DIGESTIBLE ENERGY AS A CRITERION FOR THE DEVELOPMENT OF DIETS FOR THE AFRICAN SHARPTOOTH CATFISH, CLARIAS GARIEPINUS (PISCES: CLARIIDAE)

THESIS
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by

QURBAN ALI ROUHANI

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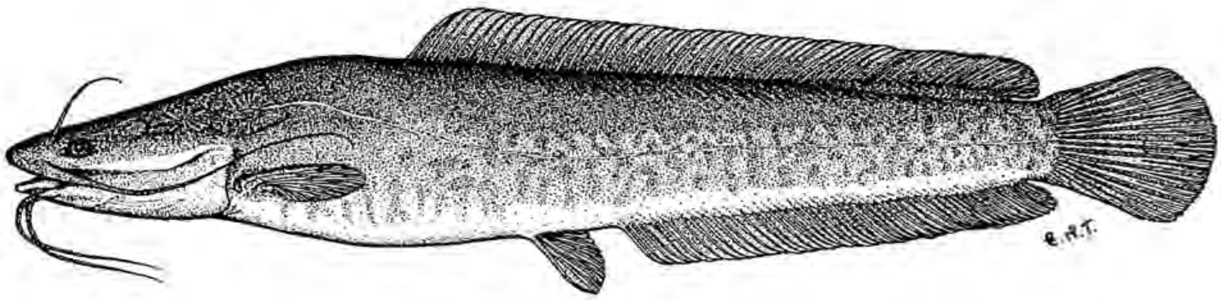

The African sharptooth catfish, *Clarias gariepinus*.

This thesis is dedicated to my father, mother and sisters.

CONTENTS

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ABSTRACT

Based on a number of attributes, the African sharptooth catfish, *Clarias gariepinus* is an ideal aquaculture species. Much work has been undertaken on developing the technology for the commercial culture of the species inclusive of determining the nutritional requirements for cost effective feed formulation. However, digestible energy (DE) values for specific feed ingredients were unknown. As DE values are not interchangeable between species, this study set out to determine the DE values for conventional and unconventional feed-ingredients for the formulation of an optimal feed. The ultimate goal in feed formulation is to produce a diet that supports the maximum production at the least cost.

The indirect method was found to be most suitable in obtaining DE values for the catfish. Feed samples were marked by adding 2% chromic oxide as an inert indicator. The fish were then force-fed and faecal samples were obtained from the hindgut by stripping the fish 10 hours after feeding as this was found to be when the hind-gut was at its fullest and the feed maximally digested.

A linear based computer programme was used to formulate experimental diets using the established DE values as an additional variable. The formulated feed was tested and compared to a commercial catfish diet and a standard reference diet (H-440). All of the newly formulated feeds were found to be cheaper to manufacture (by between 5 and 10%). Statistically (ANOVA), all of the experimental diets resulted in higher specific growth rates (SGR) than the standard reference diet. The commercial diet also resulted in a lower SGR than those experimental diets with similar protein levels.

CHAPTER 1: INTRODUCTION.

If you have an apple and I have an apple and we exchange the apples then you and I will still each have one apple. But if you have an idea and I have an idea and we exchange these ideas, then each of us will have two ideas.

George Bernard Shaw

The biology and ecology of the African sharptooth catfish, *Clarias gariepinus*, has been intensively studied for a long time (Greenwood 1955; Blache 1964; Bell-Cross 1976; Gaigher 1977; Clay 1977; Bruton 1979 a, b & c and others). These studies laid the foundation for the development of the culture technology for the species. Although some studies on the culture of catfish were undertaken as long ago as the late 1950's (El Bolok and Koura 1959), it was only since the late 1970's that a concerted scientific effort was launched to realise the culture potential of the species (Micha 1971, 1975, 1976; De Kimpe and Micha 1974; van der Waal 1972; Richter 1976; Hogendoorn 1977, 1979, 1980; Schoonbee *et al.* 1980; Hecht 1981; Christensen 1981).

On examination of the literature, it indeed becomes evident that the African catfish is an ideal aquaculture species on the basis of a number of attributes (Greenwood 1955; van der Waal 1972; Clay 1977; Babiker 1984; Quick and Bruton 1984; Viveen *et al.* 1985; Huisman 1985; Hecht and Britz. 1990). Apart from the attributes mentioned in the studies of the above authors, the species is highly adaptive to adverse environmental conditions, it has a high handling (Hecht and Britz 1990). The African catfish, as in other species of the family Clariidae, is adapted to breathing atmospheric air by means of a suprabranchial organ (Moussa 1957; Bruton 1979c). The capacity for air-breathing gradually develops with age, constituting 50-60% of the total oxygen consumption of mature fish over 400g (Babiker 1979). Babiker (1979), however, acknowledges that the rate of air breathing is dependent upon the oxygen content of the water. The high fecundity of the catfish (Micha 1973; Gaigher 1977; Hogendoorn 1979; Bruton 1979b; Haylor 1989) makes it ideal for large scale natural spawning in captivity, with the potential for year round induction of final maturation (Janssen 1984). Gonadal maturation is associated with increasing water temperature and photoperiod from July to September (Bruton 1979a). In a study on the Indian catfish *Heteropneustes fossilis* (Vasal and Sundararaj 1976) under laboratory conditions, oogenesis was stimulated at 30°C regardless of photoperiod. Egg and larval development is rapid and the larvae are capable of swimming strongly within 48 hours of fertilisation. There is no parental care of the young (Bruton 1979a). The African catfish has a suitably fast growth rate and reaches maturity towards the end of the second year of growth (350 mm Total Length) (Bruton 1979a). In 1965, Munro defined the African catfish as an omnivorous scavenger. This non-specificity in its dietary requirement, enables the species to consume a wide range of plant and animal food (van der Waal 1972; Bruton 1979c, 1979d; Clay 1981; Haylor 1989, 1992).

The emphasis on this thesis is on aspects of the nutritional physiology of the animal and in particular on the development of an optimal feed based on available published information and on the determination of digestible energy values of basic ingredients. A considerable amount of work on the nutrition of African catfish has been published in the past (Clay 1979; Hogendoorn 1981; Hogendoorn *et al.* 1983; Uys 1984, 1989; Uys and Hecht 1985;

Henken *et al.* 1985; Degani *et al.* 1988; Appelbaum and van Damme 1988; Uys 1989), upon which diets have been formulated and manufactured. However, contrary to recommended nutritional practise no research has been carried out to date which has incorporated information on the digestibility of feed ingredients into a diet formulation for the African catfish (Uys 1989).

As feed cost is often the single most important factor in fish production (Ekpo and Bender 1989), it was rather surprising to find that very little work has been undertaken on digestibility in the species. Digestible energy (DE) of each ingredient to be used in a diet formulation must be determined in order to ensure that the diet can be digested and absorbed to its maximum level (Lovell 1977; Wellborn and Tucker 1985; Brown and Robinson 1989; Uys 1989), as excess nutrients over and above the requirements of the fish is uneconomical and can also lead to poor water quality (Alsted 1991). Nutrient requirements and DE may differ between fish of different species. It is important therefore to calculate these values before formulating a species specific diet (Jobling 1983; Hardy 1989). The correct formulation of a diet is important, as it lays the foundation upon which diets can be manufactured. The first step in formulating a diet (which is a mixture of many components, including nutrients and non-nutrients (NRC 1981, 1983; Hardy 1989)), is to determine the nutritional requirements for the fish in question. An excess or deficiency of either protein or total feed energy can result in reduced growth rates of the fish (NRC 1983).

Acceptability of feed is also an important factor in the formulation of a diet. This depends on a number of characteristics, such as texture, pellet size, colour, the effect of water on the feed (leaching) and whether the pellets float on the water surface or whether they sink. There are different methods available for making pellets with distinct characteristics (NRC 1981, 1983; Pigott and Tucker 1989), depending upon the feeding behaviour and preferences of the fish for which the diet is being formulated. These diets must comply to certain physical and chemical properties, such as water stability. In fact one of the most important requirements of an optimal feed is that it remains intact, with a minimal loss of nutrient components (leaching), for a specified period after it is placed into water. Requirements for water stability is variable and depends on the feeding behaviour of the fish. For example, some fish feed at the surface so water stability of pellets would not be so important as the pellets would be eaten very quickly. On the other hand, fish feeding on the substratum would require a pellet that does not disintegrate quickly (Pigott and Tucker 1989). Another important property of a feed is that of particle density, as this is important in maintaining the position of the pellet in the water column as different fish species feed at different. The organoleptic properties of the diet are also extremely important for attracting the fish to consume the feed. As different fish species are stimulated by different odours, the manufactured diet must be tailored to suit individual species. Particle texture, size and shape requirement varies between fish species and also play an important role in the acceptability of a diet (Pigott and Tucker 1989). Storage stability is essential if large quantities of a diet are to be manufactured and stored for any length of time. So pellets must be hard enough not to disintegrate during storage, yet soft enough for the fish to accept them. Diets may also have to be altered and modified during the different growth stages and development of the fish. Before fish are harvested, "finishing diets" may be required in order to produce certain flesh pigmentations and taste (Pigott and Tucker 1989).

The determination of the nutritional requirements of fish and shell-fish is a science confounded by confusion (Jobling 1983; Baker 1986; Parker 1987). It was therefore thought to be necessary to review the literature to illustrate the use of loose, interchangeable and sometimes contested definitions of terms. Perhaps an adequate analysis of this situation was made by Castell *et al.* (1981) who reviewed the subject in detail: "One of the discouraging aspects of conducting this review was the number of published papers that we felt could not be cited due to lack of adequate information on dietary ingredients, environment parameters such as water temperature, salinity, photoperiod, and other factors relating to adequate experimental design and reporting". New (1976) made various recommendations on the standardization for methodology of crustacean nutrition. Based on his views, the World Mariculture Society formed a Nutrition Task Force (Castell *et al.* 1981). The European Inland Fishery Advisory Commission (EIFAC), International Union of Nutritional Sciences (IUNS), and the International Council for Exploration of the Sea (ICES) also joined this task force (Castell *et al.* 1981). The aim of this task force was to standardise the terminology used in nutrition and bioenergetics research. As this is a broad topic, only the terminology which is pertinent to this study is discussed in this section.

Standard calorific coefficients are not standard at all but vary according to the whim of the author (Jobling 1983; Devendra 1989). Jobling (1983), states that fish nutritionists are primarily concerned with the relationship between the amount of dietary energy available in feed and the production of fish flesh. This has led to the introduction of a hierarchy of terms for expressing dietary energy content:

Total (Gross) Energy

Digestible Energy

Metabolizable Energy

The general bioenergetic terminology is summarized by Hepher (1988) (Figure 1.1). However, Jobling (1983) argues that the term Metabolizable Energy (ME) should be abandoned in fish nutrition studies, for several reasons. The method of calculating ME of a dietary formulation is based upon the gross chemical composition of the diet using assumed digestibility values of various chemical components (e.g. 90% for protein, 85% for lipids and 40% for starch). Moreover, metabolizable energy of a diet is also not constant but depends on the level of feeding.

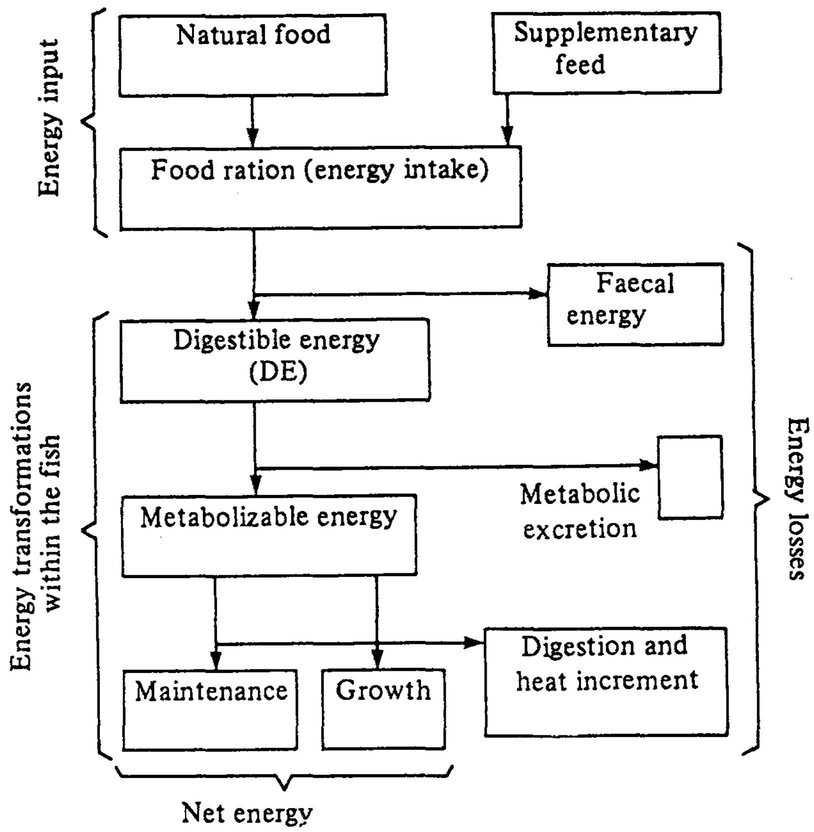

Figure 1.1: Flow chart of energy transformation and losses in fish (from Hepher 1988).

Jobling (1983) considers metabolizable energy to be a 'mythical' unit which can never be accurately measured. Instead of ME Jobling (1983) recommends that only digestible energy be used as a valid and quantifiable unit as it is a more realistic unit upon which to base nutrition and production studies. Based on these arguments, this study has used digestible energy (DE) as a criterion for the development of a diet for the African catfish, *Clarias gariepinus*.

The term digestibility which is the portion of the feed which is not excreted in the faeces but absorbed by the animal, is also often expressed as Digestible Energy (DE) (the portion of the gross food energy minus the faecal energy that has been absorbed) (Matty 1988). In this study, digestibility is expressed as a percentage (%DE), and digestible energy in kilo joules per gram of feed (KJ/g). Both of these terms (DE and digestibility) can however be used interchangeably, depending upon the way the researcher would like to express his results. For example, if diet X has a calorific value (CV) of 100 KJ/g, but the fish can digest only 80% of it, then the diet has a DE (digestible energy) of 80KJ/g, or a digestibility of 80 % (Matty 1988).

Although many fish nutritionists still express energy in kilo calories (Kcal), it is being replaced universally in the scientific literature by KJ (Kcal can however be easily converted to KJ by multiplying by 4.184) (Jobling 1983).

Unfortunately, the controversy regarding terminology in fish nutrition and bioenergetics does not stop here. It is now being debated whether the term 'feed conversion', (defined as the weight of feed converted into weight of fish), provides any information on the relation of energy input to energy output and is often considered to be an unacceptable form of data presentation (Parker 1987). In fact, Baker (1986), argued that " the decision to use gain : food, or food : gain should not be left to the discretion of authors, and journals should require that authors use output - input for all expressions of efficiency". The definition of Food Conversion Ratios (FCR) is influenced by many factors, including: species, age or size, sex, reproductive state, stocking rate, water temperature, photoperiod, feeding rate, feeding frequency, palatability or acceptance of feed, season of the year and time of day when fish are fed (Parker 1987), thus comparing results between different authors becomes difficult.

