# ASPECTS OF THE NUTRITIONAL PHYSIOLOGY AND DIETARY REQUIREMENTS OF JUVENILE AND ADULT SHARPTOOTH CATFISH, *CLARIAS GARIEPINUS* (PISCES: CLARIIDAE).

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CONTENTS

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## ABSTRACT

Past and current research on the biology and culture of the sharptooth catfish (Clarias gariepinus) has stimulated the development of its commercial production. At the inception of this project in 1985, it was decided that the development of an optimal dry feed was the most important step in developing a more complete culture technology for the species. The objectives of the project were, therefore, to investigate aspects of the nutritional physiology and dietary requirements of the species to provide the necessary information for the formulation of optimum economical feeds. A review of the natural feeding biology of the species provided valuable information with regard to its food preferences. From this information, and by estimating the nutritional composition of its preferred natural diet, it could be concluded that the animal requires a relatively high dietary protein content. This conclusion was substantiated by studies of the functional morphology of the digestive system (gross and fine structure). The characteristics of the digestive system and external anatomy were found to be typical of an opportunistic, omnivorous predator. The ontogenetic development of the digestive system is relatively fast, and enables the utilization of a variety of diets efficiently from an early stage. Since the ability of an animal to digest a given substance is predominantly dependent on the presence of appropriate enzymes the quantification and characterization of C. gariepinus digestive enzyme activities were investigated on an experimental basis. This work revealed that the animal has relatively high levels of digestive enzyme activities, and that its proteolytic enzyme activities correspond with those of other carnivorous fishes, while its starch digesting abilities correspond with those of specialized herbivores. Also, the high levels of lysozyme and alkaline phosphatase indicate an adaptation to detritivory, making this fish a truly opportunistic omnivore. Evidence was found to suggest that sharptooth catfish are able to re-absorb

secreted digestive enzymes in the posterior section of its intestinal tract. It was shown that digestive enzyme activities were induced by food intake, and that no inherent rhythmicity in digestive activities seemed to occur. The development of digestive enzyme systems in the early life history of this animal was found to be also extremely rapid (complete within 10-16 days after hatching). It was also shown that artificial dry feed stimulates higher levels of digestive enzyme activities in larvae and juveniles than natural food. Although increased cellulase activity was found in larvae and juveniles that were fed on live zooplankton, the relative contribution of exogenous prey enzymes to protein digestion could not be ascertained. Specific nutritional requirements were investigated by means of feeding trials. The results of these trials confirmed the above indications regarding the dietary requirements of sharptooth catfish. It was concluded that for optimal growth and production performance, this animal requires a dietary protein content of 40-42%, a dietary lipid content of 10-12% and that diets should contain 14-16kJ/g digestible energy. The optimum protein-to-energy ratio was found to be 26-29mg protein per kJ of digestible energy. Dietary requirements are evidently the same for juveniles and adults, except that younger fish require higher relative feeding levels. Finally, practical feed formulations were evaluated in terms of their economic feasibility. The results of these trials are presented along with recommendations on the formulation of commercial feeds for C. gariepinus. A computer program with which to implement least cost feed formulation by means of linear programming is provided.

# CHAPTER 1

# INTRODUCTION

Intensive commercial warmwater fish culture in Africa is characterized by a history of hope and endeavor rather than actual successes. Yet, indigenous African freshwater fishes such as the larger cichlid and clariid species possess favourable attributes for aquaculture (Van der Waal 1972; Clay, 1977; 1979; Hecht, 1981, 1982; Babiker, 1984; Safriel and Bruton, 1984; Huisman and Richter, 1987). In South Africa in particular, warmwater aquaculture is overshadowed by a well developed and efficient marine fishery (1987 catch by South African and Namibian fisheries totaling 1.4 million tons (Stuttaford, 1989)). Relatively low market prices for farm produced fish and high prices of feed ingredients (Table 1.1), require that intensive aquaculture ventures be based on a sound system of efficient feed conversion, or more appropriately from an economical point of view, a system of profitably converting money spent on feed, into income from sales of fish products.

The sharptooth catfish Clarias gariepinus (Burchell, 1802) qualifies as one of the most suitable species for intensive aquaculture in Africa, and it has long been regarded as such by several workers (El Bolock and Koura, 1960; Micha, 1971, 1975; Van der Waal, 1972; De Kimpe and Micha, 1974; Richter, 1976; Hogendoorn, 1979; Hecht 1985). Feed conversion ratios of one (dry feed/wet weight gain), and better, have been achieved under intensive pond culture conditions in South Africa (this study) and in the Netherlands (Hogendoorn, 1983). The other major advantage which has a bearing on the culture potential of C. gariepinus is that it can be kept at high densities per unit of water volume and flow (Huisman and Richter, 1987; Bolnick et al., in press.). Under culture conditions it can reach a marketable size of 800 - 1000g in its first year (Hecht, 1985).

Table 1.1
Fish price - feed cost ratios in South Africa, compared to other countries.

| Country | Species | Fish price per kg | Feed price per kg | Price ratio (Fish/feed) |
|---|---|---|---|---|
| South Africa | C. gariepinus | R2.20 (own obs. Jan 1988) | R0.68 (own obs. Jan 1988) | 3.2 |
| South Africa | C. gariepinus | R3.00 (own obs. Jun 1989) | R0.98 (own obs Jun 1989) | 3.1 |
| South Africa | Parasalmo mykiss | R6.00 (Van der Merwe, pers. com., 1989) | R1.40 (Van der Merwe[1], pers. comm., 1989) | 4.3 |
| USA | Ictalurus punctatus | $1.25 (Nash, 1988) $1.76 (Urch, 1989) | $0.25 (Ray[2], pers comm., 1988) | 5.0 to 7.0 |
| UK | Salmo salar | £2.99 (Urch, 1988) | £0.47-£0.54 (McCormack[3], pers. comm., 1986) | 6.4 to 5.5 |
| Taiwan ROC | Oreochromis mossambicus | NT$46.66 (Lee, pers. comm., 1986) | NT$8.45 (Lee[4], pers. comm., 1986) | 5.5 |

[1]Lunsklip Fisheries, PO Box 309, Lydenburg 1120, South Africa.
[2]Fish Breeders of Idaho Inc., Box 234, Buhl, Idaho 83316, USA.
[3]BP Nutrition (UK), Wincham, Northwich, Chesire CW9 6DF, UK.
[4]Research Institute of Agricultural Economics, Chung Hsing University, Taichung, Taiwan ROC.

Table 1.2
Maximum weight and length of C. gariepinus attained within its first year under natural or culture conditions.

| Weight (g) | Length (mm TL) | Age (months) | Conditions | Reference |
|---|---|---|---|---|
| 431* | 400 | 12 | natural | Van der Waal, 1972 |
| 1100* | 496 | 12 | natural | Van der Waal, 1976 |
| 97 | 240 | 12 | natural | Bruton and Allanson, 1979 |
| 238 | 340 | 12 | natural | Quick and Bruton, 1984 |
| 622* | 450 | 12 | culture | Van der Waal, 1972 |
| 765 | 480* | 6 | culture | Christensen, 1981 |
| 550 | 432* | 8 | culture | Clay, 1984 |
| 1200 | 540 | 8 | culture | This study |

* estimates by the length-weight relationship for <1 year old C. gariepinus:
228 Weight(cm) = $L^{3.117}$ (Van der Waal, 1972)

Specific growth rates as high as 9.7% of body weight/day for juveniles with an initial body weight of 1g and 2.9% of body weight/day for 100g (initial weight) fishes have been recorded by Hogendoorn (1983) under experimental conditions. Some relevant weights or lengths attained by C. gariepinus in its first year under natural or culture conditions are listed in Table 1.2.

The culture potential of this species becomes more evident when one regards its attributes in terms of its natural distribution, biology and ecology: Clarias gariepinus, of which C. lazera and C. mossambicus are junior synonyms (Teugels, 1986), has the greatest latitudinal range of all known freshwater fish (Bruton, 1988). It occurs from the Orange River in the south, northwards through central, west and north Africa, through the Middle East and into Eastern Europe (Teugels, 1986). Within this distributional range C. gariepinus thrives in a wide variety of environments from temperate to tropical streams, rivers and swamps, and from shallow, turbid to deep, clear lakes.

The animal is highly fecund (Gaigher, 1977; Hogendoorn, 1979; Bruton, 1979b), is tolerant to parasitic infections (Van der Waal, 1972), and can exploit a wide range of dietary items of plant and animal origin (Van der Waal, 1972; Bruton, 1979c, 1979d; Clay, 1981). One of the more striking features of clariid species is their ability to utilize atmospheric oxygen with the aid of their suprabranchial organs (Moussa, 1957; Bruton, 1979a). Furthermore, C. gariepinus is well renowned for its tolerance of adverse environmental conditions (Greenwood, 1955; Clay, 1977; Babiker, 1984; Quick and Bruton, 1984). It is therefore not surprising that this ubiquitous fish makes up an estimated 20% of the catch by traditional subsistence and feral African freshwater fisheries (Clay, 1977).

The development of C. gariepinus culture was initially hampered by the lack of propagation techniques suitable for large scale production of fry and fingerlings (Hogendoorn, 1977; Hecht,

1985). This constraint was therefore addressed by several workers during the past decade. Induced spawning of C. gariepinus by hypophyzation or by means of injecting commercially available gonadotrophic hormones has consequently been well researched and reviewed by various workers (Hogendoorn and Vismans, 1980; Schoonbee et al., 1980; Hecht et al., 1982; Hecht, 1985; Van Oordt and Goos, 1987). After adapting their techniques to suit local conditions, it was found that C. gariepinus females could be induced to spawn by hypophyzation alone (this study).

Successful techniques for the intensive rearing of larvae and early juveniles have been developed by Hogendoorn (1980, 1981), Hecht (1981, 1982), Hecht and Appelbaum (1987) and Polling et al. (1987). Investigations into the dietary requirements of C. gariepinus larvae (Uys and Hecht, 1985) culminated in the formulation of an artificial dry feed which, when used alone, or in combination with live food, yields excellent growth and survival during the first two weeks of primary nursing. Further improvements in dry feeds for larvae were recently developed by Appelbaum and van Damme (1988). Dutch workers favour live food organisms such as Artemia during the primary nursing phase (Hogendoorn, 1980, 1981). More recently, they have shown that decapsulated and dried Artemia cysts can also be used as a sole primary feed (Verreth et al., 1987).

During the last three years, remarkable progress has been made in research aimed at refining production techniques and furthering the understanding of the biology of the species. Notably, the work of Britz and Hecht (1987) and Britz (1988) advanced our understanding of the environmental requirements for the hatchery rearing of C. gariepinus larvae and juveniles. Given the present state of development it may safely be stated that hatchery techniques for primary nursing of C. gariepinus can no longer be regarded as a major factor impeding the development of commercial sharptooth catfish culture.

Other investigations which have recently made important contributions to a more complete culture technology for this species are: cryopreservation of sperm (Steyn and Van Vuren, 1987); reproductive endocrinology (Van Oordt and Goos; 1987); sibling cannibalism in larvae and juveniles (Hecht and Appelbaum, 1988); growth modeling (Machiels and Henken, 1986,1987; Machiels and Van Dam, 1987); intensive rearing in water recirculating systems (Bovendeur et al., 1987); health problems (Boon et al., 1987) and nutritional aspects of rearing juveniles and sub-adults (Machiels and Henken, 1985, 1986, 1987; Henken et al., 1986; Machiels and Van Dam, 1987, Uys and Hecht, 1987; Uys et al., 1987) .

From a commercial producer's point of view, the technology is now sufficiently advanced (Hecht et al., 1988) to initiate the first intensive catfish production ventures under controlled conditions. It has in fact been demonstrated that existing techniques can be applied on a commercial scale in Zambia (Hecht, 1985). Moreover in The Netherlands, there are currently more than 30 sharptooth catfish farms, each with a production capacity varying between 1 and 50 tons per year (Huisman and Richter, 1987). Chinese farmers in Guangdong Province have to a large extent recently also switched from C. fuscus and C. batrachus to growing C. gariepinus (Wembiao et al., 1988).

A catfish growers association has been formed in South Africa with a membership of over 100 as of August 1989. Based on a questionnaire completed by members of the association, production is destined to increase from 137 tons in 1988, to 420 tons in 1989 and at least 1 300 tons per annum thereafter (Uys, 1989).

Now that private entrepreneurs have entered the field, it can be expected that more rapid advances will be made in the development of improved production techniques. However, many questions regarding the optimization of culture techniques still have to be addressed in an orderly, scientific fashion.

Since feeding usually represents the single most expensive production cost in intensive aquaculture (Shang, 1981), the development of optimal dry feeds is considered one of the major tasks in ongoing aquaculture research (Ghittino, 1972; Tiews et al., 1979). At the inception of this project in 1985, it was considered that the development of an optimal dry feed for production was the most important step in developing a more complete intensive culture technology for the species. This view is substantiated by the number of nutrition related research projects on C. gariepinus, which have since been conducted in The Netherlands (Machiels and Henken, 1985, 1986, 1987; Henken et al., 1986; Machiels, 1987; Machiels and Van Dam, 1987).

In order to place fish nutrition research in historic and scientific perspective, it should be noted that the nutrition of terrestrial animals has been intensively studied for many years, whereas research concerning the nutrition of fishes is of relatively recent origin. Before 1950, hatchery production diets consisted of fresh or frozen abattoir by-products and raw fish (Nose, 1979). Diet formulation development was mainly empirical, with little regard for optimization. Nutrition studies on salmonids escalated in the 1950's and early 1960's (Halver, 1953,1957a,b; Philips, 1956). The subsequent development of commercial moist feeds (Hublou, 1963) and dry feeds (Hastings and Dupree, 1969; Fowler and Barrows, 1971; Halver, 1972) has aided intensive fish husbandry towards real industrialization.

The 1970's saw a great number of research papers on fish nutrition, reflecting the increasingly important role which nutrition and feed technology began to play in the, by then, rapidly developing global aquaculture industry. In 1972, two major syntheses on fish nutrition appeared in tandem, one of which, was a compilation of review papers edited by Halver (1972) and the other a detailed review by Cowey and Sargent (1972). Most of the work, however, dealt with salmonids, and a sound

fundamental knowledge had been established regarding their dietary requirements for energy (Philips, 1972), protein and amino acids (Mertz, 1972), vitamins (Halver, 1972), and lipids (Lee and Sinnhuber, 1972). Most of the information had been established with feeding trials. Growth and feed conversion resulting from different test diets were the most important considerations at the time (Hastings and Dickie, 1972). The reviewers realized that especially protein was to play an important role in fish feed formulation (Hastings and Dickie, 1972). The complexity of dietary requirements and their interaction with non-nutritive factors were also realized by these workers.

As the aquaculture industry developed and diversified in the 1970's, especially with the advent of channel catfish (Ictalurus punctatus) culture in the USA, an increasing demand for commercial fish feeds necessitated increased research efforts. The channel catfish industry in the USA has been supported from it inception, by a well organized, intensive research effort, especially in the field of nutrition. Information on the formulation of commercial feeds for channel catfish was available as early as 1969 (Hastings and Dupree, 1969). Since then, the information has been frequently revised and updated by means of the combined efforts of well coordinated teams of researchers. Their progress was reported in the form of compiled review papers edited by Stickney and Lovell (1977) and Robinson and Lovell (1984) which dealt with the various aspects of channel catfish nutrition such as requirements for specific nutrients, feed formulation, feed processing, feeding practices and antinutritional factors.

The National Research Council of the USA was also instrumental in directing research efforts in fish nutrition and under its auspices three major reference works in fish nutrition were published. The NRC (1977) report on the "nutrient requirements of warmwater fishes" was complimented with the "nutrient

requirements of coldwater fishes" (NRC, 1981) and subsequently updated with the "nutrient requirements of warmwater fishes and shellfishes" (NRC, 1983). These works systematically reviewed the advances made in fish nutrition studies worldwide and are widely used as standard references in fish nutrition studies.

It is evident from the Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology (Halver and Tiews, 1979), that a shift in emphasis then occurred towards the economics of fish feeding. Feed formulation studies in the 1970's mainly dealt with substitution of expensive, conventional protein sources such as fishmeal, with less expensive ingredients (Higgs et al., 1979; Spinelli et al., 1979; Tiews et al., 1979). Attention was also given to feed manufacturing technology (Csavas et al., 1979; Meyers, 1979; Van Limborgh, 1979).

Since 1979, the expanded information base, and the increased sophistication in approach to economical optimization of fish feeds, led to computerized least costing techniques being widely applied in commercial fish feed manufacture (Chow et al., 1980; Crampton, 1985; see also Appendix). Nowadays, the application of nutritional findings to the economic optimization of fish feeds has become a highly specialized field. The emphasis is not merely on replacing expensive ingredients with cheaper ingredients but to accomplish the conversion of feed into fish as quickly and efficiently as possible. To advance this principle even further, the relative importance of growth rate and feed conversion efficiency are assessed in terms of the cost of the feed in relation to the value of the fish, i.e. the closer the feed price approaches the price obtained for the fish by the producer, the more emphasis is placed on efficient conversion, while growth rate becomes of secondary importance (Crampton, 1985).

Due to the increasing complexity of fish nutrition studies, an appeal was made for the standardization of methods and

terminology (Gropp, 1979). This resulted in a widely accepted report on the standardization of methodology by Castell and Tiews (1980), and was followed by a review and critique of the methodology in fish nutrition studies by Jobling (1983). As far as possible, the methodology and technology proposed by the Castell and Tiews (1980)-paper were applied to this project.

Several extensive review papers dealing with the nutritional requirements of fish have already appeared in the 1980's, most notably were those by Millikin (1982), Piper et al. (1982) and Cowey et al. (1985).

This research project was designed to compliment the existing works on fish nutrition. The modus operandi was to firstly review the fundamental aspects of C. gariepinus nutrition in order to establish the groundwork on which to base further fundamental, and then applied experiments. In turn, the findings of these experiments were applied to the formulation of practical diets. Lastly, a series of practical diets were field tested on a commercial scale and economically evaluated.

The overall objectives of this project, therefore, were to investigate the nutritional physiology and dietary requirements of Clarias gariepinus, with which to provide information on the formulation of optimum economical feeds for use in the intensive, commercial culture of this species. The following paragraphs describe the approaches followed to achieve these goals and elaborate on the rationale behind these approaches:

Five key areas of research were identified. The first approach was to review the natural feeding biology of C. gariepinus (Chapter 3). This provided the background information concerning the food preferences of the species and its abilities to utilize various nutrients.

The second approach was to investigate the functional morphology of the digestive tract and associated organs of C. gariepinus, and the development thereof, during early life history stages (Chapter 4). The rationale here was that the development of the digestive tract and associated organs serve to indicate the development of the fish's nutritional and digestive functions. Also, comparison with other fishes of which the dietary requirements are known, could also yield conclusions as to its dietary requirements in general.

Since the ability of an organism to digest a given substance is predominantly dependent on the presence of appropriate enzymes (Smith, 1980), the quantification and characterization C. gariepinus digestive enzyme activities were investigated on an experimental basis in Chapter 5. The reasoning here, was that information regarding the digestive abilities of the fish could, in turn, be applied in subsequent feed formulation studies.

In Chapter 6, specific nutritional requirements were investigated by means of feeding trials. This is the more conventional way of quantifying the nutritional requirements of fishes (and other animals), yielding directly applicable results.

Finally, practical feed formulations based on the collective information from the previous four research areas, were evaluated (Chapter 7). In this chapter, economical considerations played the major role in assessing the suitability of different feed formulations and led to the development of an IBM PC compatible least costing programme (see Appendix). The software is provided in the back inside cover. In conclusion, recommendations were made with regard to the formulation and application of commercial feeds for C. gariepinus.

# CHAPTER 2

# GENERAL METHODS

The two experimental phases of this study consisted of the digestive enzyme experiments (Chapter 5) and the feeding trials (Chapters 6 and 7). The digestive enzyme investigations were conducted at the experimental fish farm and laboratories of the Department of Ichthyology and Fisheries Science, Rhodes University, during the two summers of 1985-'86 and 1986-'87. The feeding trials were conducted at a commercial fish farm in the Transvaal Lowveld (Blyde River Aquaculture), during the two summers of 1986-'87 and 1987-'88. Part of the fish farm was specifically constructed to accommodate the experiments.

## ORIGINS OF FISH STOCKS

The fish which were used for the digestive enzyme investigation, during the 1985-'86 season, were second generation, year-old descendants of fish collected in the PK Le Roux Dam on the Orange River, Cape Province. These fish were spawned and reared at the Amalinda Provincial Fish Station near East London and were transferred to the Experimental Fish Rearing Facility at Rhodes University three months prior to the commencement of experiments. During this time they were kept in two circular, plastic pools (2m dia.) which make up part of one of the recirculating systems at Rhodes University (Figure 2.1).

Fish used for the feeding trials were first and second generation descendants of broodstock collected from impoundments in the Olifants, Klaserie and Blyde Rivers (all part of the Olifants River Drainage System) in the Eastern Transvaal Lowveld. The offspring were spawned and reared in the course of commercial production activities at the Blyde River fish farm. The facilities used for this study are shown in Figures 2.2 and 2.3. Fish used for experimental purposes were selected from commercial

stocks and transferred to experimental rearing tanks at least two weeks prior to the commencement of each feeding trial. Fish used for the digestive enzyme experiments during the 1986-'87 season were also selected from these stocks, but were transferred to tanks in the Rhodes University hatchery (Figure 2.4).

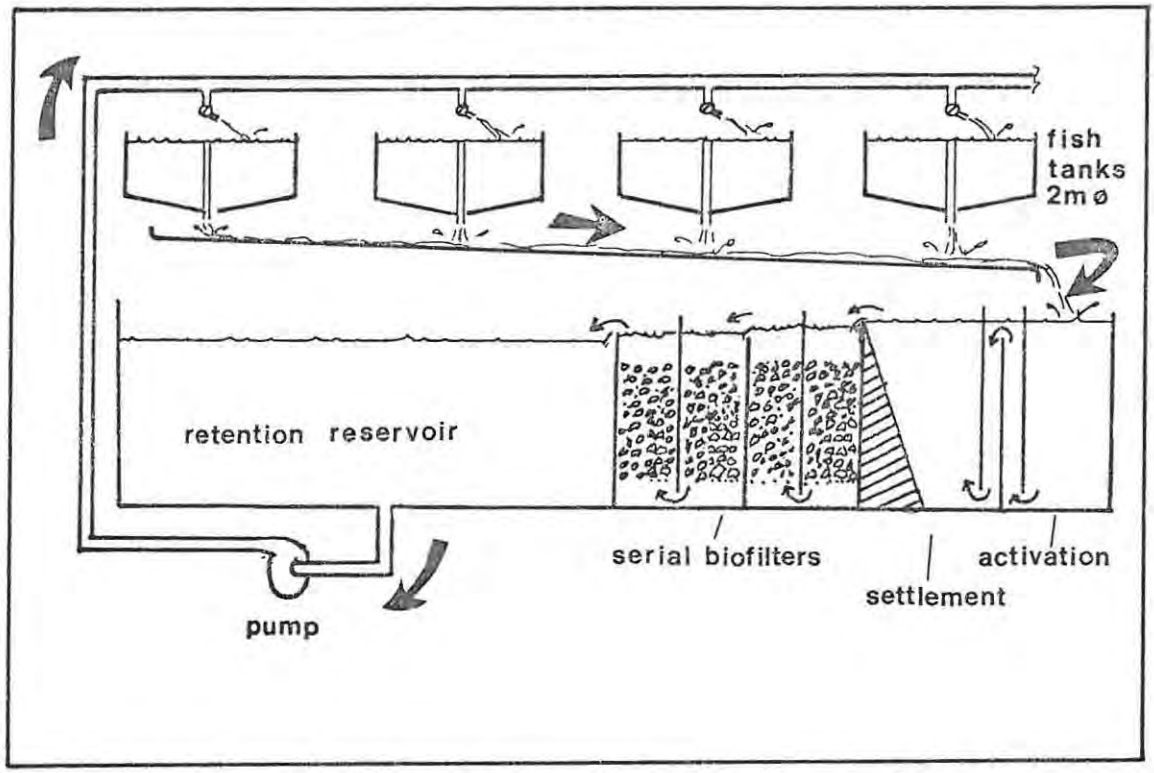


Figure 2.1 Schematic diagram of the outdoor, water recirculating, fish rearing facility at Rhodes University.

ARTIFICIAL PROPAGATION AND REARING TECHNIQUES

The procedures described by Britz and Hecht (1988) were employed for the mass production of fry at the Blyde River hatchery: Broodstock were conditioned by feeding them a high protein (>40%) diet in earthen ponds of 1000m2 by 1m deep. The fish were found to be in spawning condition from September to April, when ambient water temperatures exceeded 26°C. Gravid females were induced to spawn by hypophyzation, while sperm was obtained by dissecting the testes out of freshly sacrificed males. Eggs from one or more females were hand stripped into a glass bowl, until 400-500g was obtained. Approximately 0.5ml of semen was added to this

quantity of eggs and gently mixed for 30 seconds, whereupon a small quantity of hatchery water, enough to inundate the eggs, was added. The gametes were then gently mixed for 30-60 seconds while hydration occurred. The fertilized eggs were gently spread in a monolayer on nylon mesh frames (40 x 15cm) which were then suspended vertically in 60 liter incubation troughs. Each trough had a through flow (4l/min.) of heated (26-30°C) water from the Blyde River irrigation canal.

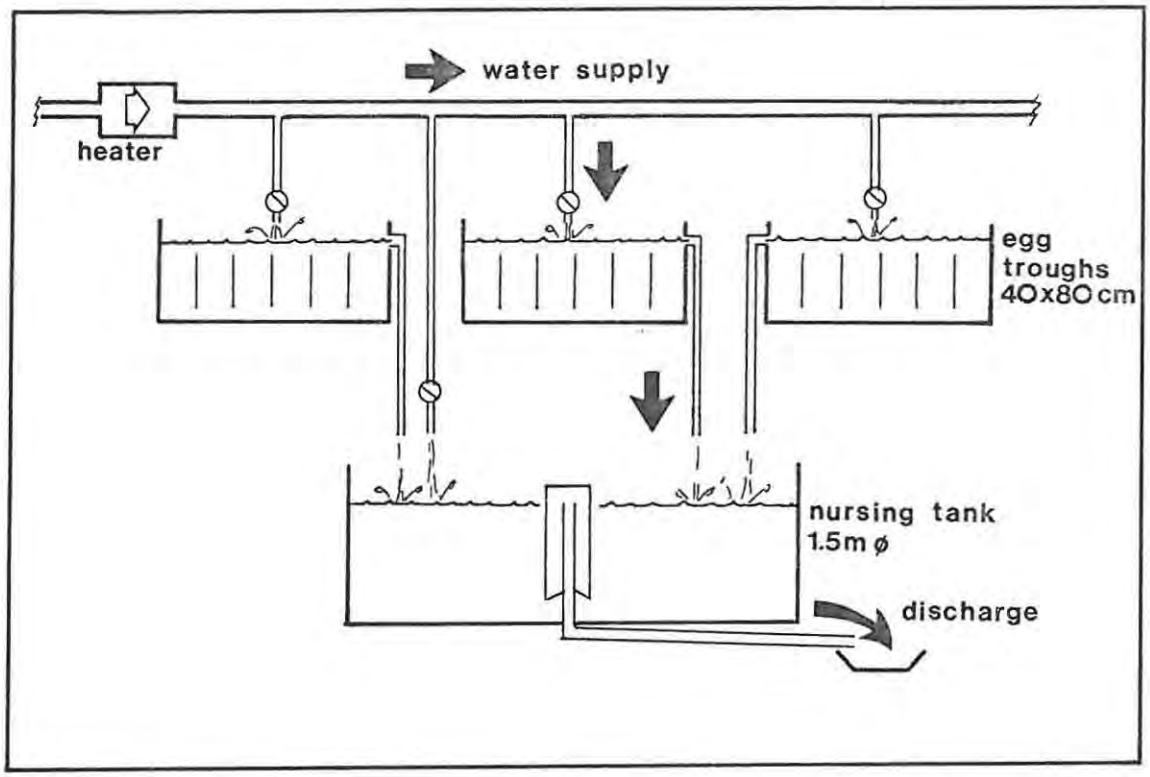


Figure 2.2 Schematic diagram of the flow-through hatchery system used for the mass production of catfish juveniles at the Blyde River facilities.

Eggs usually hatched 20-28 hours after fertilization. Hatching success was usually between 60 and 90%. Once the viable eggs had hatched, the mesh frames were removed from the system, along with the dead eggs and empty egg cases. Free embryos, swimming to the surface, passed naturally with the effluent water from the incubation troughs into the larval rearing tanks (Figure 2.2). The larvae were kept in these tanks for 12 days, during which time they were fed by hand every two hours, with a six hour lapse

at night, on a formulated, dry feed (Uys and Hecht, 1985). The dry feed was given at a ration of 100% of body weight per day. Once a day, they were also given a supplement of either natural live food harvested from ponds, or live Artemia nauplii, as recommended by Hecht and Appelbaum (1987). The larvae were kept at densities of 100 to 200 per liter. The relatively poor survival during this phase (mean = 60%) was mainly due to cannibalism, which is a common occurrence with catfish juveniles (Hecht and Appelbaum, 1988). After the 12 day primary nursing period, they were transferred to outdoor nursing ponds (150m2 by 1m deep).

In the outdoor nursing ponds, the juveniles were fed four times per day, with a 1:1 mixture of 38% crude protein feed crumbles and fish meal (resulting in 51% crude protein and 11.5% fat), at a rate of 25% of body weight per day. The fish also had access to natural food organisms, especially chironomid larvae and planktonic crustaceans (mainly cladocerans). The nursing ponds were stocked at densities of 100 to 200 fish per m2. Upon reaching a total length of four to six cm (± 5 weeks old), the juveniles were collected from the nursing ponds and brought to the hatchery. Here, they were sorted by size to prevent further cannibalism, and were either transferred to production ponds (1000m2 by 1m deep) for further on-growing, or directly into the experimental rearing containers.

The production ponds were stocked at a density of 10 fish per m2. The fish were fed 36% crude protein pellets at a ration of 10% of body weight per day for fish of up to 10g. As the fish grew their ration was gradually reduced to 4% of body per day by the time they reached a mean body weight of 200g. As the results of the feeding experiments became available, the feeding strategies and feed formulations were adapted accordingly.

The experimental designs, analysis of data, and the particular conditions and feeding regimes under which the experimental fish were kept are described in the various chapters.

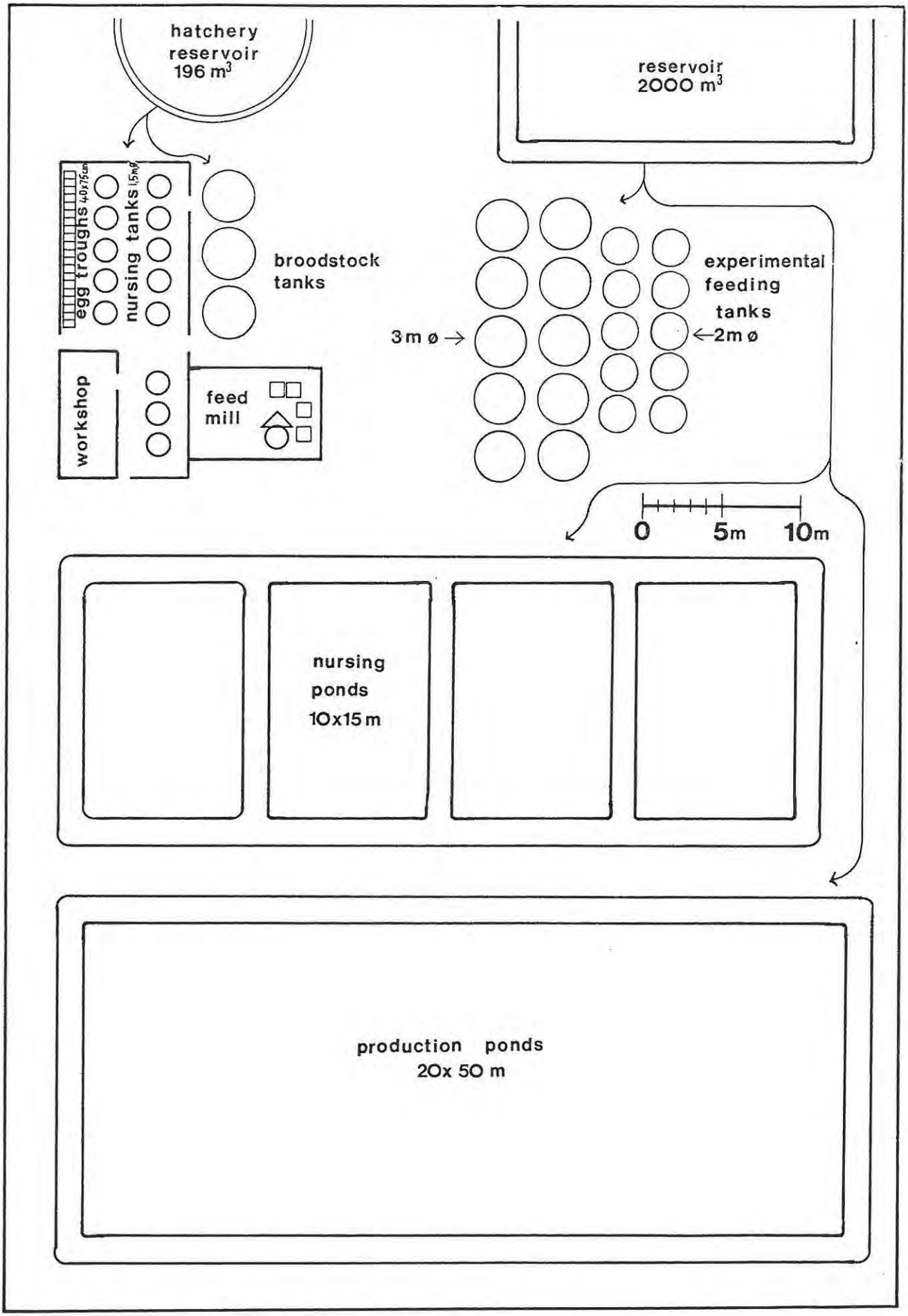


Figure 2.3 Floor plan of the hatchery, earthen ponds and experimental tanks used for this study at the Blyde River facility.

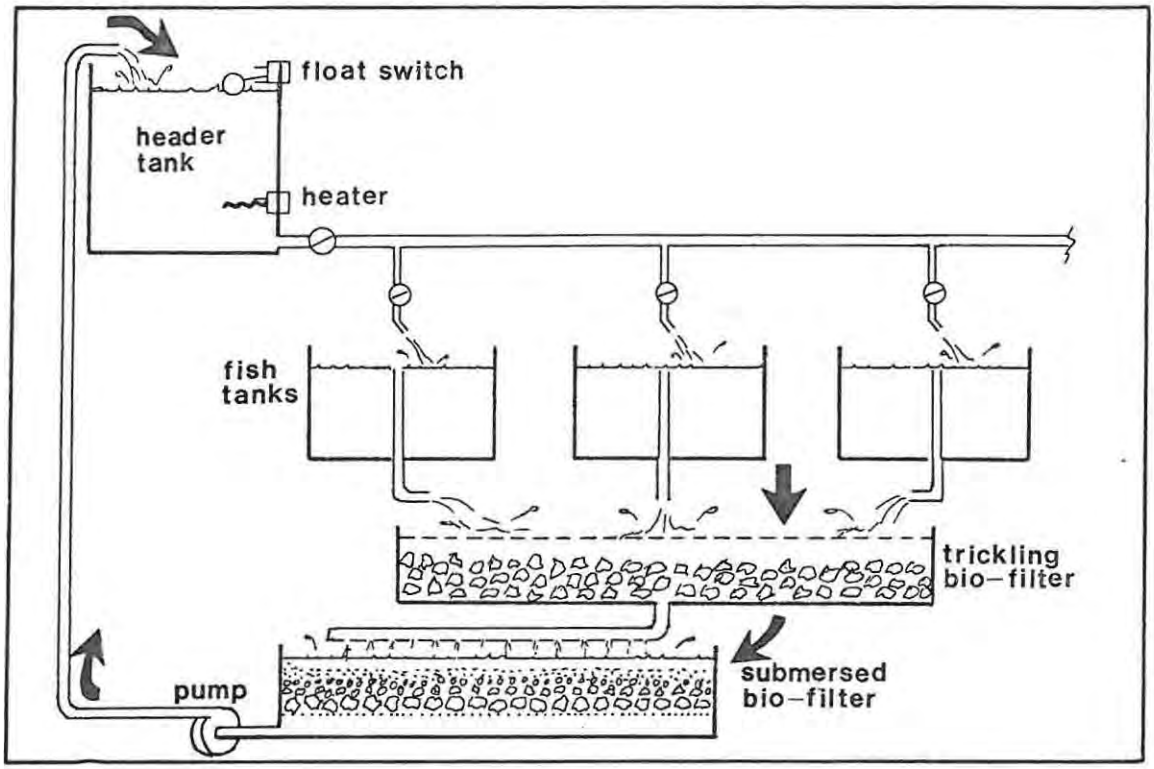


Figure 2.4 Schematic diagram showing the part of the recirculating system in the hatchery at Rhodes University which was used for this study.

# CHAPTER 3

# A SYNTHESIS OF THE FEEDING BIOLOGY OF *CLARIAS GARIEPINUS* AND THE IMPLICATIONS FOR AQUACULTURE

## INTRODUCTION

If *Clarias gariepinus* is physiologically, morphologically and behaviorally adapted to best exploit its natural environmental food resources, then a study of what and how it eats should also shed light on what type of formulated diet will best suit the animal's nutritional requirements. This hypothesis will be tested at the conclusion of this study by comparing the findings of this chapter, to the indications provided by the information and data in the subsequent chapters.

What and how the animal eats, can be examined in terms of:

-What are its food preferences in an environment which presents a wide choice of food resources ?

-What food resources can it utilize if the preferred ones are scarce or unobtainable ?

-How does it go about collecting either of the above?

-What is the nutritional (proximate) composition of its preferred or alternative natural diet?

In this chapter the above questions are answered in the form of a literature review, and on the assumption that the stated hypothesis is true, tentative conclusions are made as to the nutritional requirements of *Clarias gariepinus*.

