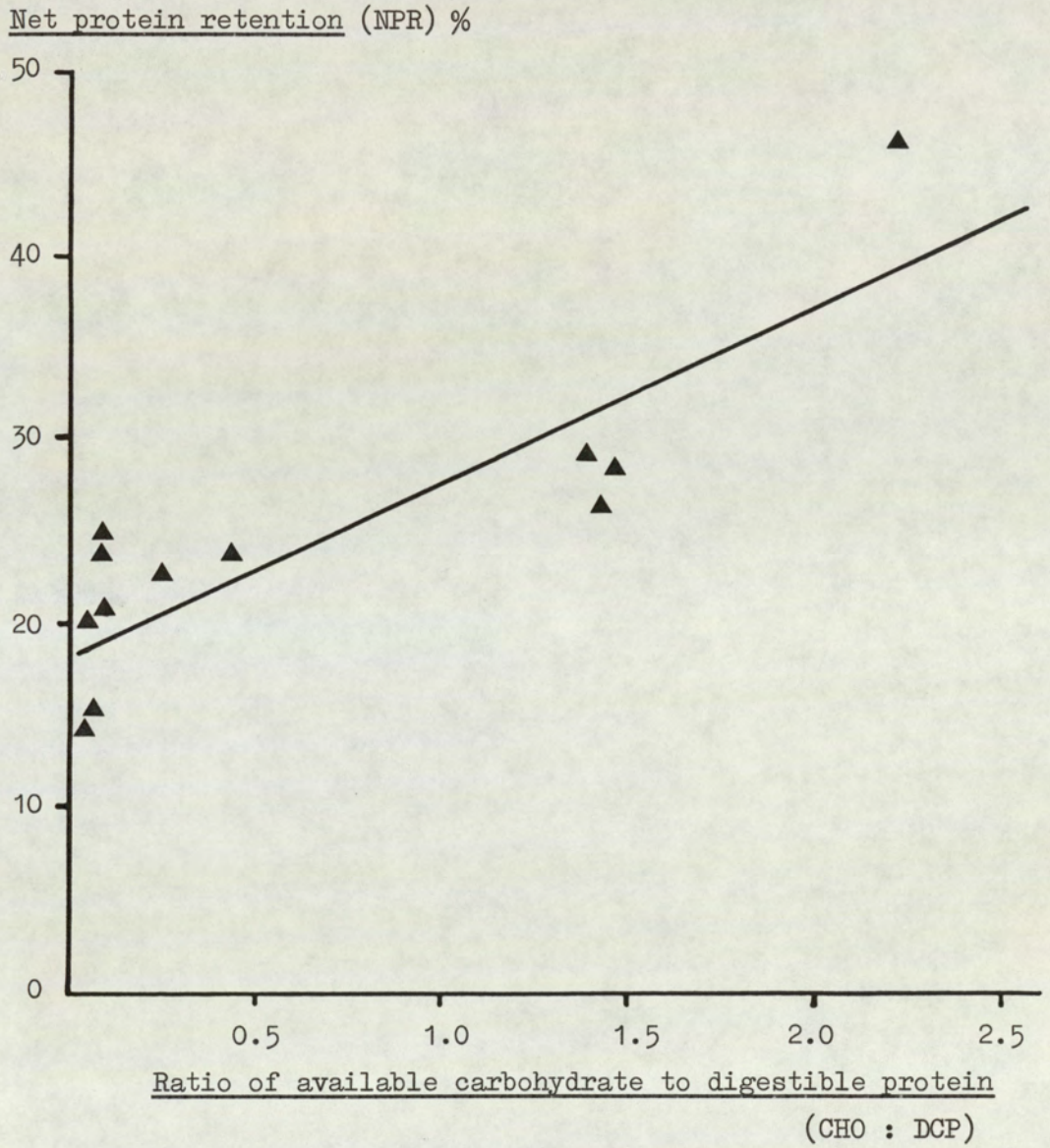
Carbohydrate energy as a percentage of digestible energy (CHO E/DE X 100%)

Each data point represents the mean carcass fat value of 12 fish

A. Liver glycogen = 4.14 + 0.16 (CHO E/DE) $r = 0.92$, $P < 0.01$
Residual standard deviation = 1.35

B. Carcass fat = 2.49 + 0.095 (CHO E/DE) $r = 0.82$, $P < 0.02$
Residual standard deviation = 1.31

FIGURE 7.5 NET PROTEIN RETENTION AS A FUNCTION OF THE CARBOHYDRATE TO PROTEIN RATIO IN DIETS CONTAINING PLANT-PRODUCT FEEDS

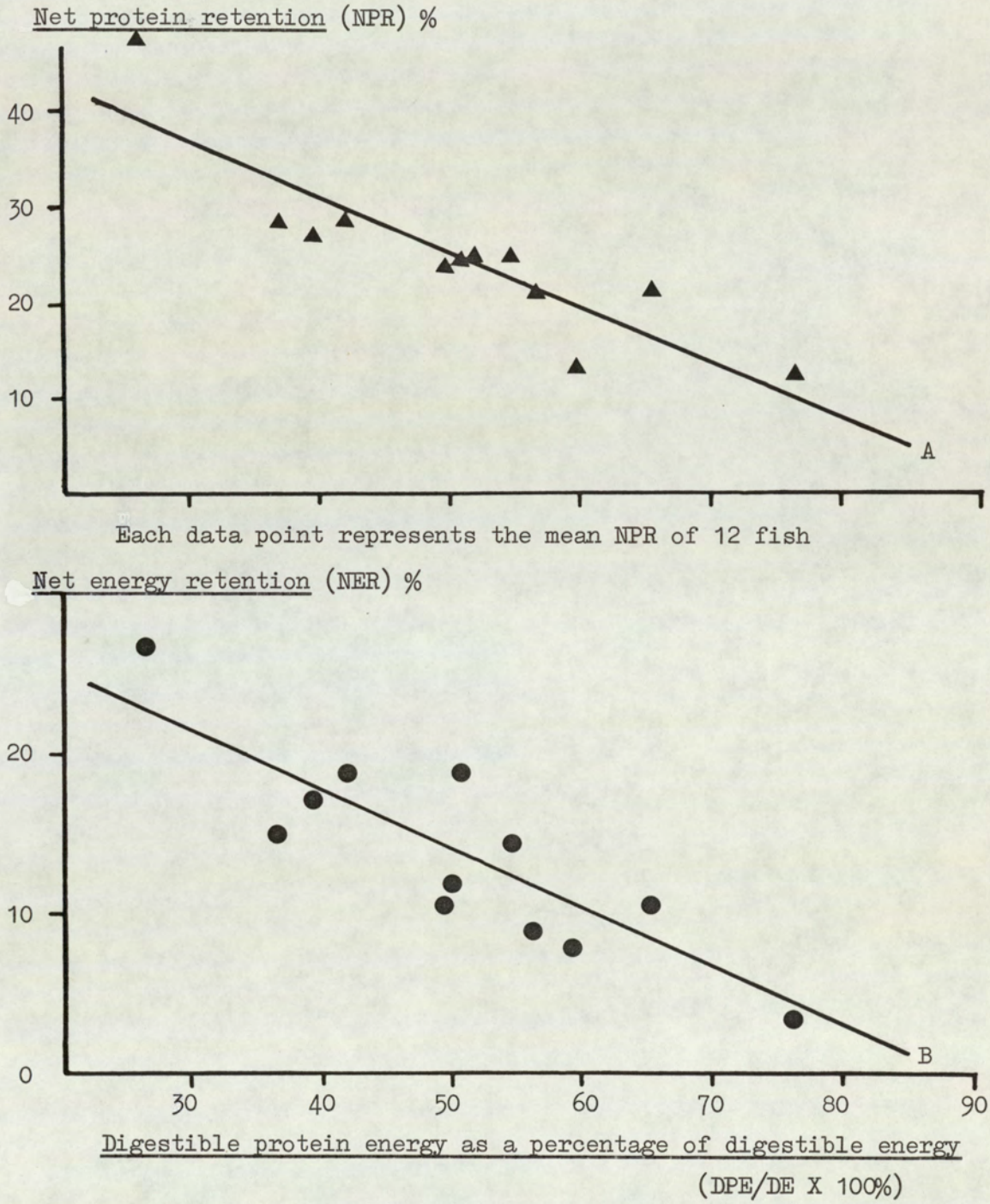


Each data point represents the mean NPR of 12 fish

NPR = 18.67 + 9.41 (CHO : DCP) $r = 0.84$, $P < 0.001$

Residual standard deviation = 5.16

FIGURE 7.6 NET PROTEIN RETENTION AND NET ENERGY RETENTION AS FUNCTIONS OF THE LEVEL OF PROTEIN ENERGY IN DIETS CONTAINING PLANT-PRODUCT FEEDS

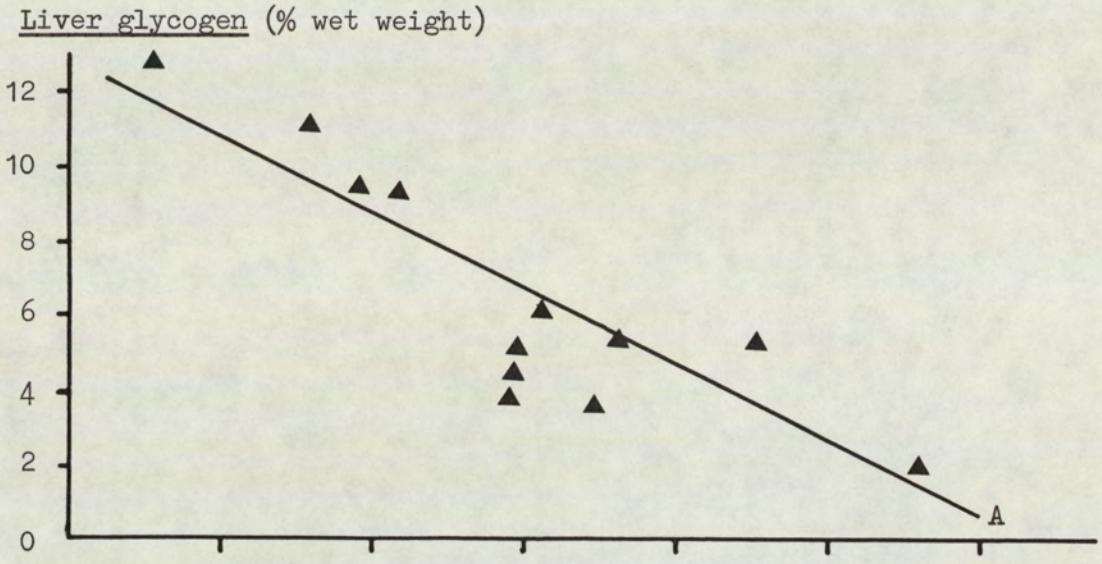


Each data point represents the mean NER of 12 fish

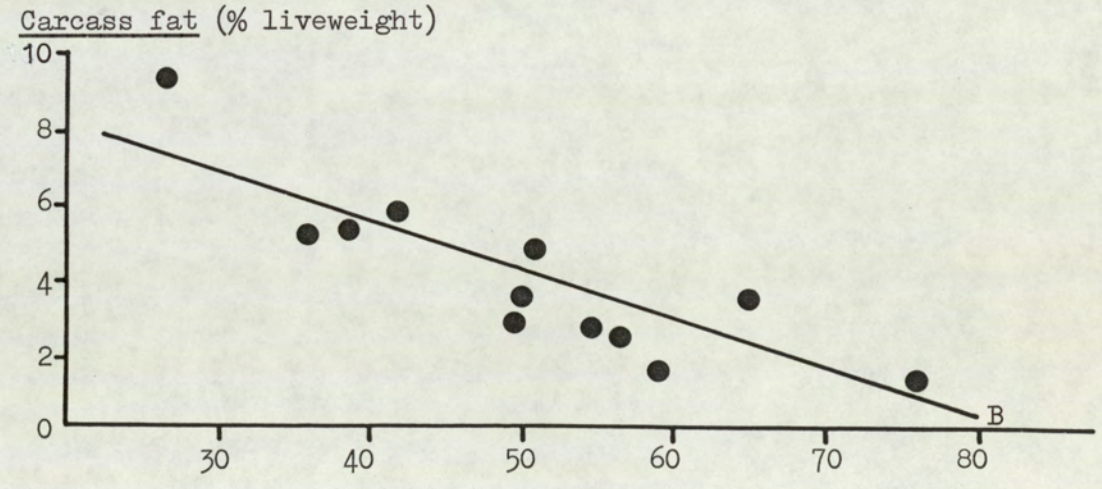
A. NPR = 53.8 - 0.58 (DPE/DE) $r = -0.90$, $P < 0.001$
Residual standard deviation = 4.25