Therefore this study will not use FCR for comparison between diets, but will use Specific Growth Rate (SGR) (see Castell *et al.* 1981), a universally used and accepted index of comparative performance.

DIET GUIDELINES

Hardy (1989) discusses the enormous changes that have occurred during the last few decades in the formulation and the preparation of feeds for farmed fish, methods of feed and ingredient manufacture, feed ingredients, availability and quality of ingredients used in feeds and advances in our knowledge of fish nutritional requirements. Manufacturing a diet is fundamentally a compromise between the ideal situation and practical considerations, as the ultimate goal is to produce a feed that supports the maximum production (ideal) at the lowest possible cost (practical).

Hardy (1989) stipulated goals of what a production diet must exhibit in a practical situation. These are:

1. The production diet must be economical to manufacture, ship, store and feed.

2. The production diet must be accepted by the target fish.
3. The production diet must be stable in water until the fish have eaten it , so as to minimise leaching and water pollution by the disintegration of the pellet.

Moreover, "having a clear goal is of great value in diet preparation because it influences the way in which feed ingredients are chosen, prepared, combined and processed" (Hardy 1989).

Throughout this study it has been attempted to clearly explain what is meant by any particular nutritional term, so as to avoid any confusion. Furthermore, a glossary of terms has been added at the end of the thesis. The diets produced for the African catfish in this study, using digestible energy (DE) as one of the principal criteria have been clearly described showing ingredient content and quantities, in order to facilitate further study in this area and also to make the results comparable with other research done on this particular species

A SYNOPSIS OF THE DEVELOPMENT OF AFRICAN CATFISH CULTURE.

The development of the technology for the farming of African catfish in South Africa has gone through its teething problems during the previous two decades (Hecht and Britz 1990) Among the first problems that needed to be overcome was the lack of a technique to reliably spawn the fish to produce adequate numbers of fry and fingerlings (Hogendoorn 1977; Schoonbee *et al.* 1980; Hecht *et al.* 1982; Hecht 1985). This search was a reflection of a world wide trend in trying to establish controlled seed production, as a constant and adequate supply of fingerlings was seen as a major factor inhibiting large scale culture of various fish species (EIFAC 1976; Huisman 1976; Coche and Bianchi 1979).

The problem was solved by injecting the fish with gonadotropic hormones, to induce spawning with reliable results. As this is a large topic and well reviewed in the literature, only a brief outline is given here. Carreon *et al.* (1973, 1976), Hecht et. al. (1982) and Britz (1991) used fresh homoplastic hypophysation to induce spawning by injecting about 1.5 pituitary glands per female fish. Schoonbee *et al.* (1980) used alcohol preserved homoplastic hypophysation to induce spawning similar to the methods of Carreon *et al.* (1973, 1976). Acetone dried carp pituitary extracts (ADCP), used by Hogendoorn (1979), Hogendoorn and Vismans (1980), Adigun (1986) and Richter and van der Hurk (1982) had good results when a single intraperitoneal injection was administered to the female fish. Human Chorionic Gonadotropin (HCG) also proved to be successful when used on its own (Carreon *et al.* 1973, 1976; Eding *et al.* 1976; Mollah and Tan 1983), or when mixed with fresh homoplastic hypophysation (Hecht *et al.* 1982). HCG and ADCP can also be used in combination (Schoonbee *et al.* 1980).

Steroid hormones, such as 17 α hydroxyprogesterone (Richter *et al.* 1987) and 11-deoxycorticosterone-acetone (DOCA) (Carreon *et al.* 1976; Hogendoorn 1979 and Richter and Van der Hurk 1982) induced spawning in the female African catfish after adminstration of a single intraperitoneal injection.

Similar research done on the Asian catfish *Clarias batrachus* (L.), a fish "on the threshold of becoming an important species for aquaculture" (Zonneveld *et al.* 1988), produced encouraging results. When injected with carp pituitary suspension, at a dosage of 6 mg/Kg body weight in order to induce oocyte maturation and ovulation, the Asian catfish produced 82.5 % normal larvae 17 hours after being injected (Zonneveld *et al.* 1988).

The development of a reliable spawning technique and the cryopreservation of sperm (Steyn and van Vuuren 1987) has allowed the fish farmer to plan ahead in terms of getting fish to spawn and relieved him of the dependency on natural spawning behaviour or cycle of the fish, which is in turn dependent upon environmental factors such as season, temperature and photoperiod (Bruton 1979a). Induced spawning, either by hormonal glycoproteins (HCG, ADCP, FSH and Oxytocin) or steroid hormones (DOCA) has meant that disease transmission from broodstock to offspring and predation by adults is minimised, as fish larvae would no longer be in direct contact with their parents. Size grading of batches of juvenile fingerlings is more easily performed when fingerlings are separated from broodstock, thereby minimising sibling cannibalism (a threat in juvenile rearing) (Hecht 1986; Hecht & Appelbaum 1988). Prophylaxis and treatment of disease for juveniles is also more effective when the young are separated from the adults. Hatchery rearing eliminates losses which would be attributed to natural predators, so greater numbers of juveniles could be reared. Induced spawning has also meant that more efficient management is possible in terms of feeding, inventories, growth rate data and mortality records.

Once the fish could be reliably spawned, the development of a weaning and a production diet was necessary. As on any intensive fish farm, the single most expensive production component is that of feed (Cowey 1992), and this is frequently responsible for more than 50% of operating expenditure (Lovell 1989; Logan and Johnston 1992). Thus, the development of an optimal dry feed was essential. Uys (1989) studied the nutritional physiology and dietary requirements of the catfish. This study laid the foundation for the development of a formulated feed for African catfish. Moreover, based on the work of Uys (*op. cit.*), such a feed is now made locally.

Artemia or *Daphnia* are usually fed to larvae after the onset of exogenous feeding (Hecht *et al.* 1980; Msiska 1981), as live feed enhances growth (Msiska 1981). However, in intensive culture of larvae, it is not possible to feed the larvae with live feed for an extended period of time. A weaning diet, (which is relatively easier to produce than live feed) must be presented to the fish as soon as possible (Verreth and Tongeren 1989). A study by Charlon and Bergot (1984) showed that dry feed could insure high survival and growth rates and Uys and Hecht (1985), formulated an artificial dry feed based on the dietary requirements of the African catfish larvae. This yielded good results when used either alone or when supplemented with live food. Appelbaum and van Damme (1988) also worked on the feasibility of using an exclusively artificial dry feed for the rearing of larvae and fry. Nutritional aspects in rearing juveniles and sub-adults has been well documented by, amongst others, Machiels and Henken 1985, 1986, 1987; Henken *et al.* 1986; Machiels and van Damme 1987; Uys and Hecht, 1987,1988; Uys 1989; Uys *et al.* 1987; Verreth *et al.* 1987 a & b; Appelbaum and van Damme 1988.

The possibilities of raising African catfish at high densities has now also been investigated by researchers (Carreon *et al.* 1976; Meske 1984; Bovender *et al.* 1987). In a recent study, Hecht and Bolnick (in press) was able to rear adult fish at a density 300 Kg per cubic meter of water. Because of its ability to breathe air, the African catfish can live without the prerequisite of pond aeration or high water exchange rates (Sidthimunka 1972; Huisman and Richter 1987), and high stocking densities have been attained as this fish is tolerable to relatively poor water quality (Babiker 1984, Huisman and Richter 1987).

However, scientific and technological breakthroughs are not the only breaks needed in an emerging industry. According to Hecht and Britz (in press a), who have monitored the progress of catfish farming in South Africa, since the first ten tons were produced in 1986 / 1987, there has been a 23 % fall in production of catfish (recorded over the period 1990 to 1991). This slump in production was not anticipated, especially as there had been an 87% increase in production bringing it to ca. 1400 tons over the 1989 to 1990 period. Two factors were responsible for this unexpected decline in production. Firstly, there was a severe drought over the entire southern African region which has resulted in many farmers having to sell off stock because of reduced water availability. Secondly, and also the main reason, it would seem that African catfish farmers have hit a bottleneck in marketing. Unless a considerable marketing campaign is undertaken to alleviate this problem, no solution is seen. The financial implications of such a task seems to be a major constraint for the farmers. Perhaps an example that South African catfish farmers could follow is that of the development of the American channel catfish industry, where effective marketing techniques (Waldrop 1981; Dupree and Huner 1984) helped to increase the annual processed weight from 1 million Kg in 1970 to approximately 70 million Kg in 1982 (Wellborn and Tucker 1985). It is suggested that farmers in South Africa could also experience such rapid and constant growth if the marketing strategies were revised and if the necessary finance is available to guard such a marketing strategy.

OBJECTIVES OF THE PRESENT WORK

As no information is available on the digestibility of various feed ingredients of the African Catfish (Uys 1989; Hecht in press a), DE values for the American channel catfish *Ictalurus punctatus* have been used to calculate the energy requirement of the African catfish, even though DE values are not interchangeable between species (Jobling 1983).

Therefore this study, using digestible energy (DE) as a criterion for the development of a diet for the African catfish, *Clarias gariepinus,* was divided into two parts. The first involved the development and identification of a suitable method for obtaining digestible energy (DE) values for the catfish, determining gut evacuation times and determining the rate of nutrient leaching from faecal and feed samples. DE values from different segments of the intestine were also determined and compared.

The second part of the research was concerned with the determination of DE values of various conventional and unconventional feed ingredients. The DE data were then used in the modelling of new diets using a linear based computer programme. The new diet formulations were then manufactured and tested by means of comparative growth trials. All the experimental work was conducted at the experimental fish farm, hatchery and laboratories of the Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, South Africa.

As mentioned previously, the study by Uys (1989) laid the foundation for the development of a formulated African catfish feed. He recommended a dietary protein level of 40 - 42 %, a lipid level of 10 - 12 % and a total digestible energy of the feed at between 14 - 16 KJ/g. However, no comprehensive data is available on the digestible energy (DE) values of individual feed ingredients of African catfish .

EXPERIMENTAL GUIDELINES

Castell *et al.* (1981) laid down certain protocols which need to be observed in evaluating dietary nutrients and for reporting on results, so that studies by different researchers are compatible. The guidelines were as follows:

1. All diets should be defined as clearly as possible, i.e. contents lists, proximate analyses and sources of the various ingredients.
2. Diets should be administered quantitatively and, if the fish are fed on an *ad libitum* basis, consumption should be noted.
3. Measurements of a diet's success or failure (feed conversion[1]) should be reflected in a representative manner, not only in wet weight gain and in survival, but also by means of dry weight as this represents true growth more accurately (this point is discussed in greater detail on page 7 as its use has been contested (Parker 1987)).
4. Access to naturally occurring food should be avoided. There is little sense in testing the value of a diet if the animals can obtain equally good or superior nutrition from their surroundings.

In some instances, researchers have submitted the relevant information, concerning biotic or abiotic conditions, but have neglected to employ standard techniques and methods. This results in data which are largely independent and non-comparable. Great care was exercised in the execution of this project to remain within these experimental guidelines.

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[1]Feed conversion is also known as 'conversion factor', 'absolute food quotient', 'nutrient quotient', and 'utilization coefficient' (Hastings and Dickie 1972).

CHAPTER 2: GENERAL METHODS.

EXPERIMENTAL LAYOUT

Tank and Biological Filter Setup

All the experiments conducted in this research were done in a closed recirculating system. The system included a header tank of 700 litre capacity. An immersion heater of 4 KW was installed in the header tank to regulate water temperature at a constant 28 ± 1^0C. The two out-flow pipes from the header tank supplied one or two sets of glass tanks. One set, consisted of nine glass tanks of 30 x 70 x 90 cm dimensions (in which the large fish were kept). The flow rate in this set of tanks was 4 litres per minute per tank, effecting a complete water exchange every 45 minutes. The other line supplied ten glass tanks of 30 x 30 x 60 cm dimensions (in which the fingerlings were kept). Each of the fingerling tanks was divided by a plastic gauze covered frame to make two equal sections. The gauze allowed for the mixing of water but not of fish between compartments. The flow rate to this set of tanks was 1.5 litres per min, with a complete water change every 36 minutes. However, the flow rate to each glass tank could be adjusted individually. The overflow from all the tanks spilled into a sediment collector (18 x 40 x 150 cm), which in turn spilled into a dry-trickle biological filter (0.6 x 1.2 x 6 meters). Once the water had passed through the trickle filter it was collected in a 400 litre tank from where it was pumped by means of two pumps (at the rate of 11,000 litres per hour) back to the header tank (the second pump was incorporated into the system as a safety measure). An overflow pipe delivered excess water in the header tank back to the sump. Municipal tap water was used to top up the header tank, as there was some water loss due to evaporation and spillage.

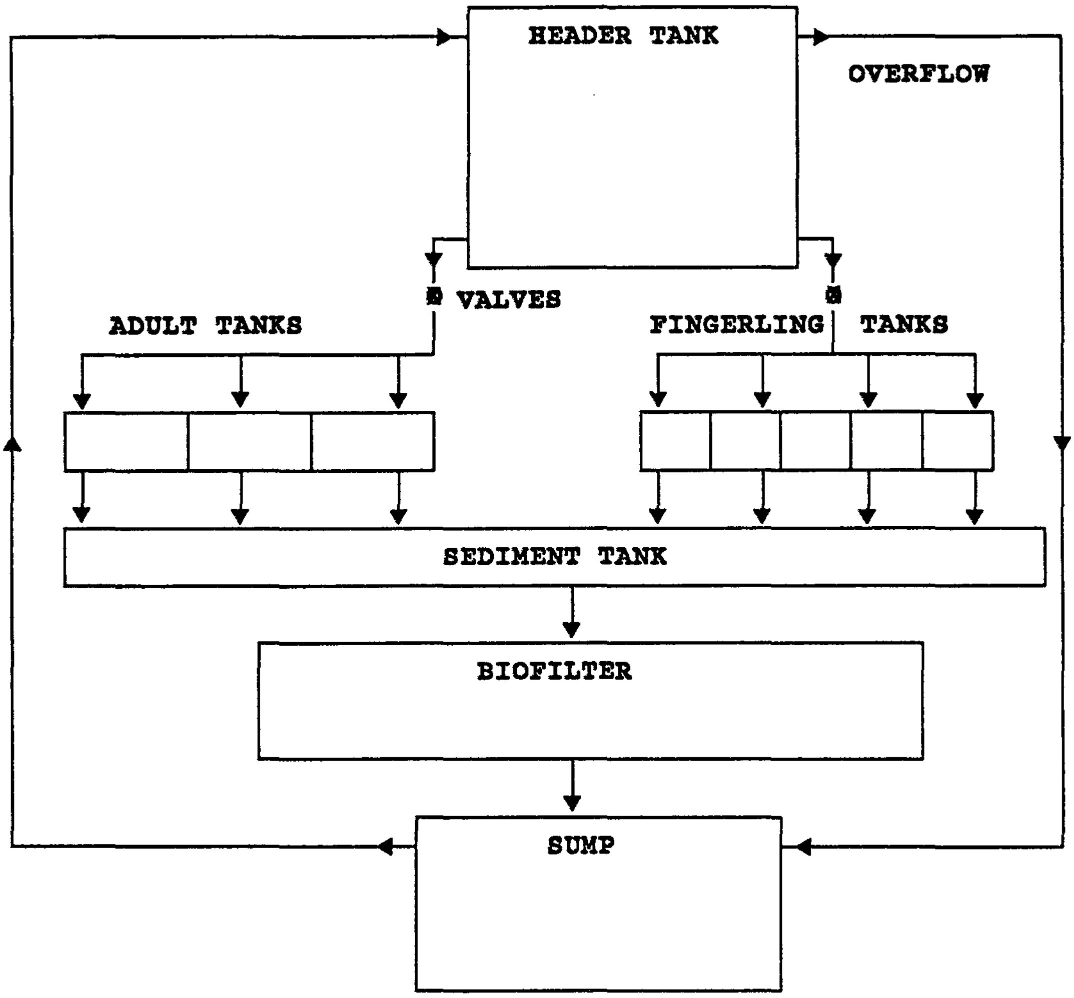

Figure 1.1 Design of the system.