TABLE 2.1
Review of the food preferences of Clarias gariepinus juveniles and sub-adults in various habitats.

| Case No. | Survey location | Catfish length (cm) | Method of stomach analysis | Dominant food items in terms of method of analysis employed | Secondary food items (in order of importance) | Number of fish examined | Author |
|---|---|---|---|---|---|---|---|
| 1 | Lake Malawi (open waters) | not given | Frequency | Fish (Haplochromis spp.) | Fish (cichlids and small Clarias) | ? | Lowe (1952) |
| 2 | Lake Malawi (sheltered creek) | 330-570 | Frequency | Insects (terrestrial) | Seeds, fish | 8 | Fryer (1959) |
| 3 | Lake Victoria (stream) | 7-870 | Frequency | Insects (Chironomid larvae) | Ostracods, Anisopteran larvae | 93 | Corbet (1961), quoted in Bruton (1979) |
| 4 | Lake Victoria (main lake) | 240-900 | Frequency | Fish (Haplochromis spp.) | Plants, molluscs and shrimps | 216 | Corbet (1961), quoted in Bruton (1979) |
| 5 | Victoria Nile | 100-240 | Frequency | Insects (lithophilic insects) | Ostracods, chironomid larvae | 5 | Corbet (1961), quoted in Bruton (1979) |
| | | 770-900 | Frequency | Crustaceans (Potamon sp.) | Fish (Barbus altianalis) | 2 | |
| 6 | Jukskei river (Transvaal, SA.) | 124-228 | Frequency | Insects (Entemostracans and Chironomids) | Detritus | | Groenewald (1964) |
| 7 | Barberspan (Transvaal, SA.) | 124-228 | Frequency | Fish (C. gariepinus) | Other fish, plankton | total 104 | Groenewald (1964) |
| 8 | Vaal River (Transvaal, SA.) | 124-228 | Frequency | Terrestrial invertebrates | Fish | | Groenewald (1964) |
| 9 | Lake McIlwaine (Zimbabwe) | 200-400 | Volumetric | Insects | Molluscs, plankton, fish, non-animal material | ? | Munro (1965) |
| | | 400-600 | Volumetric | Insects | Plankton, molluscs, fish, non-animal material | ? | |
| | | 600+ | Volumetric | Plankton | Insects, non-animal material, molluscs, fish | ? | |
| 10 | Lochinvar lagoon (Kafue r., Zambia) | not given | Frequency | Fish (100%) | -- | 2 | Tait (1965) |
| 11 | Uganda | not given | Frequency | Fish (Haplochromis spp.) | Insect larvae, molluscs, plants | ? | Greenwood (1966) |
| 12 | Lake Nuguna (Ghana) | not given | Volumetric | Insects | Non-animal material, plankton, fish | ? | Thomas (1966), quoted in Clay (1977) |
| 13 | Cleveland dam (Zimbabwe) | not given | Frequency | Insects | Non-animal material, fish | ? | Toots (1968), quoted in Clay (1977) |

TABLE 2.1 -continued

| Case No. | Survey location | Catfish length (cm) | Method of stomach analysis | Dominant food items in terms of method of analysis employed | Secondary food items (in order of importance) | Number of fish examined | Author |
|---|---|---|---|---|---|---|---|
| 14 | Ngondoma dam (Zimbabwe) | not given | Frequency | Plankton | Insects, fish, non-animal material, molluscs | ? | Toots (1969a), quoted in Clay (1977) |
| 15 | Nyambuia dam (Zimbabwe) | not given | Frequency | Non-animal material | Insects, molluscs | ? | Toots (1969b), quoted in Clay (1977) |
| 16 | Incomati river (Transvaal, SA.) | 350 | Volumetric | Fish (during summer) | Plants, non-animal material | ? | Gaigher (1969) quoted in Clay (1977) |
| | | 350 | Volumetric | Molluscs (during winter) | Insects, fish, non-animal material | | |
| 17 | Lake Chilwa (Malawi) | not given | Frequency | Fish (Barbus paludinosus) | Aquatic insects and plant detritus | ? | Kirk (1976) |
| 18 | Lake McIlwaine (Zimbabwe) | 200-400 | Volumetric | Insects (Chironomid pupae) | Insect larvae, molluscs and terrestrial insects | 102 | Munro (1967) |
| | | 400-600 | Volumetric | Insects (Chironomid pupae) | Zooplankton, molluscs | 358 | |
| | | 600+ | Volumetric | Zooplankton | Insects (Chironomid pupae) | 105 | |
| 19 | Lake Sibaya (Zululand) | 300-700 | Numeric | Crabs (Hymenosoma orbiculare) | Fish (small cichlids) | 53 | Minshull (1969) |
| 20 | Barberspan (Transvaal, SA.) | 0-200 | Volumetric | Fish (unspecified) | Insects (Ephemeropterans) | 37 | Schoonbee (1969) |
| | | 200-400 | Volumetric | Fish (unspecified) | Zooplankton (Cladocerans) | 4 | |
| | | 400+ | Volumetric | Zooplankton (Cladocerans) | Fish (unspecified) | 68 | |
| 21 | Olifants river (Marble Hall, SA.) | 180+ | Volumetric | Fish (unspecified) | Plants (filamentous algae) | 16 | Van der Waal (1972) |
| 22 | Farm reservoir, (Marble Hall, SA.) | 180+ | Volumetric | Fruit (grapes) | Fish (unspecified) | 10 | Van der Waal (1972) |
| 23 | Farm reservoir, (Groblersdal, SA.) | 180+ | Volumetric | Fish (unspecified) | Plant material (Nymphaea seeds and Typha stems) | 19 | Van der Waal (1972) |
| 24 | Lolamontes dam (Marble Hall, SA.) | 180+ | Volumetric | Fish (unspecified) | Insect larvae (Chironomids) and detritus | 13 | Van der Waal (1972) |

TABLE 2.1 -continued

| Case No. | Survey location | Catfish length (cm) | Method of stomach analysis | Dominant food items in terms of method of analysis employed | Secondary food items (in order of importance) | Number of fish examined | Author |
|---|---|---|---|---|---|---|---|
| 25 | Leeupan (W. Transvaal, SA.) | 180+ | Volumetric | Fish (unspecified) | Insects (terrestrial), zooplankton (Copepods) | 15 | Van der Waal (1972) |
| 26 | Kareepan (Wolamarnstad, SA.) | 180+ | Volumetric | Fish (unspecified) | Insect larvae(Chironomids), plant material | 16 | Van der Waal (1972) |
| 27 | Elands river (Marble Hall, SA.) | 180+ | Frequency | Fish (unspecified) | Insects (terrestrial), detritus, sand | 299 | Van der Waal (1972) |
| 28 | Lepalana dam (Lebowa) | 70-180 | Volumetric | Insect larvae (benthic) | Zooplankton (Cladocerans), insects, detritus | 15 | Van der Waal (1972) |
| 29 | Krokodilheuwel dam (Lebowa) | 70-180 | Volumetric | Insects (terrestrial) | Insects (larvae and nymphs) and detritus | 11 | Van der Waal (1972) |
| 30 | Lake Kariba (open waters) | 216-865 | Frequency | Zooplankton (Cladocerans) | Zooplankton (Copepods) | 34 | Bowmaker (1973) |
| 31 | Lake Kariba (margins amongst Salvinia) | 49-542 | Frequency | Insect larvae (*Povilla adusta*) | Zooplankton (Conchostraca), Chironomid larvae | 37 | Mitchell (1976) |
| 32 | Hardap dam (South West Africa) | 242-1482 | Frequency | Fish | Zooplankton | 69 | Gaigher (1977) |
| 33 | Lake St Lucia (Zululand) | 353-462 | Volumetric | Fish | Molluscs | 15 | Whitfield and Blaber (1978) |
| | | | Frequency | Molluscs | Fish | 15 | Whitfield and Blaber (1978) |
| 34 | Lake Sibaya (Zululand) | 20-50 | Frequency | Small crustaceans(*Grandidierella lignorum*) | Insect larvae (Chironomids), shrimps (*Caridina nilotica*) | 19 | Bruton (1979c) |
| | | | Numerical | Small crustaceans (*G. lignorum*) | Insect larvae (Chironomids), shrimps (*C. nilotica*) | | |
| | | | Dry weight | Insects (Odonatan nymphs) | Shrimps (*C. nilotica*), Chironomid larvae | | |
| | | | Composite | Small crustaceans (*G. lignorum*) | Insect larvae (Chironomids), shrimps (*C. nilotica*) | | |

TABLE 2.1 -continued

| Case No. | Survey location | Catfish length (cm) | Method of stomach analysis | Dominant food items in terms of method of analysis employed | Secondary food items (in order of importance) | Number of fish examined | Author |
|---|---|---|---|---|---|---|---|
| 34 | -continued | 60-100 | Frequency | Insect larvae (Povilla adusta) | Shrimps (C. nilotica) Chironomid larvae | 37 | Bruton (1979c) |
| | | | Numerical | Small crustaceans (G. lignorum) | Insect larvae (Chironomids), shrimps (C. nilotica) | | |
| | | | Dry weight | Insects (Odonatan nymphs) | Fish fry (Cichlids), insect larvae (P. adusta) | | |
| | | | Composite | Insect larvae (P. adusta) | Crustaceans (C. nilotica, G. lignorum), fish fry | | |
| | | 100-300 | Frequency | Shrimps (Caridina nilotica) | Insects (Odonatan nymphs, P. adusta) | 93 | |
| | | | Numerical | Small crustaceans (G. lignorum) | Shrimps (C. nilotica), fish (small Cichlids) | | |
| | | | Dry weight | Fish (small Cichlids) | Fish (small Cichlids) | | |
| | | | Composite | Shrimps (Caridina nilotica) | Insects (Odonatan nymphs), fish (small Cichlids) | | |
| | | 300-700 | Frequency | Crabs (Hymenosoma orbiculare) | Crustaceans (G. lignorum), fish (O. mossambicus) | 291 | |
| | | | Numerical | Crabs (H. orbiculare) | Crustaceans (G. lignorum, C. nilotica), molluscs | | |
| | | | Dry weight | Fish (Oreochromis mossambicus) | Crabs (Potamon sydneyi, H. orbiculare), fish | | |
| | | | Composite | Crabs (H. orbiculare) | Fish (O. mossambicus), crustaceans (G. lignorum, P. sidneyi) | | |
| | | 700+ | Frequency | Fish (O. mossambicus) | Molluscs (Bellamya capillata), crabs (P. sidneyi) | 29 | |
| | | | Numerical | Fish (O. mossambicus) | Crabs (H. orbiculare), Insect larvae (Chironomids), molluscs | | |
| | | | Dry weight | Fish (O. mossambicus) | Crabs (P. sidneyi), fish (Glossogobius giurus) | | |
| | | | Composite | Fish (O. mossambicus) | Crabs (P. sidneyi), molluscs (B. capillata) | | |

TABLE 2.1 -continued

| Case No. | Survey location | Catfish length (cm) | Method of stomach analysis | Dominant food items in terms of method of analysis employed | Secondary food items (in order of importance) | Number of fish examined | Author |
|---|---|---|---|---|---|---|---|
| 35 | Lake McIlwaine (Zimbabwe) | all sizes | Frequency | Fish and insects (during summer) | Molluscs | 34 | Clay (1979) |
| | | all sizes | Frequency | Insects (during winter) | Non-animal material, fish, plankton, molluscs | 54 | |
| 36 | Lake Kyle (Zimbabwe) | 200+ | Frequency | Insects (during summer) | Molluscs, fish | 174 | Clay (1979) |
| | | 200+ | Frequency | Fish (during winter) | Insects, molluscs, plankton non-animal material | 117 | |
| 37 | Mazoe dam (Zimbabwe) | all sizes | Frequency | Insects (during summer) | Fish | 10 | Clay (1979) |
| | | all sizes | Frequency | Insects (during winter) | Non-animal material | 15 | |
| 38 | Savory dam (Zimbabwe) summer | 0-200 | Frequency | Insects 100% (benthic) | -- | 2 | Clay (1979) |
| | | 200-500 | Frequency | Insects (benthic) | Other insects, non-animal material, plankton, fish | 47 | |
| | | 500+ | Frequency | Insects (mainly benthic) | Non-animal material, molluscs, plankton, fish | 42 | |
| | | 0-200 | Frequency | Plankton | Insects (mainly benthic), fish, molluscs | 17 | |
| | winter | 200-500 | Frequency | Plankton | Insects, fish, non-animal material | 17 | |
| | | 500+ | Frequency | Plankton | Fish, insects, molloscs, non-animal material | 14 | |
| 39 | Lake Kinneret (Israel) | 238-830 | Frequency | Fish (more than 12 species) | Insects (various), molluscs, plant material | 264 | Spataru et. al. (1987) |

FOOD PREFERENCES

In Table 2.1 the food preferences of Clarias gariepinus from 39 studies by various authors are summarized. The different methods used for the analysis of stomach contents were; (a) frequency of occurrence, (b) numerical, and (c) volumetric/gravimetric (Hynes, 1950; Hyslop, 1980).

Since the present study is concerned with the nutritional quality of the food, the volumetric- or dry weight method is the most relevant, even though it is biased towards large food items. Food preference in terms of the numerical method does not give a good representation of the relative importance of food types because of large differences in the size of the food items. Frequency of occurrence, too, is biased in favor of small food items. As can be seen in Table 2.1, when the frequency, or numerical methods are used, fish (which are relatively large food items) are seldom the dominant food items. In terms of volume and, therefore, in terms of actual quantities of food ingested, fish is definitely the most important food category (in 12 of the 25 cases in which volumetric methods were used, fish was the dominant item). Aquatic insects and the larvae, pupae and imagoes of terrestrial insects, collectively make up the must numerous and most often encountered food organisms in C. gariepinus. Benthic insect larvae, especially chironomids (Van der Waal, 1972; Clay, 1979), are almost omnipresent in all size classes of Clarias gariepinus, and it is surprising that large fish (>180mm) would devote effort to collecting these relatively small prey organisms. With the smaller size classes (<180mm), chironomid larvae and other benthos, as well as small crustaceans (benthic & planktonic) are the dominant food items. With the single exception of a sample of 10 C. gariepinus taken from a farm reservoir (Case 22, Van der Waal, 1972) in which grapes were the dominant food, plant materials never seems to be a preferred food class. Some authors regard C. gariepinus as an indiscriminate feeder and are of the opinion that plant material in its diet is purely incidental

(Groenewald, 1964; Spataru et al., 1987). This view is refuted by Munro (1967) and Van der Waal (1972), who found that C. gariepinus will actively consume large quantities of plant materials when animal prey is scarce.

Perhaps the most illuminating study on the feeding habits of C. gariepinus in a particular habitat was that by Bruton (1979c) in Lake Sibaya, South Africa. He describes Clarias gariepinus as an omnivorous predator and theorizes that two feeding strategies are possible: (a) the predator is euryphagic and feeds on a variety of organisms according to their availability; (b) the predator is stenophagic and feeds on a narrow range of organisms which are available at all lake levels. Bruton (1979c) goes on to show that Clarias gariepinus uses the first strategy. Lake Sibaya had a wide variety of food resources at any given time of the year. This consisted of a rich benthos, abundant vegetation and small fish, and also epifauna, plankton, detritus and flotsam. Because of the wide variety of available food types this is the ideal habitat for studying the food preferences of C. gariepinus. The following is a brief account of Bruton's (1979c) findings:

Crustaceans were the most important food items in terms of frequency of occurrence, followed by fish (mainly small cichlids), insects and mollusks. The most numerous prey species were Grandidierella lignorum, Hymenosoma orbiculare, Caridina nilotica, Povilla adusta, cichlid fry, chironomid larvae and odonatan nymphs. As a class, fishes contributed the greatest proportion of prey dry weight (75%) followed by crustaceans, insects and mollusks. Zooplankton was almost entirely absent in Bruton's (1979c) study. This is in contrast with the other works listed in Table 2.1. On analyzing food preferences (dry weight method) according to predator length , the following progression of dominant prey organisms becomes evident:

| | | |
|---|---|---|
| For fingerling C. gariepinus | (20-50mm): | Crustaceans |
| juveniles | (50-100mm): | Insects |
| sub-adults and adults | (100mm + ): | Fish |

The most evident correlation which could be drawn from this progression was the increase in prey size with predator size. Using SCUBA, Bruton (1979c) classified the accessibility of different prey organisms to catfish as low, moderate or high. He found, that with a few exceptions, highly accessible prey was taken in preference to prey with low or moderate accessibility. Also, the relative importance of food classes varied according to availability with the change of seasons, reflecting the opportunistic habits of C. gariepinus. Generally, feeding intensity and condition factors were lower in winter than in summer. That feeding intensity and condition factor of C. gariepinus varies with the seasons, irrespective of food abundance is supported by Spataru et al. (1987). This would suggest that temperature has a direct effect on the feeding activity of catfish.

Cannibalism is reported by several of the workers (Corbet, 1961, quoted in Bruton 1979c; Groenewald, 1964; Bruton, 1979c), but it does not seem to be a common occurrence. The low incidence of cannibalism under natural conditions is probably due to the relatively low densities at which they occur and the resulting low probability of an encounter between two fish. Under high density culture conditions, cannibalism and even sibling cannibalism is a common occurrence (Hecht and Appelbaum, 1988).

What confounds the problem of trying to determine the food preferences of C. gariepinus, is the extreme diversity of food items on which it feeds in any given habitat. Corbet (1961, quoted in Bruton, 1979c) found as many as six different prey species in a single stomach. In the population that Bruton (1979c) studied, over 55 different food species or groups were identified and individual catfish contained up to 10, sometimes 13 different food species (average 3.1). In the Lake Kinneret C. gariepinus population, Spataru et al. (1987) identified more than 50 species of plants and animals. On the other hand, C. gariepinus will at times feed exclusively on a single species of

prey organism if opportunity, in the form of over-abundance, presents itself (Munro, 1967; Clay, 1979).

From the above discussion it can be concluded that fish is the most important food of sub-adult and adult (>100 mm TL) C. gariepinus (Lowe, 1952; Corbet, 1961, quoted in Bruton, 1979c; Van der Waal 1972, Gaigher, 1977; Whitfield and Blaber, 1978; Bruton, 1979c; Spataru et al., 1987). It is also evident from Table 2.1 that insects and crustaceans as a group, is the second most important food source which can replace fish prey altogether, if the latter is scarce (Van der Waal, 1972; Munro, 1967). Insects and crustaceans as a group, is the preferred food of juvenile (<100mm TL) C. gariepinus. The term "preferred" can be misleading here, since the small catfish would most likely also prefer fish prey, but are not yet able to capture fishes effectively. Mollusks rank a close third in preference, even being a dominant prey item in certain instances (Gaigher, 1969 as quoted in Clay, 1977). Detritus and plant materials seem to be the least preferred foods, even where these are abundant. It should be noted, however, that plant material and detritus is almost always present in small quantities in the stomachs of C. gariepinus (Van der Waal, 1972; Bruton, 1979c; Spataru, et al., 1987), perhaps indicating some minor but indispensable role in catfish nutrition.

FEEDING BEHAVIOUR

The feeding behaviour of C. gariepinus is as diverse as the food it consumes. At least five distinct modes of feeding can be identified. These are briefly described in the following paragraphs:

-**Foraging** is done by individual catfish and is the normal mode of feeding in terrace and profundal habitats and plant beds (Bruton, 1979c). The predator swims along slowly with barbels extended and lunges for any prey organisms that are detected or flushed. As Bruton (1979c) remarks, this mode of feeding tends

to select for the most abundant and mobile prey organisms.

-Shoveling is also done by individual catfish and is concentrated in areas with abundant detritus (Bruton, 1979c). The object of this behaviour seems to be the capture of organisms which dwell in the detritus, rather than collecting detritus itself.

-Surface feeding is practiced by individuals (Bruton, 1979c; own observation) as well as by shoals (Bowmaker, 1973; Bruton, 1979c). Small catfish (<200m TL) occasionally feed by positioning the body perpendicularly (Fig. 2.1b), with the mouth at the water surface (Bruton, 1979c). A current of water is drawn into the mouth from the water surface, and expelled through the gill openings, thus filtering out any food items. Larger fish surface feed at a $\pm$ 60° angle (Fig. 2.1a) while remaining stationary (Bruton, 1979c), or they advance slowly, skimming the surface film and presumably filtering out any food items (Bowmaker, 1973; own observation).

-Formation feeding is practiced in shallow water by large (400-800mm TL) catfish (Pooley, 1972; Bruton, 1979c). The tightly knit formation, sometimes numbering up to a hundred fish (Pooley, 1972) herd and eventually surround or trap a shoal of small fish in shallow water, whereupon the catfish set about capturing the massed and disorientated small fish. Several variations of this technique are reported by other workers (Van der Waal, pers. comm., 1988*; Merron, in prep.).

-Ambush. When stationary, C. gariepinus distends it barbels in a cone shape in front of its head. Any prey being detected by the barbels is immediately lunged at and is usually caught (Jubb, 1965; Bruton, 1979c; own observation). It is, of course, debatable whether this is a valid category, since the intent of being stationery might be to rest rather than to hunt. Nevertheless, catfish have been observed (own observation) to remain stationery in places such as shallow water below a cover of flotsam, where the probability of encountering prey organisms is higher than in deep, open water. Anglers often exploit this phenomenon by lure fishing for catfish on top of flotsam.

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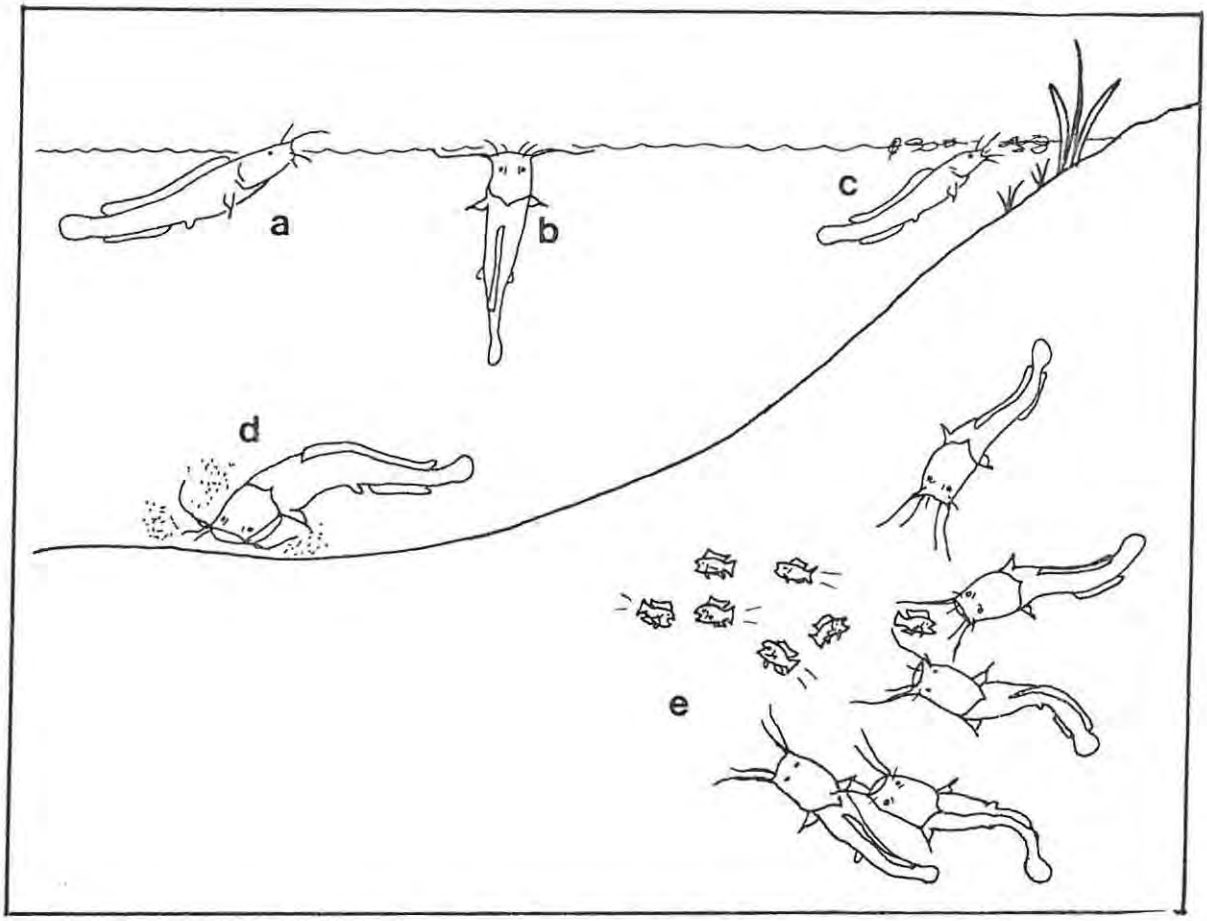


Figure 2.1. Different modes of feeding by C. gariepinus. (a)Horizontal surface feeding. (b)Perpendicular surface feeding. (c)Ambush. (d)Individual foraging and shoveling. (e)Formation feeding. (Redrawn from Bruton, 1979c).

When conditions are unsuitable for capturing fish prey, catfish switch their mode of feeding in order to capture crustaceans and insects. If a concentration of vulnerable fish is found, the predator readily switches back, and then resumes invertebrate feeding as soon as necessary (Van der Waal, 1972; Bruton, 1979c).

It is also important to note that C. gariepinus feeds intermittently and seems to be capable of utilizing infrequent, large meals (Spataru, 1987). This is also reflected in the high percentage of empty stomachs encountered under natural conditions, 11-76%, by Van der Waal (1972) and 31% by Bruton, 1979c).

## ESTIMATION OF THE NUTRITIONAL COMPOSITION OF NATURAL DIETS

In order for a tentative conclusion to be drawn from the above data with regard to the nutritional requirements of C.

gariepinus, it was thought appropriate to estimate the nutritional (proximate) composition of its natural diet. With the use of published data on the proximate analysis of natural fish food organisms similar to, or related to those taken by C. gariepinus (Yurkowski and Tabacheck, 1979), Table 3.2 was compiled. With the aid of Table 3.2, in turn, the proximate analysis of the natural diets of C. gariepinus could be estimated by calculation (Table 3.3). Two extreme case histories were chosen for this purpose. The first being Bruton's (1979c) data from Lake Sibaya (Kwazulu), owing to the high diversity of available food items in that lake. The second case was Van der Waal's (1972) data from the Olifants river near Marble Hall (Transvaal), which is representative of a habitat where "preferred" food items were scarce.

The results in Table 3.3 should be regarded as speculative because of the large variation in proximate composition of natural food organisms, even within a species (Yurkowski and Tabacheck, 1979). This source of error is, however, somewhat offset by the fact that mid-range values were used for the proximate analysis of each species or group of food items. Therefore, even though the results of the calculations might not accurately reflect the proximate composition of the diets at the time of sampling, they do give a fair approximation of the nutritional composition of the natural diet of C. gariepinus.

The results in Table 3.3 indicate that the Lake Sibaya catfish fed on a diet consisting of approximately 57% protein, 19% lipid, 7% carbohydrates, 11-12% chitin and fiber, with a metabolizable energy (ME) value of 18 kJ/g, on a dry weight basis. Similarly, the Olifants river diet consisted of approximately 43% protein, 16% lipid, 15% carbohydrates, 13% chitin and fiber, and had a ME value of $\pm$ 16 kJ/g. Two interesting trends become evident from these data: (a) The preferred diet (Lake Sibaya) has a higher protein, lipid and energy content than the ersatz (Olifants river) diet. (b) The Olifants river catfish had

a higher incidence of stomach fullness at 89% (Van der Waal, 1972) than the Lake Sibaya catfish at 69% (Bruton, 1979c), thereby presumably making up in quantity, what their available diet lacked in quality.

Table 3.2 Estimated proximate composition of natural food organisms commonly taken by Clarias gariepinus (to serve as an index for calculations in Table 3.3).

| Index No. | Organism or group of organisms | Protein % | | Lipid % | | Carbo-hydrate % | | Chitin or fiber (% | | Metabolizable energy kJ/g |
|---|---|---|---|---|---|---|---|---|---|---|
| | | range | m.r. | range | m.r. | range | m.r. | range | m.r. | estimated |
| 1 | Fish | 58-61 | 60 | 19-24 | 22 | 6-7 | 7 | 10-14 | 12 | 19.1 |
| | **Crustaceans** | | | | | | | | | |
| 2 | composite | 29-74 | 52 | 6-17 | 12 | 7-15 | 11 | 4-7 | 6 | 15.0 |
| 3 | large crabs (estimated) | | 48 | | 10 | | 9 | | 12 | 13.2 |
| 4 | shrimps | | 60 | | 17 | | 7 | | 4 | 17.5 |
| 5 | amphipods | 40-50 | 45 | 6-11 | 9 | 12-15 | 14 | | 7 | 13.2 |
| 6 | zooplankters | 29-74 | 52 | 11-24 | 18 | 4-12 | 8 | | 7 | 16.7 |
| | **Insects** | | | | | | | | | |
| 7 | composite | 34-66 | 50 | 13-32 | 23 | 2-23 | 13 | 4-14 | 9 | 19.1 |
| 8 | Odonata nymphs | 55-63 | 59 | 13-14 | 14 | 9-10 | 10 | | 10 | 16.7 |
| 9 | chironomid larv. | 48-62 | 55 | 2-14 | 8 | 4-23 | 14 | | 4 | 14.5 |
| 10 | **Mollusks** | | 60 | 6-12 | 8 | | <1 | | <1 | 13.0 |
| 11 | **Aquatic macrophytes** | 10-17 | 14 | | 5 | | 57 | | 12 | 13.0 |
| 12 | **Green algae** | 6-13 | 10 | | 10 | | 34 | | 23 | 11.7 |

All values (rounded to nearest %) based on those given for related natural food organisms by Yurkowski and Tabacheck (1979) except values for mollusks from Long (1961). In each case, range (if available) and mid-range (m.r.) are given. Only the m.r. values were used in calculations for Table 3.3. Estimates of metabolizable energy were calculated using 16.7 kJ/g total protein, 37.6 kJ/g lipid and 16.7 kJ/g carbohydrate on a total dry solids basis (Philips, 1972).

Table 3.3 Calculated proximate composition of the natural diet of C. gariepinus in two different habitats.

| Diet composition of 469 catfish Lake Sibaya (Bruton, 1979) | | | Nutritional value contributed by item to diet as a whole (% in diet x nutritional composition/100) | | | | |
|---|---|---|---|---|---|---|---|
| Item | % in diet (dry weight) | Index re. Tab. 3.2 | Protein % | Lipid % | Carbohydrate % | Chitin or fiber % | Metabolizable energy kJ/g |
| Fish | 75 | 1 | 44.6 | 16.1 | 5.3 | 9.0 | 14.4 |
| Crustaceans: | | | | | | | |
| H. orbiculare | 7.1 | 3 | 3.4 | 0.7 | 0.6 | 0.9 | 0.9 |
| C. nilotica | 0.6 | 4 | 0.4 | 0.1 | 0.0 | 0.0 | 0.1 |
| P. sidneyi | 10.7 | 3 | 5.1 | 1.1 | 1.0 | 1.3 | 1.4 |
| G. lignorum | 0.1 | 5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| P. latipes | 0.04 | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| C. carinata | 0.07 | 2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Insects | | | | | | | |
| Odonata nymphs | 2 | 8 | 1.2 | 0.3 | 0.2 | 0.2 | 0.3 |
| chironomid larvae | 0.1 | 9 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| P. adusta | 0.7 | 7 | 0.4 | 0.2 | 0.1 | 0.1 | 0.1 |
| terrestrials | 1.7 | 7 | 0.9 | 0.4 | 0.2 | 0.2 | 0.3 |
| Mollusks | 1.9 | 10 | 1.1 | 0.2 | 0.0 | 0.0 | 0.2 |
| Calculated proximate analyis: (totals) | | | 57.2 | 19.0 | 7.4 | 11.6 | 17.9 |
| Diet composition of 16 catfish, Olifants river (Van der Waal, 1972) | | | | | | | |
| Fish | 52.1 | 1 | 31.0 | 11.2 | 3.6 | 6.3 | 10.0 |
| Crustaceans: | | | | | | | |
| zooplankton | 1.9 | 6 | 1.0 | 0.3 | 0.2 | 0.1 | 0.3 |
| C. nilotica | 1 | 4 | 0.6 | 0.2 | 0.1 | 0.0 | 0.2 |
| Insects | | | | | | | |
| Odonata nymphs | 0.1 | 8 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| chironomid larvae | 0.1 | 9 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| terrestrials | 3.2 | 7 | 1.6 | 0.7 | 0.4 | 0.3 | 0.6 |
| Mollusks | 9.2 | 10 | 5.5 | 0.7 | 0.0 | 0.0 | 1.2 |
| Macrophytes | 4 | 11 | 0.6 | 0.2 | 2.3 | 0.5 | 0.5 |
| Green algae | 24.8 | 12 | 2.5 | 2.5 | 8.4 | 5.7 | 2.9 |
| Detritus and sand | 3.6 | * | | | | | |
| Calculated proximate analysis: (totals) | | | 42.9 | 15.9 | 15.0 | 12.9 | 15.8 |

* Detritus and sand assumed to have negligible nutritional value for sake of calculation

To pursue the second correlation even further (admittedly at the risk of being too speculative), the extent to which the incidence of stomach fullness seems to compensate for the deficiency in dietary quality is remarkably precise: Based on the incidence of stomach fullness data, the Olifants river -catfish consumed 1.3 times as much food as the Sibaya population. If the nutritional values of the Olifants river diet are multiplied by this factor of 1.3, then protein, lipid and energy values become highly similar to the respective values for the Lake Sibaya catfish. This can be demonstrated as follows:

| Proximate composition | | Olifants river diet | Olifants river diet multiplied by 1.3 | Lake Sibaya diet |
|---|---|---|---|---|
| protein | % | 42.9 | 55.8 | 57.2 |
| lipid | % | 15.9 | 20.7 | 19.0 |
| ME | kJ/g | 15.8 | 20.5 | 17.9 |

CONCLUSIONS

From a commercial culture point of view, the euryphagic nature of C. gariepinus is advantageous, since it would indicate that a wide variety of feed ingredients from plant and animal origin might be considered in formulating practical feeds for this animal. Even though plant materials appeared not to be preferred items in the diet of C. gariepinus, the fact that they were nevertheless often consumed, seems to indicate that this species is at least physiologically equipped to digest and utilize plant proteins and carbohydrates.

The best description of the feeding habits of C. gariepinus is that it is an opportunistic, omnivorous predator with the ability to utilize alternative food sources, such as plants and detritus when prey animals are scarce. Its apparent preference for fish indicates a high dietary protein and lipid requirement, presumably in excess of 40% and 12% on a dry weight basis respectively.

# CHAPTER 4

# DEVELOPMENT AND FUNCTIONAL MORPHOLOGY OF THE DIGESTIVE TRACT AND ASSOCIATED ORGANS

The morphology of piscine digestive systems in relation to their feeding habits is a topic which has attracted quite some attention since the earlier studies by Al-Hussaini (1949a,b). Gross anatomic characteristics of digestive systems and, especially, relative gut lengths (RGL) are aspects which have been shown to relate at least loosely to feeding habits and trophic levels of fishes (Al Hussaini, 1949a,b; Kruger and Mulder, 1973; Kapoor et al., 1975; Ribble and Smith, 1983). Studies on the ultrastructure of digestive organs and tissues have helped reveal their functions and have shed light on the ability of fishes to utilize a great variety of foods in spite of the fact that their alimentary canals are generally simpler than those of higher vertebrates (Kapoor et al., 1975; Fänge and Grove, 1979; Ferraris and Ahearn, 1984; Junger et al., 1989).

In this chapter the morphological feeding adaptations and gross and fine structure of the animals alimentary organs, as well as their development, are presented and briefly described.

## EXTERNAL MORPHOLOGICAL ADAPTATIONS TO FEEDING

The large, rounded caudal fin of C. gariepinus is typical of a fish that ambushes its prey, since it enables the fish to execute a fast, forward lunge, rather than persistent high speed pursuit. Its wide mouth, vertical gape, long circumoral barbels and ability to depress the hypobranchial apparatus are adaptations which further facilitate a benthic feeding mode in low visibility (Bruton, 1979c). Prey and food detection is largely facilitated by the tactile and chemosensory functions of the four pairs of barbels, and most likely also by the lateral line system.

The many taste buds which occur on the circumoral barbels of C. gariepinus have been shown to share many morphological (Nel and Nel, 1985) and ultrastructural (Geyer et al., 1985) characteristics with vertebrate taste buds in general. A study by Nel and Geyer (1985) has revealed that each taste bud has a single granulated basal cell as well as several classic gustatory receptor cells. The basal cell and the gustatory receptor cells innervate with a single plexus in the taste bud. This would suggest that gustatory information is processed periphally to some extent, and that a complex signal is relayed to the afferent gustatory nerves.

Visual observations made during the course of this study suggest that the barbels (and taste buds) play a vital role in locating artificial feed pellets. When feed pellets are introduced to a rearing tank or pond, resting catfish are immediately alerted (probably by being conditioned to the sound of pellets hitting the water surface as much as by chemical stimuli) and proceed swimming about in a random fashion with their barbels extended. Even under clear water conditions, a catfish can only locate a feed pellet when its circumoral barbels come into virtual direct contact with it.

## GROSS MORPHOLOGY OF THE ALIMENTARY TRACT

C. gariepinus has a wide, subterminal mouth with a large vertical gape for engulfing large prey or large volumes of water during filter feeding. Bands of fine, sharp, recurved teeth on the premaxillary and dentary bones enables the fish to grasp its prey and prevent its escape. The vomerine and pharyngeal tooth bands also perform this function but also serve to incapacitate the prey by crushing. Some mastication may be performed, especially by the blunter teeth on the vomerine band, but large prey is swallowed whole.

The anterior margins of the five branchial arches are equipped with long gill rakers, and the posterior margins of the third and fourth arches have gill rakers that interdigitate with those on the anterior margin of the next arch (Bruton 1979c). The gill rakers form a straining apparatus which enables the fish to filter-feed on midwater organisms and surface scum. Under pond culture conditions catfish were observed to filter-feed on floating fine material which results from broken feed pellets.

The oesophagus is short and dilatable, and allows the passage of whole prey to the highly expandable stomach which is differentiated into corpus and pyloric regions. The stomach is muscular and, according to Bruton (1979c), can crush and tumble the food to facilitate digestion. The intestine is simple, relatively short and thin walled. The walls of the terminal portion of the intestine (rectum) are slightly thicker and more muscular than those of the rest of the intestine.