B. NER = 32.66 - 0.37 (DPE/DE) $r = -0.84$, $P < 0.01$
Residual standard deviation = 3.48

FIGURE 7.7 LIVER GLYCOGEN AND CARCASS FAT AS FUNCTIONS OF THE LEVEL OF PROTEIN ENERGY IN DIETS CONTAINING PLANT-PRODUCT FEEDS



Each data point represents the mean liver glycogen value of 6 fish



Each data point represents the mean carcass fat value of 12 fish

A. Liver glycogen = 16.81 - 0.20 (DPE/DE) $r = -0.88$, $P < 0.001$
Residual standard deviation = 1.57

B. Carcass fat = 10.76 - 0.13 (DPE/DE) $r = -0.87$, $P < 0.001$
Residual standard deviation = 1.12

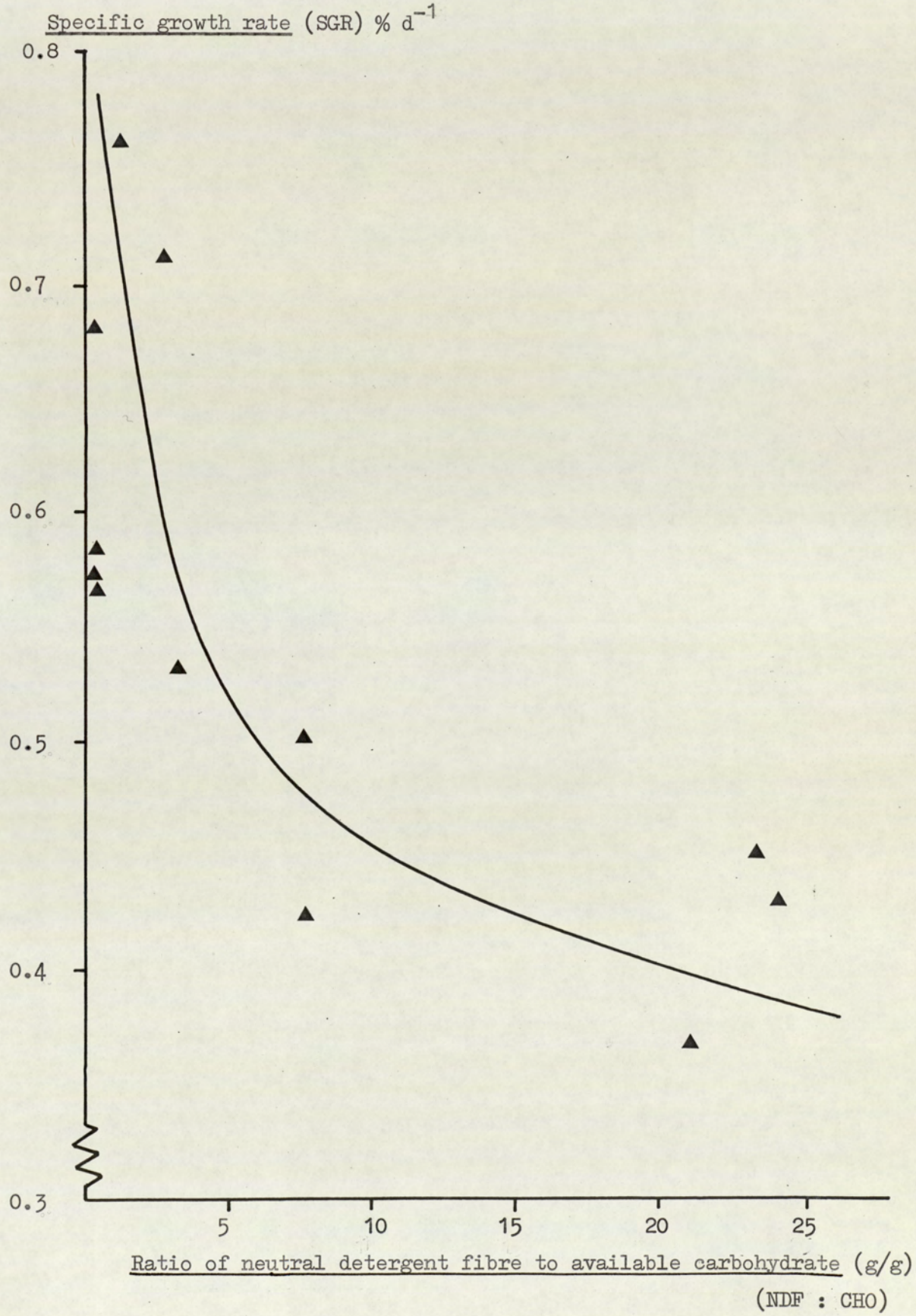
was a decrease in growth rate (Fig. 7.8). This reduction was not linear and there were too few data to determine the exact form of this relationship. However, it is clear that the reduction in growth with increasing fibre to available carbohydrate ratio was more pronounced at low fibre levels than at high levels. The reduction in specific growth rate was particularly noticeable as the ratio of fibre to available carbohydrate in the diets increased from 0:1 to 5:1. Subsequent increases in the ratio of fibre to available carbohydrate caused relatively smaller reductions in growth.

Section 7.4 Discussion

The results of this feeding trial demonstrate a clear relationship between the digestible energy value of plant-product feeds and the rate of tilapia growth. As the DE of the diets increased, so growth increased and this applied over a wide range of dietary protein, available carbohydrate and fibre levels (Fig. 7.1). The diets all shared a common basal mixture and differed only in their source of plant material. Thus, fat levels being similar in all diets, the differences in diet DE contents were directly due to the effect of different protein, available carbohydrate and fibre levels in the individual feeds as demonstrated in Chapter 6.

The plant-product feeds were essentially of 2 types: those in which most of the DE was provided by protein (soya, groundnut, sunflower, rapeseed) and those containing greater levels of available carbohydrate and fibre and lower levels of protein (maize, wheat bran, cassava, rice bran, sorghum, wheat grain, copra and palm kernel). Growth rates were highest on the diets containing the high-protein, low

FIGURE 7.8 SPECIFIC GROWTH RATE AS A FUNCTION OF THE FIBRE TO AVAILABLE CARBOHYDRATE RATIO IN PLANT-PRODUCT FEEDS



fibre feeds soya and groundnut. The protein/energy ratios of these two diets were 106 and 100 mg crude protein/kcal DE respectively. These values are close to the optimal range of 111 - 125 mg protein/kcal DE reported for tilapia in the literature, as summarised in Section 1.3.4. The other high-protein diets (sunflower and rapeseed) did not produce such good growth, possibly because of their greater levels of fibre and this will be discussed further. With the remaining diets, the growth of tilapia was very closely correlated with the level of available carbohydrate energy ($r = 0.96$, Fig. 7.2).

As the level of available carbohydrate energy increased relative to overall DE in the carbohydrate diets, so growth improved. This correlation was stronger than the correlation between overall DE and growth and clearly demonstrates that the carbohydrate fraction of cereals, roots and cereal by-products can be utilised as a major source of dietary energy by tilapia.

Increasing levels of available carbohydrate not only influenced growth, but also affected the energy balance of tilapia. Thus, as more DE was provided by carbohydrates, so net energy retention (NER) increased (Fig. 7.3). Clearly, less dietary energy was wasted and more retained by the fish when proteins were replaced by carbohydrate. Much of the increase in carcass energy levels was, evidently, due to an accumulation of lipid, as the results indicate a close correlation between dietary carbohydrate and carcass fat (Fig. 7.4). This suggests an increased rate of lipogenesis upon consumption of carbohydrates. Details of the metabolic pathway by which carbohydrates are converted to lipid have not been reported for tilapia and a study of the activity of lipogenic enzymes and the conversion of protein to lipid in response to dietary carbohydrate could form the basis of further studies.

Nevertheless, the practical implications of the present results are clear: high levels of dietary carbohydrate can produce fatty fish. Excessively fatty fish may be unacceptable to human consumers and from a commercial viewpoint, this alone places a constraint on the use of large amounts of carbohydrate in tilapia diets. According to Balarin and Hatton (1979) the liveweight fat content of wild tilapia is typically 5 - 6%. The maximum level recorded in the present study was 9.1% (cassava diet), but otherwise ranged from 1.5% - 5.7%. These carcass fat levels cannot be considered excessive and in this respect, the dietary carbohyrate levels reported for the present diets (1.4 - 39.4%) may represent an acceptable range in commercial diets for tilapia. The influence of dietary carbohyrate level on growth and carcass quality is, nevertheless, of considerable importance and this is investigated further in the following Chapter.

From a physiological viewpoint, it is perhaps of more significance that liver glycogen levels were positively correlated with dietary carbohydrate levels (Fig. 7.4). The accumulation of excessive levels of glycogen in salmonid livers has recently been shown to impair liver function. Thus, trout with high liver glycogen levels were more susceptible to the toxic effects of waterborne copper (Dixon and Hilton, 1981) and less resistant to drug treatment (Hilton and Dixon, 1982). Under intensive culture conditions, this impairment of liver function could render fish more susceptible to stress and this may be the reason for the increased mortality rates which have been reported for salmonids fed on high carbohydrate diets (Phillips et al, 1948, Austreng et al, 1977; Refstie and Austreng, 1981). However, as an herbivorous fish, tilapia may be better adapted to dietary carbohydrates than carnivorous fish for the physiological and metabolic reasons given in Section

1.3. Unlike carnivorous fish, such as eels, which mobilise tissue fat or protein in preference to glycogen when starved (Inui and Yokote, 1974), tilapia appear to rely more on glycogenolysis as a mechanism for maintaining blood sugar when starved. Thus, when tilapia are deprived of food, there is a rapid decrease in liver glyogen (Swallow and Fleming, 1969; Caulton and Bursell, 1977). This, together with the glycogenic response to dietary carbohydrate observed in the present study suggests that glycogen metabolism is relatively well developed in tilapia. If so, the liver in tilapia should be adapted to withstand larger fluctuations in glycogen content than in carnivorous fish, and may be capable of tolerating higher levels of glycogen before suffering damage. This remains to be verified, but the present trial at least showed that there were no obvious pathological effects related to liver glycogen within the limits studied. Liver glycogen was significantly higher in tilapia fed on diets containing cereal grains and cassava than in those containing protein-rich oilseeds or high fibre diets and at the highest recorded level of glycogen (12.6%) fish grew as well as, if not better, than those with lower glycogen levels. Thus, whilst liver glycogen levels were sensitive to an increase in dietary carbohydrate, this was not considered to be a problem even with the diet containing the greatest amount of available carbohydrate (39.4%).