Water Quality

The following water quality constraints and conditions were maintained for all the experimental work done in this study.

Temperature 28 $\pm$1 ^{0}C

pH 7 $\pm$0.5

Ammonia <0.025 mg/l NH_3

Nitrite 0.1 mgN / L

Photoperiod 12L / 12D

Water temperature was maintained at 28 ±1 ^{0}C, as Hogendoorn *et al.* (1983), Britz and Hecht (1987) and Degani *et al.* (1988) found that the optimum temperature for small African catfish was between 27 ^{0}C and 30 ^{0}C. For larger fish (125g), the optimum is 25 ^{0}C (Hogendoorn *et al.* 1983), but as most of the fish used in this study were small, and both large and small fish had to be kept simultaneously , a temperature of 28 ^{0}C was chosen and maintained.

ORIGIN OF THE EXPERIMENTAL FISH

The catfish used in this research comprised fish from two sources. One group of catfish were spawned at the research hatchery, Rhodes University. These fish were used as adults. The second group of African catfish were fingerlings which were transported from a commercial fish farm (Blyde River Aquaculture) in the Transvaal Lowveld. The catfish fingerlings (average length, 70mm), were obtained in two batches. The first batch of 300 fish arrived in April 1991, and the second batch of 500 fish in July 1992. Catfish from different sources and even different batches were always kept separate. The reason for having fish of different size groups, was that the fish used for a specific experiment depended upon which size was most suitable to work with. For example, to determine gastric evacuation time (GET), or stripping (to obtain faecal samples) large fish were used. For all growth experiments, small fish (fingerlings) were used, as growth is more pronounced in smaller fish. In the ensuing chapters, the size of fish used for any specific experiment is stated.

ARTIFICIAL SPAWNING, FERTILISATION AND INCUBATION OF EGGS.

The procedure for the artificial propagation of sharptooth catfish was conducted as described by Britz and Hecht (1988). For the fish that were spawned at the Rhodes University hatchery, broodstock was taken from a resident stock of fish maintained in an outdoor solar tunnel system. Ripe females with extended abdomens were chosen to avoid conditioning before hypophysation. Females ranged from 2000-3000g and males between 1500-2000g. Spawning was induced by hypophysation using homeoplastic pituitary glands. The pituitary gland was homogenised in a glass tube homogeniser with distilled water. A dose of 1.5 pituitary gland per female fish was used, provided the donor and recipient were of approximate equivalent weight. The resulting solution was injected intramuscularly in the nape region of the female catfish. The needle was withdrawn slowly and the area gently massaged to prevent exudation of the pituitary gland homogenate. Within 12-14 hours after hypophysation, the fish were ready for spawning. After this time, eggs would be free-running from the genital pore whereupon the females would be stripped and the eggs collected in a plastic container. Sperm of a freshly sacrificed male would be mixed gently with the eggs. A small quantity of water was added to the gametes to effect fertilization and to allow the fertilised eggs to swell and to become sticky. As the eggs became sticky, a monolayer of eggs was gently spread onto plastic gauze frames which were suspended vertically in the incubation trough. Hatching occurred 23-28 hours after fertilization at 23-27 ^{0}C. Once the embryos hatched, water flow carried them into a larval rearing tank. At the onset of exogenous feeding, they were fed on *Daphnia* for a period of 4 days. After this period, crumbles of a formulated starter diet (particle size 250 - 500 µm) was fed to the larvae (Uys and Hecht 1985).

CHROMIC OXIDE ANALYSIS

The chromic oxide method of determining digestibility, developed by Bolin *et al.* (1952) was used in this study. Chromic oxide is undigestible, therefore when added to a diet the ratio of the marker (chrome oxide) to energy in food and faeces can be used to calculate digestibility. This method is quick and able to yield accurate results and is still very much in use today. After feeding the fish with feeds containing 2 % chromic oxide, the faecal sample was collected (see Chapter 3 for method used). The faecal sample was dried until a constant weight was reached at 40 °C in a drying oven and then weighed. About 25 mg of the sample was then placed in a Kjeldahl flask along with boiling chips. Never more than 500 mg of sample should be used (Bolin *et al.* 1952), as the result can be explosive! Ten ml of nitric acid was added to the sample in the Kjeldahl flask, plus a 15 ml solution of one part sulphuric acid (70-72 %) and three parts perchloric acid. The Kjeldahl flask was then placed in a fume chamber and heated with a bunsen burner until the solution (green in colour due to chromic oxide) boiled. Heavy fumes appeared on the solution surface, which then lifted off, at which point the colour of the solution changes from green to a yellow. This colour change indicated that all the chrome was now free in the solution, and could be measured. The solution was then cooled, decanted into a 100ml conical flask, which was then topped up with distilled water to a total volume of 100 ml. The solution was then further diluted by 1:10 with distilled water. The diluted sample was placed in an atomic absorbtion spectrometer and read at 440 *u*. The following equation was used to determine chromic oxide in the sample:

$$\left\{ \frac{r \text{ x vol x } 1000}{10^6 \text{ x sample weight}} \right\} \text{x } 1.4616 = \% \ Cr_2O_3$$

where *r* is the reading from the atomic absorption spectrometer, *vol* is the volume of the flask

(100ml), and *weight* is the mass of original sample in grammes (Jobling 1983).

CALORIFIC VALUE

To measure the calorific value (CV) of feed ingredients and faecal samples, a CP400 Calorimeter Systems apparatus was used. The machine was calibrated at a standard calorific value of 26.454 MJ/Kg (this is the CV of benzoic acid). Samples to be tested for CV were first dried and weighed, then placed individually in the 'bomb'. Prior to ignition pure oxygen at a pressure of 30-35 bars was pumped into the bomb. The temperature of the bomb was then stabilised in a cooling unit before the sample in it was 'fired'. A reading from the machine then produced the CV of the sample in MJ/Kg.

This method (bomb calorimetry) was shown by Henken *et al.* (1986) to be the most accurate, when he compared it to the dichromate wet oxidation and chemical composition methods.

FEEDING REGIME

Hogendoorn *et al.* (1983), proposed a size and temperature related model for the estimation of daily ration for the African catfish. Based on their data, as well as those of Uys and Hecht (1988), a size and temperature related feeding schedule has been developed, which is generally used by South African farmers to obtain optimal FCR's. All feeding schedules in this study were in accordance with the above mentioned feeding programme (this schedule is listed at the end of the thesis in the glossary).

CHAPTER 3: COLLECTION OF FAECES FOR THE DETERMINATION OF DIGESTIBLE ENERGY VALUES.

INTRODUCTION

In nutritional and bioenergetic studies in fishes, the qualitative and quantitative collection and analysis of faeces, is problematic (Hardy 1989). The problems include the leaching of nutrients from the faeces and stress imposed on the fishes (Ogino *et al.* 1973; Possompes 1973; Possompes *et al.* 1975; Austreng, 1978; Windell *et al.* 1978; Choubert *et al.* 1979; Smith *et al.* 1980; Brown and Robinson 1989). Therefore, many different techniques have been suggested and used to counter these problems.

Two general methods exist for establishing digestibility coefficients of diets (Tunison *et al.* 1944; Post *et al.* 1965; Ogino *et al.* 1973; Hardy 1989). These are the direct and the indirect methods. Each of these methods have different approaches and both have advantages and disadvantages, depending among other things on, the species of fish on which the study is being undertaken, the aim of the study and the financial considerations. These points are discussed below.

Direct Method

The direct method requires the quantitative collection of the faecal excreta. This method is usually associated with some sort of mechanical device used to collect all faecal material excreted by the fish. Digestible energy is then calculated as total energy in the feed given to the fish at that particular meal, minus total energy of the faecal matter. This method has limitations in that it is difficult to be certain that all faecal matter excreted is collected (Noue & Choubert 1986). Another uncertainty involves the leaching of nutrients from the faecal sample. It is also difficult to correlate the faeces collected with the exact amount of feed the fish has consumed. If the fish are starved prior to faecal collection, digestibility is affected (Smith 1989), and if the fish are continuously fed, it is difficult to ensure that the sample is complete on collection of the faeces. For this reason this method was not employed.

Indirect Method

An indirect method was developed to avoid the problems associated with the complete quantitative collection of faecal matter. The method involves the incorporation of an indigestible marker in the feed. In this study, chromic oxide (Cr_2O_3) was used as an indicator. Chromic oxide is widely used in fish nutrition studies (Jobling 1989). Digestibility is then calculated by the ratio of the marker to energy in food and faeces (Maynard and Loosli 1969; Hardy 1989). Other indigestible indicators which can be used are titanium oxide, rare earth elements, celite and some natural markers such as lignin, chromogen and ash (Jobling 1989).

Collection of Faeces

In the actual process of collecting faeces, various techniques have been proposed: continuous filtration of faecal material from the fish tank (Possompes 1973; Kaushik and Louquet 1976; Choubert *et al.* 1979), settling out and then collecting the faeces (Cho and Slinger 1979), use of a metabolic chamber (Post *et al.* 1965; Choubert *et al.* 1982), dissection (Phillips *et al.* 1948; Nose 1967; Austreng 1978; Spyridakis *et al.* 1989), anal suction (Windell *et al.* 1978; Spyridakis *et al.* 1989), and abdominal pressure (Nose 1967; Spyridakis *et al.* 1989).

As mentioned previously, methods vary from simple procedures to expensive machines designed by Choubert *et al.* (1982) which automatically collect and rapidly separate faeces from water. However, the final choice of method to be used to collect faecal matter is largely dependent upon the species being studied, as fish behaviour and morphology play an important role in the success of a particular method. Financial constraints also play an important role, as some of the methods discussed above require costly apparatus.

In this study, faecal collection was initially done by means of both the direct and the indirect methods to find the most suitable one for the African catfish.

MATERIALS AND METHODS

The experiments were carried out in 162 litre glass tanks of 30 x 60 x 90 cm dimensions. Temperature was maintained at 28 $\pm 1^0$C. The average weight of the fish was 295 g. Fish density was maintained at five fish per tank. The fish used, were of those spawned and reared at the Rhodes University hatchery. The fish were fed twice daily at a rate of 3.5 % body weight per day. The feed was a commercial catfish pellet with 45% protein and a calorific value (CV) of 17.482 MJ/Kg. All the feed administered to the catfish contained 2 % chrome oxide by weight. The pellet was first passed through a hammer mill to pulverise the pellets, whereafter the chrome oxide was added at 2 % of feed weight. The mixture was mixed manually to break any clumps of chrome oxide and then passed through the hammer mill five times to ensure a homogeneous pulverised mixture. The feed powder was then made into a "putty" texture by adding water and a 1 % solution of sodium lignite, a binding agent, as the next stage involved putting the "putty" through a meat mincer. After this the feed was dried in a drying oven at 40 °C overnight.

Assays for the determination of chrome oxide in the faeces and calorific values of both feed and faeces was conducted as explained in Chapter 2.

Faecal Collection

1) Dissection

Ten hours after feeding, three fish were sacrificed by partial decapitation by cutting through the vertebral column. This proved the best method as catfish have a strong, highly ossified skull. Other methods such as that of delivering a blow to the head, a method commonly used by fish researchers, did not prove successful.

Uys (1989), when studying the nutritional and dietary requirements of the African catfish, divided the gastrointestinal tract into four parts. These were the stomach, the fore-gut, the mid-gut and the hind-gut. To distinguish between the fore-gut, mid-gut and the hind-gut when sampling for the faecal material and to keep the results constant, the total length of the gut was measured from the anus to the sphincter muscle leading into the stomach and this was divided by three. Each segment then represented the approximate appropriate section of the gastrointestinal tract. The stomach was considered the region between the cardiac and pyloric sphincters.

A longitudinal incision was made along the length of the abdomen from the anus to the base of the mouth. The gastrointestinal tract was then carefully removed so as not to squeeze the contents from one section of the gastrointestinal tract to another. The hind part of the gastrointestinal tract was then severed from the mid-gut. A longitudinal incision was then made in the hind-gut so that the contents could be removed. Care was taken not to scrape remains and dead cells, to be incorporated in the sample. Faecal material was placed in a sterile vial which was then placed in a drying oven at 40 °C until constant weight was achieved.

2) Stripping

Due to the air breathing capacity of catfish, they did not have to be anaesthetized when taken out of their tanks for faecal sampling, a common procedure for non-air breathing fish such as, for example, trout, channel catfish, and the European sea bass (Spyridakis *et al.* 1989). Before the actual sampling began, the fish would be given trial runs of faecal sampling by stripping as this conditioned the fish to handling. It was observed that if this procedure was undertaken the fish 'appeared' to be less stressed when the actual sampling was done. The fish was placed on a moist, smooth surface while being handled and stripped for faecal samples. With one hand firmly, but not tightly, around the base of the skull, the other hand gently stroked the abdominal region, in an anterior - posterior direction from the head towards the anus. The faecal matter would then be excreted from the anus in the form of a pellet. Care was taken not to get any of the skin secretion into the sampling vial. The faecal sample was then dried to constant weight in an oven at 40 °C.

3) Anal Suction

For this method, a glass tube was inserted into the anus of the fish. In this study the internal diameter of the glass tube was three millimetres. The glass tube was lubricated to facilitate insertion. A slight suction was applied (by mouth) and the faecal matter would be sucked into the tube. The faecal sample was then blown out of the glass tube into the sample vial.

During this procedure, the fish was held firmly around the base of the skull. The faecal sample would then be dried to constant weight in the drying oven at 40 °C.