The pancreatic tissue is diffuse and consists of nodules of Brockman bodies situated in the mesenteries associated with the stomach, anterior intestine, gall bladder, liver and spleen. There is, however, a concentration of pancreatic tissue which forms an elongate, compact battery of Brockman bodies attached to the serosa of the pyloric region of the stomach. Careful examination revealed that, in spite of their scattered nature, all of the Brockman bodies finally duct into a large pancreatic duct which opens into the anterior intestine just posterior to the common bile duct. The relatively large gall bladder is especially full in starved catfish, suggesting an ability to cope with large, infrequent meals.

RELATIVE GUT LENGTH

In this study, relative gut length (RGL) is expressed as gut length divided by total fish length (or fork length where reference is made to fish with forked tails). Studies on a wide

variety of fish species have revealed that RGL has a fairly reliable predictive value with regard to the feeding ecology of a species (Al Hussaini, 1949a,b; Kruger and Mulder, 1973; Kapoor et al., 1975; Ribble and Smith, 1983; Junger et al., 1989). The trend is for fishes at lower trophic levels to have longer intestines, but since single fish species often have overlapping trophic levels, it is more practical to rank feeding habits in terms of detritivory, herbivory, omnivory and carnivory (Table 4.1). Apart from an increased gut length, increased mucosal folding is also a means of increasing exposure to food. The degree of mucosal folding in a species, however, seems to have a lesser predictive value with regard to its feeding ecology (Junger et al., 1989).

The evidence in Table 4.1 seems to indicate that fishes with an RGL of less than unity, are strictly carnivorous. Those with RGL's between 1.0 and 1.2 are either carnivorous or omnivorous (including C. gariepinus) and those with RGL's in the range 1.2 to 2.2 are strictly omnivorous. Fish with extremely long intestines are herbivorous (RGL ±7.0 to 8.0) or detritivorous (RGL 10 and higher). According to this ranking, C. gariepinus is adapted for omnivory and carnivory, a conclusion which is substantiated by the findings in the other chapters of this thesis. Note that the RGL of C. gariepinus seems to show some intraspecific variation (range 0.45 - 1.18). It can be speculated that a certain degree of phenotypic plasticity may be reflected by the variation in RGL of catfish from different localities (and, therefore, different feeding habits and food availabili), but it may also be that the techniques used by the various authors were not compatible.

Table 4.1
Fish species ranked according to their mean relative gut lengths (RGL) with reference to their feeding habits

| Species | RGL | Feeding ecology according to authors | Author |
|---|---|---|---|
| Labeo rosae | 17.30 | Detritivore | Kruger and Mulder, 1973 |
| Labeo capensis | 14.90 | Detritivore | Kruger and Mulder, 1973 |
| Labeo umbratus | 10.00 | Detritivore | Kruger and Mulder, 1973 |
| Oreochromis mossambicus | 7.90 | Herbivore | Kruger and Mulder, 1973 |
| Tilapia rendalli | 7.20 | Herbivore | Kruger and Mulder, 1973 |
| Cyprinus carpio | 2.20 | Omnivore | Kruger and Mulder, 1973 |
| Chondostromus nasus | 2.05 | Omnivore | Junger et al, 1989 |
| Erimyzon sucetta | 1.76 | Omnivore | Ribble and Smith, 1983 |
| Barbus holubi | 1.70 | Omnivore | Kruger and Mulder, 1973 |
| Rutilis Rutilis | 1.25 | Omnivore | Junger et al, 1989 |
| Abramis brama | 1.19 | Omnivore | Junger et al, 1989 |
| Clarias gariepinus | 1.18 | Carnivore | Kruger and Mulder, 1973 |
| Barbus kimberleyensis | 1.14 | Carnivore | Kruger and Mulder, 1973 |
| Tinca tinca | 1.13 | Carnivore | Junger et al, 1989 |
| Blicca bjoerkna | 1.08 | Omnivore | Junger et al, 1989 |
| Clarias gariepinus | 1.07 | Carnivore | Kruger and Mulder, 1973 |
| Rutilis rutilis | 1.05 | Omnivore | Al Hussaini, 1949a |
| Eutropius depressirostris | 1.04 | Carnivore | Kruger and Mulder, 1973 |
| Leusciscus cephalus | 1.01 | Omnivore | Junger et al, 1989 |
| Barbus matozzi | 0.96 | Carnivore | Kruger and Mulder, 1973 |
| Clarias gariepinus | 0.96 | Carnivore | This study |
| Lepomis auritus | 0.91 | Carnivore | Ribble and Smith, 1983 |
| Abramis ballerus | 0.82 | Carnivore | Junger et al, 1989 |
| Aspius aspius | 0.79 | Carnivore | Junger et al, 1989 |
| Ictalurus natalis | 0.78 | Carnivore | Ribble and Smith, 1983 |
| Pelecus cultratus | 0.78 | Carnivore | Junger et al, 1989 |
| Gobio gobio | 0.72 | Carnivore | Al Hussaini, 1949a |
| Micropterus salmoides | 0.72 | Carnivore | Ribble and Smith, 1983 |
| Esox americanus | 0.58 | Carnivore | Ribble and Smith, 1983 |
| Clarias gariepinus | 0.45 | Carnivore | Stroband and Kroon,1981 |
| Aphredoderus sayanus | 0.40 | Carnivore | Ribble and Smith, 1983 |
| Percina nigrofasciata | 0.37 | Carnivore | Ribble and Smith, 1983 |
| Anguilla rostrata | 0.31 | Carnivore | Ribble and Smith, 1983 |

FINE STRUCTURE OF DIGESTIVE TRACT AND PANCREATIC TISSUE

Histological methods and techniques

The methods used in preparing the various tissues for electron microscopy followed closely those outlined by Cross (1979). For transmission electron microscopy, tissue samples (±1mm dia.) were collected from freshly sacrificed specimens and were fixed in chilled, buffered glutaraldehyde (18hrs.). Osmium tetroxide was used as a secondary fixative (90min.), wherafter the tissue samples were dehydrated in a series of alcohol baths of increasing concentration. Propylene oxide was used at the last stage of dehydration and it served as a transitional solvent for embedding the tissue samples with a mixture of TAAB 812 epoxy resin and Araldite. Ultra thin (60-100nm) sections were cut with a 50° glass knife at a cutting speed of 2mm/sec. The sections were stained with uranyl acetate (50 min.) and subsequently with Reynolds lead citrate (3 min.). The sections were viewed with a Jeol J.E.M. 100 CX 11 transmission electron microspcope.

Tissue samples (±2mm diameter) were also prepared for scanning electron microscopy. These samples were also fixed twice and dehydrated with alcohol as described above, but were then placed in a series of baths of increasing concentrations of amyl acetate (finally 100%). The tissues were dried in a Polaron critical point dryer and were finally sputter coated with gold and observed in a Jeol JSM-U3 scanning electron microscope.

Observations

The "J" -shaped stomach of C. gariepinus is distinctly divided into a descending glandular portion (corpus) and ascending non-glandular portion (pyloric region). In this respect, and in respect of the histology of these two regions, C. gariepinus is almost identical to Ictalurus punctatus as described by Sis et al. (1979). The inner lining of the stomach is made up of mucus secreting, columnar epithelial cells. These cells are

characterized by many mucoid granules throughout the cytoplasm, especially at the apex of each cell, where mucus vesicles are secreted into the lumen of the stomach. The cells also contain a distinct golgi apparatus and many strands of granular endoplasmic reticulum (ER). The lining has several large folds (rugae) which are subdivided into extensive networks of secondary folds (Fig. 4.1). This accounts for the enormous distensibility of the stomach when large, whole prey is ingested. The surface area of the epithelial lining is further increased by microvilli which protrude into the lumen.

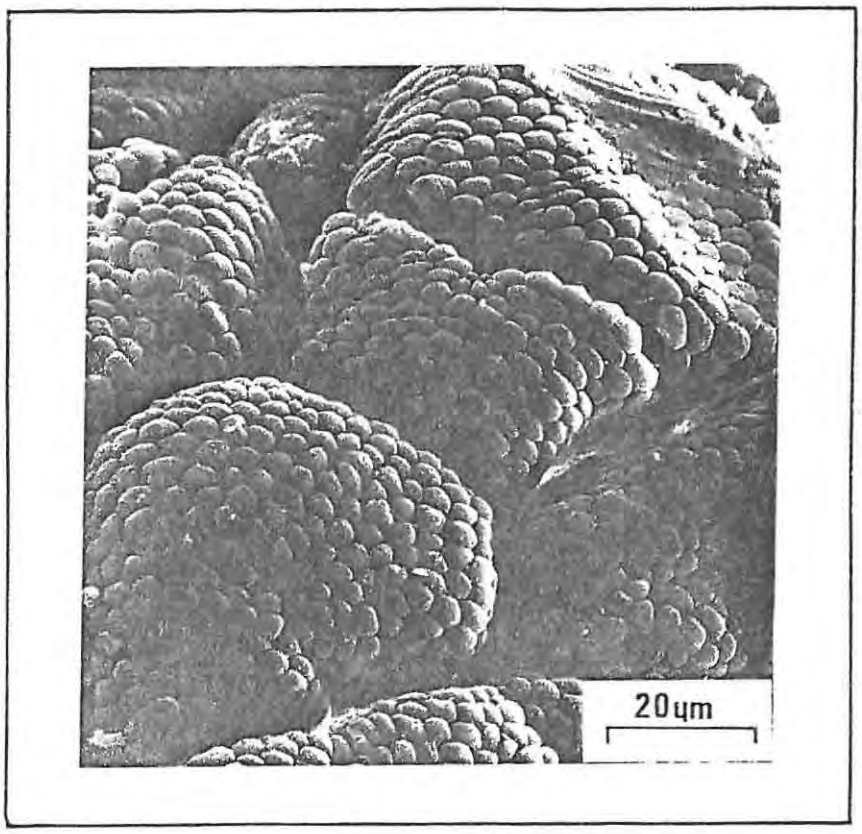


Figure 4.1 Scanning electron micrograph of the gastric lining of sub-adult Clarias gariepinus, showing densely packed columnar epithelium with mucus vesicles being secreted (small white specks).

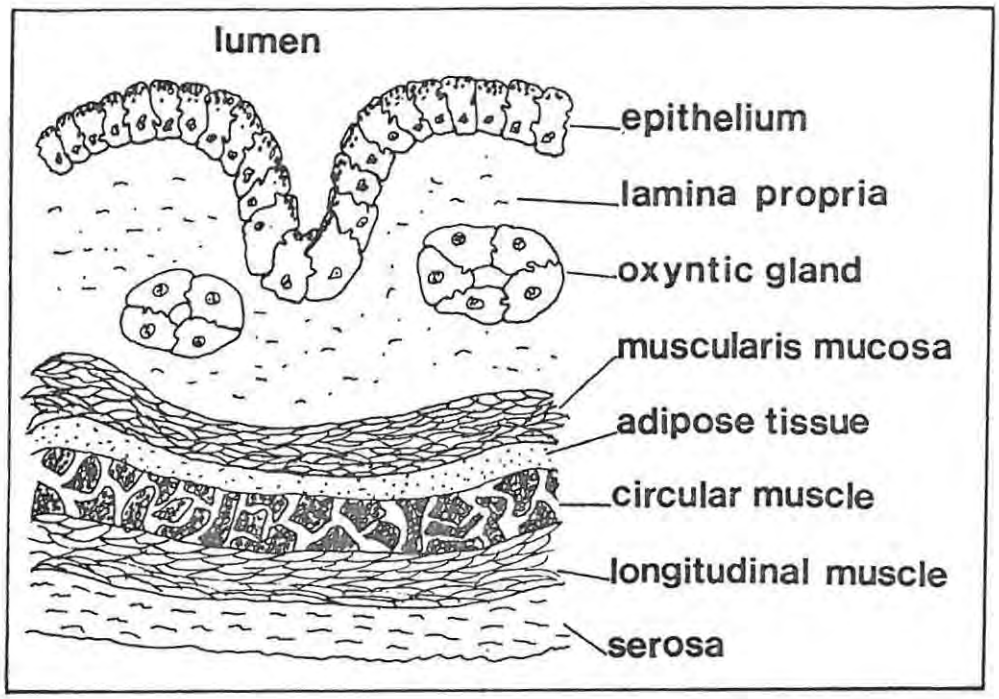


Figure 4.2 General characteristics of the corpus region of the gastric mucosa of Clarias gariepinus. The oxyntic glands are tubular and open into the stomach lumen through gastric pits. The pyloric region is similar but the glands are absent and the mucosa muscularis is much thicker.

The epithelial lining cells show complex interdigitations of the cellular membranes with neighboring cells, which probably ensure that the mucosa maintains its integrity when it is stretched. The underlying lamina propria contains tubular oxyntic glands (Figure 4.2) formed by the epithelial gland cells (only in the corpus region). These gland cells are of a single kind, and due to their similarity with the gastric gland cells of other teleosts, presumably secrete both pepsinogen and hydrochloric acid (Stroband and Kroon, 1981). Also, zymogen granules are scattered throughout the cytoplasm of these cells and exocytosis can be observed. The cytoplasm of an oxyntic gland cell also contains a number of smooth vesicles in the apical area and many mitochondria. A well developed golgi apparatus is associated with the nucleus which contains a prominent nucleolus.

Apart from the mucus secreting- and glandular cells, the epithelium also contains endocrine cells. The endocrine cells

are scarce and are similar to the type II gastric endocrine cells identified by Ezeasor (1981) in trout.


Figure 4.3 Transmission electron micrograph showing epithelial absorptive enterocytes in the mucosa of the first gut segment of juvenile Clarias gariepinus. The densely packed microvilli (mv) protruding into the lumen can be seen clearly. Underlying this is the terminal web area (tw) which is relatively free of organelles. An apical (ap) pit is present at the base of the microvilli. Epihelial cells are attached to the basement memrane (bm).

Histologically, the intestine of C. gariepinus can be divided lengthwise into three regions (Stroband and Kroon, 1981): The first segment is recognized by the absence of pinocytotic vesicles in the absorptive enterocytes (epithelial cells) and abundant cholymicrons are associated with the smooth and granular ER as well as with the Golgi bodies. Cholymicrons are also found in the intercellular spaces of the enterocytes. The cellular function of this segment is, therefore, mainly that of lipid absorption. The enterocytes of the second section have a predominance of pinocytotic vesicles which is indicative of massive pinocytotic activity. Cholymicrons are absent here, but a few fat droplets are present in the cytoplasm. The third (terminal) segment does not have the characteristics of the first and second segments; its enterocytes lack lipid droplets, cholymicrons, and pinocytotic vesicles. The microvilli are relatively short and less densely packed. As with other teleosts, food absorption does not seem to be an important function in the cells of the hindgut.

The nodules of pancreatic tissue (Brockman bodies) typically consist of two tissue types (Figure 4.5); the pale endocrine cells and the dark exocrine cells or acinar cells. The cytoplasm of acinar cells is characterized by dense granular ER, high concentrations of ribosomes, prominent mitochondria and dark (electron dense) zymogen granules (Figure 4.6). The large number of zymogen granules that in the acinar cells are indicative of digestive enzyme secretions. One can, therefore, assume that the exocrine pancreatic tissue of C. gariepinus functions in much the same way as in other animals. The typical process of digestive enzyme and enzyme precursor formation by acinar cells were demonstrated by Caro and Palade (1964) in a radiolabelling study: The proteins are synthesized in the areas of dense granular ER and transported to the Golgi apparatus where they are packed into zymogen granules. Finally, the zymogen granules move to the cell apex where release into the glandular lamina occurs upon stimulation.

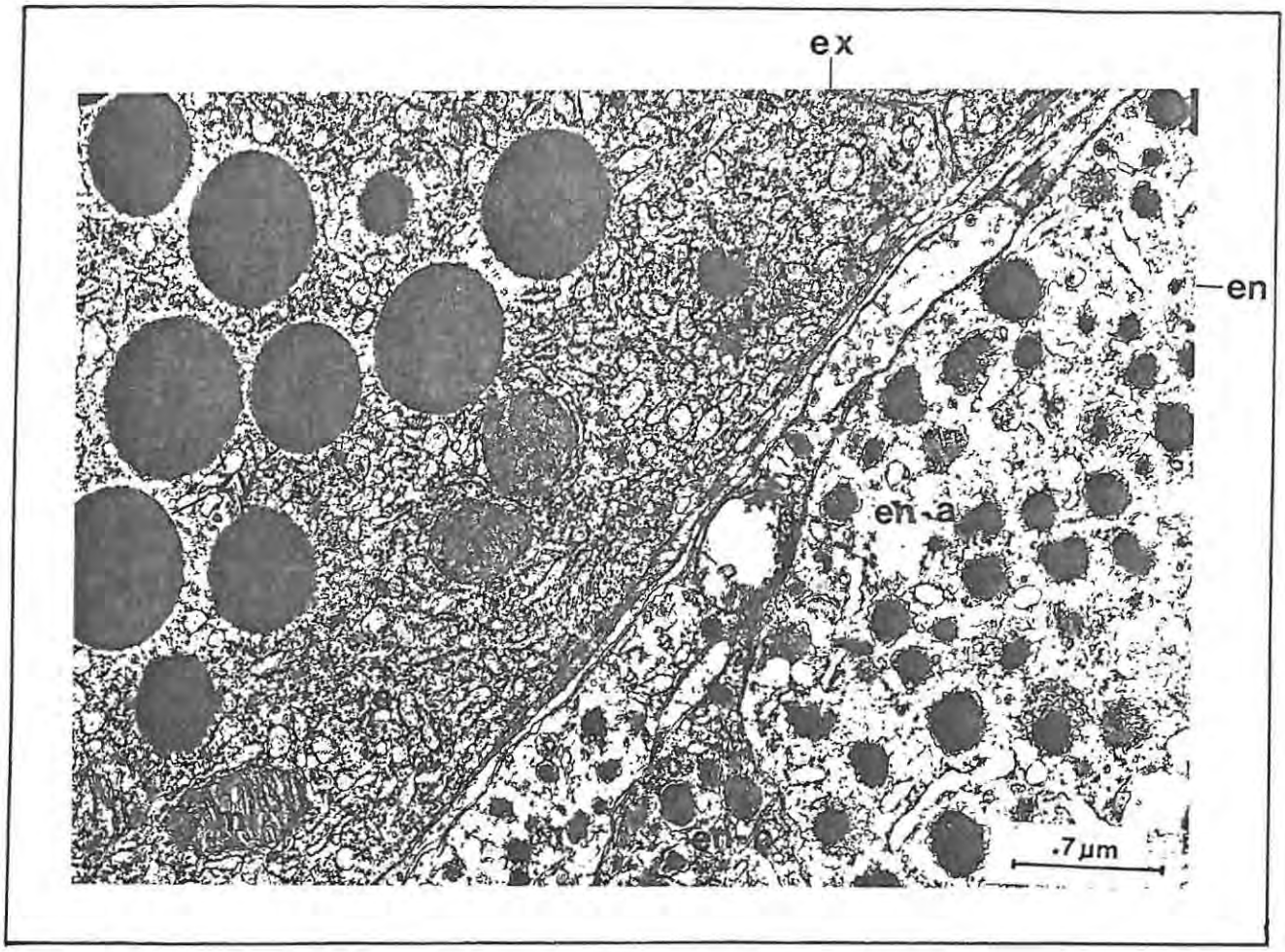


Figure 4.5 Transmission electron micrograph showing comparison of exocrine (ex) and endocrine (en) cells in the pancreatic tissue of juvenile Clarias gariepinus. Two endocrine cells are visible; and alpha cell (en-a) and a beta-cell (en-b), both containing secretory granules. Zymogen granules (zy), mitochondria (mi) and dense granular ER (re) are present in the exocrine (acinar) cell.

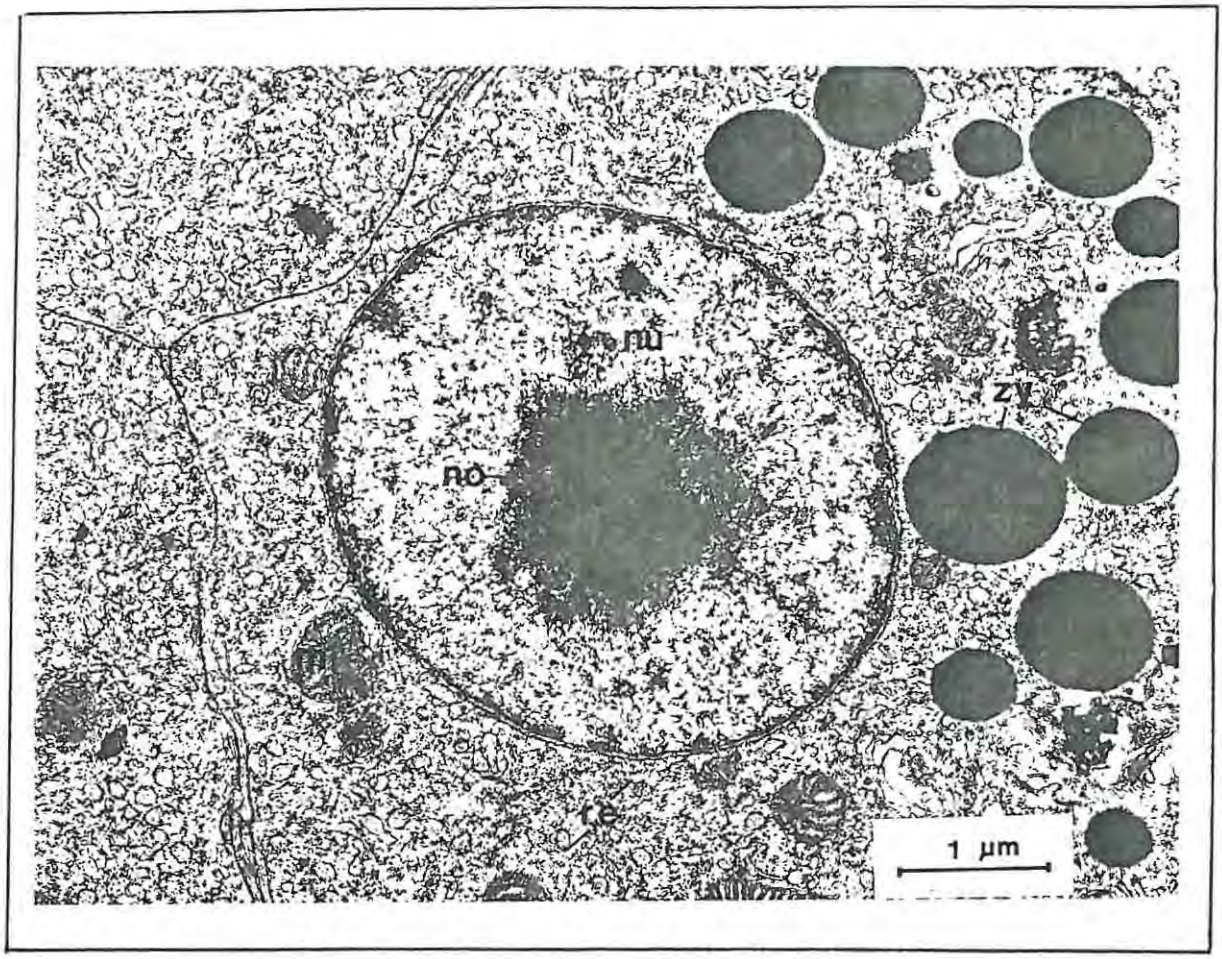


Figure 4.6 Transmission electron micrograph of an acinar cell in the pancreatic tissue of juvenile Clarias gariepinus, showing circular nucleus (nu), prominent nucleoli (no), electron dense zymogen granules (zy), mitochondria (mi) and dense, granular ER (re).

MORPHOLOGICAL DEVELOPMENT OF DIGESTIVE SYSTEM

At the onset of exogenous feeding in C. gariepinus larvae, (50-80hrs. post-hatching), the digestive tube is simple, and no stomach is evident. On about the fifth day (depending on temperature), an incipient stomach develops in the most caudal part of the "esophagus". This is characterized by the formation of oxyntic glands in the thickened mucosa (Stroband and Kroon, 1981; this study) and also by the presence of peptic activity in the digestive tract (Chapter 5). By about the 10th day, the stomach is macroscopically distinct (this study) and according the Stroband and Kroon (1981), the pyloric region of the stomach

is histologically fully developed by approximately the 15th day. After this stage, no important changes seem to take place in the morphology of the digestive tract. The relative length of the third intestinal segment is shorter in adults, but the relative lengths of the other segments, and the overall RGL, remain more or less the same throughout its life (Stroband and Kroon, 1981). In most other fish species, however (Stroband and Dabrowski, 1981), the RGL increases with age, and is probably related to a gradual change in feeding habits.

A striking feature of the ontogenetic development of C. gariepinus, is its rapidity. In Coregonus pollan, it takes up to 60 days for the fish to develop a morphologically functional stomach (Dabrowski, 1984). The rapid development of C. gariepinus larvae is in part a reflection of the species' reproductive strategy, which is for the adults to leave their spawn in freshly inundated flood pans. The offspring are then usually faced with a relatively short period of living in the "nursery area", whereupon they have to return to the main water body before the nursery dries up.

## CONCLUSIONS

Al Hussaini (1949a,b) observed that the histology of the teleostean digestive system is one of the simplest among vertebrates. There also seems to be very little interspecific variation in the digestive histology of teleosts (Stroband and Kroon, 1981). The main interspecific differences lie in the gross morphological adaptations (e.g. presence or absence of stomach, RGL etc.) and in the rapidity of the functional development of the digestive organs in early life history stages.

In C. gariepinus the fine structure of the various parts of its digestive system does not seem to differ in any important way from other fish species. This fish has a generalized digestive system which develops relatively fast, and enables it to utilize

a variety of diets efficiently from an early stage. The gross anatomical adaptations are all indicative of this species being an opportunistic, omnivorous predator (which is in agreement with the conclusion drawn in the other chapters of this work).

# CHAPTER 5

# DIGESTIVE ENZYME STUDIES

## INTRODUCTION

The ability of an organism to digest a given substance is predominantly dependent on the presence of appropriate enzymes, and on whether the required conditions for the functioning of those enzymes exist (Smith, 1980). This premise implies that the nutritional requirements of animals are to some extent reflected in their digestive enzyme patterns and that problems with feed formulation can perhaps be solved more readily by first investigating nutritional processes at a more fundamental (i.e. enzymatic) level. This approach was advocated by Dabrowski and Glogowski (1977a,b) and by Dabrowska *et al.* (1979). In fact, much has since been learnt of digestion in fish by studying fundamental physiological systems (Dabrowski, 1984; Lauff and Hofer, 1984). It is within this premise that the experiments described in this chapter were undertaken.

Only a single reference to digestive enzymes of *C. gariepinus* could be found (Cockson and Bourne 1972). They did a comparative study on the proteolytic and amylolytic activities in the stomach and intestine of catfish and tilapia and also characterized these enzymes in terms of their pH dependence (this work is referred to again later).

There are, however, numerous reports in the literature on the digestive enzymes of other species representing several families of marine and fresh water fishes. Several of these works are concerned mainly with the identification of digestive enzymes and their distribution in the alimentary canal of various fish species (Kawai and Ikeda, 1971; Cockson and Bourne, 1973; Overnell, 1973; Olatunde and Ogunbiyi, 1977; Yoshinaka *et al.*, 1984; Bitterlich, 1985). From these studies, it became evident

that fish digestive enzyme systems do not differ much from other monogastric vertebrates in general. In some studies attention was given to the correlation of digestive enzyme activities with the feeding habits of the fish. In most cases positive correlations were, indeed, found i.e. carnivorous fishes have higher proteolytic enzyme activities than herbivorous fishes, which in turn have higher carbohydrase activities (Fish, 1960; Nagayama and Saito, 1968; Hsu and Wu, 1979; Hofer and Schiemer, 1981; Hofer et al., 1982; Jonas et al., 1983; Kirilenko and Chigrinzkaya, 1984). Some of the above investigators, as well as Onishi and Murayama (1969), Jany (1974) and Cohen et al. (1981a) also determined the pH dependence of the digestive enzymes they worked with.

Seasonal variations in digestive enzyme activities were shown to occur in several fish species (Ananichev, 1959; Gelman et al., 1984). This is not surprising, since acclimation temperature (Hofer, 1982; McLeese & Stevens, 1982) and food availability (Kirilenko and Chigrinzkaya, 1984; Takii et al., 1985; Jobling, 1986) were shown to affect digestive enzyme secretions. It is also important to note that the digestive enzyme patterns of fish change along with ontogenesis, especially in the early life history stages (Kawai and Ikeda, 1973; Stroband and Dabrowski, 1981; Dabrowski, 1984; Lauff and Hofer, 1984; Lindsay, 1985; Baragi and Lovell, 1986). These changes can be explained in terms of functional morphological development, and they result in changes in dietary requirements of the growing fish (Stroband and Dabrowski, 1981; Dabrowski, 1984).

Since ration and feeding frequency can strongly influence the growth rate and conversion efficiency of cultured fish (Dupree, 1984; Lovell, 1980; Singh & Srivastava, 1984, 1985), the opinion has been expressed that more attention should be given to elucidating the physiological basis for the relationship between feeding frequency and feed utilization (Foltz, 1984). A means of studying this physiological premise is to determine the

rhythmicity of digestive and other metabolic processes in relation to feeding frequency. De Silva and Perera (1983, 1984) observed that the digestibility of dry matter and protein in two cichlid species varied from day to day, depicting a rhythmic pattern. De Silva (1985), subsequently found that feeding Oreochromis niloticus alternately on high and low protein content feeds resulted in better protein utilization, and therefore, reduced feeding costs.

Work on the response of digestive enzyme activity to feed ingestion has also elucidated the relationship between temporal digestive processes and feeding frequency in fishes: Onishi et al. (1973a,b, 1976) investigated the sequence of digestive enzyme levels in Cyprinus carpio after feeding. Their results have shown that there is a five hour delay between feed ingestion and peak digestive enzyme activity. Takii et al. (1985) observed changes in digestive enzyme activities in eels (Anguilla japonica) after feeding, and found that protease activities in the intestinal content reached a peak after 5 hours. Amylase had a slower response time, its activity increasing up to the 12th hour after feeding. The above observations suggest that infrequent meals will result in poor food utilization due to the slow secretory response of digestive enzymes in these fishes.

The stated premise that digestive enzyme patterns in fish reflect their nutritional requirements is somewhat complicated by the fact that exogenous enzymes, originating from live food organism which are ingested, can play an important role in a fish's ability to digest its food (Reichenbach-Klinke, 1972; Dabrowski & Glogowski 1977a,b). It seems that not only can exogenous enzymes act as activators of fish zymogens (Jancarik, 1964), but they can also complement a fish's digestive capabilities (Dabrowski, 1984). Moreover, enzyme inhibitors such as soybean trypsin inhibitor (Kalac, 1978; Cohen et al., 1981b) and the presence of tapeworm, Botriocephalus sp. (Reichenbach-Klinke, 1972; Matskasi, 1978 quoted in Dabrowski, 1982) were shown to inhibit

endogenous enzyme (trypsin) activity. Also, Hofer and Sturmbauer (1985) showed that wheat can inhibit amylase activities in carp and trout.

In this study, assays were run to establish the presence of certain digestive enzymes and to determine their relative activities and distribution in the digestive tract and associated organs of C. gariepinus. The important enzymes were partially characterized by establishing their temperature and pH dependent profiles, and the secretory response time of sub-adults was studied by measuring digestive enzyme levels at intervals after feeding. The development of digestive enzymes patterns along with fish ontogeny was also investigated and, finally, an attempt was made to establish the contribution of exogenous (prey) enzymes to the fish's digestive processes. Enzymes which were investigated are combined (alkaline) proteases, acid protease (pepsin), trypsin, chymotrypsin, amylase, alkaline phosphatase, lysozyme and cellulase. The reasons for choosing each of these enzymes are discussed in the following paragraphs:

In fish that possess a true stomach, protein digestion occurs in two distinct phases, viz. gastric and intestinal (Ash, 1985). Proteolytic activity starts in the stomach where pepsin (an endopeptidase) acts on non-terminal peptide bonds. Pepsin, secreted in zymogen form (Kapoor et al., 1975), is activated by HCl and is only effective in an acid environment. Once the food passes into the intestinal lumen, which has a neutral or alkaline pH, other endopeptidases (trypsins and chymotrypsins) as well as ectopeptidases, tri- and dipeptidases cause further cleavage of proteins and peptides (Ash, 1985). "Combined protease" was chosen, since the assay technique is simple and at a neutral or alkaline pH, it demonstrates the combined activity of the proteolytic enzymes which occur in the intestinal lumen. With a small adaptation, the same technique could be used for assaying pepsin activity. With pepsin being the key enzyme of gastric proteolysis, trypsin and chymotrypsin were chosen as specific

enzymes representative of intestinal proteolysis. Moreover, these four groups of enzymes (alkaline proteases, pepsin, trypsin and chymotrypsin) are well covered in the literature dealing with the digestive enzymes of other fish species.

Amylase was chosen since it is a key enzyme in carbohydrate digestion. It is responsible for the hydrolysis of starch or glycogen, which are important fuel molecules of animals and plants. The utilization of starch and other carbohydrates is still relatively poorly understood in fish nutrition (NRC, 1983), and different fish species show variation in their abilities to utilize starch. Pieper and Pfeffer (1979), listed "insufficient enzymatic break-down in the digestive tract", as one of three possible reasons why carbohydrate is poorly utilized by fish. Since starch is one of the least expensive energy sources in practical feeds, and since it is an important binder in the extrusion process of manufacturing fish feeds, it was thought necessary to determine whether C. gariepinus has sufficient amylase activity to digest this nutrient. Amylase acts on the glucosidic bonds of polysaccharides (starch or glycogen) to produce smaller oligosaccharides. It produces first dextrins, which are secondarily cleaved to yield maltose (87%), glucose (10%) and branched oligosaccharides (3%).

Lipids, waxes and other esters are split by digestive enzymes that belong to the group of serine esterases. These esterases have turned out to be more diverse than probably any other enzyme system and are difficult to categorize due to their overlapping substrate specificity. Esterases are involved in many other physiological processes in vertebrates, but they are omnipresent in animal digestive tracts (Gordon et al., 1977). Indeed, alkaline phosphatase (a monophosphate esterase) has been associated with the digestion and absorption of high energy metabolites (lipids and sugars) in several fish species (Overnell, 1973; Fraisse et al., 1981; Gelman et al., 1984). Although the specific role of alkaline phosphatase in digestion

is not well understood, it was elected as a representative esterase for this study, since the occurrence of high activities of this enzyme in the intestine of other fish species, including Clarias batrachus (Shaffi et al., 1974), implies its importance as a digestive enzyme.

Lysozyme, which hydrolyzes bacterial cell walls, is normally not associated with nutrition in higher animals, but was chosen as an enzyme to be investigated, since catfish often ingest detritus and partially decomposed material which contain large quantities of bacteria (Bowen, 1976; Hofer et al., 1982). Lysozyme is a widely occurring hydrolase in invertebrates as well as vertebrates. In the latter it is normally associated with defense against bacterial invasion and its distribution patterns in fish, particularly, suggest a defensive rather than digestive function (Lindsay. 1986). It was thought that this premise should be tested for C. gariepinus.

Finally, cellulase was chosen since fish (and for that matter all vertebrates) are presumed to lack the ability to form this enzyme (Stickney and Shumway, 1974) and its presence would indicate that exogenous enzymes or enzymes from bacterial flora contribute towards the digestive capabilities of the fish.

## MATERIALS AND METHODS

### A note on terminology

In this study, as is the case with the majority of other works dealing with fish digestive enzymes, no attempt was made to unequivocally identify the specific enzymes which were encountered. Enzymes were named, for the sake of clarity, according to the reactions they catalyzed, the conditions under which they functioned optimally, and the nature of their substrates. For instance; if an enzyme (or perhaps group of enzymes) was found to catalyze a pepsin-like reaction it was named pepsin, even though it might not have been the exact enzyme

"pepsin" which is well characterized and assigned the international Enzyme Commission number, EC3.4.23.1

Experimental animals

Two hundred yearling catfish (350-600g body weight, origins described in Chapter 2) were kept in two circular plastic pools of 2m diameter and 1m depth, in the experimental recirculating fish culture system at Rhodes University (Figure 2.1, Chapter2). The fish were acclimated for three months and during this time were fed twice a day on 38% crude protein (Epol) trout pellets at a ration of 4% of body weight per day. Water temperature was ambient at 23 $\pm$ 2.5°C. This population was used for all of the assays, except for the investigations on the development of digestive enzyme patterns during the early life history, and the possible contribution of exogenous enzymes. For these latter experiments, fertilized eggs as well as larvae and juveniles of various age groups (1-25 days) were translocated from the Blyde River Hatchery to the Rhodes University Hatchery (Figure 2.4, Chapter 2). Here, they were kept for three days, in separate containers and under different feeding regimes as shown in Table 5.1, before being sacrificed.

Sample preparation

Fish were sacrificed four hours after feeding, since it was established that peak digestive enzyme activities occurred at this time. In each case at least four fish were sacrificed for replicate samples for each condition. Their alimentary tracts and associated organs were quickly excised and placed on ice. Ingested food was collected from the stomach, foregut, midgut and hindgut. Tissue samples were collected from the pancreas and liver. Bile was collected by piercing and draining the gall bladder with a hypodermic syringe. Care was taken to prevent contamination between samples. All the samples except the bile were homogenized in ice cold, distilled water with a Potter-Elvehjem homogenizer, followed by centrifugation at 7000 x g for 5 minutes. Each supernatant was then diluted with an appropriate buffer solution and kept on ice until the assays were run shortly afterwards. Bile samples were assayed without dilution. Buffer solutions were prepared in accordance with the specifications for their use in enzyme studies by Gomori (1955). With the last experiments, where the fish were less than 16 days old (Table 5.1, groups 1-6), samples for assays were prepared by homogenizing four replicate 1g samples of whole fish from each group (it was impractical to dissect out digestive organs from these small fish). In the fish that were 16 and 28 days old (groups 7-9), the alimentary tracts with their associated organs were dissected out for sample preparation. The organs were pooled in order to obtain four replicate 1g samples. In order to tie in the digestive enzyme activities as determined in whole fish, with the activities determined in alimentary tracts alone, four samples were also prepared from whole, 16 day old fish.