As discussed earlier, the influence of dietary carbohydrate energy on growth and food conversion should be considered in relation to the influence of dietary protein energy (Section 1.3.4). In Chapter 6, it was shown that the DE of a diet depends more on the balance of protein and carbohydrate in plant-product feeds, than on the absolute level of either nutrient alone. In the present chapter, this observation was extended further by the fact that the ratio of available carbohydrate

energy to digestible protein was positively correlated with the net protein retention (NPR) over the entire range of diets (Fig. 7.5). Thus, as carbohydrates replaced protein in the diets, a greater proportion of the dietary protein was retained by the fish. The inference here is that fewer amino acids were catabolised for energy and more used in protein synthesis as increasing levels of dietary energy were provided by carbohydrates. In other words, dietary carbohydrate was used to spare protein.

The sparing of protein by non-protein energy sources (usually lipids) has been reported in the literature for other species of fish (e.g. Halver, 1972; Jauncey and Ross, 1982; Section 1.3.1). Protein sparing has been recommended as a possible means of reducing the amount of fishmeal currently used in fish diets and this is a necessity if diet costs are to be reduced (Section 1.3.1). Most studies have claimed to investigate the effect of non-protein energy in semi-purified isonitrogenous and isoenergetic diets, to ensure that the influences of fats and carbohydrates are not confounded by changes in dietary protein level. However, as Jobling (1983) points out, there are doubts as to whether or not these diets are truly isoenergetic. Certainly, it is possible to formulate diets to similar energy levels on the basis of the gross or digestible energy values of their ingredients, but it is doubtful that such diets are isoenergetic with respect to metabolisable energy (Jobling, 1983). The same problem applies to balancing dietary protein levels, because the digestibility of this nutrient has often been assumed to be a constant for all diets, but as shown in Chapter 5, this assumption is invalid. Thus, whilst diets may be similar with respect to crude protein levels, they may differ considerably with respect to digestible crude protein. For these reasons, it was

considered more valid in the present trial, to consider the ratio or available carbohydrate to digestible protein.

The priority here was to demonstrate the utilisation of carbohydrate energy in plant-product feeds. It was not possible to manipulate the energy or protein content of these feeds. Instead, the gross effects of whole feeds on growth and food conversion was investigated by substituting these feeds in a series of diets which shared a common basal diet. This basal diet contained no carbohydrates or fibre and so the only source of these components in the final diets was from the plant-product feeds themselves. Additionally, the basal diet on its own was sufficient to satisfy the nutrient requirements of tilapia. Nutritional responses to the diets could, therefore, be ascribed to the additional protein, carbohydrate and fibre provided by the plant-product feeds, but the result of this formulation strategy was that the final diets were anisoenergetic and anisonitrogenous. This problem was circumvented by expressing the energy contributions of protein and carbohydrate as proportions of DE, as recommended by Jobling (1983). Additionally, NPR was expressed as a function of the dietary carbohydrate to protein ratio, thereby compensating for changes in dietary protein levels in the present demonstration of the protein sparing effect of carbohydrates. The observation that NPR, NER, carcass fat and liver glycogen could be equally well correlated with a decrease in protein energy (Figs. 7.6 and 7.7) as with an increase in carbohydrate energy is, therefore, only further demonstration that nutritional responses to protein and carbohydrate were reciprocal functions of one another. The essential point is that growth, NPR, NER, carcass fat and liver glycogen all increased as protein was replaced by carbohydrate in the diets.

Apart from protein and available carbohydrate, the only other component to vary in the diets was fibre. This component (measured as NDF), consists mainly of cellulose, hemicellulose and lignin, all of which are resistant to digestion by tilapia (Section 1.2.3). The carbohydrate content of plant-product feeds can, therefore, be split into two categories: available carbohydrate (sugars and starches) and indigestible carbohydrate (fibre).

As the amount of fibre relative to available carbohydrate increased in the present diets, so SGR decreased (Fig. 7.8). The most obvious explanation for this is that less energy was available for growth as cellulose, hemicellulose and lignin replaced sugars and starches. Chapter 6 showed that increasing levels of dietary fibre caused a linear decrease in DE. The present chapter has extended this observation by showing that a reduction in DE causes a linear decrease in growth rate. By inference, therefore, increasing levels of fibre could be expected to cause a linear reduction in growth. However, the relationship between dietary fibre and growth was non-linear (Fig. 7.8). This suggests that the dilution of dietary energy is not the only reason for the observed reduction in growth rates with increasing dietary fibre levels. It is possible that fibre not only replaced energy yielding nutrients, but in some way interfered with the availability of the remaining nutrients in a diet. This cannot be proved with the present results, but the idea is investigated further in the following chapter.

However, for now, it is sufficient that increasing levels of dietary fibre have been shown to reduce the growth of tilapia. The practical implication is that whilst a feed may have relatively high levels of available carbohydrate, it may be of limited value in complete

diets for tilapia if it also contains substantial amounts of fibre.

The feeds containing the highest level of available carbohydrate per unit of fibre, were the cereals maize, sorghum and wheat grain and the root cassava. All contained similar levels of available carbohydrate (36 - 40%) and fibre (5 - 12% NDF). However, the response of tilapia to these different carbohydrate feeds was not the same. NPR, NER, liver glycogen and carcass fat were all lower in fish fed on the cereal grains than in those fed on cassava. The different values for each of these parameters of food utilisation are summarised below.

Diet	Net protein retention (%)	Net energy retention (%)	Liver glycogen (% wet liver)	Carcass fat (% DM)
Maize	26.5	17.5	9.3	5.4
Sorghum	29.4	19.5	9.6	5.7
Wheat grain	28.2	14.7	11.2	5.1
Cassava	47.0	26.9	12.6	9.1

Thus, more of the carbohydrate energy was retained and more used to spare protein in tilapia fed on the cassava diet than in those fed on the cereal grain diets. Clearly, the carbohydrates in cassava are of greater energy value to tilapia than the carbohydrates in cereal grains.

The available carbohydrates in all plant-product feeds are mainly starches and sugars, but the relative levels of these two classes of carbohydrate depends on feed type. Cassava, for example, is an underground storage organ, whilst cereal grain feeds are derived from plant seeds. The carbohydrate profile of these two types of feed would, therefore, be expected to differ and this could account for the observed differences in their energy values. Indirectly, this would

suggest that the mono-, di-, oligo- and polysaccharide components of a feed's available carbohydrate fraction are not all equally available to tilapia. Differences in the availability of these different types of carbohydrate could affect their protein sparing capacity and this is the subject of the following chapter.

In summary, the present chapter has demonstrated the value of carbohydrates in plant-product feeds as a source of DE for tilapia. Carbohydrate-rich feeds such as cassava and cereal grains can be used to spare protein in practical diets and attention should be given to this when formulating least-cost diets for tilapia. Protein-rich feeds such as soya and groundnut promoted the most rapid growth, but these feeds were inefficient with regard to protein retention. The provision of carbohydrate-rich feeds in a complete diet could considerably reduce feed costs with only a small reduction in growth rate. However, the maximum tolerable level of carbohydrate should first be determined and this is one of the considerations of the following chapter.

CHAPTER 8

THE UTILISATION OF DIFFERENT TYPES OF AVAILABLE CARBOHYDRATE FOR DIETARY ENERGY AND THE EFFECTS OF DIETARY FIBRE ON FOOD CONVERSION AND GROWTH

Section 8.1 Introduction

Chapter 7 demonstrated the ability of tilapia to utilise dietary carbohydrate for energy. The significance of this for reducing feed costs was outlined and the effects of available carbohydrate on carcass quality were examined. However, it was also indicated that the energy value of a carbohydrate depends on its feed source. Available carbohydrate is present in plant-prodct feeds mainly as simple sugars, dextrins, and starches, and their proportions can vary considerably in different feeds (cf. sugar cane with wheat grain). These different carbohydrates do not all have the same energy value, for whilst their gross energy contents are similar, their digestibilities vary considerably. In fact, with trout, carbohydrate digestibility has been shown to be inversely related to the degree of molecular complexity; monosaccharides are absorbed completely, whilst crude starch is poorly absorbed (Section 1.2.2, Millikin, 1982). Thus, the simpler a carbohydrate molecule, the greater is its DE value.

However, highly digestible carbohydrates are not necessarily the most suitable form in practical diets. For example, Furuichi and Yone (1982b) have recently shown that whilst α-starch is less digestible than dextrin and glucose, it is nevertheless a better carbohydrate source for carp on the basis of growth and food conversion efficiency. Similarly, Hilton and Slinger (1983) have shown that trout grow better on diets in

which carbohydrate energy is reduced by replacing wheat middlings with wheat bran.

There would appear to be a limit to the amount of available carbohydrate energy that can be tolerated by a fish, be it from small amounts of glucose or large amounts of starch and, as suggested in Section 1.3, this may be determined by fish species. Thus, herbivorous fish such as catfish and carp appear to be able to utilise greater amounts of carbohydrate than carnivorous salmonids (Brett and Groves, 1979). Clearly, it is necessary to measure the maximum tolerable level of available carbohydrate in diets for tilapia and to determine which types of carbohydrate are the most suitable for inclusion in production diets. The aim of the present experiment was, therefore, to evaluate the growth, food conversion and carcass composition of tilapia fed on semi-purified diets containing carbohydrates of differing molecular complexity at various levels of dietary inclusion. The carbohydrates chosen for study were, in increasing order of molecular complexity: a monosaccharide (glucose), a disaccharide (sucrose), a partially hydrolysed polysaccharide (dextrin) and an unhydrolysed polysaccharide (crude starch). In addition, an even more complex (and indigestible) carbohydrate (α-cellulose) was studied for its effects as a fibre source.

Most natural sources of available carbohydrate also contain indigestible fibre. Fibre is an assemblage of the most complex carbohydrates (Section 2.3.2) and their presence in cereals and oilseeds has the positive effect of adding structural integrity to pelleted diets (NRC, 1977). However, at dietary fibre levels greater than 8%, the growth of trout was found to be depressed (Leary and Lovell, 1975). A similar response to high-fibre diets was also found for tilapia in the

present research (Chapter 7). The reason for the growth depressing effect of fibre was not clear from the previous experiment, but it was suggested that this was due both to the inert bulk of fibre and possible interference with the availability of other dietary nutrients. The present experiment was, therefore, extended to include an examination of the effect of different levels of a pure fibre source (α-cellulose) on not only growth, but on protein retention, feed conversion and carcass composition.