4) Immediate Pipetting

Model A

Two glass containers of different designs were built for this purpose (Figure 3.1). The first container, in the shape of half a globe, had a diameter of 40 cm at the top which then narrowed down to 0.5 cm at the base. The height of the tank was 90 cm. The tapered bottom had an access nozzle through which a 5 mm diameter pipe was passed. The end of the pipe had a tap attached to it so that the water at the bottom of the glass tank could be drained out. The catfish was placed in the glass tank. This tank was then submerged in the glass holding tank (30 x 70 x 90 cm). When the fish excreted, the faeces would settle at the bottom of the tapered tank, and by opening the tap at the end of the pipe, the faeces could then be flushed into a beaker where it would be immediately filtered and then dried in an oven at 40 °C until constant weight was achieved.

Model B

The second design was of a different shape. The top section was in the shape of a box, of the dimensions 30 x 15 x 15 cm. The bottom section of the glass tank narrowed into a "V" which provided an area of "dead water", in which the faecal matter collected when excreted by the fish. As catfish are strong and vigorous swimmers, this area of "dead water" was to ensure that the faeces would not disintegrate if the fish disturbed the water. When the fish excreted, the faeces would then be immediately pipetted from the water and dried (in the oven at 40 °C) until constant weight was attained.

Figure 3.1: Faecal collecting tank - Model A.

In the experiments described above, all of the vials and glass tanks especially built for the immediate pipetting experiments, were first wiped with a sponge that was soaked in a 10 % formalin solution. This procedure was practised so as not to allow bacterial growth on the faecal samples. Moreover, before any of the samples were put into the drying oven, a 10 % solution of formalin in a petri dish was left to evaporate overnight in the drying oven, as it was noted that if this procedure was not carried out, fungal growth would begin on the moist faecal samples, resulting in contamination.

The digestibility of the food was calculated as follows (Jobling 1983):

$$\text{Digestibility (\%)} = 100 - 100 \left\{ \frac{Xa}{Xb} \times \frac{Ya}{Yb} \right\}$$

where Xa and Xb are the concentration of the marker in the food and faeces and Ya and Yb are the energy concentrations of the food and faeces, respectively.

Chromic oxide testing was done in triplicate (Bolin *et al.* 1952) and measurement for total energy was done in triplicate by using a bomb calorimeter (details of assay method in Chapter 2).

A non-parametric statistical test (Kruskal-Wallis) was used to compare the results of different methods for collecting faeces.

RESULTS AND DISCUSSION

Statistically there was no significant difference found between the methods used for collecting faeces ($P > 0.05$). However, the results (Table 3.1 and Figure 3.2) show that the most accurate and consistent method of obtaining representative samples for faecal energy sampling is that of stripping. Stripping of faeces from the African catfish resulted in the highest digestibility coefficient (83.4 %), compared with anal suction (80.2 %), dissection (80.1 %), immediate pipetting method / Model A (78.6 %) and immediate pipetting method / Model B (75.7 %). These results are compared with those of Spyridakis *et al.* (1989), who calculated crude protein digestibility of the European sea bass *Dicentrarchus labrax*. They obtained a digestibility value of 82.5 % for stripping, 84.4 % for dissection, 89.6 % for anal suction, 90.4 % for filtration and 90.6 % for immediate pipetting. In their work (Spyridakis *et al.* 1989), stripping exhibited the lowest digestibility coefficient. However, this difference in results can be explained by the fact that Spyridakis *et al.* (1989) anaesthetized their fish with ethylene glycol monophenyl ether, at 0.2 ml/l, before handling. Anaesthetizing a fish causes sudden defecation (Spyridakis *et al.* 1989), which in turn would effect digestible energy (DE) measurement. The African catfish used in this study were not anaesthetized at any time prior to stripping, as the African catfish is an air breather and can be handled for a considerable period of time out of water without showing any stress.

Table 3.1: Digestible energy values using different methods of faecal collection.

METHOD OF FAECAL SAMPLING	DIGESTIBILITY COEFFICIENT (%)
Immediate pipetting (A)	78.6 ± 1.0
Immediate pipetting (B)	75.7 ± 1.1
Dissection	80.1 ± 0.9
Anal Suction	80.2 ± 1.2
Stripping	83.4 ± 1.1

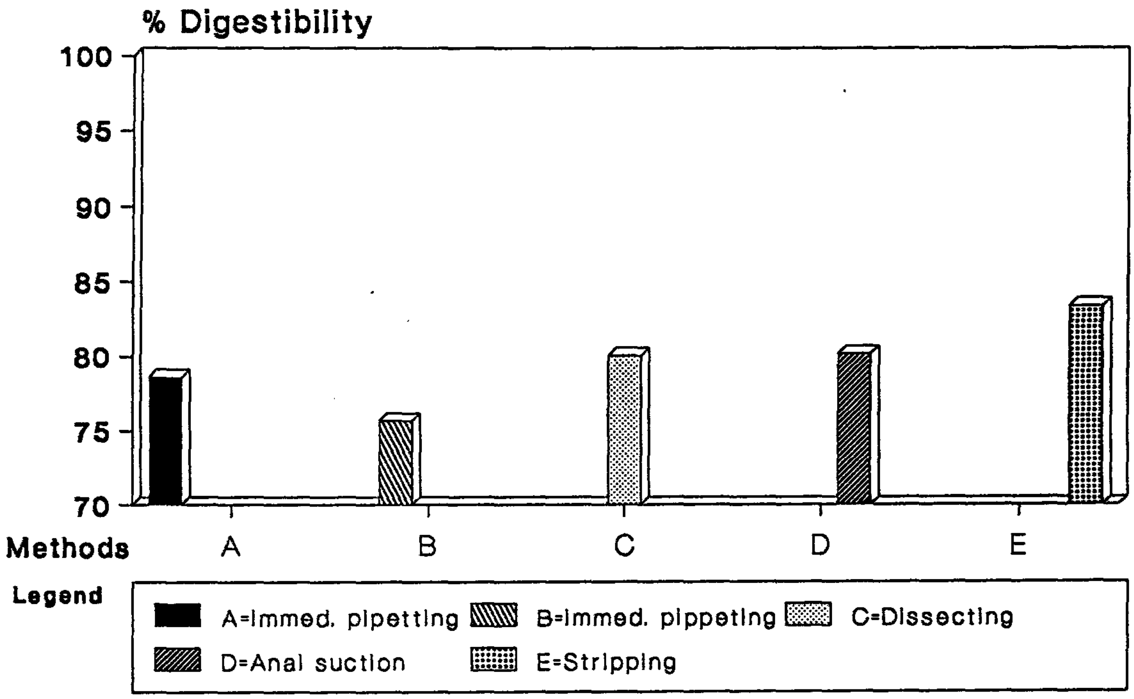

Figure 3.2: Digestible energy values as obtained by different methods of faecal collection in African catfish.

Henken *et al.* (1985), used the intestinal dissection technique in their studies on feeding level and apparent dry matter and crude protein digestibility of African catfish. They, however, suggested that other methods, such as stripping and anal suction might prove to be more efficient. Other studies on measuring DE have all been performed on non-airbreathing fishes (Windell *et al.* 1978; Spyridakis *et al.* 1989). The observed differences between these studies and the present study on African catfish are largely ascribed to stress related factors which these fish had to endure during the stripping operation.

Dissection, as a method for obtaining digestibility coefficients, yields good results (80.1 %), however this method is limiting in that fish have to be sacrificed. In this study about 30 ingredients were to be tested for DE, so the dissection method was not even considered.

The method of continuous filtration, as described by Choubert *et al.* (1979) and Schimitz *et al.* (1983), is probably the most accurate for obtaining digestibility coefficients as the fish are never handled and within seconds the faecal matter is collected from the water column. The drawback, however, is the cost of the apparatus. Use of such apparatus is only justified if extensive and routine work on nutrient digestibility is planned.

CHAPTER 4: THE EFFECT OF LEACHING ON THE DETERMINATION OF DIGESTIBLE ENERGY (DE).

INTRODUCTION

Coefficients of digestion are relatively easy to determine in terrestrial animals (Schimitz *et al.* 1983). However, conducting the same experiments with aquatic animals poses unique problems (Austreng 1978; Smith *et al.* 1980). Leaching (solubilization) of nutrients from feed and faeces in water, and stress (when handling the animals to conduct the work) are two of the major problems faced by fish nutritionists. Quantifying nutrient loss due to leaching is an important factor that needs to be considered when evaluating digestibility of feeds (Brown and Robinson 1989).

As soon as the feed or faeces comes into contact with water, leaching starts. The rate of nutrient leaching is largely dependent upon the type of feed (pellet structure) and fish feeding behaviour. Fish which are highly active when feeding disturb the water, causing the feed or faecal pellet to disintegrate more quickly.

Windell *et al.* (1978) found that apparent crude protein digestion coefficients showed a 10 % increase and apparent ether extraction digestion coefficients increased by 4 % during the first hour when trout faeces were immersed in water. There was no further leaching when faeces were immersed for longer than 24 hours. Smith *et al.* (1980), in a similar study with trout showed that digestibility coefficientts changed (increased) when faeces were immersed in water. The apparent increase in digestibility coefficients obtained in studies such as those of Windell *et al.* (1978), Smith *et al.* (1980) and Brown and Robinson (1989) is ascribed to the leaching of nutrients from either the feed or the faeces in the water, which is then falsely reflected as nutrients being absorbed by the fish.

The purpose of this experiment was to quantify changes in energy in feed and faecal matter of the African catfish when immersed in water for varying periods. The exact nutrient and energy content of feed and of faecal material is fundamental for the accurate assessment of digestibility coefficients. Thus if leaching was significant a correction factor could be applied if necessary to the DE values obtained.

MATERIALS AND METHODS

Catfish of average weight 250g, reared under recirculating conditions at a temperature of 28 ± 1^{0}C were used. Ten catfish per tank, measuring 30 x 60 x 90 cm, were fed 3.5 % of body weight per day with a semi-floating pellet twice daily. The feed consisted of the recommended 40 % protein, 10 % lipid, 14 - 16 KJ/g digestible energy and 26 mg protein per KJ of digestible energy (Uys 1989).

Faeces were stripped from the catfish approximately ten hours after feeding (see Chapter 5). Faecal matter was then dried in a drying oven at 40^{0}C until a constant weight was achieved. All the faecal material was then pooled together and random samples were taken for testing. The dried faecal and feed samples were placed in sterile glass vials containing water from the recirculating system for 7.5 sec, 15 sec, 30 sec, 1 min, 2 min, 4 min, 8 min, 16 min and 32 minutes. The temperature of the water was 28 °C and the pH 7. The experiment was done in triplicate and after the samples had been submerged in the vials, they were oven dried and reweighed. Total energy in the samples was measured by bomb calorimetry (see Chapter 2).

Regression Analysis was used to compare the relationship between % CV and % weight loss for both the pellet and faecal samples.

RESULTS AND DISCUSSION

For both the pellet and faecal samples immersed in water, there was a loss in weight which increased with immersion time (Table 4.1 & 4.2 and Figure 4.1 & 4.2). Regression analysis showed that the correlation coefficient between percent calorific value (% CV) and percent weight loss over time for the pellet was 0.995 (r^2 = 99.72 %). The correlation coefficient between % CV and % weight loss over time for faecal pellets was 0.955 (r^2= 91.22 %). These strong correlation coefficients (0.995 and 0.955) mean that weight loss of a pellet due to leaching also means a loss of nutrients. Pellet erosion after immersion in water could have contributed to the high correlation coefficients between weight and energy loss.

Within the first 30 seconds of immersing the pellet in water there was 1.2 % and 1.3 % decrease in CV and weight respectively (Table 4.3). After one minute of immersion in water the loss in both CV and weight of the pellet sample was 1.8 % in both cases. After immersion for approximately half an hour (32 minutes) the CV of the pellet decreased by 14.9 % and the pellet weight decreased by 15.2%. The faecal samples showed similar, but higher trends in the reduction of CV and weight after immersion in water (Table 4.1 and 4.2). At 30 seconds of immersion there was 4.5 % and 4.2 % reduction in CV and weight respectively and after one minute of immersion there was 6.0 % and 5.8 % reduction in CV and weight respectively.

Table 4.1: Percent calorific value (CV) and percent weight loss of pellets after immersion in water.

IMMERSION TIME OF PELLET	% CV LOSS	% WEIGHT LOSS
7.5 secs	0.5	0.4
15 secs	0.8	0.9
30 secs	1.2	1.3
1 min	1.8	1.8
2 min	3.1	3.3
4 min	4.8	4.5
8 min	7.6	7.9
16 min	11.3	11.5
32 min	14.9	15.2

Table 4.2: Percent calorific value and percent weight loss of faecal samples after immersion in water.

IMMERSION TIME OF FAECES	% CV LOSS	% WEIGHT LOSS
7.5 secs	1.4	1.6
15 secs	3.2	3.5
30 secs	4.5	4.2
1 min	6.0	5.8
2 min	8.6	8.3
4 min	11.2	10.9
8 min	12.2	12.0
16 min	15.1	14.9
32 min	15.5	15.7

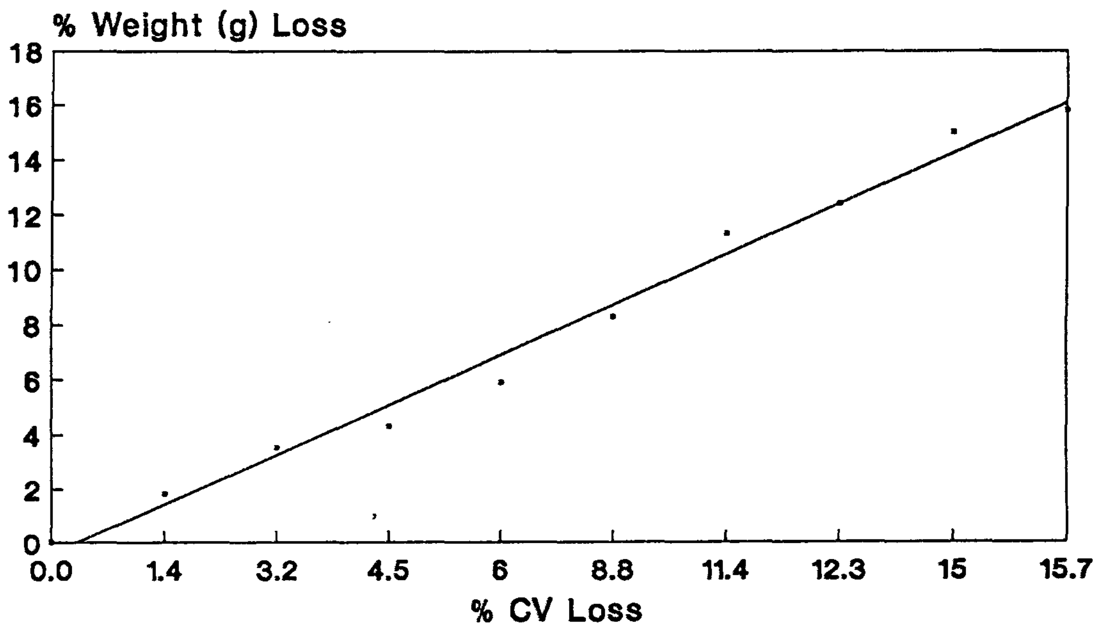

Figure 4.1: The regression showing the relationship between percent loss in calorific value and percent loss in weight for African catfish pellets.