Protein determination

Since specific enzyme activity is often expressed in terms of activity per unit weight of protein in the sample, part of each sample was analyzed for protein content, by either the "standard" or "microprotein assay" methods proposed by Bradford (1976). Her methods were chosen in preference to the more commonly used

Biuret or Folin phenol methods. All of the above methods are subject to interference and inaccuracies and all of these methods were initially used in this study. However, the Bradford (1976) -method was found to yield the most reproducible results with the relatively small samples which were available and the latter two techniques were subsequently discarded. The Bradford (1976) protein determination method involves the binding of Coomassie Brilliant Blue G-250 to protein (termed protein dye-binding) which causes a shift in the absorption maximum of the dye from 465 to 595 nm. The increase in absorption at 595 nm is directly equivalent to total protein concentration. This technique also eliminates some of the problems encountered with the Biuret and Folin-phenol methods in that it is more sensitive, faster and less subject to interference from amino acids, carbohydrates and certain laboratory chemicals (Bradford 1976; own obs.). The major drawback with the Bradford (1976) method, is variation in response with different protein types (Kresze, 1981).

The procedures for the Bradford (1976) determination of protein were as follows: 100g Coomassie Brilliant Blue G-250 (Sigma) was dissolved in 50ml 95% ethanol. To this solution was added 100ml 85% (w/v) phosphoric acid. The resulting solution was diluted with distilled water to a final volume of 1 liter. Five ml of this reagent solution was added to each 100ul of sample containing 10 to 100ug of protein (standard protein assay), or 1 ml of reagent was added to each 100ul of sample containing 1 to 10 ug of protein (microprotein assay). The absorbance at 595nm was measured after 2 min. and before 1 hr. against a blank containing 100ul buffer and 5 ml reagent. Standard calibration curves were plotted with bovine serum albumin (Sigma).

Enzyme assays

All the assay techniques were based on photometric procedures in which the rate of disappearance of substrate or the rate of

formation of product was measured. A Shimadzu, double beam spectrophotometer (model UV 150-02) was used. For measurements in the UV-range, 3ml, quartz cuvettes were used and for measurements in the visible light spectrum, disposable 3ml and 1ml cuvettes were used.

An enzyme catalyzed reaction is characterized by a linear phase, or phase of zero-order kinetics, during which time the rate of the reaction is directly equivalent to the concentration of the enzyme under a given set of conditions, or directly equivalent to the activity of the enzyme at a given enzyme concentration. Measurements were taken during linear phases of reactions to insure accurate assays. The measurements were taken either as discrete readings at intervals during an "in cuvette" reaction, or as "control" and "sample" measurements in cases where larger reaction containers were required. In the latter case, the reaction was arrested at a given time by adding a debilitating substance, and zero order kinetics were verified by repeating duplicate tests with different reaction periods. Due consideration was always given to the possibility of interfering reactions by setting up the necessary control tests.

Combined proteolytic enzymes

General proteolytic activity was measured using Walter's (1984) modification of the Anson (1939) method. This method was chosen for its simplicity and adaptability. Variations of the Anson (1939) method were used in several other fish digestive enzyme studies (Jany, 1974; Dabrowski, 1982; Hofer et al., 1982; Jonas et al., 1983). The principle of this assay technique is that the substrate, hemoglobin, is converted to peptides and free amino acids. The rate at which tyrosine (measured directly at 280nm) is liberated from the substrate, is taken as a measurement of the proteolytic activity. Hemoglobin was used because it is a reproducible substrate, and different batches are digested at the same rate by a given protease solution. The substrate solution was prepared by suspending 1g of bovine hemoglobin substrate

powder (Sigma) in 20ml of distilled water. To this was added 18g of urea and 4ml of 1M NaOH to denature the protein. After 1 hour, 5ml of 1M phosphate buffer was added. The resulting solution was acidified to pH 7.5 with 1N HCl and diluted to 50ml with distilled water. The procedure was initiated by equilibrating all solutions to 37°C, whereupon 2.5ml of substrate and 0.2ml of sample supernatant were allowed to react for exactly 10min. at 37°C. The reaction was arrested by addition of 5ml of 0.3M trichloroacetic acid, which caused the protein to precipitate. The mixture was centrifuged (4000 x g, 20min.) to obtain a clear supernatant, which was read at 280nm against distilled water. Blank samples were prepared in the same manner, but by adding the sample supernatant after the trichloroacetic acid, instead of before, thereby preventing any enzyme reaction from taking place. Calibration curves were prepared for each batch of reagents, by using tyrosine standard solutions (1-10mmol/liter) instead of sample solutions. Activities were expressed as mg of tyrosine liberated from hemoglobin by 1g of sample material in 10 min. at 37°C.

Acid protease or pepsin

The same procedure as combined protease was used, except that the reaction mixtures were acidified with HCl to pH 4 (or lower, depending on the purpose of the assay). This is the Anson (1939) method as modified by Ryle (1984) for the estimation of pepsin.

Trypsin

Trypsin activity was determined using p-toluenesulphonyl-L-arginine methyl ester (TAME) as a substrate (Hummel, 1959). This substrate has the advantage of high sensitivity and high selectivity towards trypsin. The substrate solution consisted of $1.04 \times 10^{-3}$ mol TAME (Sigma), 0.04 mol Tris(hydroxymethyl) aminomethane, 0.01 mol CaCl2, adjusted to pH 8.1 with HCl and diluted to 1 liter with distilled water. The assays were conducted directly in the cuvettes. Three ml of substrate solution was placed in each of the blank and sample cuvettes and

equilibrated at 30°C. To the blank cuvette was added 150ul of distilled water and to the sample cuvette was added an equal volume of sample supernatant. After each addition the contents of each cuvette was agitated for 10sec with a small spatula. The initial absorbance difference at 247nm (1cm light path) was set to read zero, and the change in absorbance was measured at 30sec. intervals. Under these standard conditions, trypsin activity was expressed in terms of change in absorbance units per minute caused by 1g of wet sample material.

Chymotrypsin

Chymotrypsin was assayed using N-benzoyl-L-tyrosine ethyl ester (BTEE) as a substrate which has a high sensitivity and selectivity towards chymotrypsin (Hummel, 1959). This procedure is analogous to the trypsin assay and the same procedure was followed except that the substrate solution consisted of $5x10^{-4}$ mol BTEE(Sigma), 0.035 mol Tris (hydroxymethyl) aminomethane, 0.05 mol $CaCl_2$ and 256g methanol, adjusted to pH 7.8 with HCl and diluted to 1 liter with distilled water. The change in absorbance per minute at 256nm (1cm light path) with 3ml substrate and 0.1ml sample supernatant at 30°C was used as a direct measure of chymotrypsin activity.

Amylase

Amylase activity was assayed according to the Bernfield (1955) method in which the increase in reducing power of a buffered starch solution was measured with 3,5-dinitrosalicylic acid at 540 nm. This method was chosen since it is rapid, sensitive and was favoured by other investigators working on fish digestive enzymes (Overnell, 1973; Mukhopadyay, 1977). The substrate solution was prepared by dissolving 1g of soluble starch (Sigma) in 100ml of 0.02M phosphate buffer (pH 6.9). The reagent mixture was prepared by dissolving 1g of 3,5-dinitrosalicylic acid in 20ml of 2N NaOH. To this was added 50ml of distilled water and 30g of Rochelle Salt. Once all the constituents had dissolved, the mixture was made up to 100ml with distilled water. The

procedure for the assay was to add 1ml of sample supernatant to 1ml of substrate solution, which was then incubated in a water bath at 25 or 37°C for exactly 3 min. The reaction was arrested by adding the reagent mixture and heating the resulting mixture in boiling water for 5min. After cooling with running tap water, the optical density was read at 540nm against a blank. A blank was prepared for each sample by first deactivating the sample supernatant through heat treatment and then following the same procedure as for the active sample. A calibration curve was established for each batch of solutions with a series of maltose solutions (0.2 to 2mg in 2ml of distilled water). Amylase activity was expressed in terms of mg. of maltose liberated from starch by 1g of sample material in 3 minutes at 25° C and at pH 6.9. Specific activity was expressed as amylase activity per mg of protein in the sample material.

Alkaline phosphatase

Alkaline phosphatase activity was assayed with the aid of a Sigma diagnostic test-kit (Procedure No. 104). The procedure depends upon hydrolysis of p-nitrophenyl phosphate by the enzyme, yielding p-nitrophenol and inorganic phosphate. When made alkaline, p-nitrophenol is converted to a yellow complex readily measured at 400-420nm. The intensity of color formed is proportional to phosphatase activity. Calibration curves were established with p-nitrophenol and activities were expressed in Sigma Units per gram of wet sample material. A Sigma Unit is defined as that amount of enzyme activity that will liberate 1 umol of p-nitrophenol per hour under the conditions specified for the assay. Phosphatase activity in Sigma Units/ml may be converted to International Units (U/l) by multiplying by 16.7.

Lysozyme

Lysozyme activity was determined by measuring the decrease in light absorbance of a suspension of bacterial cells Micrococcus leisodeikticus (Sigma) on which sample supernatant was allowed to act (Jolles, 1962). Unit activity (Sigma Unit) is defined as a

0.001 change in absorption at 450nm per minute at pH 6.4, and 25°C, using a suspension of Micrococcus leisodeikticus, in a 2.6ml reaction mixture (1cm light path). Lysozyme activity was, therefore, expressed as Sigma Units of activity per gram of wet sample material.

pH adjustment of assays and pH measurement of gut contents

The pH dependence of enzymatic activities was determined by adjusting the buffer systems of the reaction mixtures. Enzyme activities were assayed at intervals of 0.2 pH units. The pH values of the stomach and intestinal content were determined with a glass electrode as well as with sensitive pH test strips for comparison with the pH dependence of the various enzymes.

Temperature adjustment of assays

In order to determine the effect of incubation temperature on the various enzyme activities, use was made of thermo-regulated water baths. The effect of temperature was first determined at 2°C intervals and once an indication of optimum temperature was established, assays at 1°C intervals were run around this point to determine more closely the temperature optimum for each enzyme.

Assays for changes in digestive enzyme activities after feeding

The yearling fish in the two circular tanks were starved for 24 hours, whereupon a sample of four fish was collected (0 hour - sample). The fish in one tank were then fed to satiation while the other group remained unfed. Further samples of four fish were taken from each pool at 1, 2.5, 4, 6.5 and 10 hours after feeding. The sampled fish were sacrificed and from each fish, the stomach content, foregut content and pancreatic tissue were collected. Sample supernatants were prepared in the same manner as with the other assays. Pepsin activity was determined in the stomach content samples, while combined protease and amylase activities were determined in the pancreas and foregut samples.

Development of enzyme activities during early life history stages and contribution of exogenous enzymes

A population of cladocerans (mainly Daphnia sp.) which established itself in one of the water conditioning reservoirs at the Rhodes fish culture facility, was found to exhibit high levels of cellulase activity (this study). Since cellulase is not an endogenous enzyme of vertebrates -or is at least unlikely to be- (Yokoe and Yasumasu, 1964), this enzyme was elected as a tracer enzyme for studying the contribution of exogenous enzymes in young C. gariepinus. Therefore, in order to study the development of digestive enzyme patterns in the early life history stages of catfish and to assess the possible contribution of exogenous enzymes, pepsin, combined protease, trypsin, amylase and cellulase were assayed in samples prepared from the fish groups described in Table 5.1 as well as in samples prepared from whole cladocerans.

Cellulase assay technique

Exactly the same procedure as for the determination of amylase was followed, except that a suspension of pure, fine cellulose powder (Sigma) instead of a starch solution was used as substrate, and that the incubation time was increased to 50 min. The principal product of the hydrolyzation of cellulose by cellulase, is cellobiose (a reducing sugar), which in turn is attacked by cellobiase, if present, to yield more reducing sugars, e.g. glucose or smaller saccharide fractions (Halliwell and Halliwell, 1984). Irrespective of the presence of cellobiase, cellulytic activity is characterized by an increase in soluble sugars, some of which are reducing sugars. Cellulase activities were, therefore, expressed in terms of mg of maltose liberated from cellulose by 1g of wet sample material in 50min. at 37°C, and at pH 7.0.

Analyses of data

Means, their standard deviations (SD) and sample size (n) were

calculated for each set of measurements. This allowed computation of the significance for differences between related observations by the t-test (Sokal and Rohlf, 1981). Unless otherwise stated, a 5% significance level (in other words a maximum probability (P) level of 0.05) was accepted for stating differences between means.

## RESULTS AND DISCUSSION

### Distribution of pepsin and combined proteases

The distribution of proteolytic activities is shown in Table 5.2. and is illustrated in Figure 5.1. Tyrosine was liberated most rapidly from the hemoglobin substrate by the foregut content samples (49.6 mg tyrosine liberated by 1g of sample in 10min. at 37°C). The intestine shows a descending proximo-distal gradient in protease activity, with the activity in the hindgut being only 18% of that in the foregut. This is similar to the trend in other fish species (Fish, 1960; Hofer and Schiemer, 1981; Hofer, 1982). According to Hofer (1982), the decrease in proteolytic activity from anterior to posterior intestine cannot be ascribed to autolysis of these enzymes since they are extremely stable in neutral or alkaline solution. He concluded that proteolytic enzymes are re-absorbed in the hindgut by pinocytosis. This mechanism has indeed been shown to exist in mammals (Beynon and Kay, 1976; Diamond, 1978). If the descending proximal-distal gradient in protease activity can be ascribed to the re-absorption process alone, it implies that more than 80% of proteolytic enzymes were re-absorbed in the hindgut of *C. gariepinus*

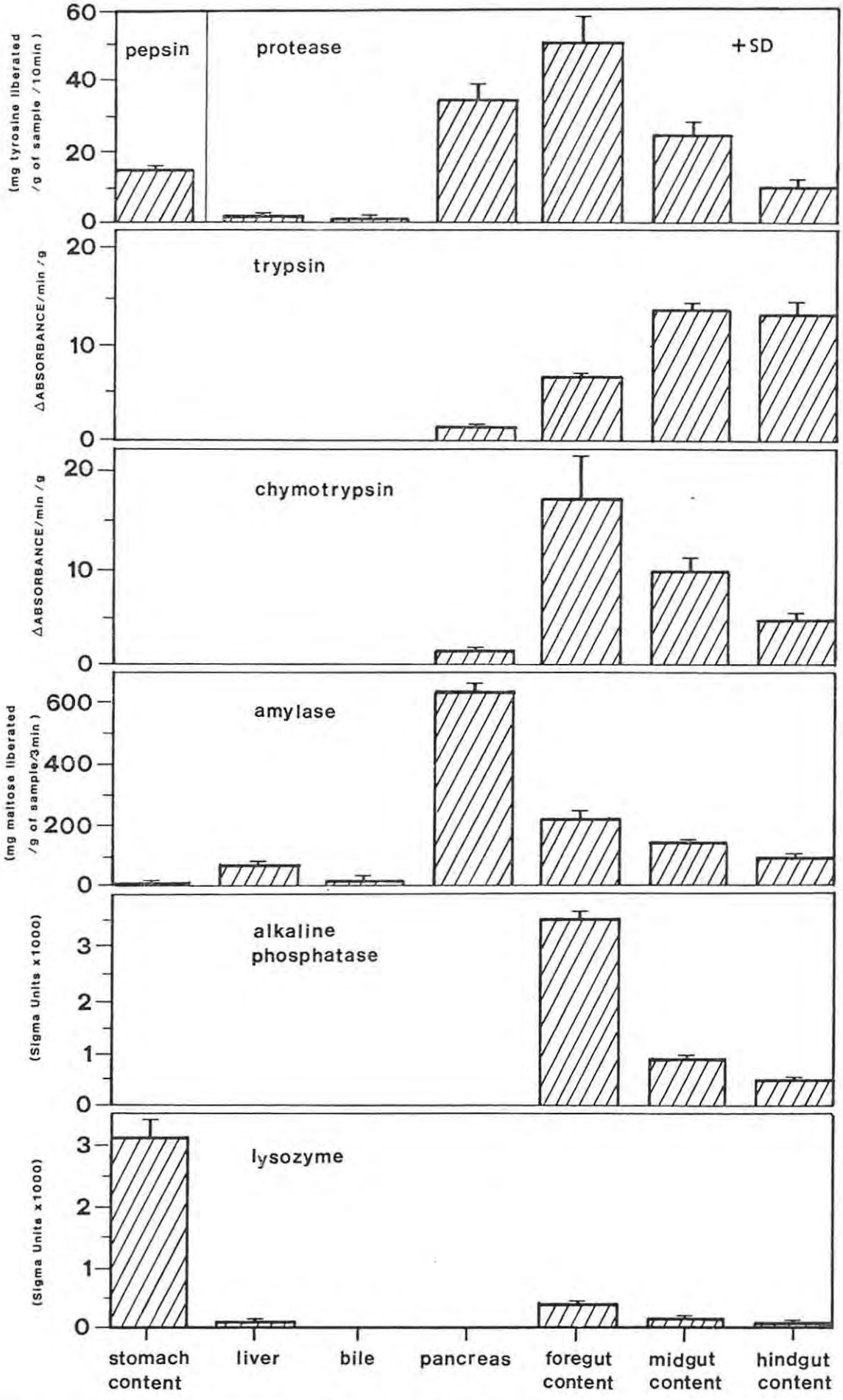


Figure 5.1 Distribution of digestive enzyme activities in the digestive tract and associated organs of Clarias gariepinus.

Mean protease activity in the stomach content was significantly lower ($P<0.01$) than combined protease activity in the foregut content. It should be noted here that the relatively low gastric protease (pepsin) activity in comparison with the intestinal proteases, could be attributed to the nature of the assay. Pepsin is only responsible for the initial and partial hydrolysis of proteins which is subsequently completed by the combined action of other peptidases when the food reaches the intestine (Ash, 1985). Since the assay technique relied on the measurement of liberated tyrosine, which is a final product of proteolysis, this measurement reflected only a portion of the proteolytic activity of pepsin. This argument also applies to the endopeptidases in the foregut lumen. However, since one can expect ectopeptidases and dipeptidases (which complete protein digestion) to be present in this area, free tyrosine should be generated more rapidly. The conclusion is, therefore, drawn that the gastric phase of protein digestion in C. gariepinus is equally as important as the intestinal phase.

Table 5.2
Distribution of proteolytic enzyme activities in the digestive tract and associated organs of Clarias gariepinus.

| Sample material | pH of assay | Protease activity* | | |
|---|---|---|---|---|
| | | Mean | SD | n |
| Stomach content (pepsin) | 3.5 | 14.7 | 1.1 | 4 |
| Liver tissue | 7.5 | trace | - | 4 |
| Bile fluid | 7.5 | 0.5 | 0.2 | 4 |
| Pancreas tissue | 7.5 | 34.0 | 4.8 | 8 |
| Foregut content | 7.5 | 49.6 | 8.9 | 7 |
| Midgut content | 7.5 | 22.9 | 5.3 | 8 |
| Hindgut content | 7.5 | 8.9 | 3.8 | 7 |

*Activities expressed in terms of mg of tyrosine liberated by 1g of wet sample material in 10 min at 37°C.

In contrast to the results of this study, Cockson and Bourne (1972), concluded that gastric protease activity in C. gariepinus was four times higher than intestinal protease activity. They

employed a different assay technique and their samples were prepared from cleansed stomach and intestinal tissue, rather than -content. Since most intestinal proteases originate from pancreatic tissue, and are secreted in zymogen form (Ash, 1985), it is surprising that they found intestinal protease activity with their methods at all. Membrane-bound enzymes and digestive enzymes associated with brush border cells (Fraisse et al., 1981) were probably responsible for the activities Cockson and Bourne (1972) demonstrated.

The second highest protease activities were measured in the samples prepared from pancreatic tissue. Since these enzymes are probably mainly present in zymogen form, and only activated upon secretion into the gut lumen, their actual concentration in the pancreatic tissue is probably much higher than inferred by the measurements of their activity. It is evident, therefore, that the pancreatic tissue is an important, if not the most important source of intestinal proteases.

The gastric protease activity (14.7mg tyrosine liberated per gram of stomach content/ 10min. can, for the sake of comparison, be transformed to roughly 5.6 units increase in optical density/min./100g body weight. This figure is on par with data presented by Hsu & Wu (1979) for other predominant carnivores, Anguilla japonica and Clarias fuscus (respectively 10.1 and 8.4 units increase in O.D./min./100g. B.W.) and it is is considerably higher than the 0.96 units recorded for the more herbivorous Oreochromis mossambicus (Hsu & Wu, 1979). The latter comparison is confirmed by Cockson and Bourne (1972), who, while finding high gastric protease levels in C. gariepinus, detected virtually no gastric protease in the predominantly herbivorous Tilapia shirana chilwae. By now, sufficient evidence exists to sustain the supposition that proteolytic activity is higher in carnivorous than in herbivorous fishes (Fish, 1960; Hofer and Schiemer, 1981; Jonas et al., 1983). Why, then, do herbivorous fishes have lower proteolytic activities, if the supply of

protein in their diets is probably one of their most limiting nutritional factors? Hofer and Schiemer (1981), based on comparative studies, offered the following explanation: Herbivorous fish generally have longer intestines and greater gut-volumes. At the same time they consume more food per day and, consequently, the food passing through the gut of herbivorous or omnivorous species is exposed to much higher proteolytic activity per day, than food in carnivorous species. If these reasons are correct, it is not surprising that C. gariepinus possesses high proteolytic enzyme activities, since it has a relatively short gut (Chapter 4), and it has strong carnivorous tendencies (Chapter 3).

Distribution of trypsin and chymotrypsin

Table 5.3 and Figure 5.1 show the distribution of trypsin and chymotrypsin in the alimentary tract and associated organs of C. gariepinus. No measurable levels were encountered in the stomach, liver and bile, but consistently high levels were found in the intestinal content.

Table 5.3
Distribution of trypsin and chymotrypsin activities in the digestive tract and associated organs of Clarias gariepinus.

| Sample material | Trypsin activity* | | | Chymotrypsin activity** | | |
|---|---|---|---|---|---|---|
| | Mean | SD | n | Mean | SD | n |
| Stomach content | 0 | - | 6 | 0 | - | 6 |
| Liver tissue | 0 | - | 6 | 0 | - | 6 |
| Bile fluid | 0 | - | 6 | 0 | - | 6 |
| Pancreas tissue | 1.33 | 0.20 | 8 | 1.29 | 0.25 | 8 |
| Foregut content | 6.40 | 0.49 | 8 | 16.80 | 4.45 | 8 |
| Midgut content | 13.38 | 0.96 | 8 | 9.50 | 1.37 | 8 |
| Hindgut content | 12.75 | 1.64 | 8 | 4.25 | 0.82 | 8 |

*Trypsin activity expressed in terms of change in absorbance per minute at 247nm, at 30 °C and pH 8.1, caused by 1g of wet sample material.
**Chymotrypsin activity expressed in terms of change in absorbance per minute at 256nm, at 30 °C and pH 7.8, caused by 1g of wet sample material.

Both trypsin and chymotrypsin were relatively inactive (although definitely present) in the pancreas, presumably because they are only activated once they enter the intestinal lumen. In fact, Yoshinaka et al., (1984), using the same assay procedure as in this study, found that trypsin and chymotrypsin activities could only be detected in pancreatic tissue of eel, A. japonica, after activation by the addition of porcine enteropeptidase sic. (enterokinase).

Fish trypsins and chymotrypsins are generally considered to be predominantly of pancreatic origin (Jany, 1974; Hsu and Wu, 1979; Cohen et al., 1981a; Fraisse et al., 1981; Hofer et al., 1982). The results of this study do not indicate any other possible source of tryptic enzymes in C. gariepinus. In contrast to the other enzyme distributions, trypsin did not show a decreasing proximo-distal gradient in the intestine. In fact its mean activity was significantly lower ($P<0.01$) in the foregut than in the midgut or hindgut. The reason for this is not readily apparent, since the post-secretion activation times of trypsin and chymotrypsin are usually not much different (Overnell, 1973),

Hsu & Wu (1979), working on a variety of freshwater fish species, concluded that trypsin plays a much less significant role in protein digestion than chymotrypsin and that only in omnivores, such as Oreochromis mossambicus do trypsin activities reach significant levels. On the other hand, Jonas et al. (1983), showed that trypsin activity in the carnivorous sheatfish (Silurus glanis) is four times higher than chymotrypsin activity. The situation is reversed in the omnivorous C. carpio and the herbivorous Hypophthalmichthys molitrix, where chymotrypsin activities were found to be almost four times higher than trypsin activities (Jonas et al., 1983). Due to the conflicting evidence, it does not seem meaningful to correlate trypsin-chymotrypsin ratios with feeding habits (and, therefore, dietary requirements). However, what does seem meaningful, is that total tryptic and chymotryptic activities in carnivorous fishes is

higher than in omnivores or herbivores (Hofer et al., 1982; Bitterlich, 1985). In C. gariepinus both these enzymes are highly active and reflect its ability to deal with a carnivorous diet.

Distribution of amylase

Table 5.4 and Figure 5.1 show the amylase activities in various parts of the alimentary tract and associated organs. The highest levels were encountered in the pancreas, which is presumed to be the principle site of origin. This also verifies that amylase is secreted by the pancreatic tissue in its active form and not as a zymogen (Grant et al., 1971 quoted in Overnell, 1973). Relatively low, but distinct levels were detected in liver tissue and bile. Although this does not seem to have any major significance, it has been speculated that enzymes of pancreatic origin may somehow find their way into bile (Kapoor et al., 1975). The slight incidence of amylase activity in the stomach is probably a result of reflux from the foregut.

Table 5.4
Distribution of amylase activities in the digestive tract and associated organs of Clarias gariepinus.

| Sample material | pH of assay | Total activity* | | | Specific activity** | | |
|---|---|---|---|---|---|---|---|
| | | Mean | SD | n | Mean | SD | n |
| Stomach content | 4.0 | 0.0 | - | 8 | | | |
| Stomach content | 6.9 | 2.8 | 2.3 | 8 | | | |
| Liver tissue | 6.9 | 65.2 | 13.3 | 8 | | | |
| Bile fluid | 6.9 | 24.1 | 3.7 | 8 | | | |
| Pancreas tissue | 6.9 | 623.1 | 44.1 | 12 | 9.58 | 0.36 | 8 |
| Foregut content | 6.9 | 210.0 | 32.0 | 8 | | | |
| Midgut content | 6.9 | 124.4 | 13.6 | 6 | | | |
| Hindgut content | 6.9 | 68.8 | 37.2 | 6 | | | |

*Total amylase activity expressed in terms of mg of maltose liberated from starch per 1g of wet sample material in 3 minutes at 25 °C.

**Specific amylase activity expressed in terms of mg of maltose liberated from starch per mg of protein in supernatant in 3 minutes at 25 °C.

The distribution of amylase in C. gariepinus as shown in Table 5.4 agrees with the work of Fänge and Grove (1979) and Fraisse et al. (1981) who have shown that starch digestion and glucose absorption occurs mainly in the anterior part of the intestine of fishes which possess a stomach, and that there is a decreasing gradient in amylase activity from the anterior towards the posterior part of the intestine. Agrawal et al. (1975) showed that amylase activity in Clarias batrachus is centered in its pancreas and Mukhopadyay (1977) reported high amylase activity in the intestine of the same species. The single exception to this pattern was reported by Cockson and Bourne (1972) who detected equal amylase activities in C. gariepinus stomach tissue, foregut tissue and hindgut tissue. Since the diffuse pancreas of C. gariepinus is also embedded in the mesenteries attached to its stomach, their samples probably included significant amounts of pancreatic nodules to cause this apparent discrepancy. It should be noted that these pancreatic nodules do not duct into the stomach.

Caution should be exercised when comparing results from different laboratories, since variations in assay techniques are commonplace (Kuz'mina, 1985) and diet composition (Nagase, 1964; Jancarik, 1964) and acclimation temperature (Hofer, 1979; Coetzee, 1982; McLeese and Stevens, 1982) influence digestive enzyme secretions. Nevertheless, the amylolytic activities which were demonstrated, are seemingly higher than those shown by similar assay procedures in omnivorous fish species such as C. carpio (Kawai and Ikeda, 1971) and even higher than for some herbivorous species such as Hypophthalmichthys molitrix (Kirilenko and Chigrinzkaya, 1984) and Tilapia nilotica (Nagayama & Saito, 1968).

Since differences in amylase activity between fish species reflect differences in feeding habits (Kitamikado & Tachino, 1960; Nagayama & Saito 1968; Hsu & Wu, 1979; Hofer et al. 1982), it can be assumed that starch plays an important role in the

natural diet of Clarias gariepinus. It also implies that starch can be well utilized as an energy source in practical diets for this species. Carnivorous fishes are ill equipped to utilize native starch as an energy source in their diet (Bergot, 1979; Cowey and Sargent, 1979). However, in omnivorous and herbivorous fishes which can utilize starch as an energy source (Anderson et al., 1984), sufficient inclusion of this relative inexpensive nutrient may have a "protein sparing effect" in practical diets (Halver, 1972; Degani and Viola, 1987).

Distribution of alkaline phosphatase

The distribution of alkaline phosphatase activities is shown in Table 5.5 and Figure 5.1. The relatively insignificant activities which were shown to occur in the liver and pancreatic tissues, probably resulted from blood serum phosphatase. Very high activities were found in the intestinal content, with a typical decreasing proximo-distal gradient being evident.

Table 5.5
Distribution of alkaline phosphatase activities in the digestive tract and associated organs of Clarias gariepinus.

| Sample material | Total activity* | | | Specific activity** | | |
|---|---|---|---|---|---|---|
| | Mean | SD | n | Mean | SD | n |
| Stomach content | 0.0 | - | 7 | | | |
| Liver tissue | 1.2 | 0.8 | 8 | | | |
| Bile fluid | 0.0 | - | 8 | | | |
| Pancreas tissue | trace | - | 8 | | | |
| Foregut content | 3485.4 | 127.3 | 7 | 282.2 | 24.4 | 7 |
| Midgut content | 868.4 | 68.9 | 8 | 248.0 | 56.3 | 8 |
| Hindgut content | 429.6 | 79.0 | 7 | 188.3 | 31.0 | 7 |

*Total alkaline phosphatase activity expressed in Sigma Units per 1g of wet sample material (Sigma Units/ml may be converted to International Units (U/l) by multiplying by 16.7).
**Specific alkaline phosphatase activity expressed as Sigma Units per mg of protein in sample supernatant.

The distribution of alkaline phosphatase, as seen in Table 5.5 is in accord with distributions of this enzyme found in Clarias batrachus (Shaffi et al., 1974), in Cyprinus carpio, and in the

catfish, Ameiurus nebulosus (Fraisse et al., 1981). The latter investigators, however, concentrated specifically on enterocyte-bound enzymes. Since Overnell (1973), working on cod (Gadus morhua), used the same assay technique as in this study, a meaningful comparison can be made. The specific alkaline phosphatase activity in total pyloric caeca extract of cod was given as 100 nanomoles p-nitrophenylphosphate liberated per minute per mg of protein in extract. This is equivalent to six Sigma Units /mg protein in sample. In this study, a much higher mean level of 282 Sigma Units/ mg protein in foregut content was demonstrated.

There are several reports in the literature on alkaline phosphatase activities in piscine digestive tracts and their associated organs (Overnell, 1973; Shaffi et al., 1974; Fraisse et al., 1981; Gelman et al., 1984). However, none of the authors did more than speculate on the exact digestive function of this esterase. That it is involved in food digestion in C. gariepinus is most certain, since its distribution pattern is consistent with that of an extracellular digestive enzyme (high levels in gut lumen, decreasing proximo-distal gradient). This is in contrast to the distribution of this enzyme in cod (Overnell, 1973) where the intestinal activity levels are not significantly higher than levels in the tissues of associated organs. It can, therefore, be inferred that alkaline phosphatase in cod plays a predominantly intracellular role. Distribution patterns similar to those in this study were detected in C. batrachus by Shaffi et al. (1974) and they speculated that alkaline phosphatase mediated the absorption of sugars, fatty acids and other metabolites through phosphorylation and dephosphorylation. These, however, are intracellular processes and do not shed light on the possible role of the enzyme in extracellular digestive processes. Fraisse et al., (1981) also associated the presence of alkaline phosphatase in fish intestine with intracellular functions.

With the evidence at hand it must be concluded that alkaline phosphatase in C. gariepinus plays an important extracellular digestive role. As stated before, serine esterases have overlapping substrate specificities, and any of the monophosphate esters which might occur as primary substrates or as intermediate digestive products in the diet of catfish may be hydrolyzed by this enzyme. It is known that high levels of energy rich monophosphate esters occur in detritus (Coetzee, 1982), and since this is a food source often utilized by sharptooth catfish it can be inferred that high levels of extracellular alkaline phosphatase is an adaptation to detritivory.

Distribution of lysozyme

In Table 5.6 the distribution of lysozyme activity is shown. It is clearly evident from these results, that disproportionately high levels of lysozyme activity occur in the stomach content.

Table 5.6
Distribution of lysozyme activities in the digestive tract and associated organs of Clarias gariepinus.

| Sample material | pH of assay | Lysozyme activity* | | |
|---|---|---|---|---|
| | | Mean | SD | n |
| Stomach content | 5.0 | 3110.3 | 345.0 | 8 |
| Liver tissue | 6.2 | 58.8 | 12.2 | 8 |
| Bile fluid | 6.2 | 0.0 | - | 5 |
| Pancreas tissue | 6.2 | 0.0 | - | 5 |
| Foregut content | 6.2 | 392.8 | 64.7 | 7 |
| Midgut content | 6.2 | 108.6 | 23.0 | 7 |
| Hindgut content | 6.2 | 68.5 | 18.4 | 7 |

*Lysozyme activity expressed in Sigma Units per 1g of wet sample material.

Bitterlich (1985), suspected that lysozyme may may be present in silver carp H. molitrix and bighead carp Aristichthys nobilis due to their habit of often feeding on detritus. He used the same assay procedure as in this study, but he could not find any evidence of lysozyme activity in the gut of these species. Lindsay (1984) could not find a correlation between lysozyme

activities in fish and their relative dependence on bacteria as a food source. In rainbow trout the distribution pattern of lysozyme implies a defense role rather than a digestive role (Lindsay, 1986). Studnicka et. al. (1986) noted that serum lysozyme levels are higher in diseased carp (C. carpio) than in healthy carp. These authors concluded, therefore, that lysozyme in fish, as in the case of other vertebrates, is associated with protection against bacterial invasion and does not play an important nutritional role.

Irrespective of whether the primary function of gastric lysozyme in C. gariepinus is defensive or not, the recorded levels (3110 Sigma Units/g of stomach contents) were sufficiently high to suggest that they play a significant role in nutrition. Under natural conditions, this fish often resorts to detritivory (up to 40% of the protein in detritus can consist of bacteria). Bowen (1976) has shown that O. mossambicus utilizes detrital bacteria through acid digestion upon which he hypothesized that fish which do not have a very low stomach pH (<2) cannot lyse bacteria. The present study has shown, however, that C. gariepinus can effectively lyse bacterial cells by enzymatic digestion. Under pond culture conditions, this fish will be able to utilize detrital bacteria, and under intensive culture conditions, bacterial cultures could be considered as feed ingredients. The use of single cell proteins (SCP's), such as yeasts and bacteria as protein sources in commercial fish feeds is becoming increasingly attractive due to the escalating cost and scarcity of fish meal. Workers such as Bergström (1979) have indeed had success in replacing fish meal partially with SCP's in fish feeds.

pH dependence of gastric and intestinal proteases.

Table 5.7 shows the pH dependent profiles of gastric protease (pepsin) and of combined proteases of pancreatic origin. The highest gastric protease (pepsin) activities occurred in the pH range 2.5 - 3.5, whereas the optimum for proteases of pancreatic

origin was in the pH range 7.5 - 8.5. Figure 5.2 is a graphic representation of this relationship and, in it, comparisons are made with the pH dependencies of proteases from other fish species. It is evident that C. gariepinus has a higher pH optimum for gastric protease than other stomach possessing species (trout and sheatfish, Figure 5.2). However, its pH range for maximal activity corresponds closely with those for human and porcine pepsins (Ryle, 1981), which function maximally at a pH of 2.9-3.2 and, characteristically, are irreversibly deactivated above a pH of 6.0. As can be seen in Figure 5.2, there was a recurrence of the typical pepsin curve in the proteases assayed in the intestinal content, which implies that pH deactivation of pepsins in catfish is not irreversible. It is also interesting to note that Carassius auratus, in spite of being an agastric fish, has two distinct pH optima for its proteases and that its profile resembles that of C. gariepinus to some extent, except that its acid protease has a higher pH optimum.