Section 8.2 Methods

8.2.1 Diets

As far as could be determined from the literature (NRC, 1977; 1983; Balarin and Haller, 1982; Jauncey and Ross, 1982), a basal premix was formulated to ensure that all the essential fatty acid, essential amino acid, mineral and vitamin requirements of tilapia were satisfied in the final diets. Pure forms of glucose, sucrose, dextrin, starch and α-cellulose were substituted in this premix at three different levels (10%, 25% and 40% w/w). All diets contained the same amount of premix (60%) and changes in the levels of carbohydrates were achieved by replacing this nutrient with different amounts of an inert bulking material (polypropylene powder). The nutrient composition of all diets were, therefore, identical in all respects other than level and type of carbohydrate. The control diet contained 40% polypropylene and no carbohydrate, but otherwise had the same nutrient composition as the experimental diets. The formulation of the 16 diets and the results of proximate analysis are given in Table 8.1. The analyses were performed as described in Section 2.3.2 and gross energy values were calculated

TABLE 8.1 Formulation and analysis of diets containing different types of available carbohydrate and α-cellulose

Ingredients (g/100 g)	Control diet	Glucose diets			Sucrose diets			Dextrin diets			Starch diets			α-cellulose diets		
Premix[1]	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60
Polypropylene[2]	40	-	15	30	-	15	30	-	15	30	-	15	30	-	15	30
Glucose[3]		40	25	10												
Sucrose[3]					40	25	10									
Dextrin[3]								40	25	10						
Starch[3]											40	25	10			
α-cellulose														40	25	10
Proximate analysis[4]																
Moisture (%)	24.5	19.9	20.0	21.9	9.7	11.9	21.4	22.8	21.9	20.6	33.6	31.5	31.5	28.5	28.0	24.5
Crude protein (% dry matter)	35.7	36.7	36.7	35.4	35.7	35.0	35.3	36.4	37.0	35.9	36.9	36.9	35.3	35.7	35.8	35.5
Ether extract (% dry matter)	14.0	16.9	17.7	18.8	17.9	16.2	18.4	16.2	16.9	16.7	18.7	18.1	16.2	15.7	15.7	14.3
Ash (% dry matter)	6.1	4.4	3.9	4.6	5.8	6.1	4.9	6.2	4.6	5.1	4.0	4.1	5.9	6.1	4.5	6.2
Gross energy (MJ/kg DM)	13.9	21.6	19.5	17.3	22.0	18.7	17.2	22.0	19.8	16.8	23.1	20.2	16.4	21.6	19.0	15.7

1 Premix contained: Fishmeal (norseamink = water, 71%; CP, 70.3%; EE, 6.9%; Ash, 17.5%), 76%; maize oil, 10%; cod liver oil 5%; carboxymethylcellulose, 2%; mineral/vitamin mix, 7%

2 In powder form (microscopic spheres), ICI grade GX 543M

3 All carbohydrates derived from maize (Sigma Chemical Co). Dextrin type III was used = 80% water soluble

4 Mean of three determinations

using the results of these analyses and the following energy equivalents: fats 39.2 MJ/kg; protein, 23.6 MJ/kg; glucose, 15.9 MJ/kg; sucrose, 16.7 MJ/kg; starch, dextrin and α-cellulose, 17.5 MJ/kg. Direct energy determinations with a bomb calorimeter were not made as in previous experiments, because the present diets contained varying levels of polypropylene, which is a combustible hydrocarbon.

Diets were pelleted in semi-moist form because their high carbohydrate contents made them excessively hard when dried. The amount of water added to each diet was varied (Table 8.1), because differences in carbohydrate solubility affected the binding characteristics of each diet. To compensate, the feeding rate was adjusted for each diet to ensure that all groups of fish received the same quantity of diet on a dry-matter basis. After mixing in an Hobart mixer and extrusion through a mincer, strings of diets were frozen and then broken in a food blender. Pellets were graded according to size (1.0 mm, 2.0 mm and 2.8 mm) to compensate for increase in fish size during the experiment. All diets were stored at -20 C.

8.2.2 Experimental procedure

Each of 16 tanks were stocked with 17 tilapia and maintained at 26 C. The mean weights and variances of the 16 groups were statistically indistinguishable at the beginning of the trial (Table 8.2). Each group was given a different diet at a rate of 3% of the group liveweight per day (dry matter diet/liveweight fish). This daily amount was presented in 3 equal portions at 0900, 1300 and 1700 h. Every 7 d fish were weighed and the food quota adjusted to maintain the feeding rate. Diets were offered for a total of 63 d and until day 49, the daily quota of all diets was consumed within 30 s of presentation.

During the last 2 weeks of the trial, slower feeding was observed, especially in larger fish, so a twice daily feeding schedule was adopted for all groups. As fish grew, the 1.0 mm pellet was replaced with the 2.0 mm and then the 2.8 mm pellet.

At the end of the trial, fish were killed, weighed and their lengths recorded. After individual drying (to constant weight at 105 C) the carcasses from each group were ground and pooled in preparation for proximate analysis as described in Section 2.4.4. A group of 7 fish from the original population was killed at the start of the trial and their carcasses also subjected to proximate analysis to enable the calculation of food conversion efficiency (FCE) and net protein retention (NPR).

During the trial, faecal traps were emptied and water quality measured every 7 d. Dissolved oxygen, nitrite, nitrate, total ammonia, pH and hardness were all stable throughout the 63 d of the trial and remained within the limits given in Table 2.1.

Section 8.3 Results

The water stability and palatability of all diets, including the 40% polypropylene control, was good and typically the feed was consumed within 30 s of being offered. This may be attributed in part to the fact that pellets were semi-moist (9.7 - 33.6% water) since the same diets were found to be less acceptable when dried at 70 C for 18 h.

Growth and feed conversion values are given in Fig. 8.1 and Table 8.2. For all carbohydrates except α-cellulose, there was improvement in specific growth rate (SGR) as the level of inclusion was increased from 0% to 40%. The reverse was observed for α-cellulose, since fish

TABLE 8.2 Growth and carcass composition of tilapia fed on diets containing different types of available carbohydrate and α-cellulose

% Inclusion of different carbohydrates

	Control	Glucose			Sucrose			Dextrin			Starch			α-cellulose		
	0	40	25	10	40	25	10	40	25	10	40	25	10	40	25	10
Initial mean wt(g)	a 2.21	a 2.45	a 2.42	a 2.35	a 2.50	a 2.33	a 2.20	a 2.43	a 2.41	a 2.30	a 2.40	a 2.48	a 2.47	a 2.40	a 2.23	a 2.28
Final wt(g)	de 6.85	abc 10.55	abc 10.27	abcde 8.56	a 11.34	abcde 8.94	abcde 8.17	ab 11.10	ab 11.04	cde 7.38	abcd 10.07	abcde 9.26	bcde 7.89	e 6.29	e 6.25	cde 7.27
Mean increase (g)	4.64	8.10	7.85	6.21	8.84	6.61	5.97	8.67	8.63	5.08	7.67	6.78	5.42	3.89	4.02	4.99
PER[1]	2.09	2.46	2.52	2.40	2.83	2.64	2.41	2.78	2.75	2.17	2.61	2.36	2.19	1.75	1.88	2.03
NPR[2]	30.65	34.07	35.08	34.41	39.95	40.36	34.35	41.22	38.42	30.93	37.27	34.67	31.61	25.61	26.14	28.94
Condition (k)	cd 2.30	abc 2.54	a 2.62	abcd 2.40	ab 2.59	abc 2.56	abcd 2.36	a 2.64	ab 2.61	abc 2.45	ab 2.58	abcd 2.41	abc 2.53	d 2.18	bcd 2.33	abcd 2.36
Carcass composition[3]																Initial group[4]
Moisture (%)	ab 75.38	abc 75.05	de 72.85	cde 73.24	abcd 74.54	cde 73.02	ab 75.57	bcde 73.47	abcd 74.39	ab 75.54	cde 73.23	e 72.02	abcd 74.63	a 75.76	a 76.32	a 75.71	de 72.63
Crude Protein (%)	14.63	13.86	13.96	14.47	14.11	15.30	14.27	14.81	13.98	14.26	14.29	14.68	14.44	14.62	13.86	14.25	15.75
Ether Extract (%)	5.17	6.00	8.17	7.26	6.54	7.70	6.17	7.11	7.19	5.73	6.00	8.22	5.21	4.91	4.48	4.98	5.66
Ash (%)	4.16	4.16	4.16	4.14	3.96	4.21	4.23	4.75	4.03	4.51	4.46	4.21	4.41	4.33	4.66	4.59	4.08