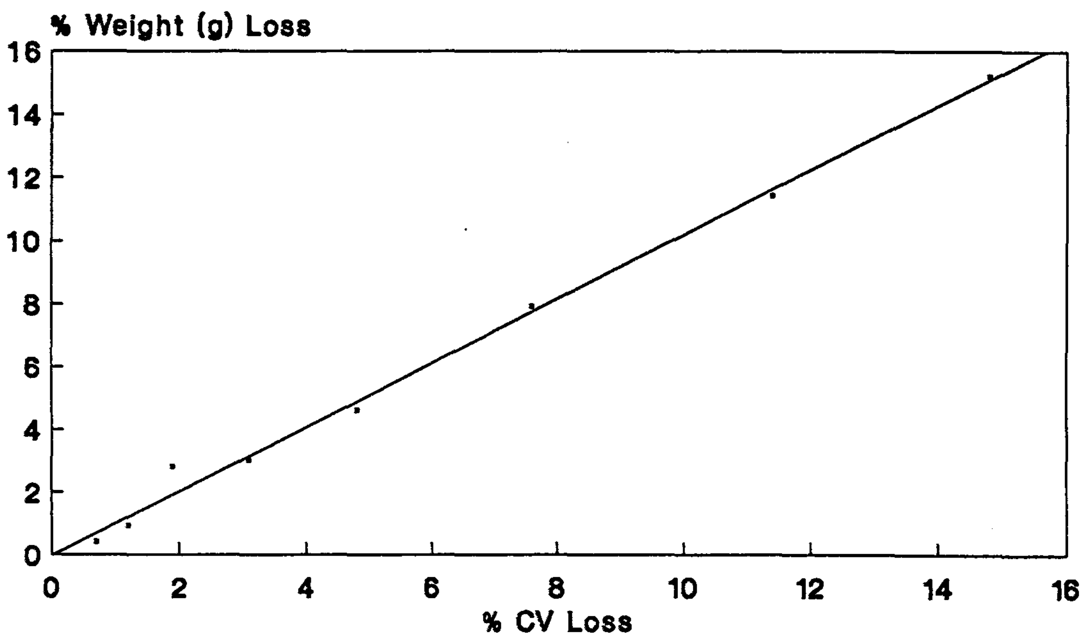

Figure 4.2: The regression showing the relationship between percent loss in calorific value and percent loss in weight for African catfish faeces after immersion in water.

The greater percentage loss of CV and weight for faecal samples compared to pellet samples after the same immersion time in water can be attributed to the differences in structure between pellets and faeces. Binding agents, such as lignin sulphonate (Kirchgessner *et al.* 1986) are used in the manufacture of pellets to effect a reduction in the disintegration of pellets for transport and when immersed in water to feed fish. When a fish ingests and then digests a pellet, the pellet no longer retains its original conformity, and thus faeces tends to be much 'softer' than pellets, which explains the increased rate of leaching in faeces.

The African catfish used in this study were trained to accept their diet by tapping the side of the glass tank, at which point the fish would congregate at a specific corner to eat their feed. Within about ten seconds almost all of the feed presented to the fish was consumed. At 7.5 seconds and 15 seconds there was 0.5 % and 0.8 % decrease in CV respectively (Table 4.3). Thus leaching of nutrients from feed within the first ten seconds of feedings in this particular study did not seem to pose too great a problem.

Brown and Robinson (1989), conducted a series of similar experiments (Table 4.3). They compared leaching between channel catfish diet (floating) and faeces, and trout diet (sinking). Dry matter loss of the sinking trout diet as opposed to the floating catfish diet was significantly greater, as sinking diets tend to lose conformity more quickly.

Based on their results, Brown and Robinson (1989) suggest that, "...faecal samples should be collected by rectal dissection or cannulation before excretion in water". This study has however shown that stripping is the most effective method in determining DE of feeds for African catfish.

Table 4.3: Mean weight loss (% dry matter) of catfish faeces and practical diets immersed in water (from Brown & Robinson 1989).

IMMERSION (MINUTES)	CATFISH FAECES	CATFISH DIET	TROUT DIET
5	9.7	3.5	5.5
10	13.4	6.7	10.6
20	14.9	8.1	13.8
40	15.0	10.3	16.0
80	18.7	12.9	18.6

In the manufacture of commercial diets, ingredients used are dependent upon the price and the availability of that specific commodity. Thus, even though recommended nutritional composition values are maintained (such as energy, protein, vitamins, and lipid levels), the composition of the various feed batches is not constant. However, the composition of different feed ingredients affect the overall texture and the ability to extrude the diet, (N. du Plessis, QUEENS FEED, pers. comm.). As a consequence the rate of leaching of a specific diet can be different from another even though they both share the same nutritional parameters. Therefore diets should all be tested individually for leaching.

CHAPTER 5: GASTRIC EVACUATION TIME (GET).

INTRODUCTION

As stripping was found to be the most suitable method for the collection of faecal matter from the African catfish, the next important step was to determine the gastric evacuation time (GET). GET is the time taken for the food ingested to move through the gastrointestinal tract of the fish (Smith 1989). The aim of this experiment was to ascertain the evacuation time in order to know when stripping should take place. This should be done when the hind-gut is at its fullest point prior to the contents being excreted, therefore yielding faecal material which had been digested to its maximum. This would then ensure that the sample of faeces obtained from the fish would reflect accurate digestibility coefficients for the specific ingredient being tested.

Gastric evacuation time (GET), is a widely discussed subject in digestive function (Smith 1989). The general rule for GET is that while small meals are processed in a shorter period of time than larger meals, the initial rate of digestion is greater for larger meals than for small meals (Jobling *et al.* 1977). Several factors, however, affect GET. These are the size of the meal, the size of the fish (Basimi and Grove 1985), and temperature (Jobling and Davies 1979; Hershey and McDonald 1985).

GER (gastric evacuation rate), a term commonly used with GET, is the rate at which digested material is evacuated from the fish. GET is the time it takes for the fish to evacuate a particular meal (Smith 1989). As the aim of this experiment was to determine the time at which the hind-gut contained most digested feed, GET was determined.

MATERIALS AND METHODS

Two approaches were used in determining gut evacuation time for the African catfish. The first involved feeding the fish with a meal containing 5% barium sulphate. The fish were then x-rayed periodically to determine the progress of the food through the gut as shown by the position of the indicator. Ross and Jauncey (1981), undertook a similar study on *Tilapia*, although they worked with a diet containing 25% barium sulphate. The reason for using a 5 % barium sulphate content in the meal in this experiment was because a dilution of the caloric value of the food might affect the GET (Spannhof and Plantikow 1983). Other indicators which may be used to mark the feed to show up on the x-ray are iron powder (Talbot and Higgins 1983) and radioisotopes such as ^{144}Ce (Peters and Hoss 1974).

The barium sulphate marker method did not prove successful, for reasons provided in the discussion. A second approach therefore had to be employed. This involved feeding the fish a diet stained with a non-nutritive dye, after which individuals were sacrificed periodically. This method was not initially considered as it involves sacrificing the fish. However it does produce accurate results. Details of these methods are discussed below.

Barium Sulphate Trial

A commercial catfish pellet (40 % protein) was made up with barium sulphate added at 5 % by weight. The barium salt, of a quality used for human patients, was obtained from the local hospital. Barium sulphate is a chemical which is not penetrated by x-rays, so it appears as a white patch on an x-ray film (Figure 6.2). Initially, four samples of feed were made with the barium at five, 10, 15 and 20 per cent concentrations. All samples showed up well on the x-ray. As a result the lowest concentration (5 %) was used for the experiment.

Four catfish of an average weight of 205 g were fed the diet containing barium sulphate for a period of one week to acclimate them to the diet. The fish were fed twice daily, in the morning and evening, at a ration of 3.5% body weight per day. At the end of the one week acclimatization period, if the fish were still in good condition and were not stressed by the barium meals (which they where not), the x-ray treatment could begin. The fish were fed a barium meal on the morning of the x-ray treatment and then transported to a nearby veterinary clinic were the x-ray equipment was housed. The catfish were then placed on a photographic plate and exposed to a low x-ray dosage. This was repeated every half hour. Between x-ray exposures the fish were placed in a holding tank. On the third x-ray, it was noticed on the film that one of the fish had regurgitated the meal containing the barium sulphate meal. The experiment was terminated as it appeared that the fish were under stress and that this might affect the rate of evacuation of the feed through the gastrointestinal tract. A week later the experiment was attempted again with a new sample of fish. This time the fish were x-rayed hourly, so as to reduce handling time and thus reduce stress. But again the fish showed signs of stress, with regurgitation of the feed. It is thought that the transportation of the fish from the laboratory to the veterinary clinic could have contributed to the fish being stressed. An alternative method had to be used.

Staining of the Food

As the barium sulphate method had failed, another approach was taken to determine the evacuation time of food through the gastrointestinal tract of the fish. In this experiment, twelve catfish of an average weight of 82 g were used. The fish were acclimated for a week by leaving them in their tanks and by not handling them before any experimental work began. The fish were fed on a commercial catfish pellet (40 % protein) at 4 % body weight per day.

However, some of the pellet diet was stained using a green, non-nutritive food colouring dye. This "coloured" feed was fed to the fish only on the day the experiment commenced. After feeding the fish the coloured diet, one of the fish would be randomly selected and sacrificed every hour for a period of twelve hours. An incision was made on the abdominal side from the anus anteriorly, so as to remove the gastrointestinal tract. The contents of the stomach, fore-gut, mid-gut, and hind-gut were separately removed and weighed. The green colouring made it easy to visually identify the experimental feed in the intestine from any residual feed from previous feeding (which was brown in colour). The contents of the gastrointestinal tract were then expressed as percentages of total faecal matter in the gastrointestinal tract.

RESULTS AND DISCUSSION

The highest percentage of faecal matter in the hind-gut, which is what this experiment was trying to determine, was found to be 10 hours after feeding (Table 5.1 and Figure 5.1). According to Uys (1989), in a similar study, the largest concentration of faecal material in the hind-gut was between 6.5 to 10 hours after feeding. This discrepancy could be explained by the fact that Uys (1989) had starved the fish for 24 hours prior to the experiment, and then taken readings of gut content at 1 hour, 2.5 hours, 4 hours, 6.5 hours and 10 hours after feeding.

The apparent increase in percentage gut content in the stomach, fore-gut, and hind-gut (Table 5.1) at 11 hours after feeding is a relative increase, as a consequence of the percentage gut content in the hind-gut decreasing as the fish excreted.

Table 5.1: The contents (expressed as percentages) of various sections of the gastrointestinal tract of African catfish over a 12 hour period.

TIME (HOURS)	STOMACH CONTENTS (%)	FORE-GUT CONTENTS (%)	MID-GUT CONTENTS (%)	HIND-GUT CONTENTS (%)
1	86.3	13.7	0	0
2	65.3	34.7	0	0
3	44.1	55.9	0	0
4	24.6	70.1	5.3	0
5	20.1	56.0	13.6	10.3
6	16.3	34.2	19.8	29.6
7	8.3	17.4	25.6	48.7
8	5.1	6.1	27.9	60.9
9	1.9	4.2	18.1	75.8
10	0.7	3.5	14.6	89.6
11	10.7	35.5	38.2	15.6
12	8.7	23.5	40.9	26.9

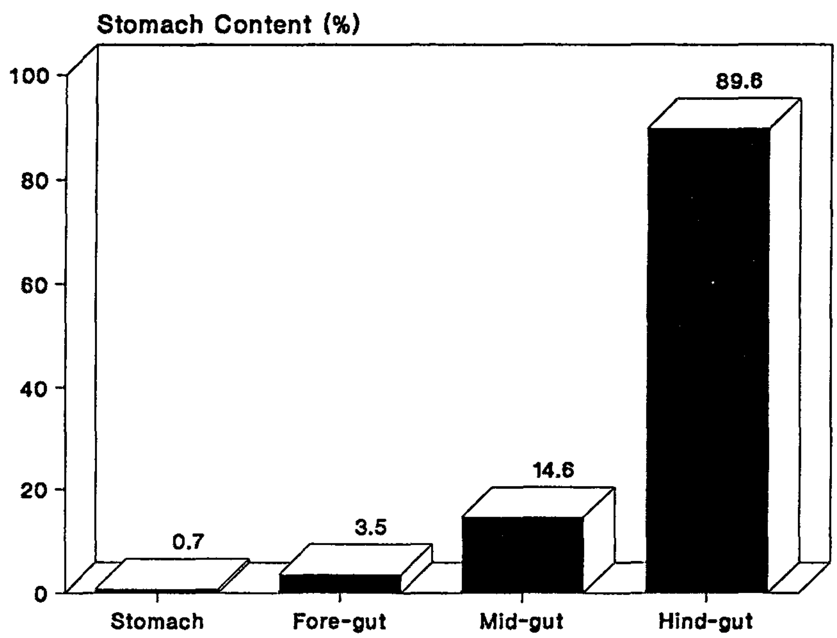

Figure 5.1: The distribution of digested feed in the gastrointestinal tract of the African catfish after 10 hours.

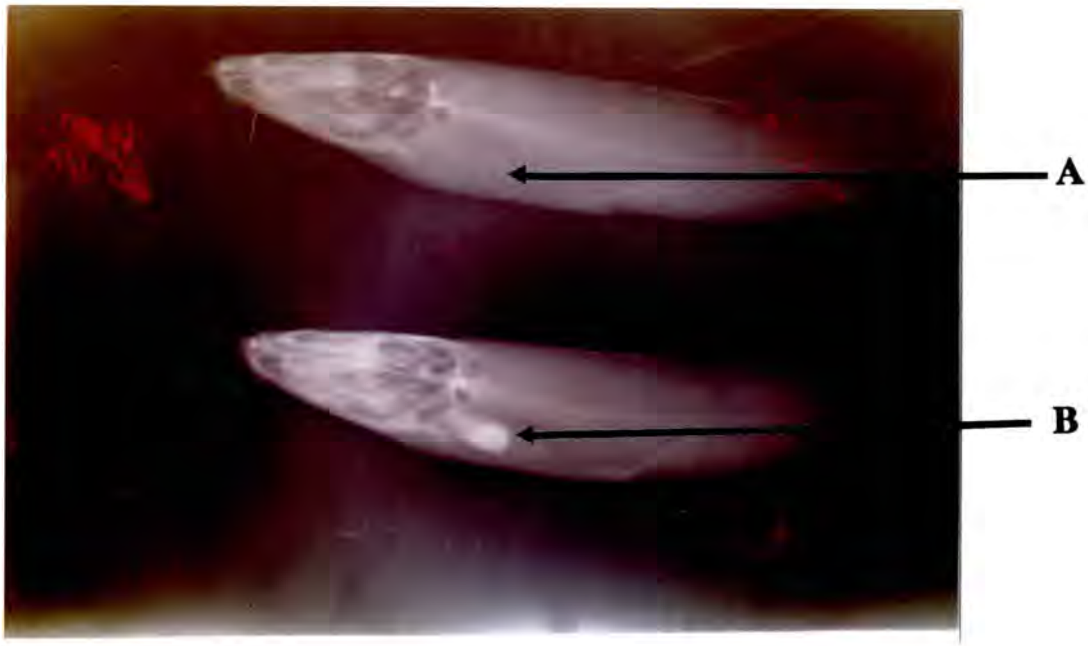

Figure 5.2: Photograph of x-ray showing a catfish with feed containing barium sulphate in the stomach (B), and a catfish that has regurgitated its food (A).