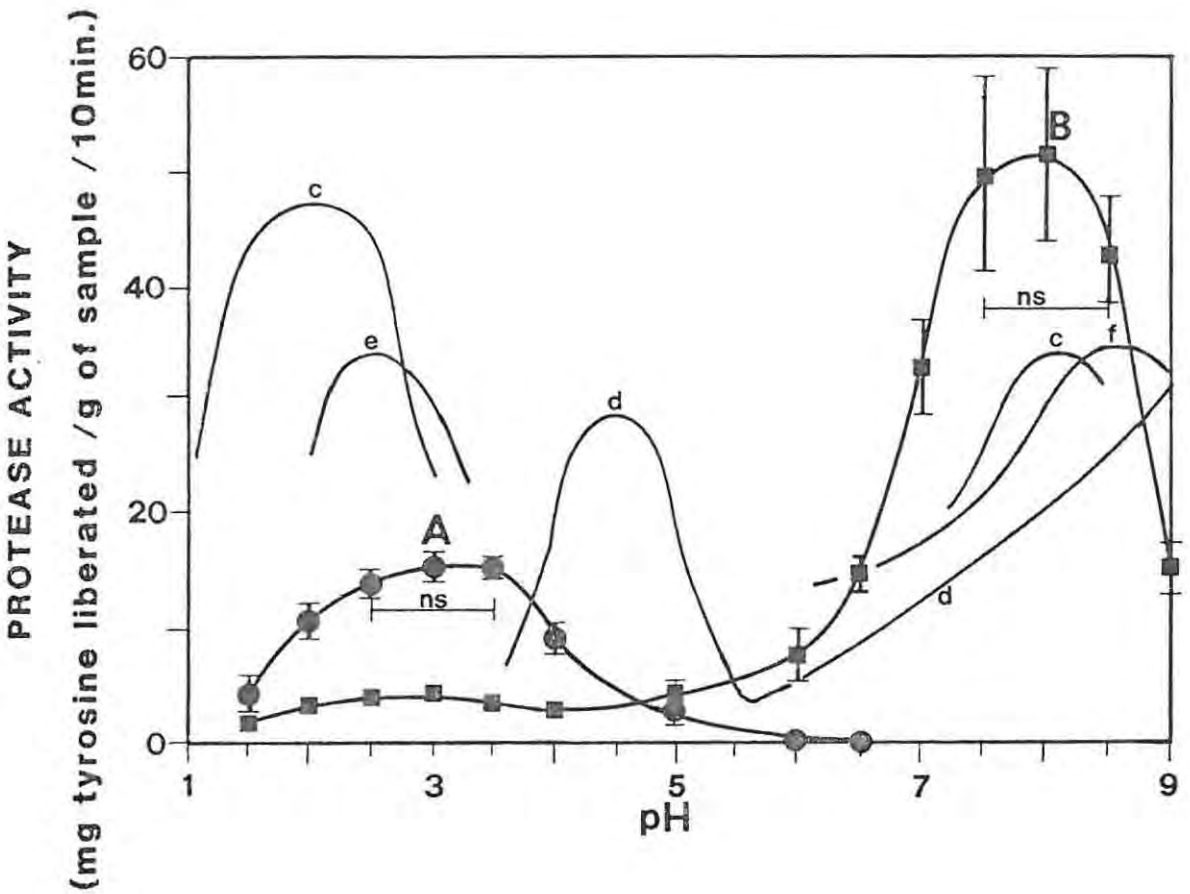


Figure 5.2 The pH dependence of gastric protease (A) and protease of pancreatic origin (B) in Clarias gariepinus (means ± SD). Horizontal lines (ns) indicate means which are not significantly different for $P<0.05$. For comparison, curves were redrawn from similar studies on other fish species. The activity scale units only apply to curves A and B. The other curves were drawn as proportions of maximum activity:
c: Silurus glanis gastric and intestinal protease (Jonas et al., 1983).
d: Carassius auratus -a stomachless fish with bimodal pH dependence of its combined proteases (Jany, 1976).
e: Rainbow trout gastric protease (Onishi and Murayama, 1969)
f: Common carp intestinal protease (Jonas et al., 1983).

Table 5.7.
The effect of pH on the activities of proteolytic enzymes occurring in the stomach and foregut contents of Clarias gariepinus.

| | Gastric protease (pepsin) | | | Pancreatic protease | | |
|---|---|---|---|---|---|---|
| pH of assay | Mean activity | SD | n | Mean activity | SD | n |
| 1.5 | 4.04 | 1.54 | 4 | 1.32 | 0.45 | 4 |
| 2 | 11.01 | 1.19 | 4 | 3.23 | 0.77 | 4 |
| 2.5 | 14.10 | 0.68 | 4 | 3.96 | 0.55 | 4 |
| 3 | 15.11 | 1.04 | 4 | 4.28 | 0.74 | 4 |
| 3.5 | 14.76 | 1.10 | 4 | 3.35 | 0.54 | 4 |
| 4 | 9.08 | 0.89 | 4 | 2.67 | 0.76 | 4 |
| 5 | 2.44 | 0.91 | 4 | 3.89 | 0.79 | 4 |
| 6 | 0.00 | 0.00 | 4 | 7.40 | 2.24 | 4 |
| 6.5 | 0.00 | 0.00 | 4 | 14.00 | 1.35 | 4 |
| 7 | | | | 32.40 | 4.49 | 4 |
| 7.5 | | | | 49.58 | 8.94 | 7 |
| 8 | | | | 51.30 | 7.76 | 4 |
| 8.5 | | | | 42.75 | 4.48 | 4 |
| 9 | | | | 15.20 | 2.51 | 4 |

Activities expressed in terms of mg of tyrosine liberated by 1g of wet sample material in 10 min. at 37 ℃

pH dependence of trypsin and chymotrypsin

Table 5.8 and Figure 5.3 show a detailed analysis of the effect of pH on the activities of trypsin and chymotrypsin. Their pH dependence profiles are highly similar to those of trypsin and chymotrypsin from C. carpio (Reichenbach-Klinke, 1972; Cohen et al., 1981b), H. molitrix and A. nobilis (Bitterlich, 1985). In fact, the correlations are so close, that plotting them into Figure 5.3 would be difficult without making the graph illegible. A comparison of their optimum pH values for the respective enzymes is as follows:

-C. gariepinus trypsin pH 8.2; chymotrypsin 7.8 (this study).
-H. molitrix and A. nobilis trypsin pH 8.3 (Bitterlich, 1985).
-C. carpio trypsin pH 8.2 (Reichenbach-Klinke, 1972).
-Carassius auratus trypsin pH 8.5; chymotrypsin pH 8.2 (Jany,1976).

Table 5.8.
The effect of pH on the activities of trypsin and chymotrypsin occurring in the intestinal content of Clarias gariepinus (trypsin sampled from midgut and chymotrypsin sampled from foregut).

| pH of assay | Trypsin activity* | | | Chymotrypsin activity** | | |
|---|---|---|---|---|---|---|
| | Mean | SD | n | Mean | SD | n |
| 7.2 | 7.40 | 1.05 | 4 | 9.93 | 0.71 | 4 |
| 7.4 | 9.37 | 0.35 | 4 | 13.08 | 0.56 | 4 |
| 7.6 | 10.11 | 1.23 | 4 | 15.18 | 1.94 | 4 |
| 7.8 | 12.09 | 1.42 | 4 | 16.23 | 3.13 | 4 |
| 8.0 | 12.46 | 0.42 | 4 | 15.37 | 0.73 | 4 |
| 8.2 | 12.70 | 0.21 | 4 | 14.61 | 1.22 | 4 |
| 8.4 | 11.72 | 0.41 | 4 | 14.32 | 0.79 | 4 |
| 8.6 | 10.11 | 1.23 | 4 | 14.03 | 0.50 | 4 |
| 8.8 | 7.52 | 0.41 | 4 | 13.27 | 0.83 | 4 |
| 9.0 | 2.34 | 0.84 | 4 | 13.08 | 0.56 | 4 |

*Trypsin activity expressed in terms of change in absorbance per minute at 247nm, and at 30 °C, caused by 1g of wet sample material.
**Chymotrypsin activity expressed in terms of change in absorbance per minute at 256nm, and at 30 °C, caused by 1g of wet sample material.

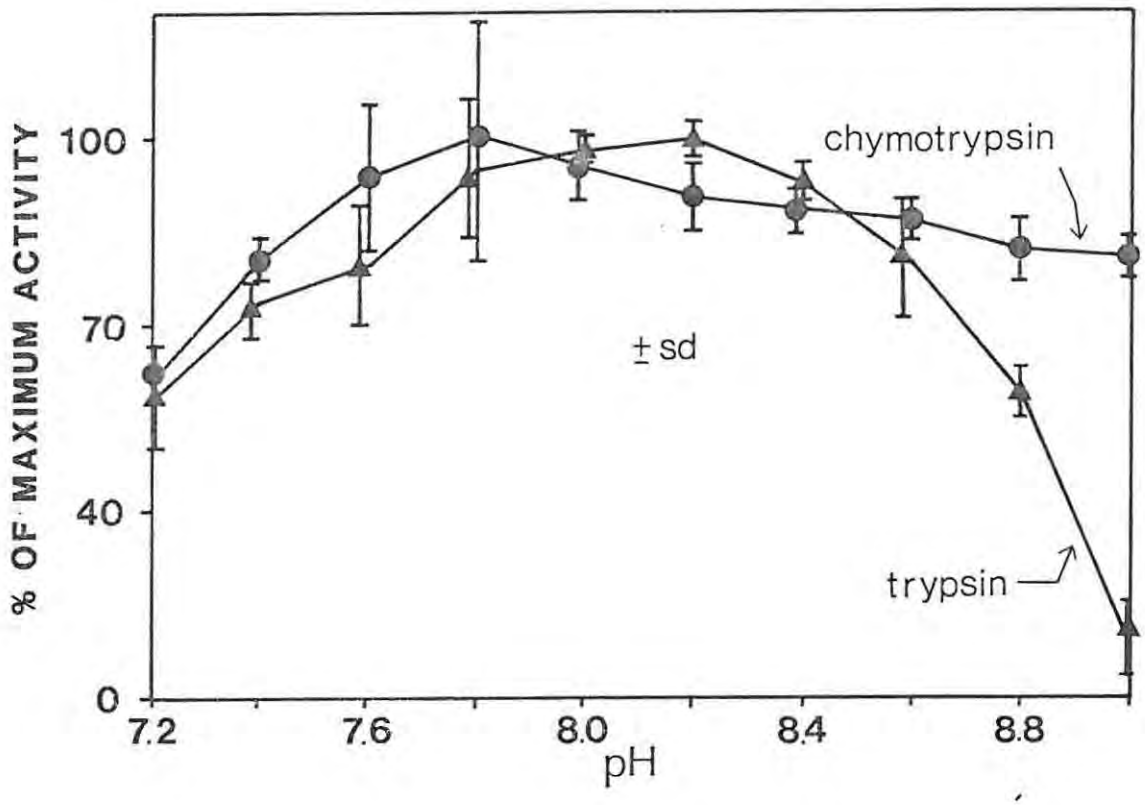


Figure 5.3. The effect of pH on the activities of trypsin and chymotrypsin found in the foregut content of Clarias gariepinus (means ± SD).

pH dependence of amylase

The effect of pH on C. gariepinus pancreatic amylase activity is listed in Table 5.9 and illustrated graphically in Figure 5.4. The optimum activity of amylase occurred at a pH of 7.8, but its activity was not much impaired either side of this point, within the pH range 6.8 to 8.4. The optimum pH for typical a-Amylase is somewhat lower at 6.9 (Bernfield, 1955), but it is not unusual for amylases to also function well into the alkaline range. Fish (1960) reported a functional pH range of 5.0 to 7.8 for O. mossambicus amylase. At the same time, he found the intestinal pH of O. mossambicus to be between 8.0 and 8.8, which does not shed much light on the usefulness of possessing an amylase which does not function well in this pH range. Agrawal et al. (1975) also reported acidic optima for piscine- (including C. batrachus) amylases, in spite of the fact that fish intestines are normally

alkaline. A reason for this apparent disparity, might be that the traditional assay technique for amylase (Bernfield, 1955) is not suitable for measurements in upper alkaline ranges. Normally, a phosphate buffer is used, and it can only be used effectively up to a pH of 8. In the present study, a second assay series was run with a TRIS (hydroxymethyl) -aminomentane buffer to extend the range beyond a pH of 8.0. If one looks at Figure 5.4 it is evident that the marked drop in amylase activity with the phosphate buffer above pH 7.8 is not repeated in the TRIS-buffer series. This drop in activity above pH 7.8 is evident in the studies mentioned above, as well as in the report by Bitterlich (1985).

It would seem to be a strong possibility, therefore, that the upper pH limit of 7.8 for piscine amylases as reported by these workers, is an artifact produced by the phosphate buffer, and that the functional pH ranges of the enzyme do, after all, overlap with the pH of the intestinal contents. In the present study, this is certainly the case, since the mean pH of C. gariepinus foregut content is 8.2, which falls well within the functional range of amylase as measured with the TRIS buffer assay series (see Table 5.10). The findings of Cockson and Bourne (1972), support the findings of the present study. They used an altogether different assay technique and detected maximal amylase activities in C. gariepinus in the pH range 9-11.

Table 5.9.
The effect of pH on the activity of amylase occurring in the pancreatic tissue of Clarias gariepinus.

| pH of assay | Amylase activity* in phosphate buffer | | | Amylase activity in TRIS buffer** | | |
|---|---|---|---|---|---|---|
| | Mean | SD | n | Mean | SD | n |
| 5.8 | 85.7 | 23.8 | 4 | | | |
| 6.2 | 187.4 | 32.8 | 4 | | | |
| 6.5 | 319.8 | 10.0 | 4 | | | |
| 6.8 | 585.9 | 56.9 | 4 | | | |
| 7.0 | 644.1 | 41.0 | 4 | | | |
| 7.2 | 715.5 | 13.0 | 4 | 316.9 | 20.3 | 4 |
| 7.4 | 788.0 | 6.0 | 4 | 432.9 | 1.9 | 4 |
| 7.6 | 824.8 | 4.8 | 4 | 512.7 | 3.8 | 4 |
| 7.8 | 834.7 | 46.2 | 4 | 528.5 | 4.5 | 4 |
| 8.0 | 698.8 | 19.7 | 4 | 513.3 | 25.3 | 4 |
| 8.4 | | | | 465.1 | 13.1 | 4 |
| 8.6 | | | | 465.4 | 27.0 | 4 |
| 9.0 | | | | 223.2 | 19.9 | 4 |

*Amylase activity expressed in terms of mg of maltose liberated from starch per 1g of wet sample material in 3 minutes at 25 °C.
**The phosphate buffer which is normally used with this assay (Bernfield, 1955), can only be adjusted to a maximum pH of 8. A 0.2M TRIS buffer was used for a second assay series in the pH range 7.2 to 9.0.

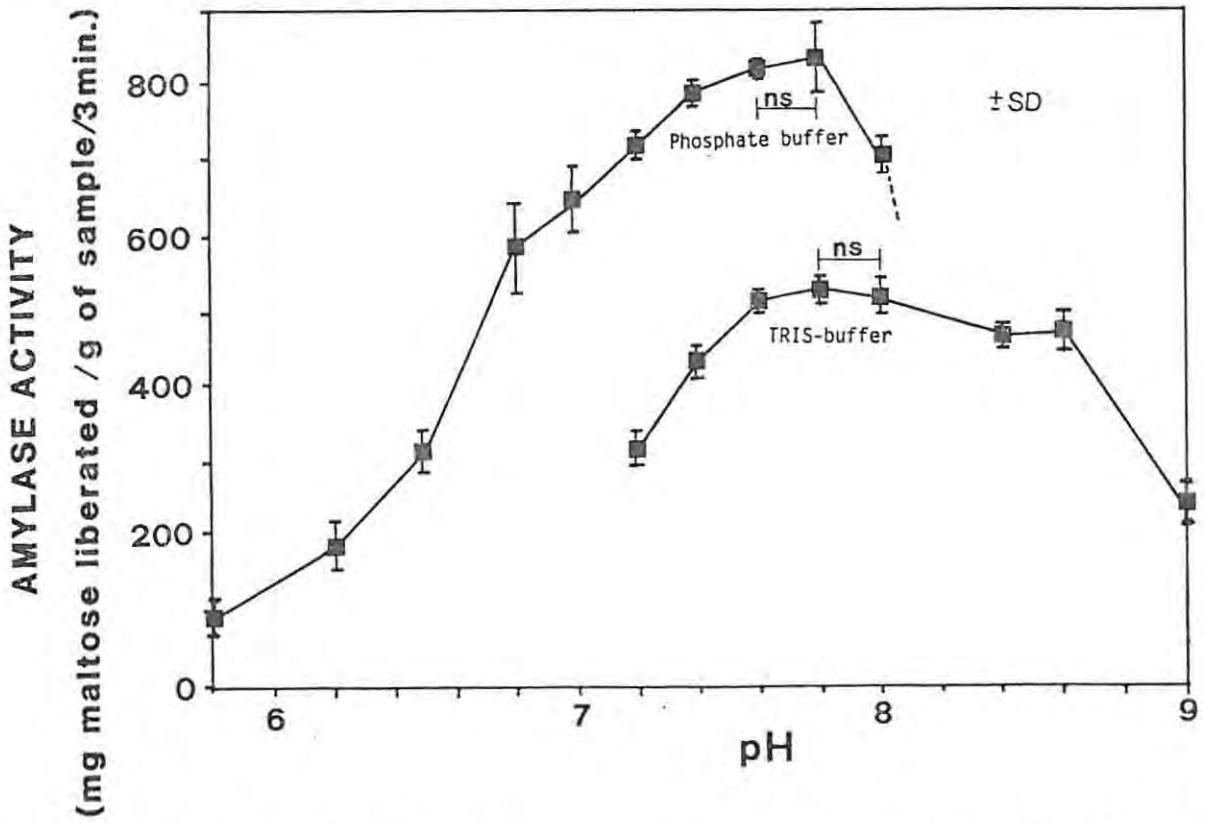


Figure 5.4. The effect of pH on pancreatic amylase activity in Clarias gariepinus (means ± SD). The horizontal lines (ns), join means which are not significantly different for P<0.05.

The relationship between pH and digestive enzyme activity

Table 5.10 presents optimum pH values for the various enzymes tested, along with the mean pH values in the stomach and gut at times of maximal digestive activity. In Figure 5.8, these relationships are presented graphically.

C. gariepinus has a thick walled, muscular stomach with relatively little acid secretion (pH of ±4). This implies that it relies on enzymatic digestion and mechanical crushing rather than acid hydrolysis of the food in its stomach. The pH dependence of its gastric proteinase being relatively less acidic than in other fish, and the relatively high levels of gastric protease and lysozyme support this premise. O. mossambicus, on the other hand, is an example of a fish which relies mainly on acid hydrolysis; it has a thin walled stomach with a pH as low as 1.14 when empty, and 2.65, four hours after feeding (Maier & Tullis, 1984).

Temperature dependence of digestive enzymes

The temperature dependence profiles of the various digestive enzymes are presented in Tables 5.11 to 5.13 and in Figures 5.6 to 5.9. It is evident from these results that elevated temperatures would greatly benefit the rate of food digestion in Clarias gariepinus. These profiles, however, have a wider base than profiles drawn for C. carpio and H. molitrix (Jonas et al., 1983), Salmo gairdneri (Kitamikado & Tachino, 1960) and Clupea harengus (Kalac, 1978). This reflects the ability of C. gariepinus to maintain high levels of digestive activity in extreme temperatures (10 to 40 °C).

Table 5.10.
Comparison of optimum pH values for digestive enzymes of Clarias gariepinus and the pH of the regions of the digestive tract in which they function.

| Enzyme | Optimum pH | Effective pH range (60% of max.) | Active region | pH of region | SD |
|---|---|---|---|---|---|
| Pepsin | 3.0 | 1.5-4.0 | Stomach | 3.97 | 0.29 |
| Trypsin | 7.8 | 7.4-8.6 | Foregut | 8.20 | 0.22 |
| Chymotrypsin | 8.2 | 7.4-9.0 | Foregut | 8.20 | 0.22 |
| Amylase | 7.8 | 6.8-8.6 | Foregut | 8.20 | 0.22 |

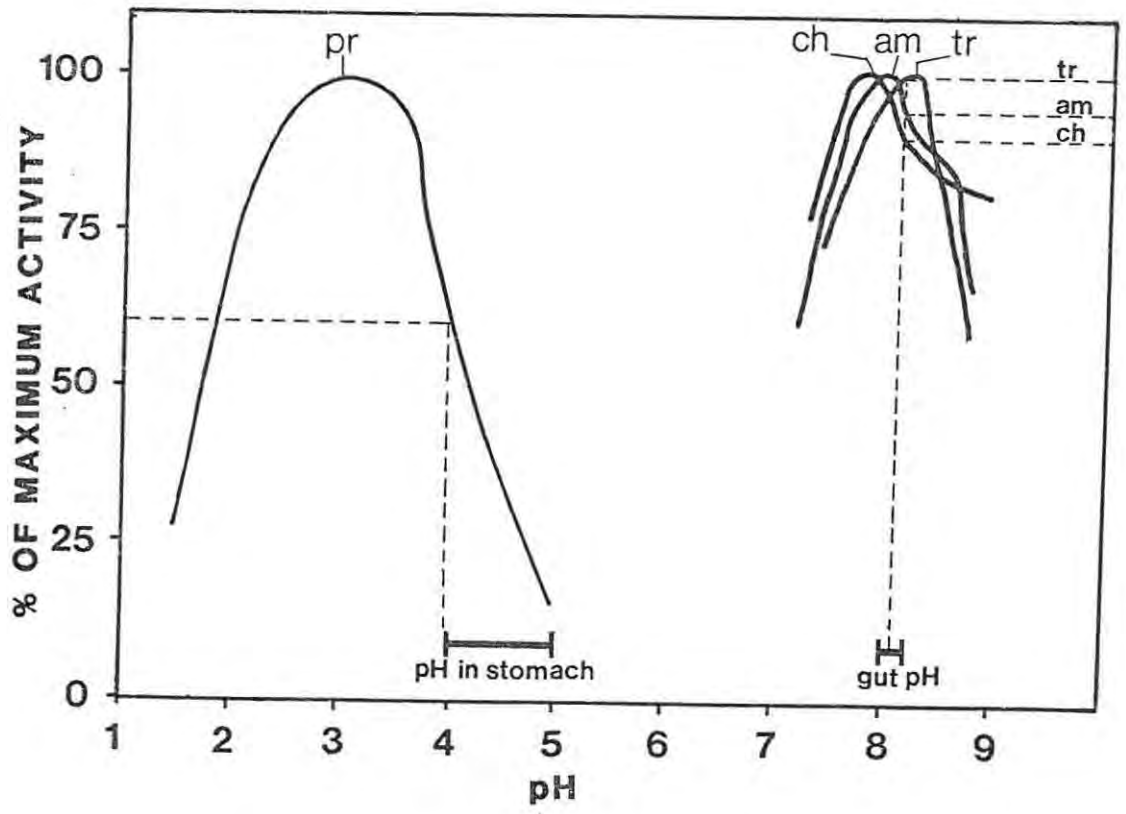


Figure 5.5. The effect of pH on the activities of digestive enzymes of Clarias gariepinus, correlated with the prevailing pH of the region in which the enzymes act upon the food. pr = gastric protease(pepsin), ch = chymotrypsin; am = amylase; tr = trypsin.

Table 5.11.
The effect of temperature on the activity of proteolytic enzyme (pepsin) occurring in the stomach of Clarias gariepinus.

| Temperature °C | Gastric protease (pepsin) activity* | | |
|---|---|---|---|
| | Mean | SD | n |
| 10 | 2.90 | 0.80 | 4 |
| 15 | 4.29 | 1.03 | 4 |
| 25 | 9.29 | 1.28 | 4 |
| 30 | 11.78 | 1.52 | 4 |
| 34 | 14.93 | 1.95 | 4 |
| 36 | 17.15 | 2.36 | 4 |
| 38 | 20.39 | 1.80 | 4 |
| 40 | 20.94 | 1.21 | 4 |
| 42 | 21.30 | 0.87 | 4 |
| 44 | 14.62 | 0.97 | 4 |
| 46 | 2.91 | 2.48 | 4 |

*Pepsin activity expressed in terms of mg of tyrosine liberated by 1g of wet sample material in 10 min. at 37°C. The pH during this assay series was 3.0.

Table 5.12
The effect of temperature on the activities of pancreatic trypsin (from midgut) and chymotrypsin occurring in the intestinal content of Clarias gariepinus.

| Temperature °C | Trypsin activity* | | | Chymotrypsin activity** | | |
|---|---|---|---|---|---|---|
| | Mean | SD | n | Mean | SD | n |
| 10 | 5.13 | 1.12 | 4 | 7.91 | 0.66 | 4 |
| 15 | 6.28 | 1.24 | 4 | 8.95 | 0.47 | 4 |
| 20 | 7.98 | 1.11 | 4 | 9.58 | 0.48 | 4 |
| 25 | 9.98 | 0.53 | 4 | 11.53 | 0.45 | 4 |
| 30 | 13.30 | 0.74 | 4 | 13.70 | 0.50 | 4 |
| 35 | 16.20 | 0.26 | 4 | 15.91 | 0.51 | 4 |
| 40 | 17.10 | 0.51 | 4 | 17.14 | 0.89 | 4 |
| 45 | 17.00 | 0.97 | 4 | 15.91 | 0.42 | 4 |
| 50 | 12.05 | 1.12 | 4 | 7.59 | 0.67 | 4 |
| 55 | 5.03 | 1.11 | 4 | 3.93 | 0.42 | 4 |

*Trypsin activity expressed in terms of change in absorbance per minute at 247nm, and at pH 8.2, caused by 1g of wet sample material.
**Chymotrypsin activity expressed in terms of change in absorbance per minute at 256nm, and at pH 7.8, caused by 1g of wet sample material.

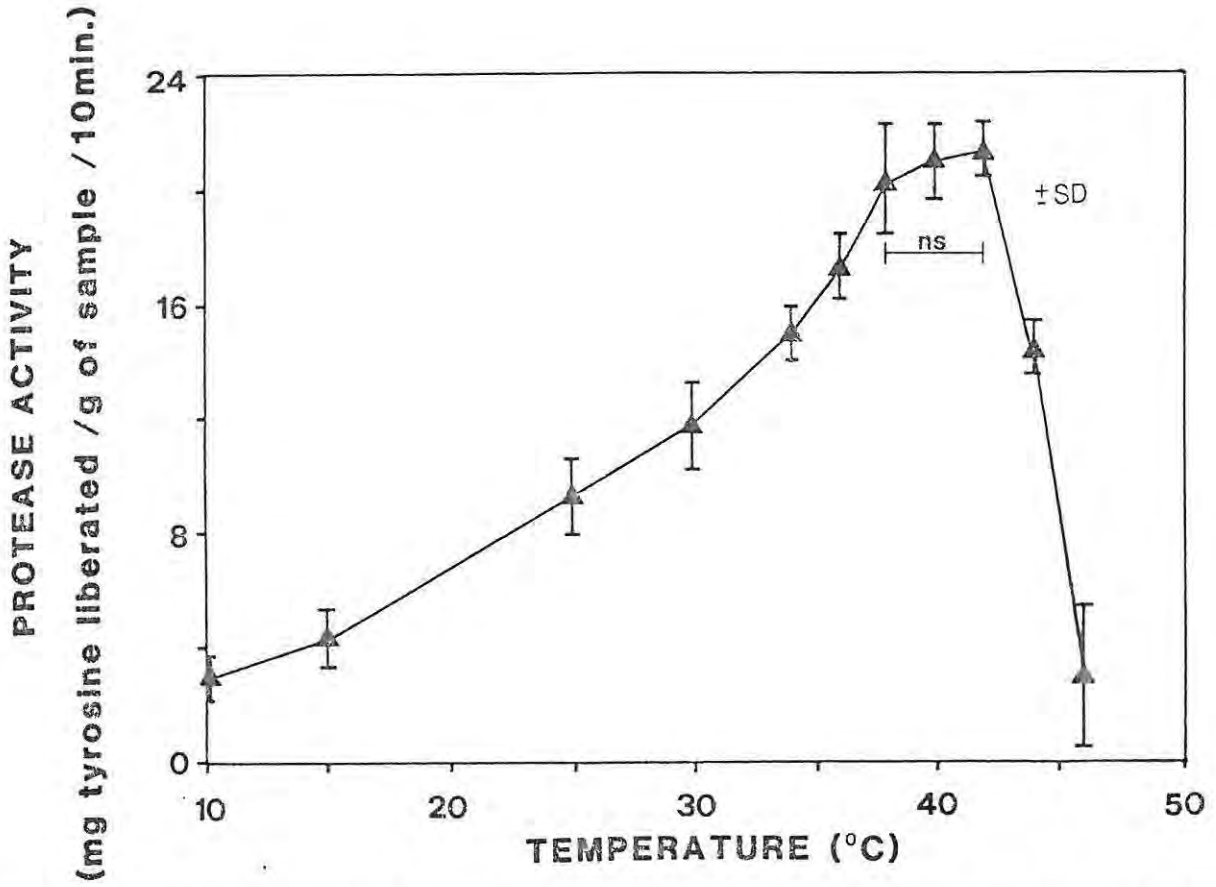


Figure 5.6. The effect of temperature on gastric protease (pepsin) activity in Clarias gariepinus (means ± SD). The horizontal line (ns) indicated means which are not significantly different for P<0.05.

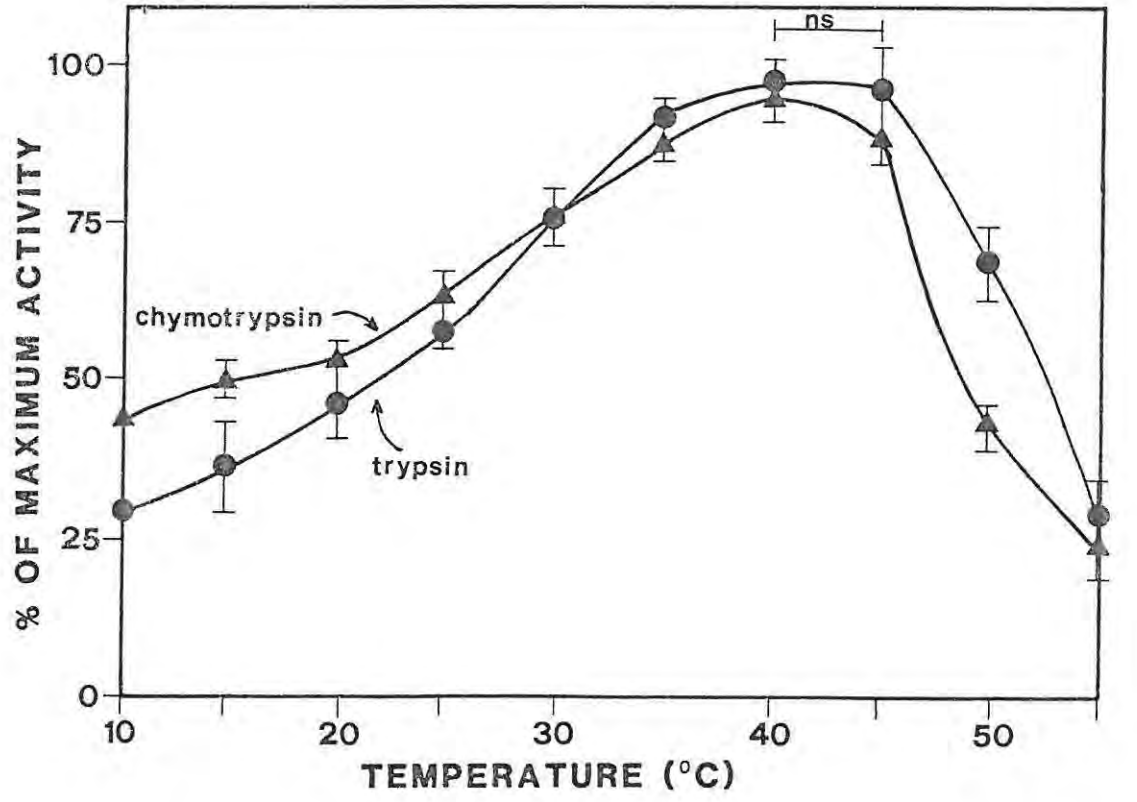


Figure 5.7. The effect of temperature on the activities of trypsin (midgut) and chymotrypsin (foregut) in Clarias gariepinus (means ±SD). The horizontal line (sd) indicates means which do not differ significantly for P<0.05.

Table 5.13
The effect of temperature on the activity of amylase occurring in the pancreatic tissue of Clarias gariepinus.

| Temperature °C | Amylase activity* | | |
|---|---|---|---|
| | Mean | SD | n |
| 10 | 317.4 | 21.4 | 4 |
| 15 | 528.7 | 10.4 | 4 |
| 20 | 730.0 | 26.3 | 4 |
| 25 | 815.5 | 15.3 | 4 |
| 28 | 821.6 | 5.0 | 4 |
| 30 | 833.8 | 6.0 | 4 |
| 33 | 846.9 | 15.7 | 4 |
| 34 | 853.2 | 11.9 | 4 |
| 35 | 854.5 | 13.1 | 4 |
| 36 | 836.5 | 15.5 | 4 |
| 37 | 789.2 | 46.7 | 4 |
| 38 | 697.8 | 36.0 | 4 |
| 39 | 638.0 | 33.0 | 4 |
| 40 | 521.8 | 46.2 | 4 |
| 42 | 301.3 | 58.6 | 4 |
| 44 | 147.8 | 52.0 | 4 |
| 46 | 10.9 | 5.5 | 4 |

*Amylase activity expressed in terms of mg of maltose liberated from starch per 1g of wet sample material in 3 minutes at pH 6.9.

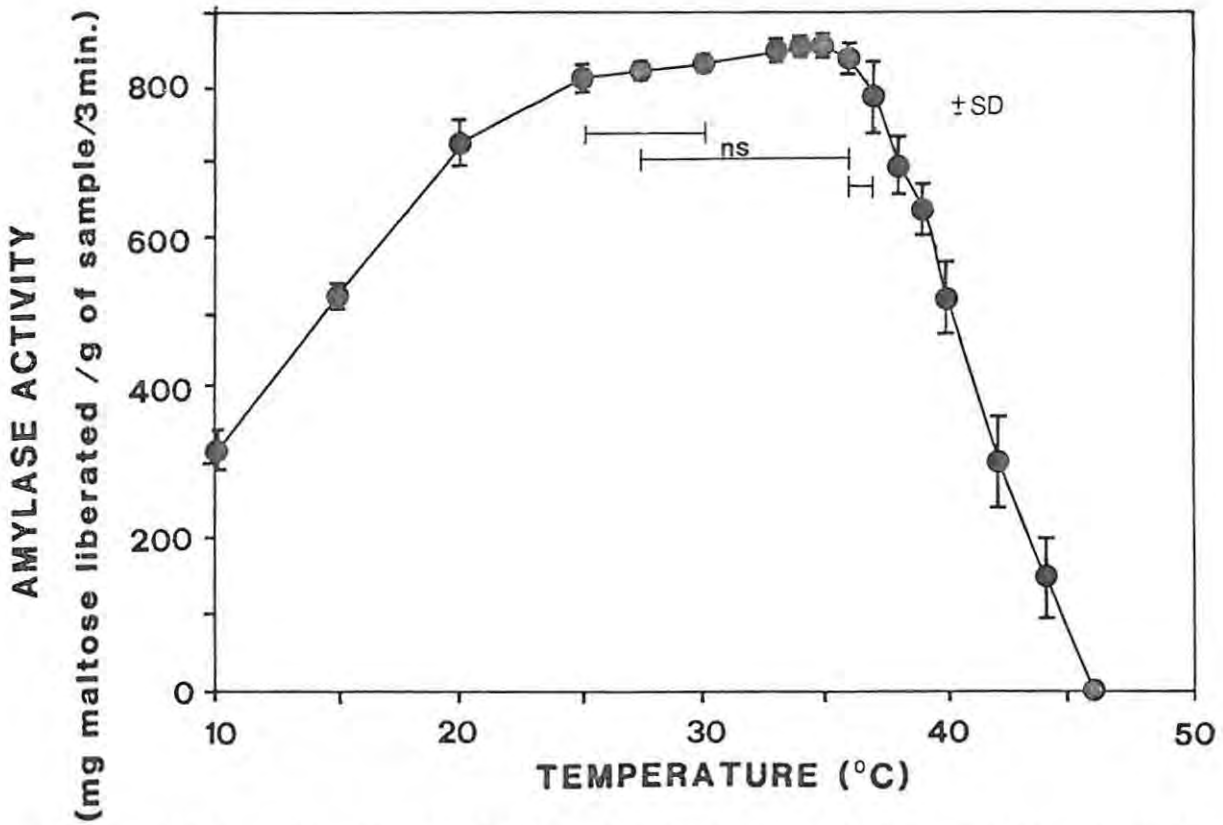


Figure 5.8. The effect of temperature on pancreatic amylase activity in Clarias gariepinus (means ±SD). The horizontal lines (ns) indicate means which do not differ significantly for P<0.05.

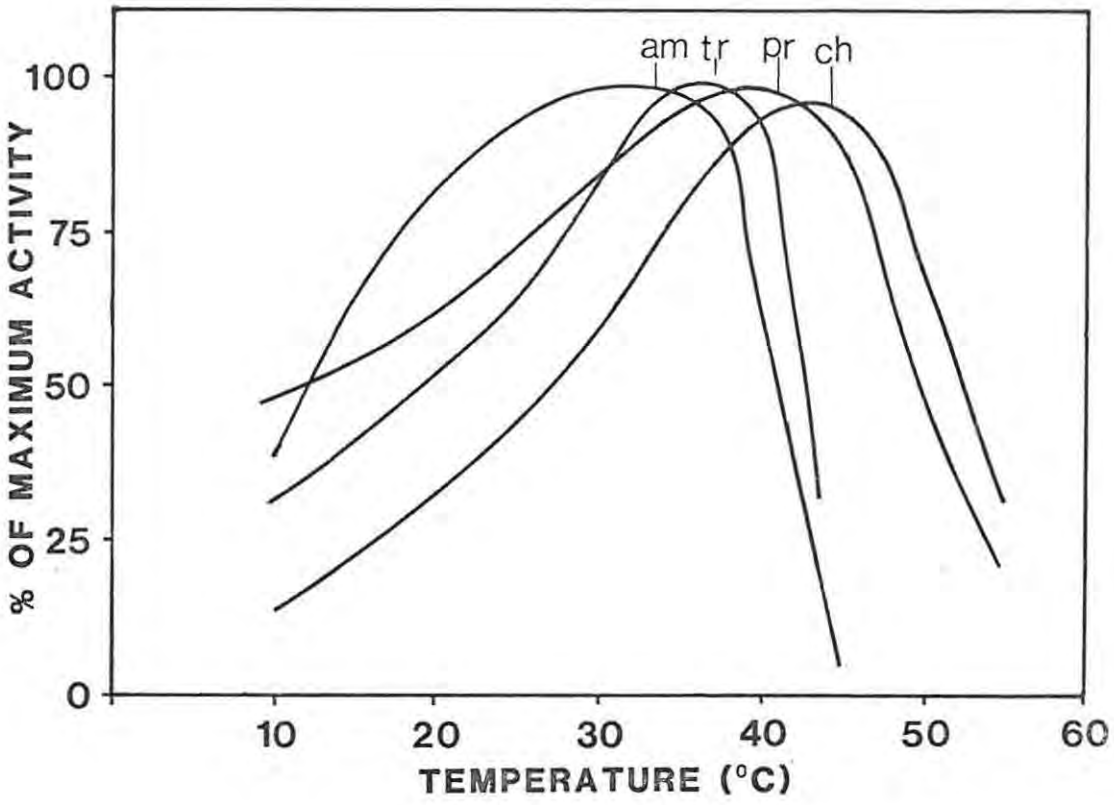


Figure 5.9. The effect of temperature on the activities of digestive enzymes of Clarias gariepinus . am = amylase; tr = trypsin; pr = gastric protease (pepsin); ch = chymotrypsin.