1. Protein efficiency ratio

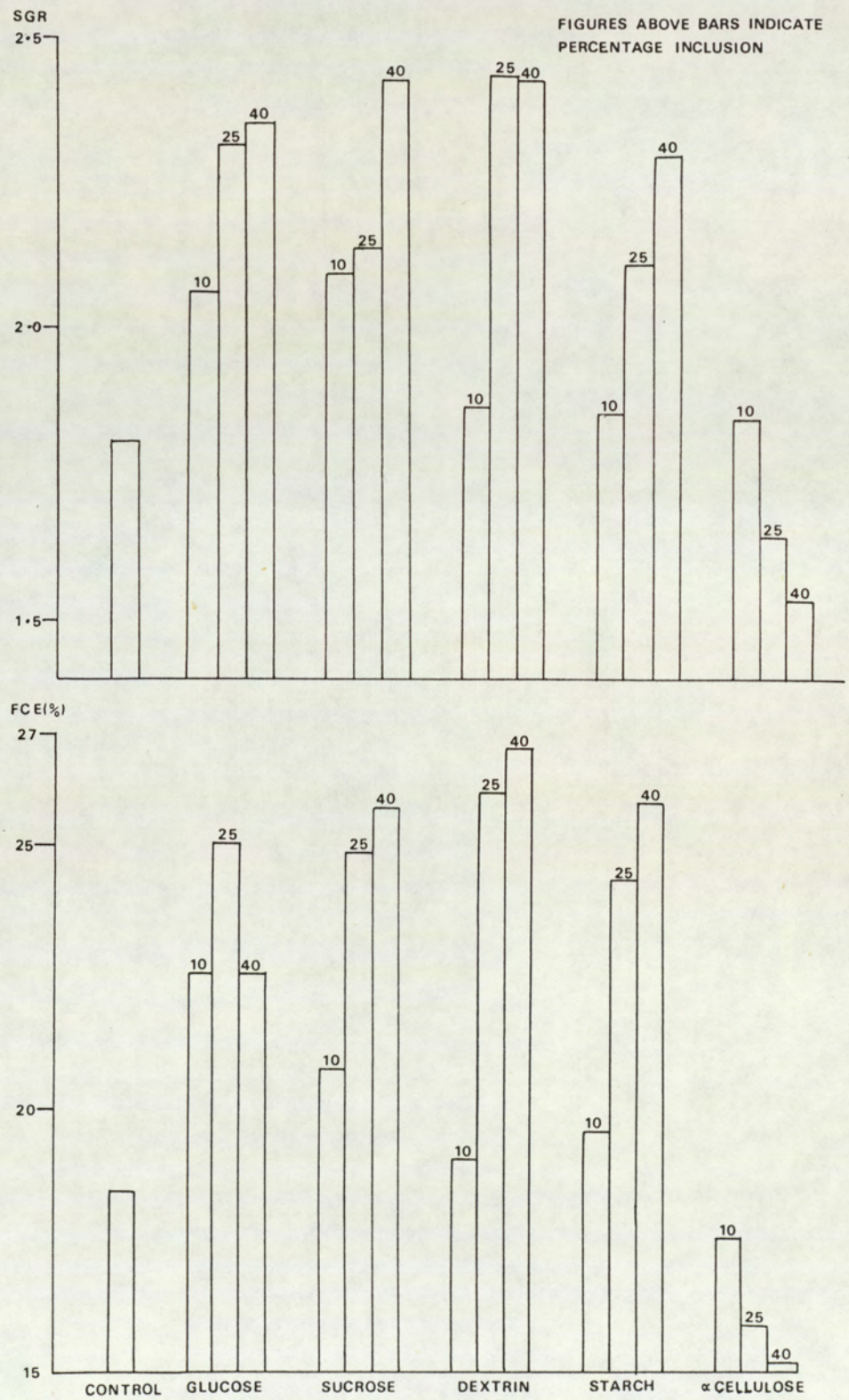
2. Net protein retention

3. Triplicate determinations on the pooled carcasses of 17 fish. Moisture values are each the mean of 17 fish.

4. Group sampled for analysis at the beginning of the trial. Mean weight 2.11 ± 0.79 g (n=7)

Figures with common superscipts in each row are not significantly different ($P > 0.05$)

FIGURE 8.1 SPECIFIC GROWTH RATE (SGR) AND FOOD CONVERSION EFFICIENCY (FCE) OF TILAPIA FED ON DIETS CONTAINING DIFFERENT LEVELS OF GLUCOSE, SUCROSE, DEXTRIN, STARCH AND α CELLULOSE

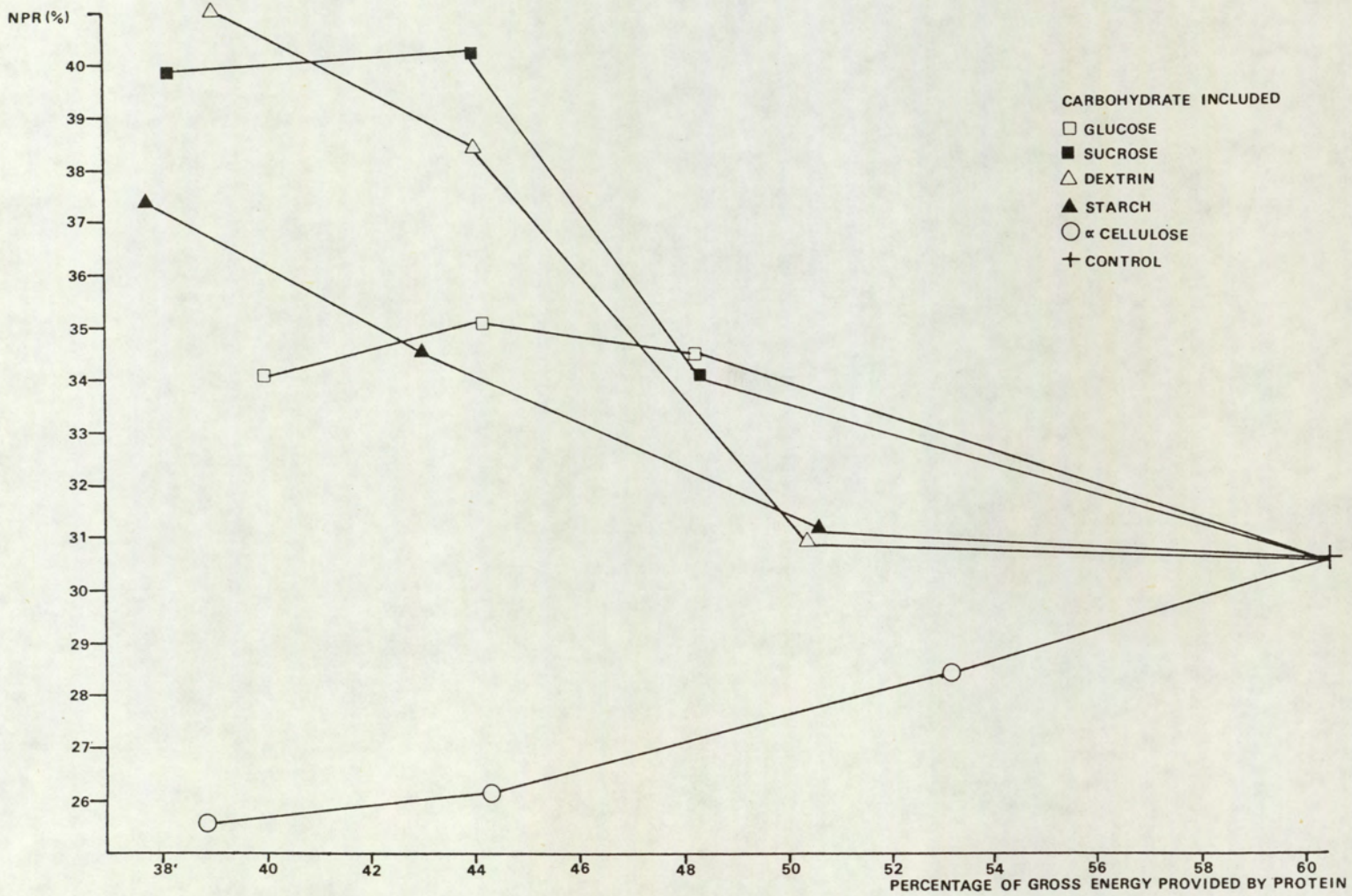


showed a progressive deterioration in growth as inclusion level was increased. Final weighings were significantly greater than the control for fish fed on diets containing sucrose at 40%, and glucose and dextrin at 40% and 25% ($P < 0.05$). FCE and NPR values were generally higher for sucrose and dextrin diets than for glucose and starch.

Since there were significant differences in carcass water between groups at the end of the experiment ($P < 0.05$), food conversion was calculated on a dry matter basis. For all available carbohydrates except glucose, there was improvement in FCE as the inclusion level was increased. However, the improvement in FCE between the 25% and 40% levels was minimal and not as large as the improvement between the 10% and 25% levels of inclusion. With glucose, the dry-matter FCE improved up to the 25% inclusion level, but thereafter decreased. Increasing the level of α-cellulose (fibre) caused a progressive decrease in FCE (Fig. 8.1).

Because of the differences in carcass water, NPR was considered a more accurate index of protein conversion than PER, but the latter is also given in Table 8.2 to enable comparison with the results of other authors. Fig. 8.2 shows that as the level of carbohydrate in the diet was increased and the ratio of protein energy to gross energy decreased, so protein retention increased for fish fed on all diets except those containing α-cellulose. This protein sparing action varied with the type of available carbohydrate included in the diet. Thus at the highest inclusion level (40%), NPR was highest on the dextrin diet and lowest on the glucose diet. On the other hand, at the lowest inclusion level (10%), the simple sugars glucose and sucrose spared more protein energy in the diet than the polysaccharides dextrin and starch. As for FCE, NPR fell as the level of α-cellulose was increased.

FIGURE 8.2 NET PROTEIN RETENTION (NPR) OF TILAPIA FED ON DIETS IN WHICH THE PROPORTION OF PROTEIN ENERGY WAS ADJUSTED WITH DIFFERENT DIETARY CARBOHYDRATES



All carbohydrates except glucose, when included at 40% produced fish with significantly higher condition values than the control or 40% α-cellulose diet ($P < 0.05$) but diets with lower carbohydrate inclusion levels (except glucose and dextrin at 25%) did not significantly increase condition with respect to the control ($P > 0.05$).

The carcass analysis in Table 8.2 shows that the fat content of fish fed on diets containing available carbohydrate was higher, whereas that of fish fed on α-cellulose diets was lower than the control. For each available carbohydrate, the 25% inclusion level produced fish with the highest body fat. Carcass fat was related to condition, and regression analysis revealed significant correlation between the two variables ($r = 0.58$, $P < 0.02$).

Carcass protein and ash were unaffected by the level or type of carbohydrate fed.

Section 8.4 Discussion

The maximum tolerable level of dietary carbohydrate may be defined as the level that can be efficiently digested and metabolised by fish without causing growth impairment or an increase in mortality rate. This differs from the optimum level of dietary carbohydrate, which is regarded as the level that is efficiently utilised as an energy source (Hilton et al, 1982). On the basis of the growth and carcass composition data (Fig. 8.1, Table 8.2), it is apparent that the maximum tolerable level of dietary carbohydrate for tilapia may be in excess of 40% (which represents 30% of gross dietary energy). 40% was the highest level studied and further work is needed to determine if higher levels can be tolerated.

The optimum level of dietary carbohydrate for tilapia may be lower than 40%, for whilst SGR was highest on diets containing 40% carbohydrate, the differences in FCE between the 25% and 40% levels were relatively small. For glucose, there was actually a reduction in FCE as glucose level was increased from 25% to 40%. Further work is needed to determine the optimum level of carbohydrate inclusion in practical diets and to assess how this depends on the balance of protein, fat and overall gross energy in a diet.

When the present results are compared with data in the literature it is apparent that tilapia are capable of tolerating more dietary carbohydrate than most other groups of fish studied to date. With carnivorous salmonids, the maximum tolerable level of digestible carbohydrate is about 25% and the optimum level is 14% (Hilton and Atkinson, 1982; Hilton et al, 1982; Section 1.3.1). With herbivorous fish such as carp, carbohydrate tolerance is generally higher than in salmonids (Section 1.3), but is is still lower than observed here for tilapia. With carp for example, the maximum tolerable level of dextrin is only 30% (Matty, 1985) and levels as high as 40% retard growth (Furuichi and Yone, 1980).

An investigation of the reasons for the greater tolerance of tilapia to dietary carbohydrate could form the basis of further work. In particular, information is needed on comparative aspects of glucose tolerance and the relative activities of key glycolytic and gluconeogenic enzymes (Section 1.3.3).

In commercial diets, carbohydrates are provided by cereals, roots and cereal by-products. Ufodike and Matty (1983) have demonstrated the use of two such feeds (rice and cassava) as carbohydrate sources in diets for carp and they found no suppression of growth with the

inclusion of these feeds in diets at levels up to 45%. In Chapter 7, it was reported that feeds such as maize, cassava, wheat grain and sorghum were included in diets for tilapia at even higher levels (60%) with no suppression of growth, increase in mortality or excessive deposition of body fat. Available carbohydrate levels in these diets did not exceed 40% (Table 7.2). Clearly, the available carbohydrate content of cereals and roots does not limit the inclusion of these feeds in practical diets at levels up to and including 60%.