Even though African catfish display minimal stress after handling (Hecht and Britz 1990), the transfer of the fish from the laboratory tanks to the veterinary clinic tank, together with the actual physical handling of the fish is thought to have caused the stress in the fish. As a consequence the fish regurgitated their food, a typical behaviour exhibited when stressed (pers. obs.). For this reason an alternative method for determining gastric evacuation time had to be sought and applied, with successful results.

There are many factors that influence GET (Jobling *et al* 1977, 1980, 1981; Basimi and Grove 1985). Some of these being meal size and water temperature. However, as all of these experiments were conducted at a constant temperature (28$\pm$1°C), and all the fish were fed according to the recommended amount per day (Chapter 2), these variables were neutralised.

GET results are not interchangeable between different species of fish as gut length, diet, water temperature and meal size, among other factors, affects GET (Smith 1989), therefore results of this study could only be compared to work done on the African catfish. Uys (1989), measured gut content of African catfish at 1, 2.5, 4, 6.5 and 10 hours after feeding, and found it to be highest between 6.5 to 10 hours after feeding (under culture conditions).

CHAPTER 6: DIFFERENTIAL DIGESTION ALONG THE GASTROINTESTINAL TRACT.

INTRODUCTION

In the evaluation of animal feeds the calculation of digestibility coefficients of component nutrients is necessary (Schimitz *et al.* 1983; Kiaerskou 1991). A quick and simple method to obtain digestibility coefficients is desirable. However, the aquatic environment makes the measurement of feed intake and faecal matter output difficult for fish (Schimitz *et al.* 1983). Methods of collecting faecal matter are also varied (as discussed in Chapter 3) as one method may prove successful with a certain species but not with another. Stripping, a process of removing faeces by applying slight pressure with the fingers between the ventral fins and anus can be done on salmonids (Inaba *et al.* 1963; Singh and Nose 1967; Austreng 1978; Windell *et al.* 1978), but cause eels to refuse to take further feed (Kuhne 1973). African catfish seemed unaffected when stripped for faecal matter (pers. obs.) and showed 'normal' activity immediately after being released into the tank. As the stripping method was chosen to obtain faeces from the African catfish in this study, it had to be known wether the contents of the hind-gut was the best representation of the digested feed.

Phillips *et al.* (1948) and Austreng (1978), force-fed trout and at various intervals sacrificed some fish, whereupon the contents of the gastrointestinal tracts were analyzed for digestibility. This method provided reliable results of digestibility of nutrients as leaching was kept to a minimum. However, as this method requires the fish to be killed to obtain the contents of the gastrointestinal tract, the usefulness of the method is limited (Austreng 1978).

Chromic oxide (Cr_2O_3), has been used as an inert indicator ever since it was proposed by

Edin in 1918. Most nutrition studies have continued to use chromic oxide (Nehring 1963; Cho *et al.* 1974, 1976; Lall and Bishop 1976; Austreng 1978; Hanley 1987; Matty 1988; Spyridakis *et al.* 1989; Degani and Revach 1991) and it was used in this study. Digestibility of the feed is measured by adding a known quantity of the indicator, chromic oxide, to the feed. All of the indicator is eaten by the fish and passes through the gastrointestinal tract appearing in the faeces. The relative change in chromic oxide percentage in the faeces compared to that in the feed represents the percentage of food digested by the fish. The formula (Jobling 1983) used to calculate digestibility is discussed in Chapter 3.

MATERIALS AND METHODS

Five catfish of average weight 250 g were kept in glass tanks measuring 30 x 60 x 90 cm in a recirculating system at 28°C. The water quality and condition are described in Chapter 2. A commercial diet of 40 % protein, 10 % lipid, 14 KJ/g digestible energy and 26mg protein per KJ of digestible energy was fed to the catfish twice daily at a ration of 3.5% body weight. The method in which chromic oxide was added to the pulverised feed is discussed in Chapter 3 (see section on materials and methods). In order to produce pellets from the pulverised feed, water was added to obtain a 'putty' like texture. The 'putty' was then passed through a meat mincer to obtain strands of feed which were dried in a drying oven overnight at 40°C. When the feed had dried the strands were broken manually into smaller, pellet-like pieces.

Ten hours after feeding the fish were killed as it had been previously determined (Chapter 5) that this was when the hind-gut was most full and when the highest digestibility values could be obtained. The fish were killed by partial decapitation through the vertebral column.

The abdomen was opened by cutting from the anus in an anterior direction in order to remove the gastrointestinal tract. (The method for distinguishing between the different segments of the gastrointestinal tract has been described in Chapter 3.) Each of the four segments was isolated by cutting through the gastrointestinal tract to give four sections. The contents of each were then collected and placed in a sterile vial and dried in a drying oven (40 °C) until constant weight was achieved.

In order to establish the calorific value (CV) of the contents of the various sections of the gastrointestinal tract, a bomb calorimeter was used (see Chapter 2). Chrome oxide assays were performed in the manner described in Chapter 2. The extent of digestion of the contents of each segment of the gastrointestinal tract was determined using the equation presented in Chapter 3.

RESULTS AND DISCUSSION

Ten hours after feeding the fish, the digestibility coefficients in the various sections of the gastrointestinal tract were as follows: stomach 22.1 %, fore-gut 53.1 %, mid-gut 77.2 % and 81.3 % in the hind-gut (Table 6.1 & Figure 6.1).

Table 6.1: The percent coefficient of digestion in different sections of the gastrointestinal tract.

SAMPLING SITE	DE %	RANGE
Hind-gut	81.3	±2.3
Mid-gut	77.2	±1.4
Fore-gut	53.1	±1.8
Stomach	22.1	±2.2

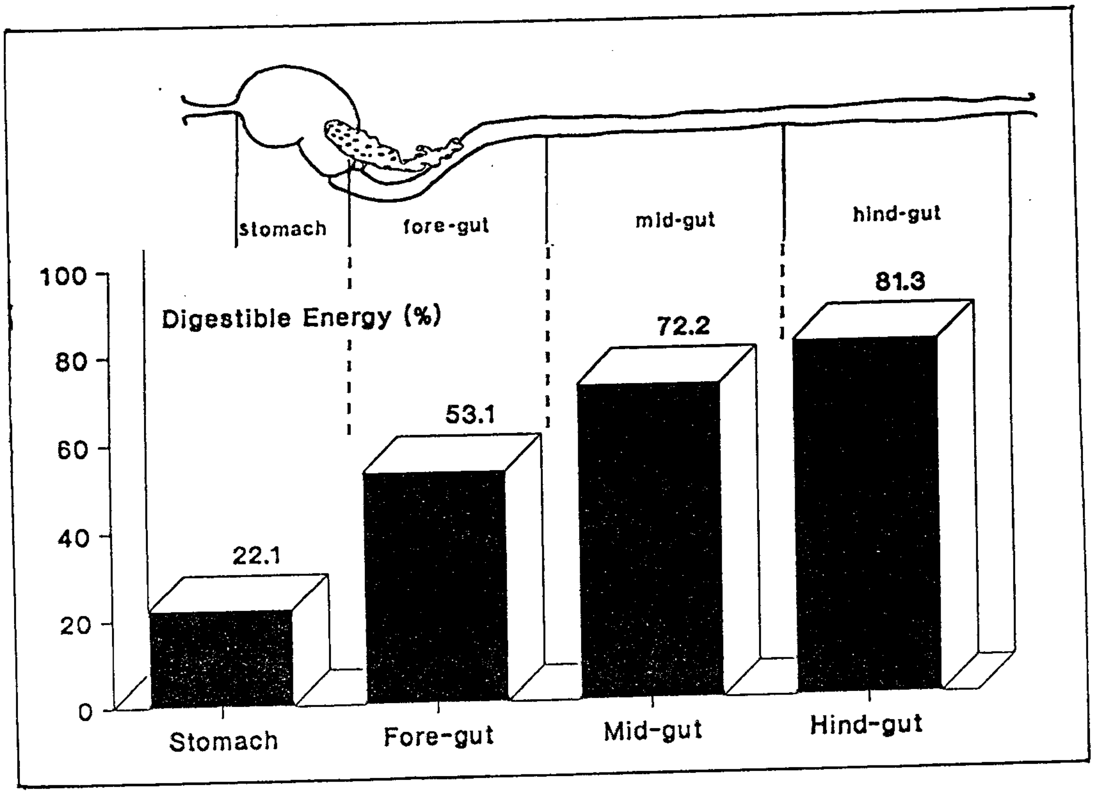

Figure 6.1: The coefficient of digestion in different sections of the gastrointestinal tract 10 hours after feeding.

In digestibility studies, it is advisable to take faecal samples from the gastrointestinal tract as close to the anus as possible (Austreng 1978). The results of this study confirm this, as the highest value for digestibility, occurred in the hind-gut (81.3 %). It was shown in Chapter 5, that the highest concentration of faeces in the hind-gut was after 10 hours. Therefore, by removing faecal material from the hind-gut after ten hours one should arrive at the most accurate DE values for the African catfish.

When collecting faecal matter from fish using the stripping method, it is thought that a significant contamination by urine occurs in the samples (Austreng 1978) and such contamination would invadvertently bias results. However, Forester and Goldstein (1969) established that only a small fraction of the total nitrogen excreted by the fish appears in the urine. Smith (1989), supplemented this by noting that about 80% of non-faecal waste nitrogen was excreted as ammonia through the gills of the fish. Therefore, Austreng (1978) suggested that in experiments designed to help in the practical evaluation of feed ingredients, the theoretical source of error of contamination of faeces samples with urine can probably be ignored.

Austreng (1978) conducted an analogous experiment on rainbow trout, *Oncorhynchus mykiss*. Some of his results are presented and discussed in relation to the present results. Austreng (1978), divided the gastrointestinal tract into the stomach (I), the anterior half of the small intestine including caeca(II), the posterior half of the small intestine(III), the anterior half of the rectum (IV) and the posterior half of the rectum(V). His results (Table 6.2) showed that digestibility coefficients increase with the passage of the experimental diet through the gastrointestinal tract.

Table 6.2: Digestibility coefficients (in percent) at different sections of the intestinal tract for the rainbow trout (from Austreng 1978).

Sampling site	Protein	Fat	Starch	Ash	Energy
I	16.5	8.5	0.4	18.7	12.7
II	47.8	47.8	2.6	16.6	34.4
III	72.2	75.6	14.4	23.1	57.8
IV	83.5	85.9	20.9	33.3	67.3
V	84.5	89.9	25.9	37.4	71.3

Higher digestibility coefficients were obtained from samples from the posterior end of the gastrointestinal tract (Tables 6.1 and 6.2). This confirms that in digestibility studies such as these, it is advisable to obtain faecal samples from as close as possible to the anus. Although for protein digestion, Austreng (1978) showed that the difference between the hind-gut and fore part of the hind-gut is only 1 %.

CHAPTER 7: DIGESTIBLE ENERGY DETERMINATION, FEED FORMULATION AND COMPARATIVE GROWTH TRIALS.

INTRODUCTION

Two objectives were pursued in this part of the study. Firstly, to establish the digestible energy (DE) of individual feed ingredients used in the commercial manufacture of pelleted fish feeds. The second objective was to formulate test diets by means of a linear based computer programme incorporating the DE values (Table 7.1) and to test the efficiency of these diets in relation to a standard reference diet (Table 7.11) and a commercial African catfish feed (Table 7.10).

To distinguish between the two experiments and procedures discussed within this chapter, the first part concerned with obtaining DE values will be referred to as Experiment A, while the second part, dealing with the formulation and testing of the feeds, will be called Experiment B.

The feed ingredients tested for their DE values consisted of conventional ingredients used in the commercial manufacture of extruded fish diets (N. du Plessis, pers. comm.), and were provided by a local fish feed factory (QUEENSFEED, Queenstown, South Africa) as well as some unconventional ingredients.

EXPERIMENT A:

MATERIALS AND METHODS

Thirty adult fish of an average weight of 305g each were used for determining DE values. Large fish were chosen for this experiment as the fish had to be force-fed, and larger fish made for easier handling with relatively little effect on the fish. The fish were maintained at 5 individuals per tank (30 x 60 x 90 cm).

Each of the dietary ingredients to be tested were first pulverised, whereupon the chromic oxide was added at 2 % of ingredient weight (as described in Chapter 2). The feed was then reconstituted into a putty-like consistency. This was necessary as the fish had to be force-fed. Force-feeding was undertaken as many of the ingredients (particulary those of plant origin) were not at all palatable to the fish. Coating these non-palatable feed ingredients with cod liver oil or monosodium glutamate (a non-nutritive food flavourant), in an attempt to make the feed more attractive, was to no avail. Therefore the fish had to be force-fed. Force-feeding was achieved by means of a silicone gun (5 cm in diameter and 20cm in length) with a nozzle on the end joined to a 30 cm soft rubber tube (translucent) marked off in centimetres. This tube was gently guided down the throat of the fish and the trigger on the gun was squeezed, extruding the putty-like feed into the oesophagus. In order to ensure that the right amount of feed (3.1 % body weight per day on a dry weight basis) was being fed to the fish, prior to feeding, a sample of feed was squeezed from the tube and weighed. This same sample was read off the tube, correlating centimetres with grams on a dry weight basis. Thus when feeding the fish, the required amount was extruded from the gun. If the addition of water to the diet (to make it into a "putty") was not compensated for, the fish would recieve less of the ingredient being tested in the diet as the ration was based on dry weight, but meal size would remain constant. However, if the additional water was compensated for in the diet, it would mean that the amount of ingredient being tested would correspond to the recommended feeding ration, but meal size would increase on account of the water being added. As meal size plays a very important factor in gastric evacuation time (Smith 1989), it was decided to keep it constant throughout this study. The fish were force-fed for three days on the ingredient before any faecal sample was stripped from them. The faeces collected was dried in a drying oven at 40 °C until constant weight was reached, then tested for CV and digestible energy using the methods discussed in Chapter 2. Sufficient faeces was collected to ensure three separate DE determinations for each sample. Those ingredients which were highly palatable to the fish, for example fish and carcass meal, were also force-fed to the fish in order to ensure experimental compatibility between results.