Changes in enzyme activities after feeding

The sequential changes in digestive enzyme activities are given in Table 5.14. In order to correlate these changes with the passage of food through the digestive tract, as well as with the pH changes in the food, these events are presented graphically in Figure 5.10.

The protease and amylase activities in the unfed fish remained unchanged ($P<0.05$) for the whole period. The fed fish, on the other hand, showed a dramatic response. Gastric protease activity responded quickly to food intake. If it is considered that the digestive juices are diluted by the food in the stomach, it is surprising that an increase in protease activity per gram of stomach contents was measured as early as 1 hour after feeding. This increase is, however, not significant for $P<0.05$. At 2.5 hours after feeding, the activity of protease in

the stomach contents reaches its maximum. This level is significantly higher than the level recorded 1 hour after feeding ($P>0.01$). After the fourth hour, the activity gradually decreased, indicating that little or no more enzyme is secreted in the stomach and that the enzymes are partially inactivated.

Table 5.14.
Changes in protease and amylase activities in Clarias gariepinus after feeding.

| Sample material | Time after feeding (h) | Protease activity* Mean | SD | n | Amylase activity** Mean | SD | n |
|---|---|---|---|---|---|---|---|
| Stomach content (pepsin) | 0.0 a | 5.43 | 0.78 | 4 | | | |
| | 1.0 | 6.55 | 0.98 | 6 | | | |
| | 2.5 | 14.03 | 3.03 | 6 | | | |
| | 4.0 | 13.60 | 1.25 | 8 | | | |
| | 6.5 | 10.10 | 1.55 | 6 | | | |
| | 10.0 | 7.00 | 2.05 | 4 | | | |
| Pancreatic tissue | 0.0 | 6.47 | 1.11 | 4 a | 655 | 43 | 6 |
| | 1.0 | 22.09 | 4.36 | 4 | 316 | 48 | 6 |
| | 2.5 | 25.48 | 3.76 | 4 | 410 | 71 | 8 |
| | 4.0 | 32.52 | 4.86 | 8 | 607 | 35 | 12 |
| | 6.5 | 28.41 | 1.82 | 4 | 649 | 58 | 7 |
| | 10.0 | 21.23 | 2.32 | 4 | 654 | 32 | 5 |
| Foregut content | 0.0 a | 22.23 | 3.60 | 4 | 112.2 | 11.4 | 4 |
| | 1.0 | 49.86 | 8.08 | 4 | 56.1 | 6.4 | 4 |
| | 2.5 | 66.30 | 6.64 | 4 | 80.6 | 8.2 | 6 |
| | 4.0 | 75.77 | 9.88 | 7 | 126.2 | 15.9 | 9 |
| | 6.5 | 69.51 | 4.42 | 5 | 113.0 | 12.0 | 4 |
| | 10.0 | 24.06 | 2.28 | 4 | 118.4 | 7.6 | 4 |

*Protease activities expressed as mg of tyrosine liberated by 1g of wet sample material in 10 min. at 25 °C. The stomach content was assayed at pH 4 and the foregut content and pancreatic tissue at pH 7.0.
**Amylase activities expressed as mg of maltose liberated from starch by 1g of wet sample material in 3 min. ant 25 C and at pH 6.9.
a- No food present. Measurements obtained from mucus scrapings in stomach and foregut.

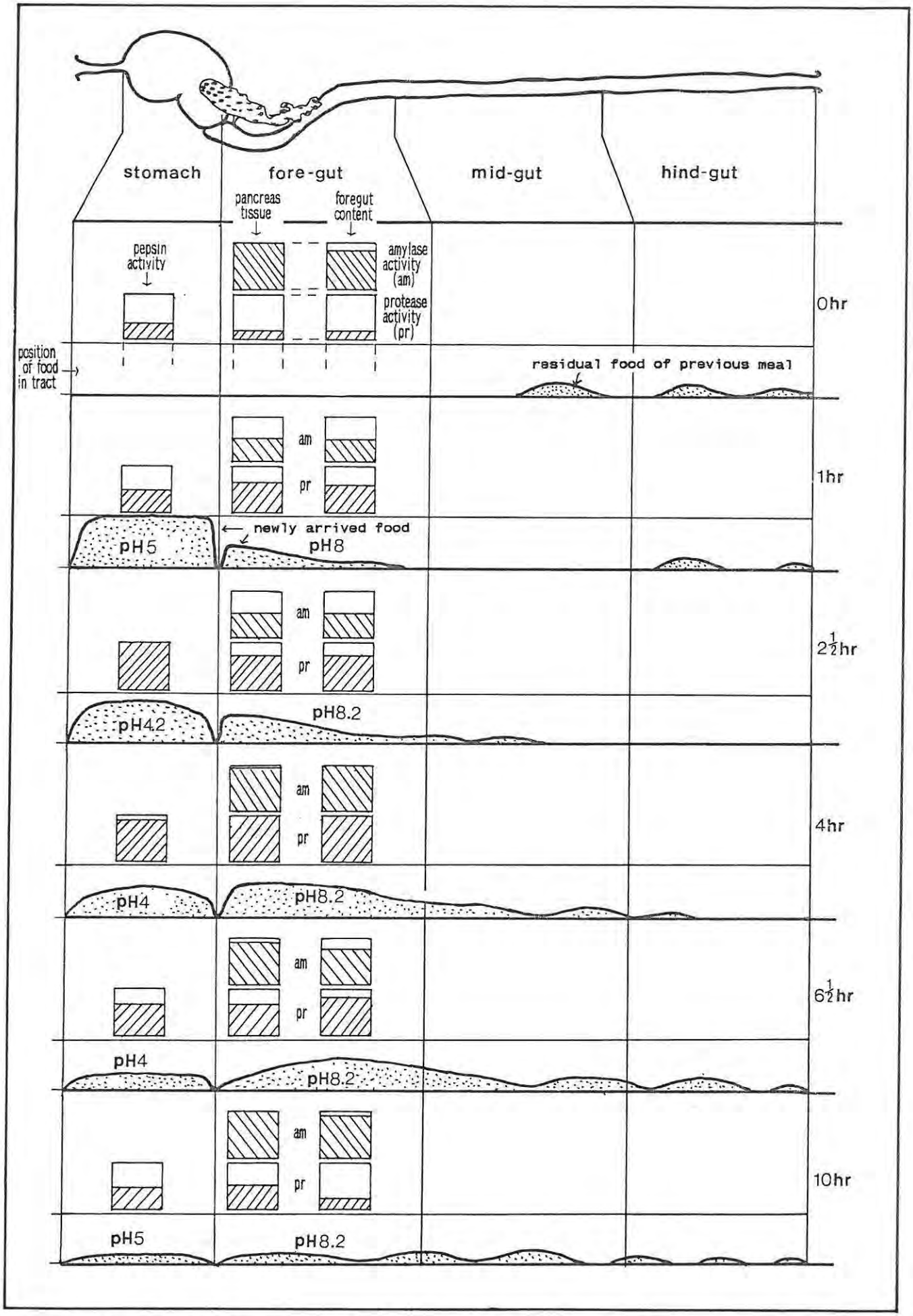


Figure 5.10. Graphic representation of the passage of food through the digestive tract of C. gariepinus, correlated with the changes in pH and digestive enzyme activities in the stomach content, pancreatic tissue and foregut content. The "0-hr." sample was taken after a 24hour starvation period and just prior to the fish being fed to satiation.

Peak protease activity in the intestine of carp occurred five hours after feeding (Onishi et al. 1973a,b, 1976). Takii et al. (1985) found that protease activity in the stomach content of A. japonica increases very gradually from the time of feeding to 12 hours after feeding, when the maximum levels were recorded. In contrast, the present study has shown that protease activity in the stomach content of catfish increases sharply after food intake, and decreases to pre-feeding levels after 10 hours.

The pattern for intestinal protease is very similar to gastric protease. The activity rose sharply after feeding, despite the dilution caused by the arrival of food, and peaked in the region of 4.5 hours after feeding. Subsequently, it decreased quite rapidly to reach pre-feeding levels 10 hours after feeding. This pattern, as well as the time of peak activity is very similar to the activity pattern for protease in the intestinal contents of carp (Onishi et al. 1973a,b, 1976), and eel (Takii et al., 1985). In both these species, protease activity in the intestinal contents peaked 5 hours after feeding

Amylase activity in the foregut of C. gariepinus follows a completely different pattern. The high pre-feeding activity indicated that high levels of amylase activity were maintained despite the absence of food in the intestine. This is in contrast with protease activity, which is relatively low while food is absent. One hour after feeding, the amylase activity had decreased sharply, probably due to dilution by the food and an inadequate rate of secretion. From 1 hour after feeding, though, it rose sharply, to parallel the rise in protease activity. By 4 hours after feeding, the amylase activity had more or less recovered to the pre-feeding level. It seems, therefore, that amylase activities in the foregut are maintained at more or less constant levels, and that the temporary decrease after feeding is caused only by the dilution effect. The decrease in activity is, however, compensated for within four hours. Onishi et al. (1976) suggested that there is a daily rhythm in digestive enzyme

activities in carp. Since no changes in enzyme activities were observed in unfed fish, it would seem that digestive enzyme activity cycles are induced by food intake alone.

The pattern of protease and amylase activities in the pancreas is very similar to the pattern in the foregut. Once again, no significant changes in pancreatic enzyme activities were measured in the unfed fish ($P<0.05$). In this case, the temporary drop in amylase activity cannot be ascribed to dilution by food. The pancreatic tissue which is very closely associated with the stomach and intestine needs to stretch considerably when the stomach and foregut distend after feeding. It was observed that this is accompanied by hydration of the pancreatic tissue, which could partially explain the apparent decrease in amylase activity. The temporary decrease in measured activity could perhaps also be ascribed to the migration of this enzyme from the pancreatic tissue towards the intestinal lumen. Pancreatic protease activity, on the other hand, increases sharply after feeding, despite whatever causes the temporary decrease in amylase activity.

Onishi *et al.* (1976) described a highly similar pattern for both these enzymes in the hepatopancreas of carp. The only difference is that in *C. gariepinus*, the increase in protease activity and the recovery of amylase activity is more rapid than in carp. In carp, the amylase activity recovers only 5 to 6 hours after feeding and protease activity peaks 7 to 7.5 hours after feeding (Onishi *et al.*, 1976).

Development of digestive enzyme activities in early life stages

The digestive enzyme activities of *C. gariepinus* at various early life history stages, fed on either live, or artificial dry feed are listed in Table 5.15. The first appearances and subsequent increases in the digestive enzyme activities of the young fish fed on dry feed only, are presented graphically in Figure 5.11.

Table 5.15

Digestive enzyme activities in Clarias gariepinus free embryos, larvae and post larvae fed on live and artificial food, and the digestive enzyme activities in their live food.

| Group No. | Age (days) | Feeding regime | Sample material | Mean enzyme activities (n=4 for each mean) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Protease | SD | Amylase | SD | Pepsin | SD | Trypsin | SD | Cellulase | SD |
| 1 | 2 | endogenous | whole fish | 0.252 | 0.01 | 7.8 | 0.2 | 0 | 0 | 0.010 | 0.002 | 0 | 0 |
| 2 | 4 | dry feed | whole fish | 0.365 | 0.01 | 16.4 | 1.1 | 0.026 | 0.00 | 0.015 | 0.010 | 0 | 0 |
| 3 | 4 | live food | whole fish | 0.300 | 0.03 | 18.4 | 1.0 | 0.030 | 0.00 | 0.027 | 0.01 | 3.710 | 0.11 |
| 4 | 7 | dry feed | whole fish | 0.663 | 0.01 | 33.1 | 1.2 | 0.864 | 0.02 | 0.265 | 0.13 | 0.847 | 0.03 |
| 5 | 7 | live food | whole fish | 0.469 | 0.01 | 33.5 | 2.9 | 0.582 | 0.02 | 0.110 | 0.03 | 3.521 | 0.37 |
| 6 | 10 | dry feed | whole fish | 0.670 | 0.01 | 34.5 | 2.1 | 1.244 | 0.03 | 0.210 | 0.14 | 0.680 | 0.03 |
| 7a | 16 | dry feed | whole fish | 0.873 | 0.05 | 32.5 | 1.9 | 0.960 | 0.06 | 0.190 | 0.11 | 0.870 | 0.02 |
| 7b | 16 | dry feed | dig.tract | 1.894 | 0.10 | 112.6 | 7.2 | 6.880 | 0.09 | 1.380 | 0.15 | 2.920 | 0.19 |
| 8 | 28 | dry feed | dig.tract | 2.079 | 0.11 | 80.4 | 8.2 | 6.498 | 0.13 | 0.960 | 0.22 | 2.663 | 0.3[illegible] |
| 9 | 28 | live food | dig.tract | 0.955 | 0.05 | 75.6 | 10.1 | 4.852 | 0.06 | 0.399 | 0.14 | 6.198 | 0.8[illegible] |
| Live food (cladocerans) | | | whole | 0.379 | 0.01 | 56.2 | 5.7 | 0 | 0 | 0.246 | 0.08 | 17.633 | 1.51 |

Protease (pH 7.5) and pepsin (pH 3.5) -activities expressed in terms of mg tyrosine liberated by 1g of wet sample material in 10 min. at 37°C

Amylase activities expressed in terms of mg of maltose liberated from starch by 1g of wet sample material in 40 min. at 37°C and pH 7.0

Trypsin activities expressed in terms of change in absorbance per minute at 247nm, at 30°C and pH 8.1, per 1g of wet sample material.

Cellulase activities expressed terms of mg of maltose liberated from cellulose by 1g of wet sample material in 50 min. at 37°C and pH 7.0

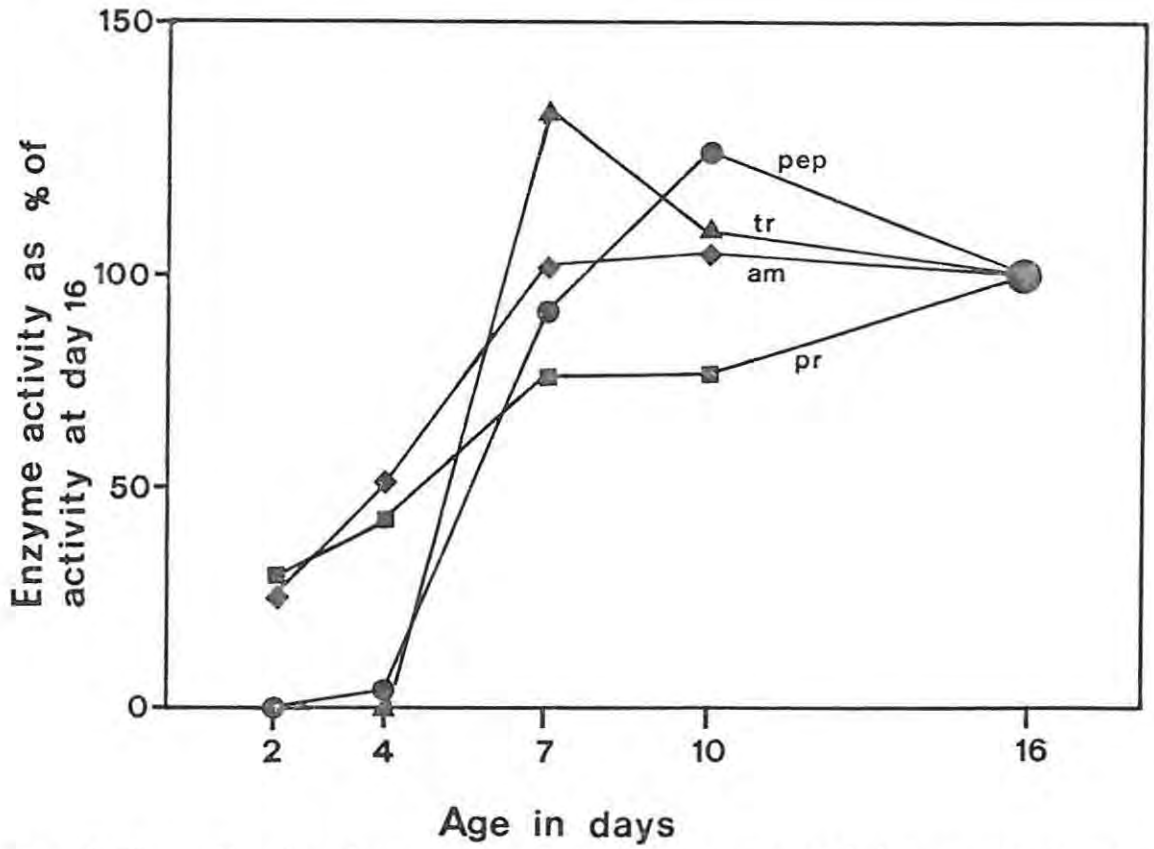


Figure 5.11. The development of digestive enzyme activities in young of Clarias gariepinus fed on artificial dry feed. Day 2 -4 = free embryo stage; day 4 -10 = larval stage; day 10 onwards = juveniles. pep =pepsin activity (acid protease); tr = trypsin activity; am = amylase activity; pr =total alkaline protease activity.

Of the endogenous enzymes tested for, only pepsin and cellulase were completely absent in the free embryo stage (day 2). Fairly high alkaline protease and amylase activities were evident in this early stage. Even though a visibly distinct stomach only appeared on about the 10th day of development, strong pepsin activities were detected in seven day old larvae and weak pepsin activities were already evident in four day old larvae. Since high alkaline protease activities were present in the free embryo stage and at the start of exogenous feeding, while trypsin activities were negligible, it must be assumed that other proteolytic enzymes, possibly aminopeptidases of mucosal origin, play an important role when exogenous feeding starts. By the 10th day of development, all the enzymes that were tested for, had reached more or less maximal levels of activity, with pepsin being the last one to do so.

Note in Table 5.15 that with the 16 day old juveniles (groups 7a and 7b), samples were prepared both from whole fish and from digestive tracts which had been excised. The enzyme activities in digestive tracts only, as percentages of their respective activities in whole fish, were as follows: Total alkaline protease 46%; amylase 29%; pepsin 14%; trypsin 10.5% and cellulase 30%. With the 28 day old juveniles, enzyme activities were assayed in the removed digestive tracts only, but based on the above percentages, it can be concluded that no important changes in digestive enzyme patterns occurred between the 16th and 28th day of development. It is, therefore, likely that the digestive enzyme patterns of C. gariepinus reach a more or less final configuration, as early as the 10th or 16th day of development. The early morphological development of the digestive tract and associated organs (Chapter 4), bears out this assumption.

Based on the morphological development of alimentary tracts and digestive enzymes Dabrowski (1984) divided larval fishes into three groups: Those that have a functional stomach before the

onset of exogenous feeding (e.g. salmonids); those that have delayed gastric development (e.g. C. gariepinus, Coregonus pollan); and those that remain stomachless throughout life (e.g. cyprinids). According to Stroband and Kroon (1981) C. gariepinus has a functional stomach when the larvae (juveniles) have approximately doubled their length after hatching. The same was found in the experiment presently discussed, since the 10-day old larvae/juveniles were exactly double the length of the free embryos. That was the first stage at which their stomachs could be discerned, and at this age their pepsin activities had reached maximum levels (see Fig. 5.11). Dabrowski (1984) went on to say that fishes having no functional stomach at the larval stage present severe problems when reared solely on dry diets (see also Lauff and Hofer, 1984). Initially, this was indeed the case with C. gariepinus since primary nursing was only successful when live food was used (Hogendoorn, 1980; Msiska,1981). However, the development of yeast-based dry feeds have since then largely solved these problems (Hecht, 1981, 1982; Uys and Hecht, 1985; Appelbaum and Van Damme, 1988). Best results are, however, still obtained if the dry feed is supplemented with small amounts of live food (Uys and Hecht, 1985; Hecht and Appelbaum, 1987; Appelbaum and Van Damme 1988).

The results of this study confirm that C. gariepinus fits into the second group of fish that Dabrowski (1984) described. Typically, larvae of this group develop tryptic activity at an early stage, and only after the onset of exogenous feeding do they develop pepsin activities. This was , indeed, the case with the larvae that were studied, since trypsin and especially other alkaline proteases were already present in the free embryo stage. Trypsin activity increased as soon as exogenous feeding started, while pepsin activities only reached a maximum level 6 days later. This emphasises the need for a nursing period of $\pm$ 10 days in the hatchery where they can be provided with plentiful and easily digestible food.

Due to the relatively low production of pancreatic proteases, fish species that exhibit delayed gastric development, seem less able to compensate for the absence of a stomach than the species which are permanently stomachless (Lauff and Hofer, 1984). C. gariepinus fits into this picture, because the total alkaline proteolytic activity was significantly lower than acid proteolytic activity (pepsin), by the time the latter got established (7th day onwards).

The success attained with the use of yeast based-diets can probably be attributed to better digestibility of the proteins in these diets by the initially underdeveloped (pepsin lacking) enzyme system of C. gariepinus larvae. This statement is justifiable if one considers, firstly, that a functional stomach facilitates protein digestion by enzymes of pancreatic origin by first denaturing and partially hydrolyzing these proteins (Lauff and Hofer, 1984), and secondly, that the yeast protein used in the synthetic feeds is lightly roasted, and thus also denatured to a large extent. In effect, therefore, the lack of a stomach is partially compensated for by these feeds.

The contribution of exogenous enzymes to digestion in larvae and early juveniles

Table 5.15 lists the development of digestive enzyme activities in C. gariepinus larvae fed on live zooplankton, as opposed to the activities in larvae fed only on dry feed. The digestive enzyme activities measured in the live zooplankton are also given. These results are presented graphically in Figure 5.12.

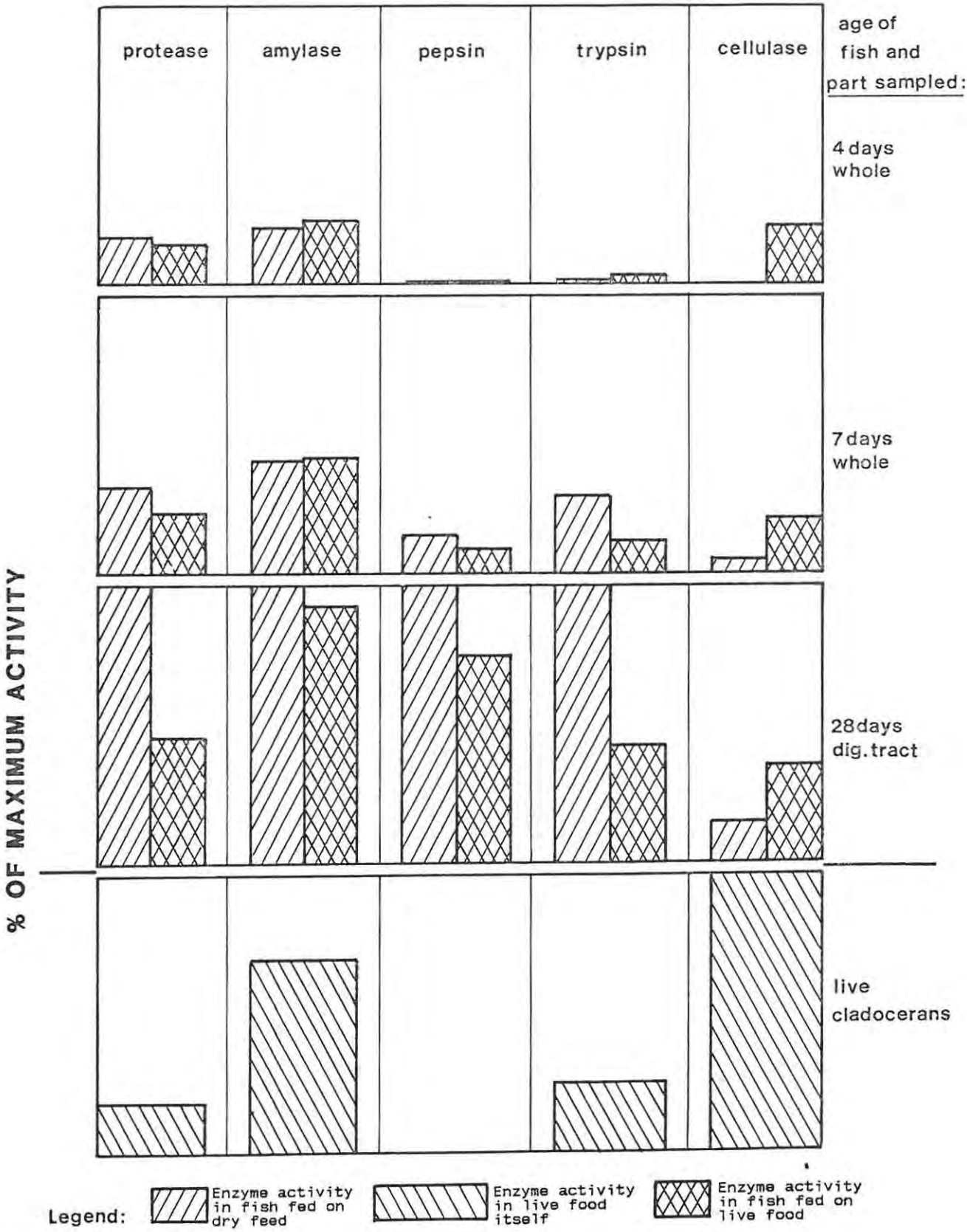


Figure 5.12 Graphic representation of the digestive enzyme activities in larval (4 and 7 day old) and juvenile (28 day old) Clarias gariepinus fed on either artificial dry feed (Uys and Hecht, 1985) or live crustacean zooplankton. The enzyme activities in the zooplankton are also shown. The activity of each enzyme is expressed as a percentage of the maximum activity measured for that particular enzyme. (Comparisons can only be made amongst measurements of the same enzyme type).

The experimental design and proposed interpretation of the results were based on the fact that the dry feed was free of enzymes, and that the live food had a given enzyme pattern. Therefore, assuming that the fish consumed equal quantities of their respective diets, the effect of live food on the fish's digestive enzyme pattern should have been reflected in the difference between the enzyme levels in the fish fed on dry feed and those fed on live food. Any exogenous enzymes contributed by bacteria or other organisms growing in the tanks, or by intestinal flora, could be discounted, since it was assumed to affect the fish fed on dry feed and live food equally.

Due to the presence of alkaline protease activities and, more specifically, trypsin activities in the zooplankton, it was expected that the activities of these enzymes in the fish fed on zooplankton, would have been enhanced. However, the seven and 28 day old juveniles which fed on zooplankton, had significantly» depressed ($P<0.05$) mean activities of these enzymes, compared to their counterparts which received dry feed only. Pepsin activities were also significantly depressed ($P<0.05$) in the fish that fed on zooplankton. Total amylase activities in the fish were not influenced by the two different diets. The only enzyme which showed enhanced levels in the fish fed on live zooplankton, was cellulase.

These unexpected results showed that the experimental design and the model for the interpretation of results as proposed above, did not suit the question that was asked. Since it was known that exogenous enzymes activate fish zymogens (Jancarik, 1964), and that exogenous enzymes from zooplankton contribute largely (up to 70%) to the total proteolytic enzyme activity in fish larvae and juveniles (Dabrowski and Glogowski, 1979b; Lauff and Hofer, 1984), it was naturally thought that elevated activities of these enzymes would result in fish larvae feeding on enzyme-rich zooplankton. However, after more thorough perusal of the literature dealing with this subject, it was realized that there

were fundamental flaws in the stated approach of this experiment.

Firstly, the estimation of the contribution of exogenous enzymes by measuring the difference in enzyme activities in fish fed enzyme free diets, and diets containing enzymes, might not be correct if the nature of the diet itself influences enzyme production in the fish (Lauff and Hofer, 1984). This was, in fact, already demonstrated by Dabrowski and Glogowski (1977b), since fish fed on diets containing bovine trypsin, had lower pancreatic proteolytic enzyme production than fish fed on an enzyme-free control diet.

Secondly, it was wrong to say that the zooplankton diet resulted in depressed digestive enzyme levels of the fish. Zooplankton is their natural diet, and the digestive enzyme levels in fish feeding on zooplankton represent the natural state of affairs.

The correct interpretation, is rather that the dry feed induced higher proteolytic enzyme levels in the fish. This is in accordance with Hofer (1985), who found that larval roach (Rutilis rutilis) fed on artificial feed had double the tryptic activity than their counterparts that were fed on Artemia. He made the interesting suggestion that this phenomenon, whatever the reason for it, could be detrimental, since it leads to an increased loss of body proteins (the relatively short gut and short retention time do not allow for re-absorption of the enzymes). If one considers that better larval catfish growth performances were obtained with dry feed than with live food in the other studies already mentioned, it would seem that such an increased loss of body proteins, if it exists, is offset by other benefits of a properly balanced and digestible dry feed.

Even though the contribution of exogenous proteolytic enzymes could not be determined with this experiment, at least one part of the initial approach was valid: Since cellulase is believed

not to be produced by any vertebrates, the difference in cellulytic activities between fish fed on live food and dry feed, can be attributed to the contribution of exogenous enzyme from the live food. As can be seen in Figure 15.12, this contribution is quite significant.

The cellulytic activities that were also detected in fish that fed only on dry feed were probably produced by intestinal microflora. This had also previously been confirmed in an interesting set of experiments by Stickney and Shumway (1974), who found that cellulytic activity in the intestine of Ictalurus punctatus fingerlings (which were fed on dry feed only), could be eliminated within 24 hours by treating the fish with an antibiotic.

SUMMARY OF CONCLUSIONS

Summaries of the preliminary results of this work have been published by Uys and Hecht (1987) and Uys et al. (1987). Owing to the variety of techniques used, accurate comparison of the enzyme activities of C. gariepinus with other species is difficult. After careful perusal of the many reports on the digestive enzymes of various fish species, it is, however, justified to make the general conclusion that C. gariepinus has relatively high digestive enzyme activities. It is perhaps more meaningful to view the result in the light of the fact that its proteolytic enzyme activities correspond with those of specialized carnivorous fishes, and that it starch digesting abilities correspond with those of herbivores. Also, the high levels of lysozyme and alkaline phosphatase, show an adaptation towards detritivory, making this fish a true opportunistic omnivore.

Its relatively un-acidic stomach provides an environment which allows efficient enzymatic digestion of its food by both pepsin and lysozyme. Acid hydrolysis of its food is therefore

sacrificed in favor of enzymatic digestion. The thick walled, muscular stomach of C. gariepinus is typical of fishes which employ this strategy.

Most digestive enzymes showed a decreasing proximo-distal gradient of distribution in the intestine of C. gariepinus. This probably reflects its ability to re-absorb its enzymes in order to conserve protein and retain the structural materials required for re-synthesis of digestive enzymes.

The optimum pH at which each digestive enzyme functions is closely correlated with the pH of the gastro-intestinal region in which it occurs. The temperature dependence of C. gariepinus dietary enzymes indicate that elevated temperatures (28 °C and higher) would benefit the rate of food digestion, but also that this species can maintain relatively high levels of digestive activity in extremes of temperatures (10-40 °C).

No evidence could be found for an inherent rhythmic cycle in digestive enzyme activities. The changes in digestive enzyme activities appeared to be induced solely by food intake. The quick and strong digestive response subsequent to feeding, implies that this fish is physiologically equipped to utilize large, infrequent and irregular meals effectively. Physiological data, therefore, provide no evidence that nocturnal feeding schedules would have to be implemented in commercial culture (behavioral data might, however, indicate otherwise).

The development of digestive enzyme systems in the early life history of C. gariepinus is as rapid as the development of its alimentary functional morphology. Free embryos are already equipped with amylase and proteolytic enzymes. However, pepsin activity and a functional stomach only develop some time after the onset of exogenous feeding (when the larvae are twice the length of free embryos). This, perhaps explains the initial difficulties that were experienced in rearing larvae of this

species on dry feeds alone.

Although increased cellulase activity was found in larvae and early juveniles that were fed on live zooplankton, the relative contribution of exogenous prey enzymes to protein digestion could not be ascertained. It was, however, shown that the artificial dry feed which is used for the initial rearing of catfish larvae induces higher levels of digestive enzyme activities.

# CHAPTER 6

# FEEDING TRIALS

## INTRODUCTION

The preceding chapters dealt with the more fundamental aspects of C. gariepinus nutrition, an understanding of which is a prerequisite for more applied research. In this chapter, a series of feeding trials are described in which the effects of different feed formulations on the growth of C. gariepinus juveniles and sub-adults were examined and evaluated.

Since fish growth and energy metabolism are of prime importance to commercial aquaculture, and since protein and energy ingredients make up the bulk of fish feeds, these two factors initially require the greatest consideration in formulating commercial fish feeds. Also, since lipids are the most concentrated source of dietary energy for fish (Stickney, 1977), the majority of feeding trials in nutritional studies deal mainly with the protein and energy factors, or the protein and lipid factors (Lee and Putnam, 1973; Dabrowski and Kozak, 1979; Mazid et al., 1979; Watanabe et al., 1979; Vens-Cappel, 1984; Zeitler et al., 1984; Watanabe, 1985; Henken et al., 1986; Machiels and Henken, 1985, 1986, 1987; Chuapoehuk, 1987). Of course there are many other vitally important nutritional factors which must also be considered, such as amino acid ratios, essential fatty acids, carbohydrates, minerals and vitamins, but one must start somewhere, and it was decided to concentrate in this investigation on protein and energy requirements of C. gariepinus and to conduct a cursory investigation into vitamin and mineral requirements.

In the first feeding trial, the total crude protein content of the different test diets were varied, while all other factors, inclusive of energy content, were kept as constant as possible.

In the second feeding trial total lipid content was the varying factor (constant protein and energy), and in the third set of trials, protein and energy levels were varied in a 3x3 factorial design (Hardy, 1980). Finally, in the fourth trial, an attempt was made to establish the optimum level of vitamin and mineral premix supplementation.

The literature on fish nutrition research is often confusing due to the variety of terminologies and techniques which have evolved. Moreover, it seems that precision in experimental design and execution is often forfeited due to uncontrollable or unforeseen circumstances. These problems are not unique to fish nutrition studies but are inherent in animal experimentation. The result is not so much failed experiments (since hypotheses might still be accepted or rejected) but rather incompatibility with other experiments and difficulty in the interpretation of results. It has, therefore, become necessary to introduce a measure of standardization in fish nutrition research, especially in experimental design, terminology and precise description of experimental conditions. In order to place this study into perspective, the methods and standards in fish nutrition research, especially those pertaining to feeding trials, are reviewed in the following section.

The classical method of quantifying the nutritional requirements of fishes (and other animals) is by trial and error based feeding trials. For each determination, a range of feeds is prepared in which one dietary factor is varied while the others are kept constant. Growth and survival responses are then taken as a measure of success. This is not as simple as it seems, since dietary factors are interrelated and one is often faced with a formidable mathematical problem when attempting to prepare a range of feeds which complies strictly with one-factor-variation.

The classical experimental design of hypothesis testing by comparing results of different treatments with a control is

usually followed. With simple hypothesis testing, the acceptance or rejection of the hypothesis is usually all that is required. However, since animal nutrition studies have to provide information to the livestock industries, experiments are carried out with the intent to generalize; if not the results, at least the conclusions. Therefore, additional evaluations are usually made which are not directly related to the hypothesis being tested.

Reports in the literature on fish feeding trials reflect the various levels of sophistication in experimental design, or the complexity of the question asked. At the lowest level are experiments which deal simply with testing two or more available foods for suitability in rearing a particular fish species (Brandt et al., 1979; Msiska, 1981). The findings of such experiments hold immediate benefits for commercial aquaculture (i.e. food A is better than food B), but do not answer any questions with regard to specific nutritional requirements, and neither do they allow generalizations (although these are often made). Such empirical methods may eventually lead to the development of an optimum diet by trial and error. However, a combination of empirical and deductive methods of research can save a considerable amount of costly experimentation (Pfeffer and Pieper, 1979), and should be undertaken if one of our objectives is to establish the nutritional requirements of a species.

In more sophisticated, deductive experiments semi-synthetic feeds are formulated and prepared specifically for the trial, using mainly conventional feed ingredients (Dabrowski and Kozak, 1979; Jackson et al., 1982). The objective of these studies is to determine the role of a specific nutritional factor (e.g. protein content), rather than simply comparing the effectiveness of available feeds. The range of feeds to be tested should be formulated in such a fashion that all nutritional factors are the same, except the one being studied (Hardy, 1980). When two factors are thought to have interrelated effects (such as the

protein to energy ratio), a two-factorial experimental design can be followed (Lee and Putnam, 1973; Gropp, 1979). The major problem with using conventional feed ingredients such as fishmeal and wheat, is that each ingredient contains a whole range of nutrients (e.g. protein, carbohydrate, lipid) and whenever an inclusion level of an ingredient is adjusted, it affects the entire range of nutrients.

Synthetic diets which are formulated with highly purified ingredients, such as casein, raw starch and cellulose (Kaushik and Dabrowski, 1983; Machiels and Henken, 1985), allow a higher degree of precision in controlling nutritional factors in a range of test feeds (Gropp, 1979). Comparison with other feed experiments is better facilitated and results are more reproducible.

Since feed comprises the most important production cost in intensive aquaculture (Tiews et al., 1979; Shang, 1981), the ultimate objective of feeding trial experiments is usually to minimize feed cost while maximizing growth rate and feed conversion efficiency. As stated before, the use of synthetic research diets with purified ingredients is probably the shortest route to determining detailed nutritional requirements (Cowey, 1976). However, the minimum feed cost objective cannot be achieved with purified ingredients alone, and ultimately, conventional feed ingredients need to be tested. This leads to another facet of feeding trial experiments, viz. the "least costing" of diets. One way of attempting to reduce the cost of an already established diet, is by systematically replacing an expensive ingredient with a similar but cheaper ingredient (empirical method). A case in point is fishmeal. It is perhaps one of the most commonly used ingredients in commercial fish feeds. It is also one of the most expensive ingredients, a fact which has led to the search for less expensive, alternative protein sources (Dabrowski and Kozak, 1979; Higgs et al., 1979; Spinelli et al., 1979; Tiews et al., 1979, Viola et al., 1981).

Ultimately, however, the objective of feed formulation studies is not merely to systematically replace expensive ingredients with cheaper ones, but to work out a set of rules by which to go about formulating an optimal combination for any given set of ingredients. Such sets of rules have indeed been worked out for different fish species and with the aid of computerized techniques are commonly applied by commercial feed manufacturers today (Chow et al., 1980; Crampton, 1985). This, so called, computerized least costing of fish feeds is further discussed in Chapter 7.