With reference to carcass quality, fat contents were higher in fish fed on diets containing available carbohydrate than for the control group. However, even at the higher carbohydrate levels (25 - 40%), carcass fat only reached 6 - 8% liveweight and this cannot be considered excessive (Section 7.4, Balarin and Hatton, 1979). On dissection, it was apparent that most of this fat accumulated around the viscera and these are easily removed when fish are prepared for human consumption. Thus, it is unlikely that the use of high carbohydrate diets in tilapia farming will be constrained by adverse changes in carcass quality. The present experiment was, however, limited to juvenile fish (up to 11 g) and only lasted for 9 weeks. Lengthier trials are needed with larger fish under practical husbandry conditions to confirm the potential for high carbohydrate diets in intensive tilapia farming. Condition factor (k) was significantly correlated with carcass fat ($p < 0.02$) and so in practical field trials, k could be a useful means of estimating carcass fat levels without killing the fish.

The principal function of dietary carbohydrate appears to be the sparing of dietary protein for growth (Section 7.4). The present results indicate that the extent of this protein sparing depends not

only on the amount of carbohydrate present, but also on their molecular complexity. As more energy was provided by carbohydrates, the proportion of gross dietary energy provided by protein decreased and this led to an increase in carcass protein retained per unit protein fed (Fig. 8.2). At low carbohydrate inclusion levels, when approximately 50% of the total energy was provided by protein, the amount of protein-sparing appeared to be related to the complexity of the carbohydrate molecule. Thus, the simple sugars glucose and sucrose had a greater sparing effect than starch and dextrin. As the level of carbohydrate was increased and the proportion of protein energy in the diets decreased, the order changed and dextrin produced the most sparing whilst glucose produced the least. Sucrose was similar to glucose in that there was no improvement in protein retention by increasing the inclusion level from 25% to 40%. However, at levels above 10%, sucrose produced greater protein retention than glucose, indicating that the disaccharide has greater potential for sparing protein in complete diets.

Increasing the level of starch and dextrin from 25% to 40% improved NPR, whilst the same increase in glucose depressed NPR. Pieper and Pfeffer (1980) found similar results when feeding sucrose, glucose and gelatinized maize starch to rainbow trout at 30% of the ration. They suggested that the poorer performance of the glucose diet might be due to a "negative physiological effect" caused by glucose saturation. As a monosaccharide, glucose requires no digestion and is rapidly absorbed across the gut, whilst starch and sucrose must undergo enzyme hydrolysis before absorption. Hence, glucose appears at gut absorption sites more rapidly than disaccharide or polysaccharide hydrolysis products and the rate of appearance of glucose is more directly related to its

concentration in the diet. The significance of this is that glucose is known to inhibit the transport of amino acids at absorption sites on the mammalian gut membrane (Alvarado, 1966; Alvarado and Robinson, 1979), and more recently the same effect has been recorded in fishes (Hokazono et al, 1979). It is possible that inhibition of amino acid transport accounts for the inferior protein retention of tilapia fed on diets containing glucose at levels greater than 25%. At present, this is pure speculation, but it is an hypothesis that could be readily tested by measuring the digestibility of protein in diets containing different levels of glucose.

The negative effect of high levels of glucose could, however, be equally due to a post-absorptive response. After glucose absorption, blood insulin levels increase to a maximum and thereafter decline. Insulin regulates the activity of glycolytic and gluconeogenic enzymes and so any carbohydrate absorbed before the post-prandial insulin peak may be poorly metabolised. Furuichi and Yone (1982b) have already shown that, with carp, the absorption of dietary glucose is so rapid that the insulin response is inadequate for efficient glucose metabolism. With starch however the absorption of glucose (the end-product of enzyme hydrolysis) was slower and more prolonged. This meant that blood insulin levels were more closely related to blood glucose and growth improved as a direct consequence.

Murai et al (1983), also found that starch produced better growth than glucose or maltose when fed to carp at 30% of the diet. These authors then went on to demonstrate that the efficiency of utilisation of glucose and maltose was dramatically improved when similar quantities of diet were given 4 times daily instead of twice daily. The conclusion was that the prolonged release of glucose into the blood

resulting from small, frequent meals containing glucose and maltose was preferable to the periodic surge of glucose resulting from larger, less frequent meals. With tilapia, a similar response to differences in carbohydrate absorption rates observed for carp by Furuichi and Yone (1982b) could explain the present differences in the utilisation of glucose and polysaccharides. Whatever the mechanism for the reduction in utilisation of carbohydrates with decreasing molecular weight and increasing inclusion level, the practical implications are clear. Starches and dextrins are the preferred energy sources in high carbohydrate diets for tilapia, but lower molecular weight carbohydrates may also be used at high inclusion levels if given to the fish in smaller, more frequent meals.

The energy values of dextrin and starch were evidently not the same. At all levels of inclusion, dextrin produced better growth, higher NPR and higher FCE's than starch, indicating that it is more available source of energy. This is probably due to a difference in the digestibilities of these two polysaccharides. It is now well established in the literature on fish nutrition that dextrin is more digestible than starch (Singh and Nose, 1967; Neuhaus and Halver, 1969; Spannhof and Plantikow, 1983; Bergot and Breque, 1983). Recently, Spannhof and Plantikow (1983) have examined the reasons for these differences in digestibility with trout and have made the following conclusions:

1. Dextrin requires less digestion than starch because it is already at an advanced stage of hydrolysis before ingestion.
2. α-amylase is adsorbed to the surface of crude (or 'native') starch and this reduces the hydrolytic activity of the enzyme. No such adsorption occurs with dextrin.

3. Crude starch, unlike dextrin, increases the volume of intestinal contents and accelerates the passage of food through the gut, thereby reducing the time available for digestion and absorption.

The practical implication of these findings, together with the present results, is that the crude starch in cereals and roots is a relatively poor source of carbohydrate energy for tilapia. However, it is now becoming apparent that the energy value of starch can be improved by gelatinisation (Bergot, 1979) and this is an option that should be considered when manufacturing diets for tilapia. Gelatinised starch is similar to dextrin in molecular structure and it has a digestibility of 87% whilst the digestibility of native starch is only 37% (Bergot and Breque, 1983). Starches can be gelatinised by cooking or extrusion processing (as described by Hilton and Slinger, 1981) and, in trout pellets, such processing slows the passage of food through the gut and increases the availability of carbohydrate energy (Hilton et al, 1981; Hilton and Slinger, 1983). With tilapia, it has been shown recently that a diet containing extruded soya passed through the gut more slowly than a similar diet containing expanded soya (De Silva and Owoyemi, 1983). However, no indication was given as to whether or not the slower passage of food improved nutrient availability, and further work is needed to verify this.

The low tolerance of trout to available carbohydrate places a limitation on the value of extrusion processing with high carbohydrate diets (Hilton et al, 1981). The same limitation is unlikely to apply to tilapia because of their greater tolerance to dietary carbohydrate. Thus, it is recommended that, when starchy feeds are included in diets for tilapia, pellets should be produced by heat extrusion to improve the energy value and hence protein sparing capacity

of the available carbohydrate fraction.

When α-cellulose was present in the diets at 10%, there was no reduction in growth with respect to the control, indicating that such low levels of fibre are acceptable in diets for tilapia. However, at higher fibre levels, FCE, NPR, condition and carcass fat were reduced below corresponding measures for the control (Figs. 8.1 and 8.2, Table 8.2). Clearly, fibre was not only acting as indigestible bulk, but it also reduced the availability of other nutrients in the diets. This is probably mediated by a change in the rate of passage of food through the gut. It is now well established that the presence of inert bulk in a diet speeds the rate at which food is evacuated from the stomach (Grove et al, 1978; Flowerdew and Grove, 1979; Jobling, 1981). In this respect, α-cellulose might be expected to have a more pronounced effect on gastric motility than other inert bulkers (such as kaolin) because fibre absorbs large amounts of water and so increases in bulk after ingestion. A reduction in gut passage time (GPT) with increasing levels of fibre could reduce digestibility by decreasing the time available for enzyme hydrolysis and absorption of food digesta. Direct evidence for this hypothesis has recently been provided by Hilton et al (1983) who showed that the depression of growth in trout fed on fibrous diets was due to a decrease in GPT and diet digestibility. A similar response by tilapia could explain the progressive decrease in SGR observed in the present study as polypropylene was substituted by α-cellulose.

α-cellulose is often used in fish nutrition experiments as an inert bulker, to adjust nutrient levels in test diets. In many such studies growth responses have been assigned to changes in the level of a test nutrient, when it is equally possible that some of the observed results

were due to the corresponding change in α-cellulose level (e.g., Page and Andrews, 1973; Adron et al, 1976). Polypropylene may be a better bulking agent. Whilst this material has a physical effect on the gut (any indigestible substance will do so), it should have a less active effect on GPT than on fibrous material since it does not absorb water. Polypropylene is uncharged and therefore should also have less effect on mineral availability than α-cellulose. In addition the amount of polypropylene in a diet can be quantitatively measured (Chandler et al, 1964) and so in digestibility studies, this material could serve a dual purpose as both an inert bulking agent and a dietary marker.

When trout are fed on an ad libitum feeding regime, they can maintain growth rates on diets containing up to 30% cellulose, by increasing the amount of food eaten (Bromley and Adkins, 1984). However, in an intensive fish farm, such high levels of fibre are undesirable because this increases the production of faeces, which in turn increases the biological oxygen demand and the levels of suspended solids and dissolved ammonia in the water (Leary and Lovell, 1975; Bergheim and Selmer-Olsen, 1978; Bergheim and Sivertsen, 1981). In addition, it is not economical to have any dietary fibre in excess of that necessary for structural integrity of the pellets because of the additional costs this incurs in baggage, transport, handling and storage of the diets (Bromley and Adkins, 1984). Thus, there are practical as well as nutritional advantages to be gained from reducing dietary fibre levels to a minimum. The conclusion from the present study is that fibre should not exceed 10% in practical diets for tilapia which is similar to the level currently recommended for commercial trout diets (Leary and Lovell, 1975; NRC, 1977, Hilton et al, 1983).

CHAPTER 9

CONCLUSIONS

This research has shown that the digestible energy (DE) value of plant-product feeds for tilapia can be predicted from their chemical composition, to an accuracy of ±1.5 MJ/kg DM. The value of such accurate prediction is that it offers an alternative to lengthy *in vivo* digestibility trials when feeds are to be evaluated for inclusion in least-cost diet formulations. In predictive regression equations, the single most useful predictor of digestibility is fibre.

Of the 3 fibre assays tested (crude (CF), acid detergent (ADF) and neutral detergent fibre (NDF)), NDF is the best predictor of digestibility because it is a more accurate measure of the material in a feed that is resistant to the digestive secretions of tilapia. In addition, NDF is an easier, more rapid assay than CF or ADF and it is now recommended that NDF should replace the more conventional CF assay when dietary fibre is measured in fish nutrition studies.