The feed ingredients used in this experiment were all of commercial grade and were supplied by a commercial feeds factory. The names of the feed ingredients used in this study correspond to the recognised terminology used in feed manufacturing. The different grades of fish meal used (Table 7.1) depend primarily on the type of fish used to make the diet and the general status (freshness and handling) of the meal. Hominy chop, an ingredient tested for DE (Table 7.1), is a mixture of molasses and various plant ingredients (for example maize, wheat, sunflower) mixed at various concentrations depending on ingredient availability and cost (N. du Plessis, pers. comm.)

RESULTS

The results of the CV and pertinent DE values of the various ingredients tested are presented in Table 7.1. There was little or no relationship between DE values for specific ingredients and their calorific values (CV). The calorific value is the total gross energy of the sample. Digestibility is not only influenced by the CV, but by many factors, including protein, fat and amino acid composition. This would explain the poor relationship (r^2 = 18.3%) between CV and digestibility. Examples are carcass meal and brewers yeast grain. Although carcass meal had a relatively low CV of 14.431 KJ/g, the DE was high at 80.4 %. On the other hand, brewers yeast grain had a CV of 19.255 KJ/g, but only a 65.3 % DE value.

Table 7.1: Calorific value (CV) and digestible energy (DE) of ingredients tested for the African catfish (These results were not corrected for leaching as the diets were force-fed and faecal matter stripped).

FEED SAMPLE	CV KJ/g (±Range)	DE % (±Range)
PRIME GLUTEN	23.611±0.8	78.3±0.5
FULL FAT SOYA (COOKED)	23.259±0.7	78.3±0.4
AFRICAN CATFISH MEAL	21.290±0.6	81.2±0.9
WHITE FISH MEAL	20.320±0.6	80.0±0.8
FISH MEAL (38% PROT.) (GRADE I)	19.955±0.8	82.3±0.6
FISH MEAL (38% PROT.) (GRADE II)	19.480±0.9	80.1±0.4
BREWERS YEAST GRAIN	19.255±0.6	65.3±0.8
IMPORTED FISH MEAL (CHILE)	18.686±0.4	78.3±0.5
GERM MEAL (YELLOW MAIZE)	18.488±0.4	63.2±0.6
FISH MEAL (34 % PROTEIN)	18.482±0.7	79.9±0.9
SOYA FLOUR (COOKED)	18.264±0.8	75.8±0.6
SUNFLOWER OIL SEED CAKE	17.800±0.7	63.8±0.4
SIFTED WHITE MAIZE MEAL	17.791±0.8	85.4±0.8
GEOTRICHUM (MYCOPROTEIN)	17.731±0.5	78.8±0.8
WHEAT (COOKED)	17.608±0.7	80.2±0.5
SUPER MAIZE MEAL (WHITE)	17.521±0.4	79.5±0.3
FISH MEAL (45 % PROTEIN)	17.511±0.6	80.4±0.8
GRAIN SORGHUM	17.443±0.8	74.3±0.9
COTTON OIL SEED CAKE	17.055±0.7	73.6±0.9
WHEATEN BRAN	17.014±0.7	76.2±0.7
SPIRULINA (SINGLE CELL ALGAE)	16.989±0.5	81.3±0.7
HOMINY CHOP	16.743±0.5	61.3±0.9
YELLOW MAIZE MEAL	16.236±0.7	75.8±0.8
SIFTED YELLOW MAIZE MEAL	16.067±0.5	73.8±0.6
DEHYDRATED LUCERNE MEAL	15.311±0.7	45.8±0.8
CARCASS MEAL	14.431±0.9	80.4±0.9

EXPERIMENT B:

MATERIALS AND METHODS

After determining the DE values of all the feed ingredients, a linear computer programme (used by the feed company that provided the feeds) was used to formulate test feeds using DE as a criterion, amongst other variables used in the programme (which could be altered for individual diets). The linear programme was instructed to formulate diets having not less than a 75% DE, which is an acceptable value (NRC 1983). The programme did not only use ingredients which this study had tested for DE when formulating test diets, but also other ingredients to enable the test diet to maintain other nutritive parametrs which may have been set (for example protein and total lipids). As such the level of minerals or vitamins in the diet did not always remain constant between different test diets, but alwats kept above the minimum requirements for warm water fish (NRC 1983).

Based on the recommendations of N. du Plessis (a commercial feed manufacturer), only seven of these diets were tested as the others were either too expensive to produce, or were not possible to produce as extruded diets. As the linear programme used for diet formulation was based on extruded values, the test diets also had to be made up as extruded diets. As only 3 Kg of each diet was required for testing, it was too small an amount to be made up by a commercial feed factory (the minimum produced for any specific diet is 1 ton). The experimental diets therefore had to be prepared in the laboratory. To make the extruded diets in the laboratory, the ingredients for each test diet were mixed with water at a ratio of one part water to 20 parts feed and then cooked in a pressure cooker at between 120 - 130°C for five minutes. The feed was then passed through a domestic meat mincer (when still hot), then sun dried and broken up manually into small pieces of about 3-4 mm in length. This method of crude laboratory-scale extrusion was recommended by A.G. McEwan (SPECIALIST ANIMAL FEEDS, pers Comm.).

The test fish used for the comparative growth trials had an average weight of 71.9g. Smaller fish (than those used in Experiment A) were used, as they have faster growth rates. The fish were size graded every two weeks for a period of 2 months prior to commencement of the experiment to achieve uniformity in fish size. Such size grading also greatly reduces the threat of cannibalism during the experimental period. These fish were maintained in 9 tanks (30 x 30 x 70 cm). Each tank was divided into two compartments by means of a plastic gauze (2mm) divider (that allowed easy mixing of water between the compartments) to allow for exact replication. In each compartment 15 fish were kept.

The experimental diets were fed to the fish twice a day at a ration of 4 % body weight per day (%bwt/day), for 28 days. At the start and termination of the experiment, the fish were individually weighed and measured. The %bwt/day feed ratio was determined from the feeding chart (see glossary). After 14 days of feeding 5 fish from each tank were weighed to determine whether a new feeding regime should be implemented. The fish used in this experiment were "trained" to accept the diet presented to them by tapping on the glass tank. They would therefore usually consume the food within the first 10 seconds of the pellet hitting the water, so leaching was negligible within this period (0.5% loss of CV after 7.5 seconds immersion in water see Chapter 3). As the criterion used in evaluating the performances for the test diets was based on SGR, which does not encompass specific nutritional values, but rather growth of the fish, the minimal leaching effects on the feed did not have to be compensated for.

The Specific Growth Rate (SGR) of the fish within the various experimental groups was calculated for all test diets, and Analysis of Variance (ANOVA), was used to determine statistically the differences in diet performance.

Two control diets were used in this study. Firstly, a standard reference diet (H-440), was used to compare the findings of this work with other work done on the African catfish (providing H-440 was used). Secondly, a commercial pellet was also used to compare the experimental diets with already established feeds, and to see if any improvement could be passed on to the commercial sector.

Diet No. 8 which is the commercial diet does not have any ingredients listed for it (Table 7.10), as ingredients vary according to cost and availability. However, the diet's nutritional levels are recorded. All the experimental formulated diets which used fish meal was that of 45% protein.

RESULTS

All the diets formulated (Tables 7.3-7.11), except Diets No. 8 and 9 were produced by the above method.

Table 7.2: Constraints (minimum value) used by the linear programme to formulate commercial feeds.

Fibre	3.4%	Calcium	1.22%
Water	10%	Protein	34%
Total Phosphorous	1.1%	Lysine	2.0%
Available Phosphorous	0.88%	Methionine	1.0%
Fat	4.3%	Digestible Energy	75%
Calorific value	17KJ/g	Tryptophan	0.39%
Methionine + cystine	1.2%	Arginine	2.2%
Threonine	1.0%	Sodium	0.29%
Potassium	0.78%	Cost (per ton)	R1100

Table 7.3: DIET 1.

Yellow maize.............	27.51	Fibre.........	3.766
Wheat midlings...........	10.00	Calcium.....	1.221
Sunflower oil seed cake..	27.80	Meth+Cystin..	1.462
Lime.....................	1.20	Protein......	39.018
Methionine...............	0.14	Total Phos...	2.446
Vitamin premix...........	3.00	Methionine...	1.036
Prime gluten.............	12.50	Fat..........	4.303
Spirulina................	20.00	Lysine.......	2.352
TOTAL....................	99.54	Avl. Phos....	2.017
		Tryptophan...	0.509
		Arginine.....	2.760
		Threonine....	1.754
		Sodium.......	1.004
		Potassium....	3.910
		DE%.........	75.90
		CV KJ/g.....	17.20

Table 7.4: DIET 2.

Yellow maize.............	30.21	Fibre	4.000
Wheat midlings..........	12.64	Calcium......	1.600
Fish meal................	15.00	Protein......	34.000
Sunflower oil seed cake..	20.63	Total Phos...	1.205
Lime.....................	0.97	Lysine.......	2.126
Methionine...............	0.37	Avl.Phos.....	0.911
Carcass meal.............	6.53	Calcium......	1.610
Vitamin premix...........	0.30	Methionine...	1.000
Prime gluten.............	3.34	Fat..........	4.458
Brewery yeast............	10.00	DE%..........	75.50
TOTAL....................	100.00	CV KJ/g......	17.20

Table 7.5: DIET 3.

Yellow maize............	24.38	Fibre........	4.000
Wheat midlings..........	3.82	Calcium......	1.600
Sunflower oil seed cake..	38.76	Protein......	38.000
Lime....................	0.75	Total Phos...	1.190
Methionine..............	3.80	Lysine.......	2.332
Vitamin premix..........	0.30	Avl. Phos....	0.886
Prime gluten............	11.96	Methionine..	1.000
Brewery yeast...........	10.00	Fat.........	4.310
TOTAL...................	100.00	DE%.........	75.50
		CV KJ/g.....	17.20

Table 7.6: DIET 4.

Yellow maize............	21.49	Fibre........	4.000
Wheat midlings..........	7.42	Calcium......	1.402
Sunflower oil seed cake..	34.44	Protein......	39.737
Lime....................	1.14	Total Phos...	1.837
Methionine..............	0.24	Lysine.......	2.426
Carcass meal............	5.00	Avl.Phos.....	1.447
Vitamin premix..........	0.30	Methionine...	1.000
Prime gluten............	10.00	Fat..........	4.408
Brewery yeast...........	10.00	Sodium.......	0.585
Spirulina...............	10.00	Potassium....	2.625
TOTAL...................	100.00	Meth+Cystine.	1.451
		Tryptophan...	0.469
		Arginine.....	2.627
		Threonine....	1.640
		DE %..........	75.60
		CV KJ/g........	17.20

Table 7.7: DIET 5.

Yellow maize............	21.21	Fibre........	4.000
Wheat midlings..........	12.64	Calcium......	1.600
Fish meal...............	10.00	Protein......	34.000
Sunflower oil seed cake..	20.63	Total phos...	1.202
Lime....................	0.97	Lysine.......	2.126
Methionine..............	0.37	Avl.Phos.....	0.911
Carcass meal............	6.53	Methionine...	1.000
Vitamin premix..........	0.30	Fat..........	4.457
Prime gluten............	8.34	DE%..........	75.50
Brewery yeast...........	10.00	CV KJ/g......	17.20
TOTAL...................	100.00		

Table 7.8: DIET 6.			
Yellow maize.............	31.47	Fibre........	4.000
Wheat germ...............	0.65	Calcium......	1.390
Wheat midlings..........	9.47	Protein......	34.000
Sunflower oil seed cake..	25.31	Total Phos...	1.136
Lime.....................	0.64	Lysine.......	2.095
Methionine...............	0.40	Avl. phos....	1.134
Vitamin premix...........	0.30	Methionine...	1.000
Prime gluten.............	15.00	Fat..........	4.319
Brewery yeast............	10.00	DE%..........	75.50
Carcass meal.............	6.72	CV KJ/g......	17.20
TOTAL....................	100.00		

Table 7.9: DIET 7.			
Yellow maize............	35.95	Fibre........	3.495
Wheat midlings.........	10.00	Calcium......	1.408
Fish meal...............	5.00	Protein......	35.081
Sunflower oil seed cake	15.50	Total.Phos...	1.893
Lime....................	0.53	Lysine.......	2.137
Methionine..............	0.23	Avl.Phos.....	1.569
Carcass meal............	5.00	Methionine...	1.000
Vitamin premix..........	0.30	Fat..........	4.861
Prime gluten............	12.50	Sodium.......	0.640
Brewery yeast...........	5.00	Potassium....	2.312
Spirulina...............	10.00	Meth+cystin..	1.376
TOTAL...................	100.00	Tryptophan...	0.395
		Arginine.....	2.244
		Threonine....	1.154
		DE %.........	76.475
		CV KJ/g......	17.200

Table 7.10: DIET 8. (Commercial pellet)

Nutrients	%
Protein............	42.0
Lipids.............	10.0
DE (KJ/g)..........	15.0

Table 7.11: DIET 9. Standard Reference Diet H-440 (Castell and Tiews 1980).

Ingredients	%	Vitamin Mixture	%
Vitamin-free casein	12.7	a-cellulose	8.888
White dextrin	9.3	Choline chlorides	5.555
Gelatin	4.0	Inositol	2.222
Maize oil	2.0	L-Ascorbic acid	1.111
Cod liver oil	1.0	Nicotinic acid	0.833
Vitamin mixture	3.0	Ca-pantothenate	0.055
Mineral mixture	1.3	Riboflavin	0.222
Water	66.7	Thiamin-HCl	0.055
		Pyridoxine-HCl	0.055
TOTAL PERCENTAGE	100.0	Menadione	0.044
		Folic acid	0.016
		Vitamin B12	0.014
		Biotin	0.006
		a-Tocopherol acetate	0.444

Mineral mixture	(g)	USP XII No.2	
USP XII No.2	100.000	Calcium biphosphate	13.58
$AlCl_3.6H_2O$	0.015	Calcium lactate	32.70
$ZnSO_4.H_2O$	0.300	Ferric citrate	2.97
CuCl	0.010	Magnesium sulphate	13.20
$MnSO_4$	0.080	Sodium chloride	4.35
Ki	0.015	Sodium biphosphate	8.72
$COCl_2.6H_2O$	0.100	Potassium phosphate dibasic	3.98

The ANOVA statistical tests (at the 95% confidence level) showed that there was no significant difference in initial weight between the fish (P=0.0205), due to size grading prior to the experiment. Therefore, as all the fish shared the same environment and were stocked at similar densities, changes in fish weight or length during the duration of the growth trials can only be attributed to the effect of the different diets.

Table 7.12 Results of the 28 day feeding trial using nine experimental diets.