## GENERAL METHODS AND TERMINOLOGY

The feeding trials were conducted over two summers (1986-1988) at a commercial warmwater fish farm (Blyde River Aquaculture) in the Eastern Transvaal Lowveld. The experimental fish were selected from stocks during the course of the normal production cycles.

### Experimental design and conditions

In each trial, a full-sibling stock of fish were divided into subgroups of equal numbers and near-equal sizes for the respective rearing containers. The minimum subgroup size (n) was 40. Due to the differential growth rates encountered in C. gariepinus siblings, a procedure by which to select a near-equal size group from a group of siblings had to be developed. Firstly, the individual weights of siblings were recorded and a frequency distribution was calculated. A typical frequency distribution by weight of 14 week old full siblings is presented in Figure 6.1. Secondly, size classes to be included in the experimental group were selected equilaterally around the modus class. The fish were weighed again before being allocated to rearing containers and only individuals belonging to the selected size classes were used. As in the case of the population represented in Fig. 6.1, four size classes on each side of the modal class had to be included (120-200g) in order to have enough

fish for a trial. Weights and total lengths were recorded for all fish at the beginning and end of each feeding trial.

The treatments (diets) were assigned to the groups (containers) according to the randomized complete block design (Hardy, 1980). In the third experiment, however, a factorial design (Hardy, 1980) was followed. The design of the experiment was determined by the question(s) asked, but in each case allowed statistical evaluation of the results.

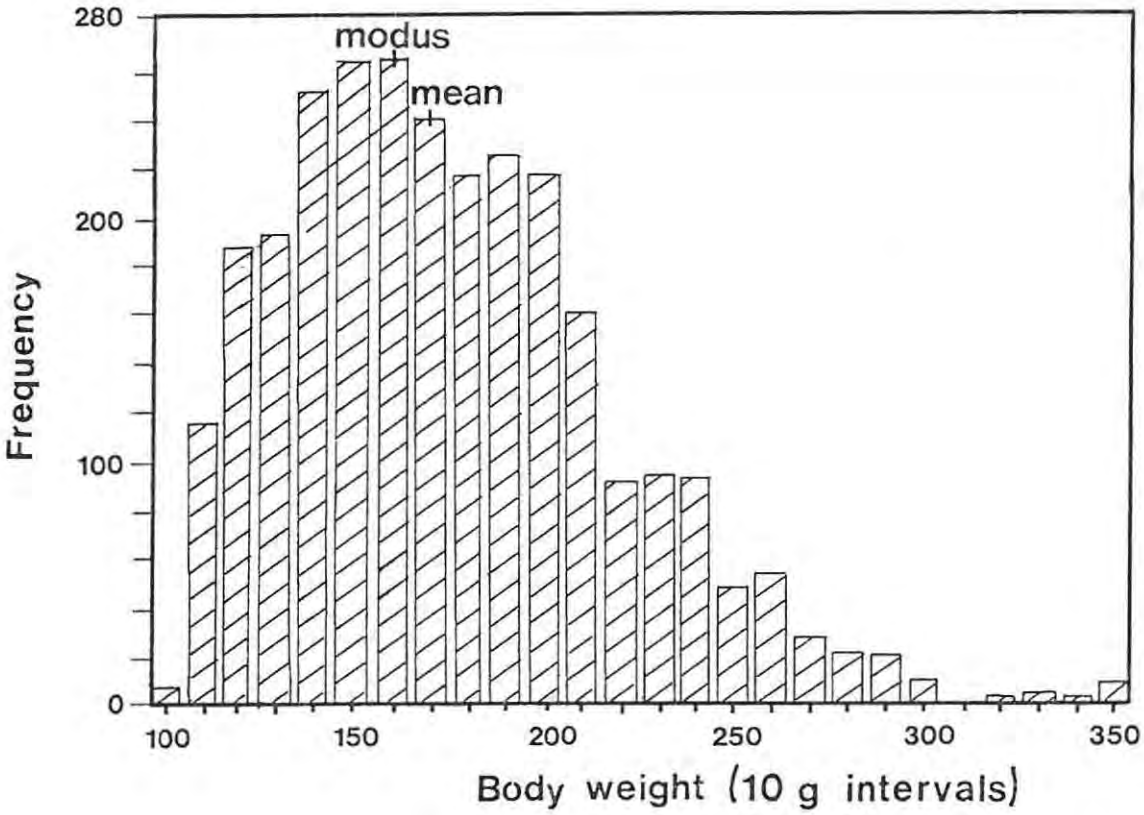


Figure 6.1. Frequency distribution by weight of 14 week old C. gariepinus full siblings.

The experimental facilities consisted of 20 outdoor, circular, plastic pools, individually supplied with untreated, through-flowing water extracted from the Blyde River (System A, See Fig. 2.3). Ten of the pools were 3m in diameter while the other ten were 2m in diameter. The water depth in all the pools was maintained at 60cm by a central upstand pipe in each pool, thereby resulting in capacities of 4200l and 1800l for the 3m and

2m pools respectively. Water flow was adjusted to effect one full exchange every 6 hours. The outdoor pools were only used during summer, when ambient water temperatures were within the range 26-34° C for the duration of each experiment. A battery of 12 circular, plastic pools (System B, diam. 1.5m, depth 0.3m, vol. 530l, flow 5l/min.) as well as 20 plastic lug boxes (System C, capacity 60l, depth 0.2m, flow 2l/min.) inside the hatchery were also used (Figures 2.2 and 2.3). These indoor rearing containers were also of the flow-through type and the supply water was maintained at above 26°C with thermostatically controlled electric- and solar heating equipment. The quality of the supply water is given in Table 6.1.

Table 6.1
Quality of supply-water used in the feeding trials.

| Parameter | Min | Max |
|---|---|---|
| pH | 6.8 | 7.65 |
| Total dissolved solids (mg/l) | 102 | 117 |
| Conductivity (mS/m) | 14 | 16 |
| Total alkalinity as $CaCO_3$ (mg/l) | 55 | 59 |
| Total hardness as $CaCO_3$ (mg/l) | 89 | 115 |
| Nitrogen as $NO_3$ (mg/l) | 0.06 | 0.18 |
| Nitrogen as $NH_3$ (mg/l) | 0.12 | 0.56 |
| Phosphorus as P (mg/l) | 0.69 | 1.32 |
| Calcium as Ca (mg/l) | 17 | 23 |
| Sodium as Na (mg/l) | 16 | 34 |
| Chloride as Cl (mg/l) | 8 | 15 |

In all the rearing containers, the supply-water was jetted in under pressure to effect aeration. Dissolved oxygen levels were not measured. Since the fish were seldom observed to surface for air breathing, it can be assumed that oxygen stress did not occur.

Diets

The experimental diets used in the study can be classified as semi-synthetic feeds, since conventional feed ingredients (e.g. fish meal, molasses powder) were used. This is in contrast to the purified ingredients (e.g. casein, dextrin) used in feeding trials on C. gariepinus by the Dutch workers (Machiels and Henken, 1985, 1987; Henken et al., 1986). As mentioned before, purified ingredients allow a higher degree of accuracy in one-factor variation in a range of test diets, but they are expensive and, therefore, impractical to use in commercial feeds. The nature of this study is, therefore, slightly less deductive, but closer to commercial conditions than the work done in The Netherlands.

All diets were prepared on a dry weight basis, and calculation of the nutritional composition of the diets was based on the analyses provided by the suppliers of the ingredients. The feed ingredients were ground with a Drotsky "C-class" agricultural hammer mill, and mixed and pelletized with a Lister combination upright mixer and pellet press with a 4mm dia. die ring.

The calculation of digestible energy (DE) and metabolizable energy (ME) values presents a problem, since these values have to be determined experimentally for each ingredient and are not interchangeable between fish species (Jobling, 1983). Such determinations have yet to be done for C. gariepinus (and will be a major study in itself). In the mean time, two alternatives have to suffice. The first is to substitute DE values as determined for channel catfish, Ictalurus punctatus (Table 6.2), and the second, a method which is favored by the Dutch workers, is to assume ME values of 16.7 kJ/g for protein and carbohydrate, and 37.7 kJ/g for lipids (also Lee and Putnam, 1973). In this study, the first alternative (substituted DE) is favored because the use of DE values is more conventional in the animal feed

industry, but the second (estimated ME) is also sometimes referred to for sake of comparison with the Dutch studies.

Table 6.2
Digestible Energy (DE) values of various feed ingredients for channel catfish, Ictalurus punctatus, as given by Lovell (1984).

| Ingredient | DE (kJ/g) |
|---|---|
| Wheat | 10.7 |
| Raw corn (maize) | 4.6 |
| Cotton oil cake | 10.7 |
| Soy oil cake | 10.8 |
| Fish meal | 16.3 |
| Meat and bone meal (carcass meal) | 14.5 |
| Alfalfa meal (lucern) | 2.8 |
| Fish oil | 36.9 |

Feeding frequency in all cases was three times daily (sunrise, noon and sunset) and feeding level was based on visually determined satiation feeding. The cumulative amount of food thus fed per tank was recorded in order to determine conversion efficiencies. Satiation feeding as opposed to restricted- and ad libitum feeding, was considered to be the best method to employ in these feeding trials. Hogendoorn et al. (1983) concluded that satiation feeding gives the most positive growth response in C. gariepinus. Moreover, it is postulated that with restricted feeding, growth responses would be biased towards the diets with the higher protein and energy levels. With satiation feeding, which is the recommended method for commercial culture, the growth responses should be most positive with the diets containing the best protein to energy ratios. It is also appropriate to note at this point, that the term feed conversion ratio (FCR) has been defined in several different ways in the literature. Utne (1979) defines it as feed intake per weight

gained and Castell and Tiews (1980) recommend the definition feed fed per weight gained. With satiation and restricted feeding regimes, these definitions have the same result, but with ad libitum feeding, intake would be difficult to determine and, feed fed would give a misleading FCR due to the unknown amount of feed wasted. In this study, since satiation feeding was applied, it was more practical to use the definition, feed fed per weight gained.

According to Castell and Tiews (1980) a basal control diet should supply all nutrients required by the test fish and allow reasonable growth and survival for the test period. The Standard Reference Diet (SRD) H-440 (also know as Halver's diet, Table 6.3) is commonly used in feeding trials and has proven successful with salmonids, carp, catfishes and several other test fish species (Castell and Tiews, 1980). The purpose of using a SRD in the present study, apart from serving as a control diet, is to allow the results of this work to be compared with the results of subsequent studies on C. gariepinus, provided of course, that the SRD-440 is also used in those studies.

It should be noted here, that instead of following the diet preparation procedure as given in the footnote to Table 6.3, the ingredients were pelleted dry in the same manner as the experimental feeds for the sake of parametric compatibility. Since no data is available yet on the requirements of C. gariepinus for dietary vitamins and minerals, the mineral and vitamin premixes as given in Table 6.3 were supplemented to all the experimental diets.

Table 6.3.
Standard Reference Diet H-440 ( from Castell and Tiews, 1980)

| Complete Test Diet | (g) | Vitamin Mixture | (g) |
|---|---|---|---|
| Vitamin-free casein | 38 | a-cellulose | 8.000 |
| White dextrin | 28 | Choline chloride | 0.500 |
| Gelatin | 12 | Inositol | 0.200 |
| Corn (=maize) oil | 6 | L-Ascorbic acid | 0.100 |
| Cod liver oil | 3 | Nicotinic acid | 0.075 |
| Vitamin mixture | 9 | Ca-pantothenate | 0.050 |
| Mineral mixture | 4 | Riboflavin | 0.020 |
| Total | 100 | Thiamin-HCl | 0.005 |
| Water | 200 | Pyridoxine-HCl | 0.005 |
| Total diet as feed | 300 | Menadione | 0.004 |
| | | Folic acid | 0.0015 |
| | | Vitamin B12 | 0.0011 |
| | | Biotin | 0.0005 |
| | | a-Tocopherol acetate | 0.040 |

| 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 | Potassium phosphate dibasic | 3.98 |
| KI | 0.015 | Sodium biphosphate | 8.72 |
| $CoCl_2.6H_2O$ | 0.100 | Sodium chloride | 4.35 |
| | 100.52 | | 99.50 |

Note on diet preparation: Dissolve gelatine in water (80°C). Mix in dextrin, casein, minerals, oils and vitamins while cooling (40°C). Pour into containers and refrigerate to harden.

## Test parameters

Whatever the intent of a feeding trial certain parameters are required by which to evaluate the response of the fish to different nutritional treatments. A great diversity of such parameters are used in the literature and there is some confusion as to their application. The parameters and their definitions used in this study are in accordance with the document on standardization of methodology in fish nutrition research by Castell and Tiews (1980).

-Mortality: percentage of animals which died per unit time.

-Growth: weight gain per unit time

-Specific Growth Rate (SGR): weight increase as a percentage of body weight per day

SGR= $(\text{Ln } W_t - \text{Ln } W_o)/t \times 100$

where $W_o$ is initial body weight and $W_t$ is body weight at time t

-Feed Conversion Ratio (FCR): weight of feed fed per unit body weight gain

-Protein Efficiency Ratio (PER): body weight gained per unit of crude protein fed

-Condition Factor : $CF = [100 \times \text{weight(g)}] / [\text{length (cm)}]^3$

-Digestible Energy (DE): gross energy of feed minus gross energy of faeces per unit weight of consumed food. DE=(Re-Fe)/Re, where Re= ration energy, Fe= faecal energy.

-Metabolizable energy (ME): gross energy of feed minus gross energy of total faeces minus gross urinary energy minus gross branchial waste energy per unit weight of food consumed

ME= [Re-(Fe+Ue+Be)]/Re, where Be=branchial waste energy, Ue= urinary energy.

**Statistical evaluation of results**

Cognizance was taken of the variety (and sometimes lack) of statistical methods employed in the many feeding trial studies in the literature. At the one end of the spectrum are the highly empirical studies (Diet A is better than Diet B), in which simple t-tests suffice to point out statistical differences between two treatments. At the other end are the highly deductive feeding trial studies on C. gariepinus by Machiels and Henken (1985) and Henken et al. (1986) in which multiple regression analysis is required.

The parameters used in this study are mostly based on body weight measurements, with the exception of CF which incorporates body length as well. Each feeding trial commenced with all the test groups at near-equal body weight. The final body weights of the

test fish form the basis of all subsequent calculations, and it is therefore appropriate that these final body weights be subjected to statistical evaluation (the second edition of 'Biometry' by Sokal and Rohlf (1981) was used as a guide for selecting and computing appropriate statistical tests and their terminology is adhered to). Analysis of variance (Anova) was computed for each set of final body weights, with subsequent multiple comparisons among pairs of means (95% confidence intervals). The specific Anova models which were used varied from one feeding trial to the other, depending on the experimental design, and are described in more detail along with the results of each experiment. Since multiple comparisons for significant differences among pairs of means in the final weight data should be regarded as "a posteriori" or unplanned (Sokal and Rohlf, 1981), the indicated test to use here is the "T-method" also known as "Tukey's honestly significant difference method" (not to be confused with the t-test).

EXPERIMENT 1

EFFECT OF DIETARY CRUDE PROTEIN LEVEL

Materials and Methods

Five test diets (Table 6.4) and the Standard Reference Diet (SRD, Table 6.3) were used in these experiments. Dietary protein content ranged from 32 to 48 % while the other dietary factors were kept as constant as possible. Note, however that DE varied from 11.1 kJ/g in the low protein diet to 12.6 kJ/g in the high protein diet. This was unavoidable, due to the high energy content of the primary protein source (fishmeal) which was used.

Table 6.4
Formulation and calculated composition of test diets containing different crude protein levels.

| Ingredient | Diet1 | Diet2 | Diet3 | Diet4 | Diet5 | Diet6 |
|---|---|---|---|---|---|---|
| Maize | 40.97 | 27.76 | 19.60 | 13.43 | 7.99 | SRD |
| Wheat Bran | 9.42 | 16.40 | 17.96 | 17.39 | 15.98 | H-440 |
| Fish meal | 31.93 | 37.85 | 42.05 | 47.42 | 51.54 | (control) |
| Carcass meal | 4.71 | 5.05 | 6.37 | 7.27 | 8.79 | |
| Blood meal | 4.71 | 5.05 | 6.37 | 7.27 | 8.79 | |
| Fish acid oil | 4.85 | 4.47 | 4.15 | 3.75 | 3.41 | |
| Vit Px | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | |
| Mineral Px | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | |
| Molasses powder | 3.01 | 3.02 | 3.10 | 3.07 | 3.10 | |
| Total | 100 | 100 | 100 | 100 | 100 | |
| Crude protein % | 32.00 | 36.00 | 40.00 | 44.00 | 48.00 | 38.0 |
| Crude fat % | 10.67 | 10.66 | 10.66 | 10.66 | 10.66 | 9.0 |
| Crude fiber % | 3.65 | 3.84 | 3.71 | 3.44 | 3.13 | |
| N-free extract | 35.12 | 30.09 | 25.54 | 21.11 | 16.74 | |
| Moisture | 9.92 | 9.94 | 9.84 | 9.83 | 9.78 | |
| Ash % | 8.64 | 9.47 | 10.25 | 10.96 | 11.70 | |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | |
| DE kJ/g * | 11.1 | 11.4 | 11.8 | 12.2 | 12.6 | ? |

* DE calculated on the basis of DE values of ingredients for Ictalurus punctatus (Lovell, 1984, Table 6.2).

Three separate trials were conducted, each with a different age group in order to establish whether protein requirements change with age. The respective ages at the start of the trials were four, six and eight weeks (rearing systems C,B and A respectively) and the trials lasted for 21 days. Each treatment (group) for each age group was replicated (two subgroups). The numbers of fish per container (or subgroup) were 50, 45 and 40 for the four, six and eight week old groups respectively.

Results

The results are presented in Table 6.5 and Figures 6.2 to 6.7. Due to an error in feed application, no replicate results were obtained for the eight week old age group. Since the same response pattern emerged for each age group (Figures 6.2 - 6.4), the trial was not repeated.

Growth rates were positive for all groups of fish concerned with the 44 and 48% protein diets showing the best responses. Neglible mortalities (<5%) occurred in the four week old fish while no moratlities were recorded for the other age groups. Analysis of variance (one way, two level nested Anova) revealed that there was a highly significant ($P< 0.001$) added variance component among treatments (groups) at all ages (Table 6.6), and that there was no evidence of a significant variance among replicate treatments (subgroups). Note that the largest variance component in each case occurred within subgroups (among individuals). This, again, illustrates the differential growth rates of *C. gariepinus* siblings.

Due to the large variance within subgroups, the 95% comparison intervals by the T-method are rather wide (Figures 6.2 - 6.4). The intervals of the final mean weights of the 40, 44 and 48% protein treatments overlapped in each of the age groups, signifying that there were no significant differences between these treatments for $P<0.05$. However, the 44 and 48% treatments

had significantly superior final mean weights to the 36 and 32% protein treatments.

Table 6.5
Growth and feed utilization of Clarias gariepinus fed on diets with different protein contents for 21 days.

| Group No. | Dietary Protein content % | Mean initial length mm | Mean initial weight g | Mean final length mm | (SD) | Mean final weight g | (SD) | Weight increase g | CF 100*wt/len3 | SGR % body wt/d | Food fed g | FCR | PER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial age 4 weeks (n=50) | | | | | | | | | | | | | |
| 1a | 32 | 49.1 | 1.1 | 103.7 | 9.5 | 8.2 | 2.8 | 7.1 | 0.73 | 9.54 | 5.7 | 0.81 | 3.87 |
| 1b | 32 | | | 100.4 | 11.8 | 7.6 | 2.9 | 6.5 | 0.75 | 9.18 | 5.7 | 0.88 | 3.54 |
| 2a | 36 | | | 108.8 | 9.4 | 9.4 | 2.7 | 8.3 | 0.73 | 10.20 | 6.0 | 0.73 | 3.82 |
| 2b | 36 | | | 110.8 | 7.9 | 9.3 | 3.2 | 8.2 | 0.68 | 10.18 | 6.0 | 0.73 | 3.81 |
| 3a | 40 | | | 113.6 | 6.9 | 11.0 | 2.1 | 9.9 | 0.75 | 10.96 | 6.5 | 0.66 | 3.81 |
| 3b | 40 | | | 116.8 | 7.3 | 12.1 | 2.4 | 11.0 | 0.76 | 11.43 | 6.5 | 0.59 | 4.24 |
| 4a | 44 | | | 116.9 | 5.3 | 14.0 | 2.1 | 12.9 | 0.88 | 12.13 | 7.7 | 0.60 | 3.82 |
| 4b | 44 | | | 115.4 | 6.6 | 13.2 | 2.7 | 12.1 | 0.86 | 11.83 | 7.7 | 0.64 | 3.57 |
| 5a | 48 | | | 120.4 | 5.4 | 13.9 | 2.2 | 12.8 | 0.80 | 12.07 | 7.7 | 0.60 | 3.46 |
| 5b | 48 | | | 118.1 | 8.5 | 13.6 | 2.6 | 12.5 | 0.83 | 11.99 | 7.7 | 0.61 | 3.39 |
| 6a | 38 | (Control) | | 111.4 | 7.9 | 9.7 | 2.7 | 8.6 | 0.70 | 10.36 | 6.6 | 0.77 | 3.42 |
| 6b | 38 | (Control) | | 116.2 | 7.6 | 11.1 | 2.0 | 10.0 | 0.71 | 11.00 | 6.8 | 0.68 | 3.86 |
| Initial age 6 weeks (n=45) | | | | | | | | | | | | | |
| 1a | 32 | 89.1 | 8.9 | 150.7 | 8.7 | 28.5 | 5.0 | 17.6 | 0.78 | 5.20 | 17.2 | 0.98 | 3.20 |
| 1b | 32 | | | 151.7 | 9.0 | 26.6 | 5.2 | 17.7 | 0.76 | 5.21 | 17.2 | 0.97 | 3.22 |
| 2a | 36 | | | 156.6 | 13.9 | 29.4 | 4.9 | 20.5 | 0.77 | 5.69 | 18.8 | 0.92 | 3.03 |
| 2b | 36 | | | 155.6 | 13.9 | 28.4 | 4.6 | 19.5 | 0.75 | 5.53 | 18.8 | 0.96 | 2.89 |
| 3a | 40 | | | 162.1 | 12.6 | 32.0 | 7.0 | 23.1 | 0.75 | 6.10 | 19.6 | 0.85 | 2.95 |
| 3b | 40 | | | 161.0 | 11.9 | 30.9 | 6.6 | 22.0 | 0.74 | 5.93 | 19.6 | 0.89 | 2.81 |
| 4a | 44 | | | 164.4 | 12.3 | 36.6 | 8.2 | 27.7 | 0.82 | 6.73 | 22.3 | 0.81 | 2.82 |
| 4b | 44 | | | 159.7 | 12.7 | 34.4 | 7.8 | 25.5 | 0.85 | 6.44 | 22.3 | 0.87 | 2.60 |
| 5a | 48 | | | 160.1 | 17.3 | 34.6 | 10.1 | 25.7 | 0.84 | 6.47 | 22.4 | 0.87 | 2.39 |
| 5b | 48 | | | 161.4 | 17.5 | 34.7 | 9.6 | 25.8 | 0.82 | 6.48 | 22.4 | 0.87 | 2.40 |
| 6a | 38 | (Control) | | 157.3 | 20.3 | 31.5 | 10.8 | 22.6 | 0.81 | 6.02 | 21.4 | 0.95 | 2.78 |
| 6b | 38 | (Control) | | 155.8 | 19.8 | 30.4 | 9.5 | 21.5 | 0.80 | 5.85 | 21.4 | 1.00 | 2.64 |
| Initial age 8 weeks (n=40) | | | | | | | | | | | | | |
| 1 | 32 | 131.2 | 17.2 | 163.3 | 18.7 | 32.3 | 13.5 | 15.1 | 0.74 | 3.00 | 15.5 | 1.02 | 3.49 |
| 2 | 36 | | | 167.5 | 17.3 | 36.9 | 11.9 | 19.7 | 0.79 | 3.64 | 17.4 | 0.88 | 3.54 |
| 3 | 40 | | | 171.8 | 26.7 | 42.7 | 18.8 | 25.5 | 0.84 | 4.33 | 22.0 | 0.86 | 3.22 |
| 4 | 44 | | | 177.6 | 18.4 | 47.6 | 15.3 | 30.4 | 0.85 | 4.85 | 22.5 | 0.74 | 3.07 |
| 5 | 48 | | | 180.5 | 26.8 | 48.9 | 20.7 | 31.7 | 0.83 | 4.98 | 22.5 | 0.71 | 2.94 |
| 6 | 38 | (Control) | | 170.4 | 18.8 | 41.3 | 11.9 | 24.1 | 0.83 | 4.17 | 20.0 | 0.83 | 3.16 |

Note: The group number postscripts (a,b) denote replicate treatments (subgroups) for the same diet . There were no replicate treatments in the 8 week old groups.

Table 6.6
Anova tables showing the sigificance of treatment effects on the final weights of fish fed on different dietary protein levels (each age group analyzed independently).

| Source of variation | Variance component as % | df | SS | MS | Fs | Significance |
|---|---|---|---|---|---|---|
| Initial age 4 weeks | | | | | | |
| Among groups (diets) | 43.7 | 5 | 1387.5 | 277.5 | 30.70 | P<0.001 |
| Among subgrps.(replicates) | 0.7 | 6 | 54.2 | 9.0 | 1.32 | not significant |
| Within subgroups | 55.6 | 288 | 1966.0 | 6.8 | | |
| Total | 100 | 299 | 3407.7 | | | |
| Initial age 6 weeks | | | | | | |
| Among groups (diets) | 14.4 | 5 | 2610.2 | 522.0 | 25.08 | P<0.001 |
| Among subgrps.(replicates) | 2.4 | 6 | 124.9 | 20.8 | 0.33 | not significant |
| Within subgroups | 83.2 | 264 | 16607.2 | 62.9 | | |
| Total | 100 | 275 | 19342.3 | | | |
| Initial age 8 weeks | | | | | | |
| Among groups (diets) | 11.7 | 5 | 7964.5 | 1592.9 | 6.30 | P<0.001 |
| Within groups | 88.3 | 234 | 59184.0 | 252.9 | | |
| Total | 100 | 239 | 67148.5 | | | |

df= degrees of freedom SS= sums of squares
MS= mean squares Fs= variance ratio

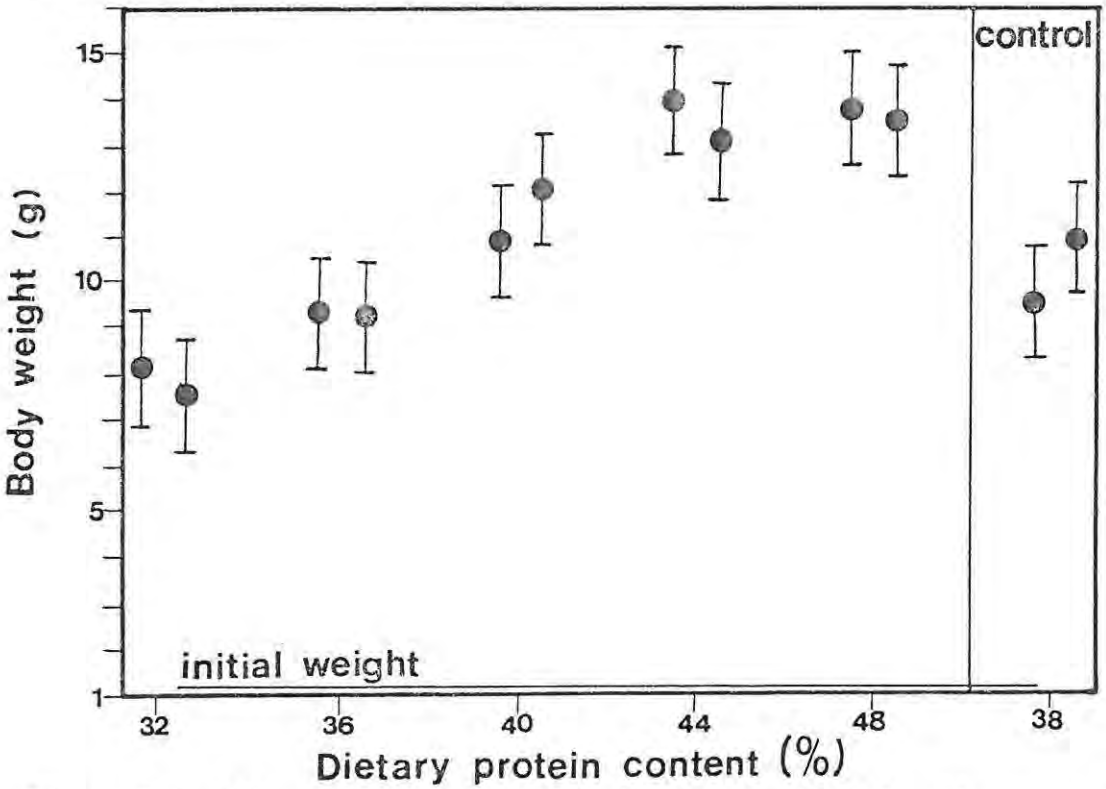


Figure 6.2. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on different dietary protein levels for 21 days. Initial age 4 weeks. Means and comparison intervals of replicate treatments are shown separately.

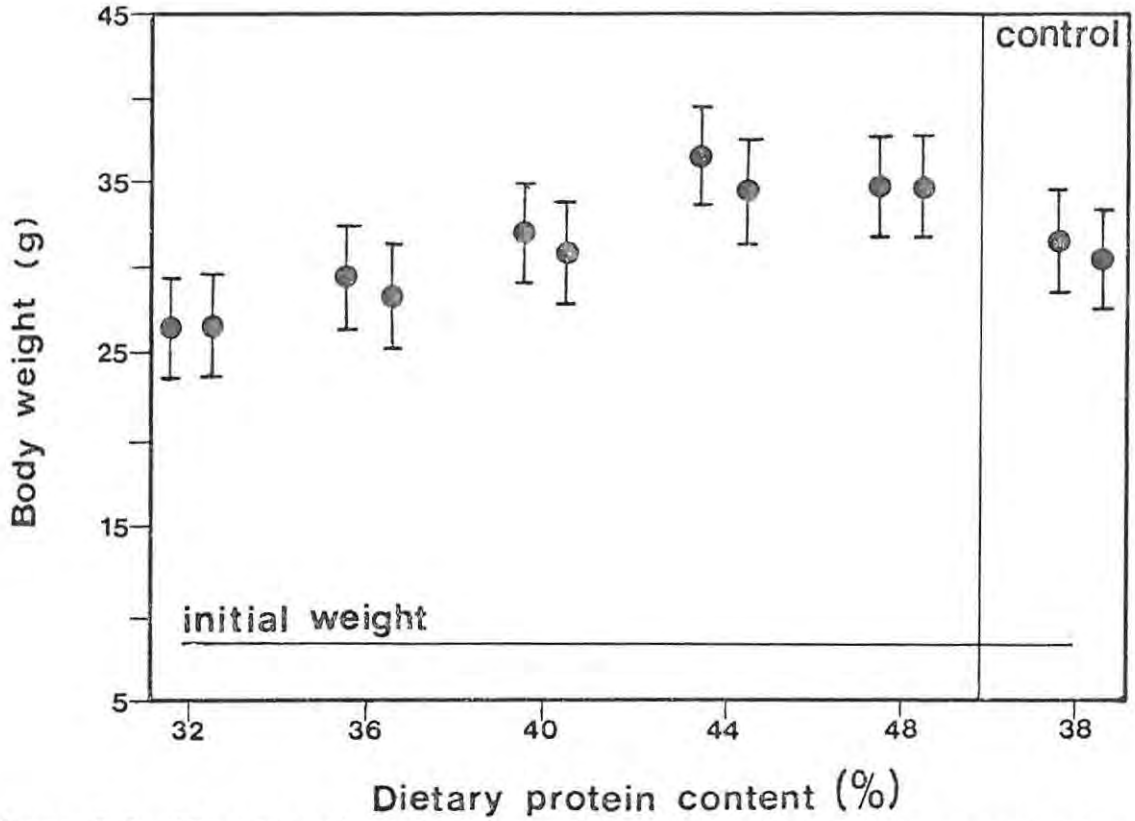


Figure 6.3. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on different dietary protein levels for 21 days. Initial age 6 weeks. Means and comparison intervals of replicate treatments are shown separately.

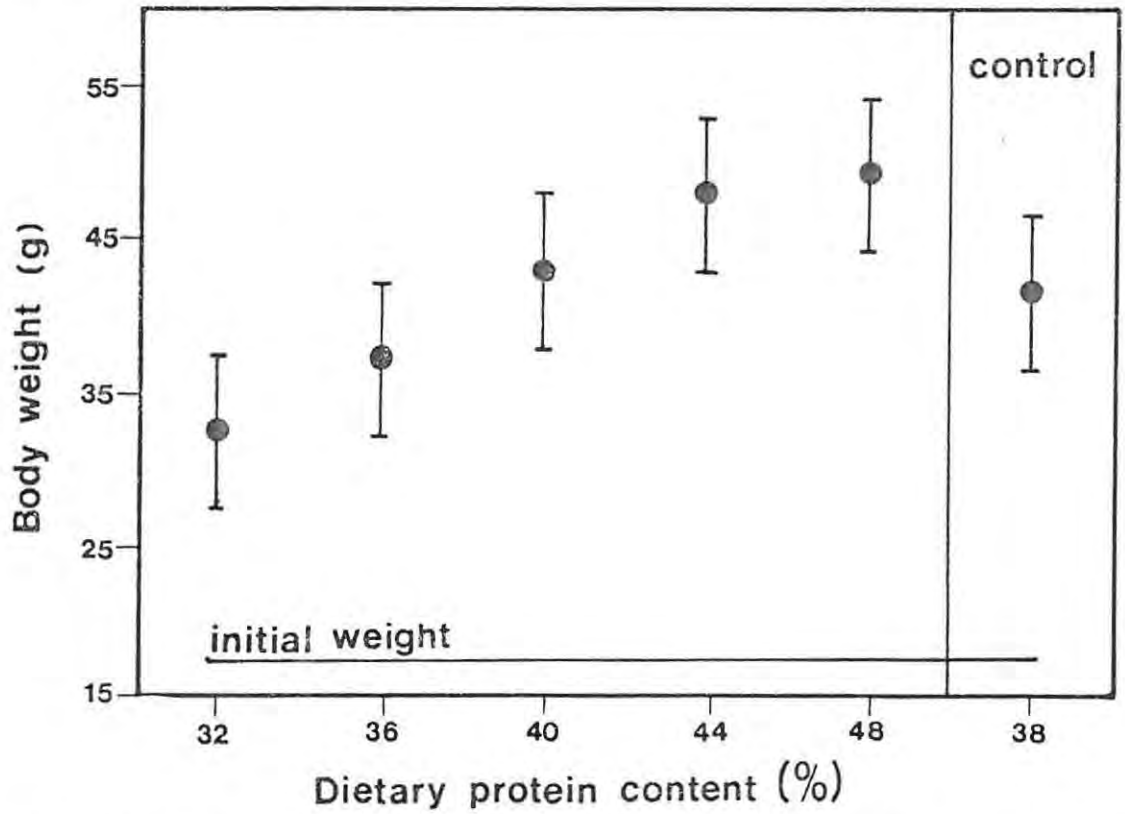


Figure 6.4. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on different dietary protein levels for 21 days. Initial age 8 weeks. No replicate treatments.

The Anova presented in Table 6.6 provides sufficient validation of the observed treatment effects to allow further parametric analysis: Increased protein levels had a positive effect on condition factors (Fig. 6.5) and feed conversion ratios (Fig. 6.6), while protein efficiency ratios (Fig. 6.7) were best with the low protein diet (32%). Condition factors showed a peak at the 44% protein level while the 44-48% protein levels resulted in the best feed conversion ratios. Age of the fish had no evident effect on condition factors (Fig. 6.5) but specific growth rates (Table 6.8), feed conversion ratios (Fig. 6.6) and protein efficiency ratios (Fig. 6.7) deteriorated markedly in the older fish.

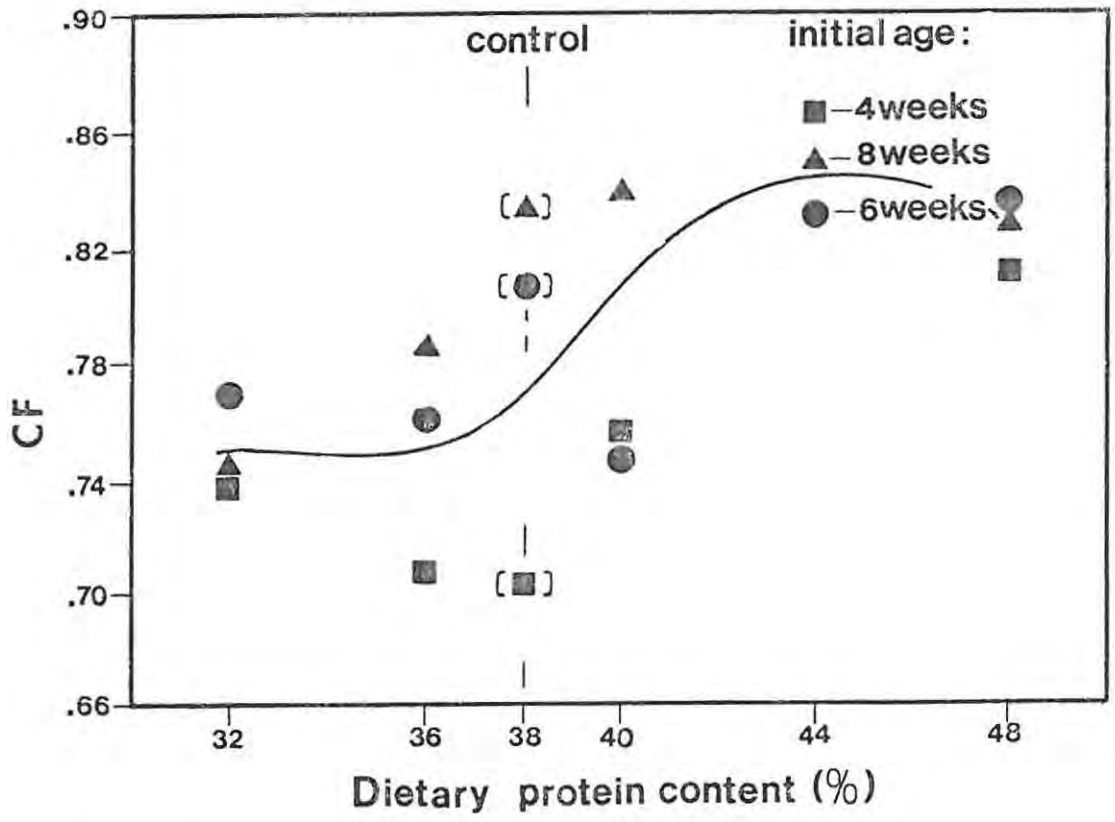


Figure 6.5. The effect of dietary protein level on the condition factors of three different age groups of C. gariepinus juveniles and sub-adults. The curve represents the general trend in all three age groups and is fitted by eye. Means of replicate treatments are pooled.