Fibre can only be used to predict DE in feeds which contain moderate levels of fibre. In feeds which contain no fibre, DE must be predicted from other feed parameters and in high-fibre feeds it has been found that the predictive power of NDF is reduced. In these cases, the influence of fat, carbohydrate and protein on DE must be considered. In a representative series of plant-product feeds, fat had no value for predicting DE, because it was present at such low levels in these feeds. In complete diets containing supplemental oils, the energy contribution of fat to DE will of course increase and predictive equations for such diets will be different to those for single feeds.

Protein and carbohydrate were more valuable than fat as predictors

of DE in the feeds studied. The method used for measuring available carbohydrate had a considerable effect on the accuracy of this feed component as a predictor of DE. The most accurate measure of available carbohydrate was a direct chemical assay using enzyme hydrolysis. The least accurate method was nitrogen-free extract (NFE), calculated by deducting the sum of the measured ash, protein, fat and crude fibre from the dry matter weight of the feed. The accuracy of the NFE estimate can, however, be considerably improved if the indigestible fraction of the feed is measured as neutral detergent fibre instead of of crude fibre.

Protein and carbohydrates were both positively correlated with DE, but protein had a relative contribution to DE that was about 1.25 times greater than that of available carbohydrate. The ratio of dietary protein to carbohydrate in a diet had more of an effect on DE and growth than the level of either nutrient alone. Clearly the level of dietary protein affects the digestion and metabolism of carbohydrate and vice-versa.

The most robust predictive equation (i.e, the one which is most accurate over a wide range of feed compositions), is a multiple regression equation which includes measures of fibre, protein and available carbohydrate, as the independent variables. Such an equation takes account of both the negative effect of fibre on DE and the positive contributions of protein and carbohydrate. It is therefore applicable to most types of plant-product feed. A full list of the most convenient equations for predicting DE is given in Section 6.5. Some of the single factor equations are more convenient to use than the multiple regression equations because they require only one measure of feed composition. Some of these single factor regressions are as

accurate as the multiple regressions, but in general their applicability is limited to certain classes of feed (e.g., fibrous or non-fibrous, oilseeds or cereals). Ultimately, the regression equation chosen to predict DE will be determined by the chemical measures available to the investigator and the type of feed to be analysed. Where possible, multiple regressions should be used in preference to single factor regressions because of their greater robustness.

Dry-matter digestibility (DMD) can also be predicted from the chemical composition of plant-product feeds. However, the only useful predictor of DMD is fibre, because protein and carbohydrate have less bearing on DMD than they do on DE. As for DE, neutral detergent fibre is the best single predictor of DMD.

The prediction of DMD is of some value for estimating food quality, but is is also useful for anticipating faecal production rates. For example, tilapia which were fed on fibrous feeds were found to produce 3 times more faeces than those fed on grain-based diets (Chapter 5). In an intensive farm, this is important because increased production of faeces reduces water quality and increases levels of suspended solids in the effluent discharge. The rate of faeces production is affected by many factors, only one of which is DMD. These factors include level of feeding, fish size, and temperature. Nevertheless, the ability to predict the DMD of a diet now makes it easier to estimate the amount of faeces that will be produced for any given set of husbandry conditions.

The digestibility of crude protein (DCP) cannot be predicted from the present results. Whilst the DCP values of the plant-product feeds do vary slightly (73 - 93%), the conclusion is that the digestibility of protein by tilapia is uniformly high and is relatively unaffected by feed composition. Clearly, tilapia are well adapted to digesting and

absorbing plant proteins. In general, the digestibility of plant proteins by tilapia is higher than for terrestrial livestock such as pigs and poultry and in this respect, tilapia are similar to many other species of fish. However, tilapia are also relatively well adapted to digesting and absorbing high-carbohydrate feeds and in this respect, they differ from most other species of farmed fish. The consequence of this is that more of the carbohydrate energy in plant material is available for sparing protein in diets for tilapia than for other fish such as trout and catfish.

With the present results, the prediction of DE and DMD is only possible for plant-product feeds. With animal-product feeds, it is not yet possible to predict digestibility from feed composition. A more extensive data base is needed for the digestibility and composition of animal-product meals before their digestibility can be predicted. The most useful predictors of digestibility in animal-product meals are likely to be crude protein and ash.

When a rapid estimate of protein digestibility is required, it may be better to rely on _in vitro_ methods. These techniques are already used widely in studies with terrestrial livestock and they are presently being pioneered for fish by scientists at the University of Innsbruck, Austria (M. Grabner, Pers. Comm., 1984). _In vitro_ techniques involve digesting feed samples under conditions which simulate the environment in the gut of the test fish. These conditions include factors such as physiological temperature optima, pH, enzyme activity and food exposure time. In common with the predictive techniques reported in this thesis, _in vitro_ methods have the attraction that they could become a valuable means of reducing the need for long-term feeding trials when estimates are required for the digestibilities of novel feeds. _In_

vitro methods can also be used to study the kinetics of specific nutrient digestion. However, much more research is necessary before physiological conditions in the gut of fish can be modelled with sufficient accuracy to provide unequivocal estimates of digestibility.

To recapitulate, the chemical composition of a feed has been shown to have a direct bearing on its DE value. The DE of a feed has in turn been shown to have a direct, positive correlation with growth. Indirectly therefore, the growth of tilapia can be predicted from the composition of a diet. Of particular importance in this respect is the relative balance of protein and carbohydrate. As carbohydrate energy replaces protein in practical diets, protein is utilised for growth more efficiently. Thus, the available carbohydrate fraction of plant-product feeds can be used in complete diets to spare dietary protein for growth. The utilisation of carbohydrate for energy appears to be more efficient in tilapia than in most of the other fish currently farmed for human consumption. The maximum tolerable level of dietary carbohdyrate appears to be in excess of 40% (which is equivalent to about 30% gross dietary energy) but the optimum level may be slightly lower than this. However, long-term feeding trials are needed to confirm sustained tolerance of tilapia to high carbohydrate diets under commercial husbandry conditions.

The source of dietary carbohydrate is of significance to the utilisation of this nutrient for energy. Thus, cassava is a more available source of carbohydrate energy than cereal grain. Cassava is also cheaper than most grains and in less developed countries, this root crop could become a valuable component of complete diets for tilapia farming. Other carbohydrate-rich feeds should also be evaluated now that the potential of carbohydrate for sparing protein has been

established. In particular, consideration should be given to the carbohydrate profile of all novel feeds, because the energy value of individual carbohydrates appears to depend on their molecular complexity.

Small molecular weight carbohydrates are rapidly absorbed across the gut, and at high dietary inclusion levels they can reduce the food conversion and protein retention efficiencies of a diet. Sugars such as glucose and sucrose should, therefore, be present in tilapia diets at levels no greater than 25%. Larger molecular weight carbohydrates such as dextrin and starches are utilised more efficiently than sugars at higher inclusion levels, and may be used in diets at levels between 25 - 40%. When the major carbohydrate in a diet is a sugar, it should be given to tilapia in small, frequent meals to reduce the deleterious effects of glucose saturation. Where possible, hydrolysed forms of starch such as dextrin should be used in tilapia diets because this has a greater energy value at high inclusion levels and does not interfere with food utilisation. Starch can be partially hydrolysed by cooking or steam extrusion. These processing methods can, therefore, increase the energy value of a feed and this should be considered when pellets are manufactured for tilapia. There is also evidence that the DE of a feed increases as tilapia adapt to a new diet (Chapter 4) and that there is daily variation in protein digestibility (De Silva and Perera, 1984). All of these factors should be considered when developing carbohydrate-rich diets and feeding practices in tilapia farming.

Differences in the energy values of different carbohydrates are probably due to differences in their digestibility. A study of the digestibility of specific carbohydrates would, therefore, be a useful adjunct to the present research. A valuable approach would be to

produce carbohydrate profiles of feed and faeces using gas-liquid chromatography. These profiles could then be compared with reference to an inert marker and digestibility estimates calculated for each of the component carbohydrates.

It is also necessary to know why tilapia are more able to digest, absorb and metabolise carbohydrates than carnivorous fish such as trout. Spannhof and Plantikow (1983) suggest that membrane-contact digestion is an important mechanism in the digestion of carbohydrates by fish. If so, this could help to explain the relatively high digestibility of carbohydrate-rich feeds by tilapia, because this fish has a comparatively long gut with a greater surface area for membrane-contact digestion than carnivorous fish. In addition, tilapia have higher amylase activity in their gut than the related but more carnivorous perch (Nagase, 1964). These two factors together could explain why tilapia are relatively well adapted (for a fish) to digesting and absorbing starchy feeds such as cassava and cereal grain. However, it remains to be established why tilapia tolerate and utilise higher levels of dietary carbohydrate than other fish. It would be useful to study the metabolism of carbohydrates in tilapia with particular reference to glucose tolerance, the fate of absorbed glucose, and the activities of key gluconeogenic, glycolytic and lipogenic enzymes.

Many plant-product feeds which contain available carbohydrate also contain structural carbohydrates such as cellulose, hemicellulose and lignin. These fibrous materials are indigestible to tilapia (they are useless bulk in a diet) and only contribute to the production of faeces and the fouling of water. At high levels of dietary inclusion, fibre not only replaces useful nutrients, but it also reduces the availability

of the remaining nutrients. For this reason, it is recommended that tilapia diets should contain no more than 10% fibre.

It is also recommended that the fibre α-cellulose is no longer used as an inert bulker to adjust nutrient levels in experimental diets for fish. This is because a change in fibre level confounds the effect of a corresponding change in the level of the test nutrient under study. A plastic bulker such as polypropylene is a better material for adjusting nutrient levels, because it does not affect nutrient availability, it does not absorb water, it is uncharged and, in the form of microscopic spheres, it has the smallest possible surface area for interference with digestion in the gut. Polypropylene (or polyethylene) also has the advantage that it can be used as a marker in digestibility studies.

Protein sparing with carbohydrate-rich feeds is only one way of reducing feed costs. Another option is to replace some of the expensive fishmeal in tilapia diets with either conventional plant-protein feeds (Jackson et al, 1982) or more novel protein sources such as coffee pulp (Bayne et al, 1976), brewing wastes (Hastings, 1973) or even green algae (Appler and Jauncey, 1983). However, both methods may be limited by factors such as the presence in feeds of toxic or growth inhibitory factors; by deficiencies of amino acids, fatty acids or minerals; or by compounds which make the feeds unpalatable. In certain circumstances, the expense of the diet is in any case, of secondary importance to the speed of fish growth and here, fishmeal would probably not be replaced at all. Rapid fish growth is of overiding importance in farms which have high financial investment and capital overheads (Crampton, 1981). All of these factors should be considered when developing diets for tilapia from locally available feeds, but perhaps

of most significance with respect to the present research would be a study of potential growth inhibitors in cereals and other high-carbohydrate feeds.

Already, it is apparent that wheat contains an albumin compound which reduces amylase activity in the gut of trout (R. Hofer, Pers. Comm., 1984). Inhibitors such as this could reduce the availability of carbohydrates and so reduce growth, although it is known that some inhibitors can be destroyed by feed processing. In the present research, diets containing wheat and other cereals all produced growth rates that were slightly lower than diets containing soybean or groundnut. However, this was probably due more to the superior utilisation of oilseeds for growth than to the effects of any growth inhibitor in the carbohydrate-rich feeds. Nevertheless, the influence of chronic growth inhibitory factors in practical high-carbohydrate feeds, should be investigated in long-term feeding trials with tilapia under commercial conditions.