DIET	A	B	C	D	E	F	G	H
1	72.5	25.0	95.4	33.5	0.43	1314	39	4.3
2	73.6	25.8	91.6	30.4	0.34	1312	34	4.6
3	72.0	22.7	92.0	28.6.	0.37	1332	38	4.3
4	70.1	25.0	93.1	30.8	0.42	1290	39.7	4.0
5	71.9	24.2	87.7	29.2	0.31	1274	34	4.5
6	71.5	24.5	87.2	29.4	0.36	1236	34	4.3
7	71.6	25.8	91.6	32.7	0.38	1289	35	4.9
8	72.0	24.0	91.8	30.6	0.38	1292	42	10
9	72.6	23.2	82.9	27.0	0.22	1257	38	9

A: initial weight (g); B: initial length (cm); C: final weight (g); D: final length (cm); E: SGR; F: total feed presented to fish (g); G: % protein in diet; H: % fat in diet.

Statistically, there was no significant difference (at the 95% confidence level) in final weight results between the two replicates that were conducted for each of the test diets (P=0.9644). Therefore, the results for each of the replicate tanks could be pooled. All diets, except Diet No. 8 (commercial pellet) and Diet No. 9 (H-440), were formulated using DE energy as a criterion. Diet No. 9 had the lowest SGR although the protein content was 38 % (Figure 7.1). It was also shown to be statistically different to all the diets except Diet No. 5 (at the 95% confidence level). Diet No. 1 had the highest SGR (Figure 7.1) although it was in the same homogeneous group (Table 7.13) as Diets No. 2, 3, 4, 6, 7 and 8. Diets No. 2, 3, 5, 6, 7 and 8, can also be grouped together (Table 7.12).

Table 7.13: MULTIPLE RANGE ANALYSIS FOR SGR (AT 95% - SCHEFFE RANGE TEST)				
DIET	% PROTEIN	HOMOGENEOUS GROUP		
9	38	X		
5	34	X	X	
2	34		X	X
6	34		X	X
3	38		X	X
7	35		X	X
8	42		X	X
4	39			X
1	39			X

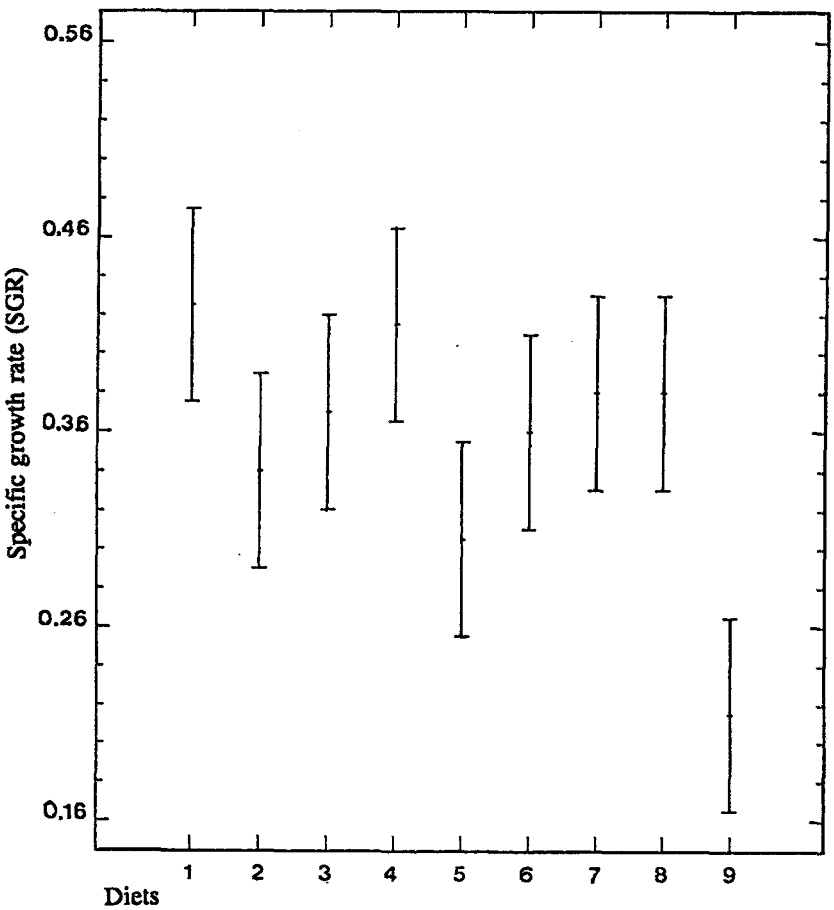

Figure 7.1: Graph showing ANOVA (95% Scheffe range test) results for SGR of the different diets tested.

On closer examination of the results, Diet No.1 which contained 39 % protein, performed better though not significantly better than Diet No. 8 (commercial pellet) which had 42 % protein. It is suggested therfore that using DE as a criterion for determining diet efficiency results in better cost effeciency in feed formulation. Diet No. 8 was within the same homogeneous group as Diets No. 2, 3, 5, 6 and 7 which contained 39 %, 38 %, 34%, 34 % and 35 % protein respectively (Table 7.13). Diet No. 5 was grouped homogeneously with Diet No. 9; this could be attributed to the fact that Diet No. 5 had 34 % protein. Although Diet No. 9 (H-440) had a relatively high protein content (38 %), the SGR was only 0.22. It is suggested that this low SGR could be due to the feed not being palatable to the fish (Chapter 1). Nevertheless, it was important to include the H-440 diet in the growth trials for comparative purposes.

DISCUSSION

These results can obviously only be used effectively when examined in relation to each other, not only in terms of DE values, but also in terms of cost. All the diets formulated in this study are cheaper to manufacture (by 5-10%) than the currently used commercial formulation. The actual prices of ingredients are not included as they are subject to change, and are considered confidential by the company that provided this information (N. du Plessis, QUEENSFEED, pers. comm.) The diet that was cheapest to produce was Diet No.6, which was 10% less expensive than the standard commercial catfish pellet. This diet (No.6) had a SGR of 0.36 compared to Diet No.8 (commercial diet) which had a very similar SGR of 0.38 (statistically both these diet are within the same homogeneous group, Table 7.13) This kind of saving by the feed factory could be passed on to the fish farmer while maintaining good fish growth.

No matter how good a diet may be nutritionally, if it is not palatable to the fish, then it is of no use. While it is relatively easy to design and manufacture a feed, one cannot be certain that the diet is palatable until it has been tested by feeding it to the fish (Pigott and Tucker 1989). Only then can one be sure of its effectiveness as a diet.

Uys (1989) compared various diet formulations, including the Standard Reference Diet H-440, using SGR as the means of comparison. His results showed that statistically there was no difference between his formulated diets and H-440. However Uys (1989) worked with fish of mean initial weight of 1.1, 8.0 and 17.2 grammes at a water temperature of 26°C.

CHAPTER 8: CONCLUSION.

It has become clear in this study that the use of DE as a criterion for the manufacture of diets for the African catfish is advantageous. This can be viewed from two perspectives. Firstly, from a biological or fish nutritionist's point of view, it is a way of enhancing growth of the fish within a given period of time (Jobling 1983; Wellborn and Tucker 1985; Brown and Robinson 1989; Uys 1989). Also, it means that water quality, a vital factor in intensive fish culture, is maintained as there would be less unabsorbed nutrients within the water column (Alsted 1991). Secondly, from a commercial farmer's point of view, DE values can be applied to formulate cheaper feeds. Overall the later consideration is the most important one, especially if it is considered that feed cost can amount to half the production expenditure of farmed fish.

However, in order to formulate a diet using digestible energy (DE) values , certain pertinent questions need to be answered. These include determining the best method for obtaining DE values (as in the direct or indirect method) for individual ingredients. If the indirect method has been chosen (as in this study), at what time should faecal sampling be taken, how should it be taken and does leaching pose a problem in maintaining the accuracy of results ? Digestible energy values for individual ingredients are obviously of no value when used singularly in diet manufacture. They have to be combined with other factors affecting diet production, such as ingredient cost, protein, lipid, fats and other factors mentioned in greater detail in Chapter 7.

There are many methods described in the literature concerning faecal collection for digestibility studies. These fall under two main methods: the direct and indirect methods

(Jobling 1983; Brown and Robinson 1989). The choice of a particular method over another depends primarily on the type of fish being studied. In this study, use of the indirect method using Cr_2O_3 as a marker proved to yield acceptable and accurate results.

Leaching of nutrients is a factor that needs to be determined and compensated for (Brown and Robinson 1989). As the majority of the work done in this study was conducted in conditions in which no leaching could occur (force-feeding and stripping of faecal material), it did not affect results. In the growth trials, where the fish were fed under normal conditions, leaching was negligible as the fish were "trained" to prehend the feed as the pellets hit the water.

Determining the gastric evacuation time (GET) for the African catfish was crucial for this study, as stripping was to be used to obtain faecal samples from the hind-gut. In using this method, one has to be certain that when stripping the fish for faeces, the sample must be a true representation of the digested material from the hind-gut. Therefore samples of gut content were measured from the stomach, fore-gut, mid-gut and hind-gut hourly over a period of 12 hours. This hourly sampling showed that the hind-gut was at its full capacity 10 hours after the fish were fed.

Moreover as fish were stripped to remove faecal samples for DE determination, it had to be established and confirmed that the hind-gut was the most suitable part of the gastrointestinal tract from which to remove faecal material. Therefore faeces were sampled 10 hours after feeding (the previously determined gastric evacuation time) from the stomach, fore-gut, mid-gut and the hind-gut. Results showed that DE was highest in the hind-gut, which meant that samples taken from that section would yield the most accurate DE values for the specific dietary ingredients being tested.

DE values of commonly used ingredients in the commercial manufacture of formulated diets were combined with other data pertinent to diet manufacture (for example, protein, fat, lipid and cost) by means of a linear computer programme. This produced formulated diets that conformed to the nutritional requirements of African catfish (Uys 1989). When these diets which were cheaper to produce were tested against a commercial pellet and a standard reference diet (H-440), using specific growth rate (SGR) as a measure of efficiency, they had a higher growth rate, suggesting that using DE for diet formulation can increase fish growth. The results also suggest that the total protein (40-42%) recommended for catfish diets (Uys 1989) can be reduced to 38% without any adverse effect on growth rates.

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GLOSSARY

Additives: Feed material such as colouring matter, flavours, hormones and medicants.

Calorie: The unit for measuring the energy in feeds.
1000 cal = 1 Kcal
1000 Kcal = 1 Mcal
1 cal = 4.184 joules (J)

Gram Calorie (gcal): The gram calorie is defined as 1.184 J. This amount of energy raises the temperature of 1 g of water from 16.5°C to 17.5° C. In practise, both the joule and the calorie are so small that nutritionists work with multiple units.

Condition factor (CF): CF= [100 x weight (g)] / $[\text{length (cm)}]^{3}$.

Crude fibre: The ash-free residue of food which remains after boiling for 30 minutes successively in 1.25 % sulphuric acid and in 1.25 % sodium hydroxide. With most foods, crude fibre represents some 90 to 95% cellulose, the remainder being hemicellulose, lignin and other plant cell wall constituents.

Crude protein: Refers to the protein component and all the nitrogen (N) in the feed. It is determined by multiplying the total N by 6.25; the latter represents the average N content in most foods.

Digestibility: Is that portion of a feed which is not excreted in the faeces and is assumed to be absorbed by the animal.

Digestible Energy: The portion of the gross food energy minus the faecal energy that has been apparently absorbed.

Digestible nutrient: The portion of the dietary nutrient which is digested and absorbed by the animal body. This usually refers to carbohydrates, fats and proteins.

Essential Amino Acids: These are the amino acids which are essential to the animal and which the animal itself cannot synthesize fast enough to meet the requirements. These include arginine, histidine, leucine, isoleucine, lysine, phenylalanine, threonine, tryptophan and valine.

Ether Extract: Also called crude fat. The material extractable with any anhydrous solvent, for example, petroleum spirit or diethyl ether. It contains neutral fats and all fat-soluble material.

Faecal Energy: is the gross energy in the faeces.

Feed efficiency: Refers to the ability with which animals can convert the feed consumed into growth.

Feed Conversion Ratio (FCR): The ratio between the weight of the food consumed and the weight gain of the fish.

Gross Energy: is the energy released as heat when an organic substance is completely oxidized to carbon dioxide and water. It is often referred to as "heat of combustion" and generally measured in an oxygen bomb calorimeter.

Intake: Refers to the amount of feed consumed or feed available for digestion. Intake is the result of either restricted or *ad libitum* feeding.

Joule (J): The joule is equal to 10^7 ergs; 1 erg is the amount of energy expended in acceleration of a mass of 1 g (gram) by 1 cm/s (centimetre per second). The international joule is defined as the energy liberated by one international ampere flowing through a resistance of one international ohm in 1 s.

Kilojoule (kJ): and kilocalories (kcal) are 10^3 times greater than the joule and the calorie, respectively.

Maintenance: State or energy equilibrium of an animal when there is no net gain or loss of energy in the body.

Meal: Describes the physical form of a feed that has been reduced to a particle size larger than that of flour.

Minerals: Are elements that have a metabolic role in the body. They include macro-minerals and microminerals; the latter include trace elements.

Net energy (NE): Is the amount of energy used for maintenance or for production, or both.

Mortality: percentage of fish death per unit time.

Non-essential amino acids: Are the amino acids which are not needed in the diet but which are essential to the animal, e.g. aspartic acid, alanine, cystine, glycine and proline.

Non-protein nitrogen (NPN): Compounds which are not true protein in nature but contain nitrogen and can be converted to protein by bacterial action, e.g. urea.

Nutrient balance: Condition which describes a diet that makes available various nutrients in the right amounts to fulfil the physiological needs of the animal, and which meets both maintenance and production requirements.

Pellets: Refer to the physical form in which a feed or combination of feeds are compacted by mechanical means. Different size pellets can be made depending on die diameter.

Pressed: Process of compaction by pressure or extraction under pressure.

Protein quality: Refers to the ratio of amino acids in the protein.

Protein supplement: Products which contain 20% or more protein from plant or animal origin.

Specific growth rate (SGR): weight increase as a percentage of body weight per day, (Ln W^t- Ln W^o)/t x 100, where Wo is initial body weight and Wt is body weight at time t.

Supplement: A feed, either alone or in combination with other feeds, which is used to increase the availability of nutrients and also performance of animals. Can be an energy, protein, mineral and /or vitamin supplement.

True protein: The portion of the protein source which is composed only of amino acids.

Recomended daily percent body weight (%bwt) feeding ration for the African catfish. These feeding values are based on body weight of the fish and water temperature (Table by W. Uys for South African Catfish Association):

TEMP °C	1g - 10g	10g - 25g	25g - 50g	50g - 100g	100g - 300g	300g - 8000g
16	1.0	0.6	0.4	0.3	0.2	0.2
18	3.0	1.6	1.0	0.8	0.6	0.5
20	5.0	3.0	2.0	1.5	1.2	1.0
22	6.8	4.5	3.0	2.4	2.0	1.7
24	8.1	6.0	4.0	3.0	2.5	2.2
26	9.5	6.6	5.1	3.6	3.2	2.8
28	10.0	7.0	5.5	4.0	3.5	3.1
30	9.8	6.8	5.3	3.7	3.2	2.9
32	9.5	6.5	5.0	3.5	3.0	2.8