Clearly, the effectiveness of least-cost diet formulation depends on the information available for the raw materials (feeds). In this respect, the main contribution of this research is that DE values can now be obtained for tilapia within 24 hours, by simply subjecting a feed to proximate analysis. However, it is still necessary to extend the amount of data on feed digestibility for tilapia and this can only be achieved, at present, with in vivo digestibility trials. Several recommendations can be made concerning the conduct of such trials. Firstly, every feed should be evaluated by substitution in a standard reference diet, at a constant level of inclusion. This is necessary because raw feeds can have a different digestibility value when they are given individually to fish than when they are mixed with supplemental

nutrients before feeding (Chapter 4). Secondly, the test feed substitution level should be as high as possible to optimise the accuracy of digestibility measurement. In the present research, experimental diets consisted of 60% test feed and 40% reference diet. Thirdly, all diets should be given to the fish for the same number of days before faeces are collected, because tilapia exhibit digestive adaptation to diet. Finally, faeces should be collected after egestion by the fish (dissection and stripping should be avoided) and samples analysed with automated micro methods such as those developed in Chapter 3. These methods enable more replicate determinations than conventional methods of faecal analysis and can be applied to the small amounts of faeces obtainable from individual fish. This increases the number of dietary treatments possible in an experiment and so improves the accuracy and sensitivity of statistical analysis.

In summary, this research has revealed quantitative relationships between the chemical composition of feeds, the digestibility of feeds and the growth of tilapia. Specific relationships between measures of feed composition and digestibility can be used to predict the digestible energy value of plant-product feeds. In addition, the relationship between dietary carbohydrate and growth has revealed that tilapia can effectively utilise relatively high levels of carbohydrate for dietary energy and that this nutrient can reduce the amount of protein used for energy in practical diets. Clearly, the cost of tilapia diets can be reduced by including high levels of cereals and roots in the diet. The digestible energy value of such feeds can be rapidly estimated with the predictive models given in this thesis, and this should enable a more flexible approach to the formulation of least-cost diets from locally available feeds in less developed countries.

APPENDIX 1

CLASSIFICATION OF TILAPIAS

Over the past 6 years, there has been controversy with regard to the taxonomy of tilapias. Much of the disagreement is based on the question of whether or not fish genera can be distinguished on non-morphological criteria. Trewavas (1973) proposed that the genus formerly referred to as Tilapia should be considered as two separate genera depending on whether the fish are mouthbrooders (Sarotherodon) or substrate spawners (Tilapia). Later, Trewavas (1982a) further subdivided Sarotherodon into two distinct genera, viz, Oreochromis in which mouthbrooding is maternal, and Sarotherodon in which mouthbrooding is paternal or biparental.

It may be argued however, that taxonomic divisions should rely on factors which prevent reproduction between separate populations. Ethological and ecological variables are selective pressures which some authors find unacceptable in this respect. An alternative classification in which the new genera proposed by Trewavas (1982a) are relegated to sub-genera may, therefore, be more acceptable (Trewavas, 1983).

The alternative classifications may be illustrated thus:

Classification 1

I	family	subfamily	tribe	genus
	Cichlidae	Tilapiinae	Tilapiini	Tilapia
				Sarotherodon
				Oreochromis

Classification 2

family	subfamily	tribe	genus	subgenus
Cichlidae	Tilapiinae	Tilapiini	Tilapia	Tilapia
				Sarotherodon
				Oreochromis

(from Trewavas, 1983)

Thus, in Classification 2, one may refer to

Tilapia sparrmanii or *Tilapia (Tilapia) sparrmanii*
Tilapia galilaea or *Tilapia (Sarotherodon) galilaea*
Tilapia nilotica or *Tilapia (Oreochromis) nilotica*

In Classification 1 these are:

Tilapia sparrmanii
Sarotherodon galilaeus
Oreochromis niloticus

Classification 1 is the system adopted in this thesis because I support the behavioural and reproductive factors proposed by Trewavas (1982) as valid speciation pressures. Under Classification 1, there are two subgenera within *Oreochromis*. These are *Oreochromis* (the nominate subgenus) and *Nyasalapia*. Thus, the formal name for the species

reported in this thesis is Oreochromis (Oreochromis) niloticus (Linn).

APPENDIX 2

A COMPARISON OF TILAPIA DE AND POULTRY ME VALUES IN THE LEAST-COST FORMULATION OF PRACTICAL DIETS

In the absence of data on the DE of feeds for specialised livestock such as tilapia, feed manufacturers often use literature values (usually poultry ME), to balance energy in least-cost formulations of commercially produced diets. However, the differences between poultry ME and tilapia DE shown in Table 5.9 suggest that diets for tilapia may be incorrectly balanced for energy when poultry ME values are used in the computation of least-cost diets. This Appendix gives a practical illustration of the change in optimum formulation of a diet caused by using DE values for tilapia (as determined in the present research) instead of literature values for poultry ME.

Least-cost formulations are computed from a data base which includes information on the nutrient levels, available energy levels and cost of competing raw materials (feeds). The diet manufacturer must first input to the computer program the required levels of energy and specific nutrients. The computer then tests many different combinations of the available raw feeds and selects the formulation which will meet the requirements of the diet for the lowest cost.

The two formulations given below were computed from data on the 16 feeds evaluated for DE in Chapter 5. Both formulations were computed from the same data base, with the exception that in diet 1 the energy values of the feeds were given to the computer as poultry ME and in diet 2 as tilapia DE.

The feed data base was as follows:

Raw materials

Feed	Crude protein (%)	Crude fibre (%)	Ether extract (fat) (%)	Tilapia DE (MJ/kg)	Poultry ME (MJ/kg)	Price* (£st/tonne)
Soybean	44.2	6.9	1.9	13.0	10.2	180
Ground maize	9.0	1.4	3.7	11.6	13.9	105
Wheat middlings	15.6	6.4	3.6	9.3	9.5	105
Wheat bran	15.4	8.8	2.4	5.4	4.6	130
Cassava	2.8	3.8	0.9	12.0	13.2	80
Sunflower	28.1	25.7	2.0	3.3	6.7	150
Rice bran	12.6	18.1	0.5	4.5	9.1	110
Sorghum	10.3	1.5	2.8	10.6	13.6	110
Wheat grain	9.9	1.9	1.4	11.5	12.3	110
Copra	20.3	13.2	7.0	5.8	6.1	130
Groundnut	50.6	3.5	9.6	16.7	12.8	150
Rapeseed	35.7	11.9	2.1	9.5	9.5	140
Palm kernel	17.2	15.8	10.9	7.9	7.9	140
Meat and bone	43.0	0	12.6	8.7	7.5	175
Poultry by-product	61.5	0	5.6	8.6	11.0	250
Fishmeal	70.0	0	6.9	15.0	12.6	340
Min/vit premix	0	0	0	0	0	2000
Vegetable oil	0	0	100	33.5	33.5	350

All values are on an "as received" (freshweight) basis adjusted from values in Tables 5.3 and 5.9.

*Feed prices vary daily. These were the prices of feeds recommended by a commercial feed mill on 04-10-84.

Diets 1 and 2 were both formulated to satisfy the following requirements.

Required nutrient levels

Crude protein	35% (At least 40% of this from fishmeal)
Crude fat	10%
Crude fibre	10% (max)
Energy	12.5 MJ/kg
Minerals/vitamins	1%

The output of the computer program can be summarised as follows:

Least-cost formulations (kg/Tonne)

1. Formulation	Diet 1 (Poultry ME)	Diet 2 Tilapia DE
Mineral/vitamin premix	10.00	10.00
Fishmeal	200.00	200.00
Vegetable oil	43.85	38.91
Groundnut	307.68	242.78
Wheat middlings	328.40	341.12
Cassava	110.07	-
Copra	-	167.19
2. Analysis		
Fat (%)	10.00	10.00
Protein (%)	35.00	35.00
Fibre (%)	3.60	5.24
ME (MJ/kg)	12.50	(11.20)
DE (MJ/kg)	(14.00)	12.50
3. Cost (£st/Tonne)	193.00	196.00

The differences between these two hypothetical diets demonstrate the importance of accurate energy values for raw feeds when they are compounded into diets on a commercial scale. When poultry ME values were applied to the present feeds the optimum diet formulation included 3 plant-product feeds; groundnut, wheat middlings and cassava (diet 1). When the more appropriate tilapia DE values were used in the computation, cassava was replaced by copra, the level of groundnut was reduced and that of wheat middlings was increased (diet 2). The result was that the tilapia DE diet was slightly more expensive than the poultry ME diet. However, poultry ME values were clearly unsuitable for formulating a tilapia diet because when they were applied to diet 2, the apparent energy value was only 11.20 MJ/kg. This is lower than the required energy level of 12.50 MJ/kg and so here, poultry ME values were inaccurately constraining the diet.

Clearly, it is important to evaluate the DE of a wide range of feeds specifically for tilapia if cheaper diets are to be accurately formulated to meet the energy requirements of this fish. Where these values cannot be measured directly, they should be predicted as described in Chapter 6 and the use of poultry ME values in the least-cost formulation of fish diets should be discontinued.

APPENDIX 3

FEED SPECIFICATIONS

Name used in thesis	Scientific name	International feed number	Feed fraction
Soybean	*Glycine max*	5-04-604	Seeds, meal, solvent extracted.
Ground maize	*Zea mays*	4-02-948	Grain.
Wheat middlings	*Triticum* sp.	4-05-205	Mixture of germ and shorts (fine bran and flour).
Wheat bran	*Triticum* sp.	4-05-190	Fibrous coating around endosperm.
Cassava	*Manihot esculenta*	-	Tubers.
Sunflower	*Helianthus* sp.	5-04-740	Seeds, meal with hulls. Solvent extracted.
Rice bran	*Oryza sativa*	4-03-928	Defatted bran, possibly some hulls present.
Sorghum	*Sorghum vulgare*	4-04-383	Grain.
Wheat grain	*Triticum* sp.	4-05-211	Grain.
Copra	*Cocos nucifera*	5-01-572	Coconut meal mechanically extracted.
Groundnut	*Arachis hypogaea*	5-03-649	Oilcake without hulls mechanically extracted.
Rapeseed	*Brassica* sp.	5-03-871	Oilmeal, solvent extracted.
Palm kernel meal	*Elaeis guineensis*	-	Kernel oilcake, mechanically extracted.
Meat and bone meal	-	5-00-388	High proportion of bones
Poultry by-product meal	-	5-03-798	Meal rendered.
Fishmeal	*Clupea harengus*	5-02-000	Herring meal (= brown fishmeal).

Feed descriptions and numbers are as recommended by Harris (1970) and NRC (1977).

APPENDIX 4

RESEARCH PUBLICATION

(Reprint of a paper concerning the

experiment reported in Chapter 8

of this thesis)

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Anderson, J., Jackson, A.J., Matty, A.J. & Capper, B.S. (1984). 'Effects of Dietary Carbohydrate and Fibre on the Tilapia *Oreochromis niloticus* (Linn.)' *Aquaculture*, 37, pgs. 303—314.

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