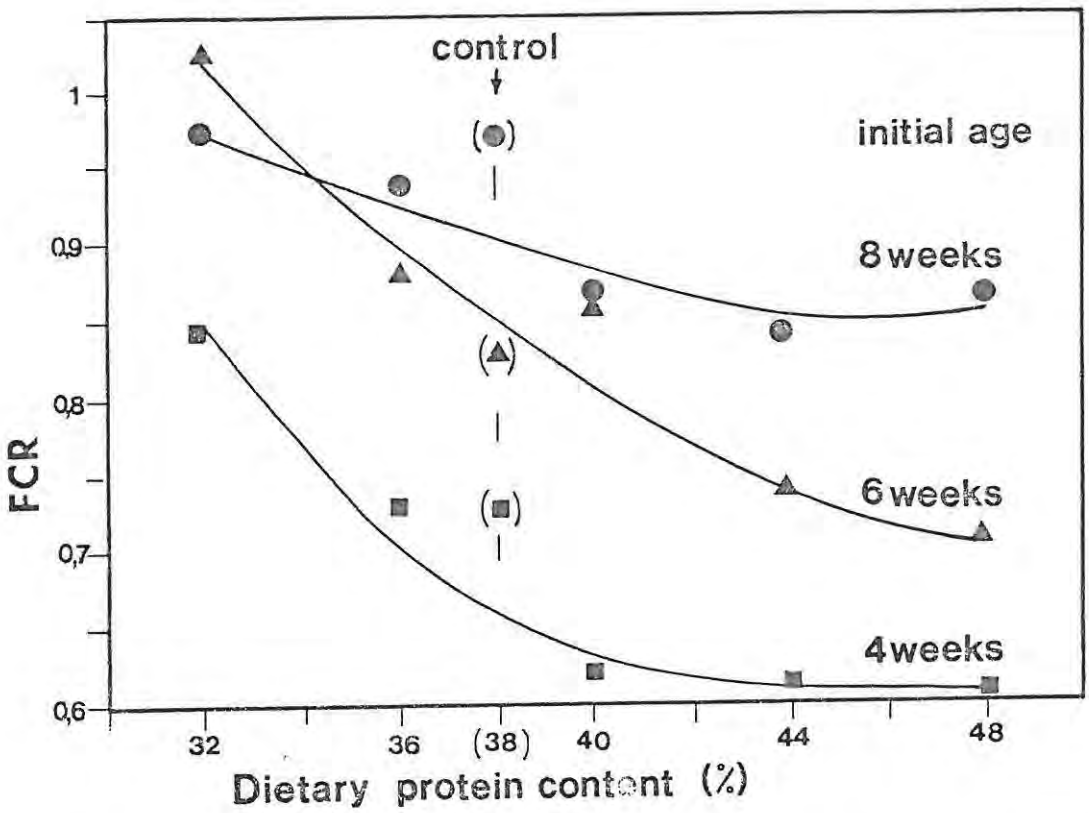


Figure 6.6. The effect of dietary protein level on feed conversion ratios of three different age groups of C. gariepinus juveniles and sub-adults. Curves fitted by eye. Means of replicate treatments are pooled.

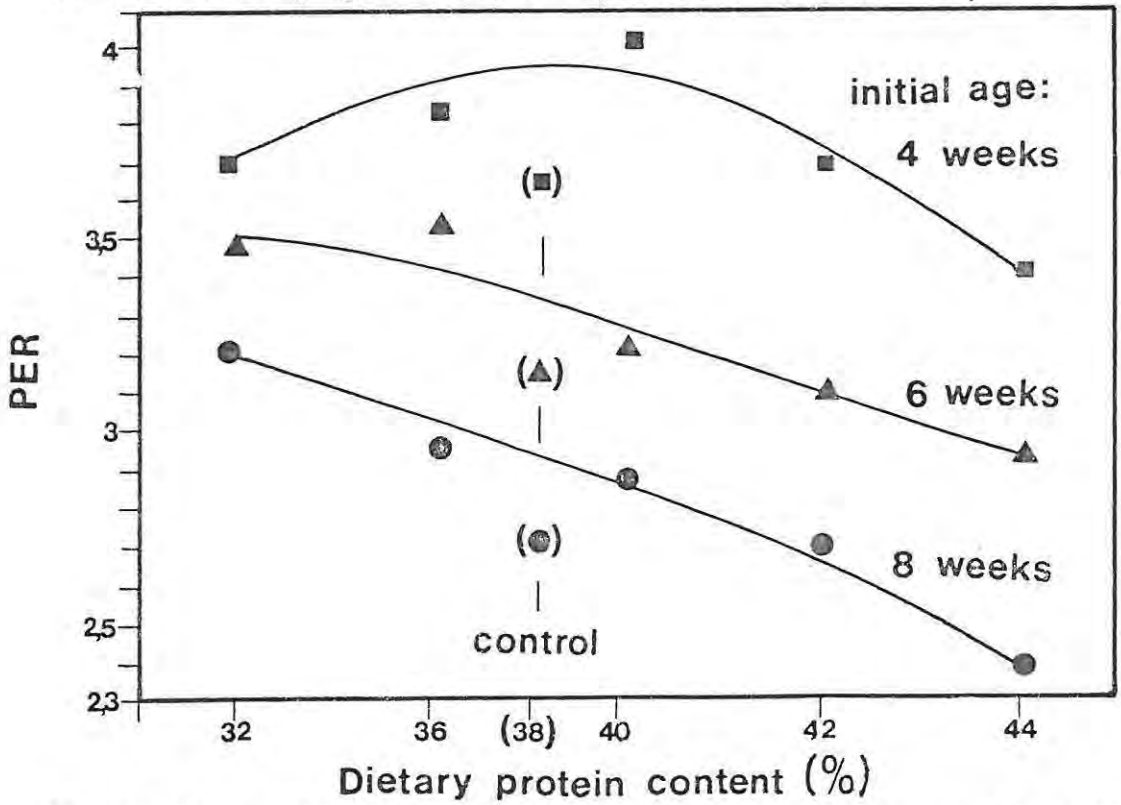


Figure 6.7. The effect of dietary protein level on protein efficiency ratios of three different age groups of C. gariepinus juveniles and sub-adults. Curves fitted by eye. Means of replicate treatments are pooled.

## Discussion

The results (subject to the limitations of the experimental framework) lead to the conclusions that *C. gariepinus* juveniles and sub-adults, seem to have a dietary protein requirement of 44 - 48%, and that this requirement does not change with the age of the fish (4-11 weeks). This is in agreement with the findings of Machiels and Henken (1985) who worked with purified feed ingredients and concluded that *C. gariepinus* requires a dietary protein content in excess of 40% for maximal growth, irrespective of dietary energy level.

Ufodike and Ekokotu (1986), using blood meal as a protein source, found that diet mixtures containing 50.2% protein resulted in better growth responses in *C. gariepinus* (initial weight ±21g) than diets containing 38.0% or 62.9% protein. Growth rates, feed conversion ratios and protein efficiency ratios were, however, far superior in the present study.

The fact that protein efficiency ratios decreased only slightly with increasing dietary protein levels is in agreement with the findings of Machiels and Henken (1985) and Henken *et al.* (1986), who concluded that *C. gariepinus* is capable of using high-protein diets very efficiently. *Ictalurus punctatus*, on the other hand, showed a more pronounced reduction in protein efficiency at higher dietary protein levels (Garling and Wilson, 1976). It should be noted that PER does not take changes in body composition of the fish into account.

It is worth noting, that the SRD, or control diet, in spite of its highly purified nature, yielded similar results to those of the semi-purified test diets. Since all that is required of a SRD is that it should contain all the nutrients, that it should be replicable and that it should yield a positive growth response, the SRD H440 is suitable for use in *C. gariepinus* growth trials.

EXPERIMENT 2.

EFFECT OF DIETARY CRUDE LIPID LEVEL

Materials and Methods

Five test diets (Table 6.7) and the Standard Reference Diet (SRD, Table 6.3) were used in this set of experiments. Dietary lipid content ranged from six to 14 % while the other dietary factors were kept as constant as possible. Based on the parametric analysis of the responses in the previous experiment a crude protein content of 42% was elected for this experiment. As a direct result of the different lipid levels, the calculated protein to energy ratios in the test diets ranged from 28.7 to 34.9 mg protein/kJ digestible energy.

Table 6.7
Formulation and calculated composition of test diets containing different crude lipid levels.

| Ingredient | Diet1 | Diet2 | Diet3 | Diet4 | Diet5 | Diet6 |
|---|---|---|---|---|---|---|
| Maize | 16.52 | 16.52 | 16.52 | 16.08 | 13.79 | SRD |
| Wheat Bran | 17.68 | 17.68 | 17.68 | 16.54 | 16.35 | H-440 |
| Fish meal | 44.74 | 44.74 | 44.74 | 44.83 | 45.45 | (control) |
| Carcass meal | 6.82 | 6.82 | 6.82 | 6.87 | 6.80 | |
| Blood meal | 6.82 | 6.82 | 6.82 | 6.87 | 6.80 | |
| Fish acid oil | 0.00 | 1.30 | 3.30 | 5.33 | 7.39 | |
| Vit Px | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | |
| Mineral Px | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | |
| Molasses powder | 3.09 | 3.09 | 3.09 | 3.07 | 3.04 | |
| Alpha cellulose | 3.95 | 2.65 | 0.65 | 0.00 | 0.00 | |
| Total | 100 | 100 | 100 | 100 | 100 | |
| Crude protein % | 42.01 | 42.01 | 42.01 | 41.94 | 42.00 | 38.0 |
| Crude fat % | 6.71 | 8.01 | 10.01 | 12.00 | 14.01 | 9.0 |
| Fibre | 5.55 | 4.90 | 3.90 | 3.43 | 3.30 | |
| N-free | 28.83 | 28.18 | 27.19 | 25.73 | 23.80 | |
| Moisture | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | |
| Ash | 6.90 | 6.90 | 6.90 | 6.90 | 6.90 | |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | |
| DE kJ/g * | 12.05 | 12.53 | 13.27 | 13.90 | 14.62 | |
| mg protein/kJ | 34.87 | 33.53 | 31.67 | 30.16 | 28.74 | |

* DE calculated on the basis of DE values of ingredients for Ictalurus punctatus (Lovell, 1984, Table 6.2)

Two separate trials were conducted, with respective ages at the start of the experiments being four and 14 weeks. The trials were run consecutively in System A, with each trial lasting 21 days. Each treatment (group) for each age group was replicated (two subgroups). The numbers of fish per container (or subgroup) were 50, and 55 for the four and 14 week old groups respectively.

Results

The resulting growth rates and calculated performance parameters are listed in Table 6.8 and presented graphically in Figures 6.8 to 6.12. Growth rates were positive for all groups of fish concerned with the 12 and 14% lipid diets showing the best responses. No mortalities occurred. Analysis of variance (one way, two level nested Anova) revealed that there was a highly significant ($P< 0.001$) added variance component among treatments (groups) at both ages (Table 6.9), and that there was no evidence of a significant variance among replicate treatments (subgroups).

The higher lipid levels also resulted in improved condition factors (Figure 6.10), feed conversion ratios (Figure 6.11) and protein efficiency ratios (Figure 6.12).

Growth and feed utilization of Clarias gariepinus fed on diets with different lipid contents for 21 days.

| Group No. | Lipid content % | Mean initial length mm | Mean initial weight g | Mean final length mm | (SD) | Mean final weight g | (SD) | Weight increase g | CF 100*wt len3 | SGR % body wt/d | Food fed g | FCR | PER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial age 4 weeks (n=50) | | | | | | | | | | | | | |
| 1a | 6.7 | 49.1 | 1.1 | 94.8 | 6.3 | 6.4 | 1.9 | 5.3 | 0.75 | 8.4 | 7.2 | 1.36 | 1.75 |
| 1b | 6.7 | | | 92.7 | 9.8 | 6.0 | 1.5 | 4.9 | 0.75 | 8.0 | 7.2 | 1.48 | 1.61 |
| 2a | 8 | | | 109.5 | 8.4 | 9.8 | 3.0 | 8.7 | 0.75 | 10.4 | 7.4 | 0.85 | 2.80 |
| 2b | 8 | | | 111.3 | 6.1 | 10.0 | 2.9 | 8.9 | 0.72 | 10.5 | 7.4 | 0.83 | 2.86 |
| 3a | 10 | | | 109.6 | 7.2 | 9.3 | 2.6 | 8.2 | 0.71 | 10.2 | 7.5 | 0.91 | 2.60 |
| 3b | 10 | | | 110.5 | 8.2 | 10.7 | 2.6 | 9.6 | 0.79 | 10.8 | 7.5 | 0.78 | 3.05 |
| 4a | 12 | | | 116.1 | 8.8 | 12.3 | 3.0 | 11.2 | 0.79 | 11.5 | 7.7 | 0.69 | 3.47 |
| 4b | 12 | | | 116.0 | 8.9 | 12.4 | 2.9 | 11.3 | 0.80 | 11.5 | 7.7 | 0.68 | 3.49 |
| 5a | 14 | | | 116.1 | 8.1 | 13.0 | 2.3 | 11.9 | 0.83 | 11.8 | 7.7 | 0.65 | 3.67 |
| 5b | 14 | | | 117.6 | 6.2 | 13.8 | 2.2 | 12.7 | 0.85 | 12.0 | 7.7 | 0.61 | 3.93 |
| 6a | 9 | (Control) | | 98.3 | 8.7 | 7.0 | 1.7 | 5.9 | 0.74 | 8.8 | 6.7 | 1.13 | 2.33 |
| 6b | 9 | (Control) | | 100.1 | 10.2 | 7.1 | 2.2 | 6.0 | 0.71 | 8.9 | 6.7 | 1.12 | 2.35 |
| Initial age 14 weeks (n=55) | | | | | | | | | | | | | |
| 1a | 6.7 | 268.5 | 159.6 | 303.5 | 8.7 | 210.6 | 15.0 | 51.0 | 0.75 | 1.3 | 102.5 | 2.01 | 1.19 |
| 1b | 6.7 | | | 304.3 | 11.0 | 214.9 | 22.2 | 55.3 | 0.76 | 1.4 | 102.5 | 1.85 | 1.28 |
| 2a | 8 | | | 312.2 | 11.9 | 245.7 | 32.2 | 86.1 | 0.81 | 2.1 | 121.8 | 1.41 | 1.68 |
| 2b | 8 | | | 315.0 | 8.8 | 251.2 | 21.1 | 91.6 | 0.80 | 2.2 | 121.8 | 1.33 | 1.79 |
| 3a | 10 | | | 323.5 | 11.0 | 287.8 | 26.8 | 128.2 | 0.85 | 2.8 | 169.1 | 1.32 | 1.80 |
| 3b | 10 | | | 321.7 | 8.4 | 279.5 | 22.6 | 119.9 | 0.84 | 2.7 | 169.1 | 1.41 | 1.69 |
| 4a | 12 | | | 324.3 | 14.0 | 288.8 | 36.0 | 129.2 | 0.85 | 2.8 | 170.0 | 1.32 | 1.81 |
| 4b | 12 | | | 326.0 | 11.5 | 294.4 | 32.0 | 134.8 | 0.85 | 2.9 | 170.0 | 1.26 | 1.89 |
| 5a | 14 | | | 326.7 | 8.8 | 303.0 | 25.0 | 143.4 | 0.87 | 3.1 | 167.3 | 1.17 | 2.04 |
| 5b | 14 | | | 322.2 | 8.7 | 293.7 | 25.2 | 134.1 | 0.88 | 2.9 | 167.3 | 1.25 | 1.91 |
| 6a | 9 | (Control) | | 311.5 | 12.0 | 243.6 | 32.8 | 84.0 | 0.81 | 2.0 | 122.0 | 1.45 | 1.81 |
| 6b | 9 | (Control) | | 312.6 | 9.9 | 249.0 | 21.7 | 89.4 | 0.82 | 2.1 | 122.0 | 1.36 | 1.93 |

Note: The group number postscripts (a,b) denote replicate treatments for the same diet .

Table 6.9
Anova tables showing the sigificance of treatment effects on the final weights of fish fed on different dietary lipid levels (each age group analyzed independently).

| Source of variation | Variance component as % | df | SS | MS | Fs | Significance |
|---|---|---|---|---|---|---|
| Initial age 4 weeks | | | | | | |
| Among groups (diets) | 46.6 | 5 | 1406.8 | 281.4 | 100.60 | P<0.001 |
| Among subgrps.(replicates) | 1.2 | 6 | 16.8 | 2.8 | 0.45 | not significant |
| Within subgroups | 52.2 | 288 | 1798.2 | 6.2 | | |
| Total | 100.0 | 299 | 3221.8 | | | |
| Initial age 14 weeks | | | | | | |
| Among groups (diets) | 59.5 | 5 | 308313.6 | 61662.7 | 99.52 | P<0.001 |
| Among subgrps.(replicates) | 0.2 | 6 | 3717.6 | 619.6 | 0.84 | not significant |
| Within subgroups | 40.3 | 324 | 239510.7 | 739.2 | | |
| Total | 100.0 | 335 | 551542.0 | | | |

df= degrees of freedom SS= sums of squares
MS= mean squares Fs= variance ratio

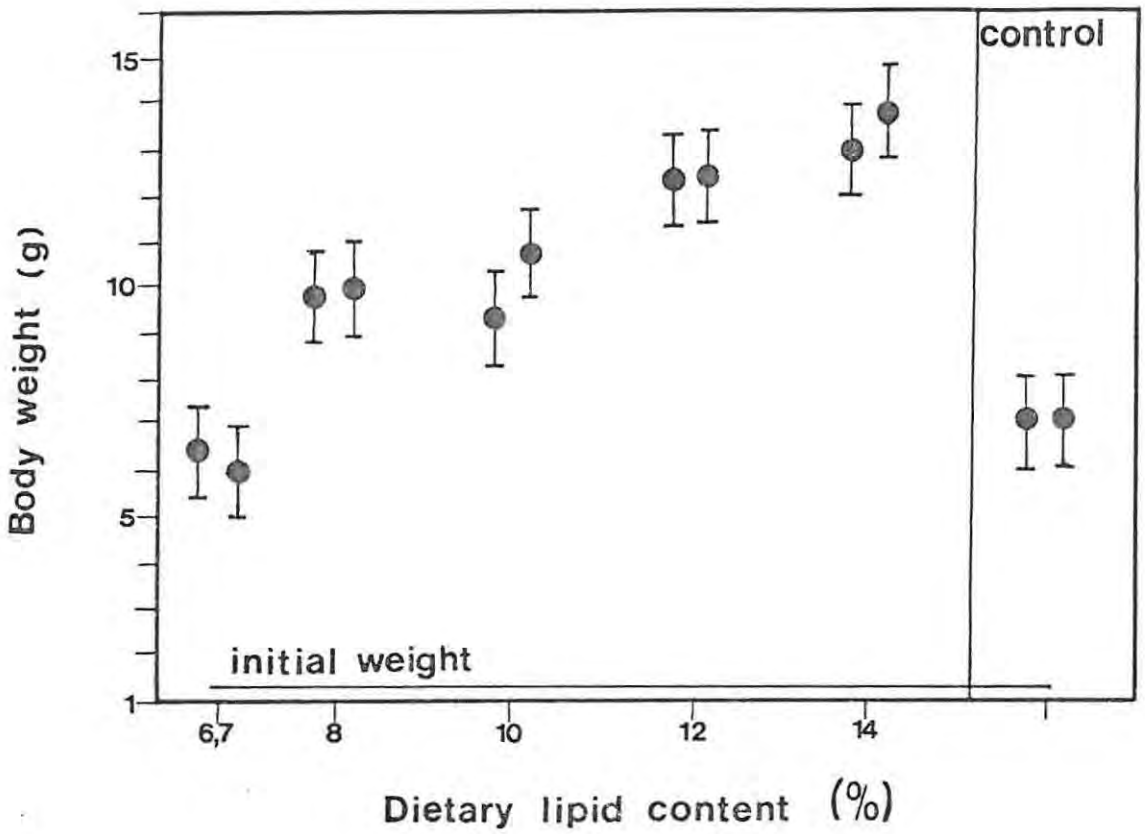


Figure 6.8. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on different dietary lipid levels for 21 days. Initial age 4 weeks. Means and comparison intervals of replicate treatments are shown separately.

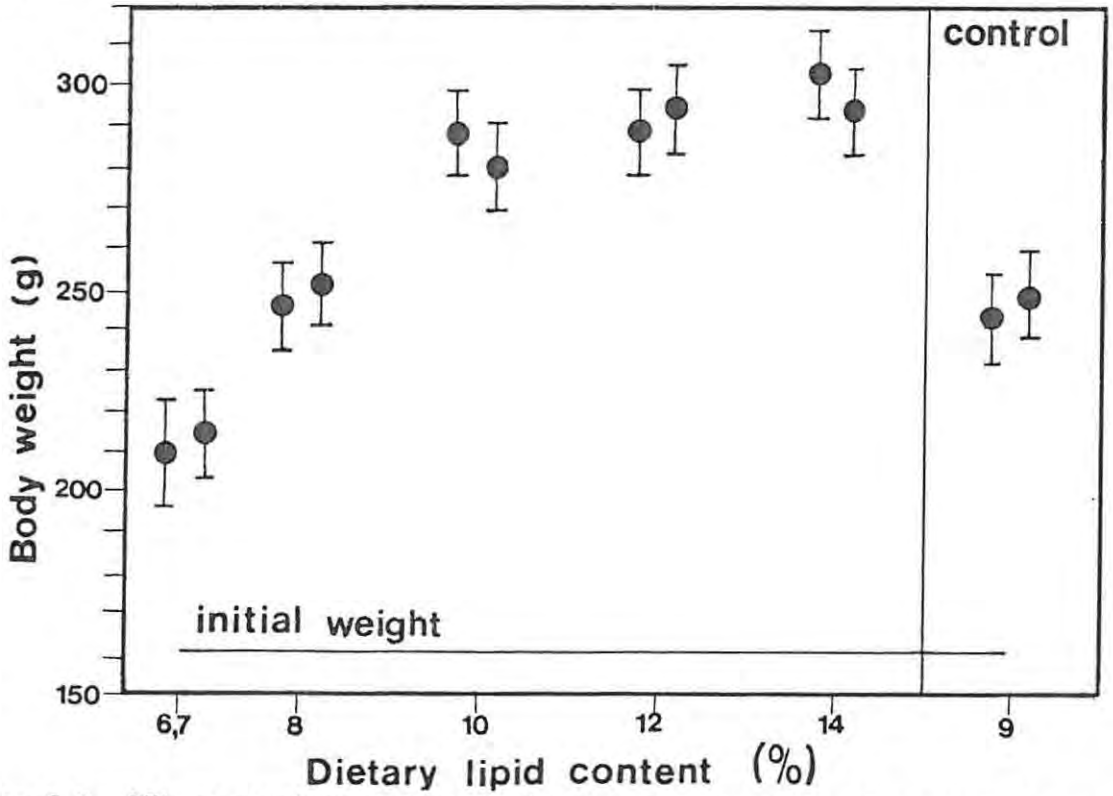


Figure 6.9. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on different dietary lipid levels for 21 days. Initial age 14 weeks. Means and comparison intervals of replicate treatments are shown separately.

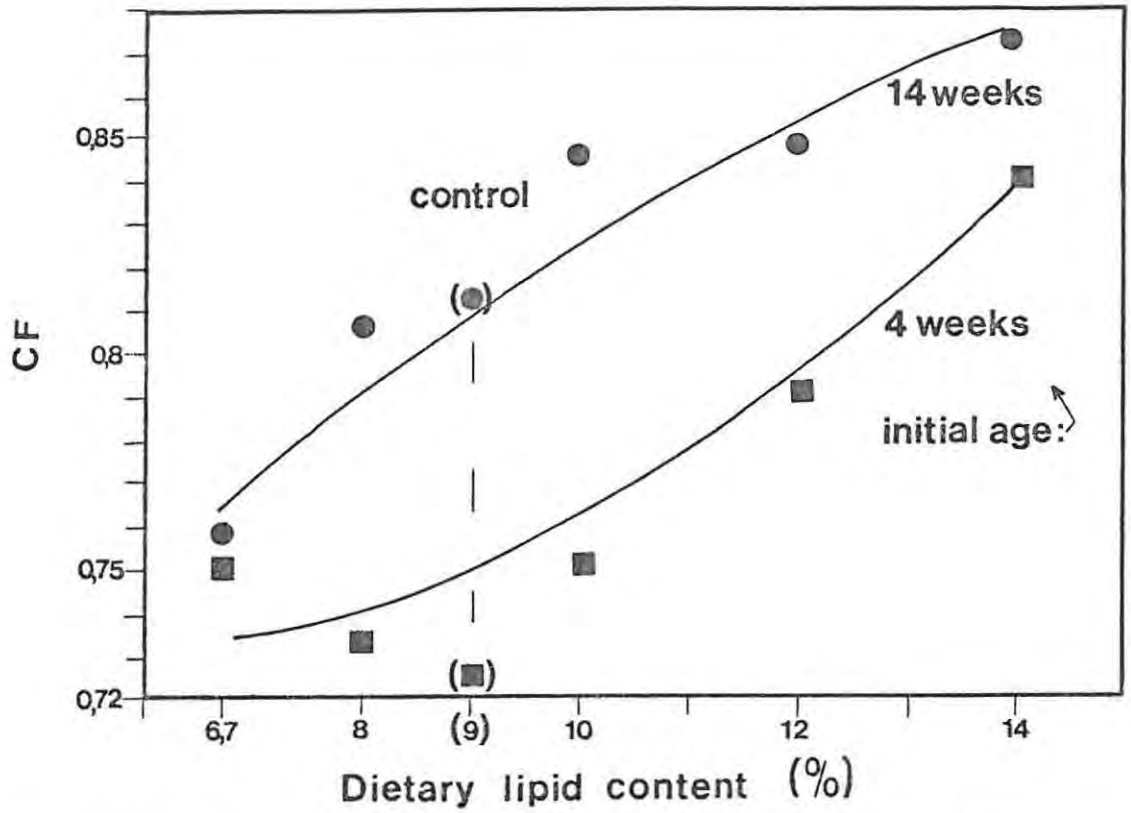


Figure 6.10. The effect of dietary lipid level on the condition factors of two different age groups of C. gariepinus juveniles and sub-adults. Curves fitted by eye. Means of replicate treatments are pooled.

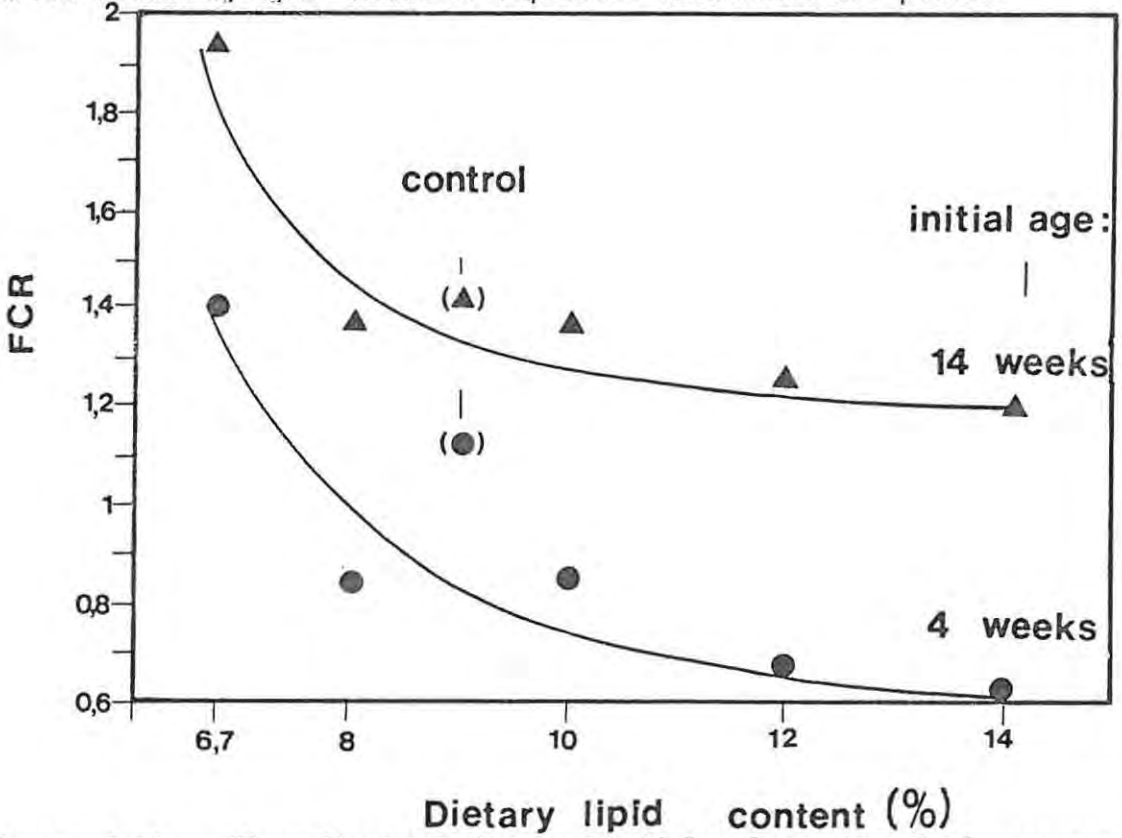


Figure 6.11. The effect of dietary lipid level on the feed conversion ratios of two different age groups of C. gariepinus juveniles and sub-adults. Curves fitted by eye. Means of replicate treatments are pooled.

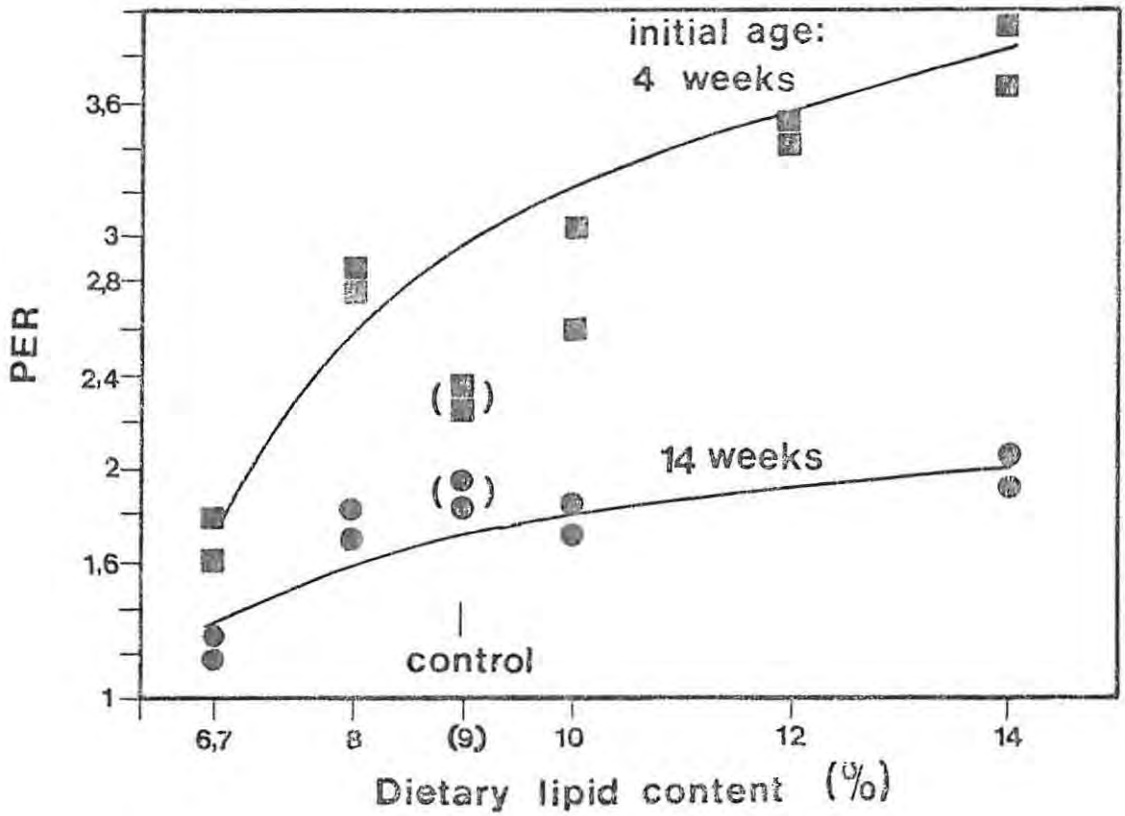


Figure 6.12. The effect of dietary lipid level on the protein efficiency ratios of two different age groups of C. gariepinus juveniles and sub-adults. Curves fitted by eye. Means of replicate treatments are pooled.

Discussion

It is quite noticible that the 4 week old (initial age) fish which were fed on the control diet performed rather poorly - even when compared with the same age group of the previous experiment. This was perhaps due to the control diet for the 4 week old fish having been stored for too long (more than 6 weeks).

The highly significant added variance effect ($P<0.001$) of different lipid levels on growth rate at a constant protein level of 42% signifies the importance of supplying sufficient energy in catfish feeds for efficient growth and feed utilization. As opposed to dietary protein levels, where a costly increase is required for a small increase in growth performance, only a small amount of added lipid can result in markedly improved growth rates and feed utilization. The cost sensitivity of protein as a dietary component increases drastically above the 40% level,

whereas the cost sensitivity of lipid below the 16% level is still relatively low. Moreover, increased dietary lipid levels result in markedly increased protein efficiency ratios (Fig. 5.12), whereas high dietary protein levels do not. This is known as the "protein sparing effect" caused by the addition of lipid or energy (Lee and Putnam, 1973; Watanabe et al., 1979; Degani, 1987). It should be stressed here, that an unproportional increase in body fat may lead to a PER which is biased in favour of high energy diets. However, since changes in dietary composition has a much greater effect on overall somatic growth than on body composition (Hogendoorn, 1983; Hogendoorn et al., 1983), the PER is still a valid method for expressing protein utilization. Machiels and Henken (1985) showed a similar effect in C. gariepinus by using purified feed ingredient. They concluded that for maximum protein synthesis and, therefore, maximum growth, sufficient energy intake is required. The implication of the "protein sparing effect" is that protein content of the diet could be lowered if fat (energy) levels were increased simultaneously (Lee and Putnam, 1973; Machiels and Henken, 1985). The following experiment was designed to test this hypothesis.

EXPERIMENT 3

EFFECT OF DIFFERENT DIETARY PROTEIN AND ENERGY COMBINATIONS

Materials and Methods

Nine test diets (Table 6.10) and the Standard Reference Diet (SRD, Table 6.3) were used in this set of experiments. Dietary protein and energy levels were varied in a three by three factorial design, while the other dietary factors were kept as constant as possible. Based on the parametric analysis of the responses in the previous experiments, crude protein levels of 38, 43 and 48% were elected, while calculated digestible energies ranged from 12.5 to 16.8 kJ/g. In order to achieve this range of energy values, the lipid levels had to be varied from 7.9 to 13.4%.

Table 6.10

Formulation and calculated composition of test diets containing a 3x3 factorial combination of protein and energy levels .

Table 6.10
Formulation and calculated composition of test diets containing a 3x3 factorial combination of protein and energy levels .

| Ingredient | Diet1 | Diet2 | Diet3 | Diet4 | Diet5 | Diet6 | Diet7 | Diet8 | Diet9 | Diet10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Maize | 18.45 | 12.60 | 6.75 | 17.35 | 11.50 | 5.40 | 15.40 | 10.30 | 5.20 | SRD |
| Wheat Bran | 18.45 | 12.60 | 6.75 | 17.35 | 11.50 | 5.40 | 15.40 | 10.30 | 5.20 | H-440 |
| Fish meal | 43.30 | 43.90 | 44.60 | 51.10 | 51.70 | 52.90 | 58.60 | 59.70 | 60.90 | (control) |
| Carcass meal | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | |
| Blood meal | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | |
| Fish acid oil | 1.40 | 4.50 | 7.50 | 0.80 | 3.90 | 6.90 | 0.20 | 3.30 | 6.30 | |
| Vit Px | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | |
| Mineral Px | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | |
| Molasses powder | 8.00 | 16.00 | 24.00 | 3.00 | 11.00 | 19.00 | 0.00 | 6.00 | 12.00 | |
| Total | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | |
| Crude protein % | 38.00 | 38.00 | 38.00 | 43.00 | 43.00 | 43.00 | 48.00 | 48.00 | 48.00 | 38.00 |
| Crude fat % | 7.85 | 10.53 | 13.12 | 7.95 | 10.63 | 13.25 | 7.96 | 10.75 | 13.44 | 9.00 |
| Crude fiber % | 3.68 | 2.79 | 1.90 | 3.55 | 2.66 | 1.74 | 3.30 | 2.53 | 1.75 | |
| N-free extract | 29.15 | 28.11 | 23.14 | 24.41 | 21.37 | 18.38 | 19.51 | 16.62 | 13.79 | |
| Moisture | 9.87 | 9.18 | 8.50 | 10.22 | 9.53 | 8.85 | 10.46 | 9.87 | 9.29 | |
| Ash % | 11.46 | 13.40 | 15.35 | 10.87 | 12.81 | 14.80 | 10.76 | 12.24 | 13.73 | |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | |
| DE kJ/g * | 12.50 | 14.58 | 16.19 | 12.85 | 14.66 | 16.32 | 13.26 | 14.76 | 16.78 | |
| mg protein/kJ | 30.4 | 26.1 | 23.5 | 33.5 | 29.3 | 26.4 | 36.2 | 32.5 | 28.6 | |

* DE calculated on the basis of DE values of ingredients for Ictalurus punctatus (Lovell, 1984, Table 6.2)

Two separate trials were conducted, with respective ages at the start of the experiments being four and 11 weeks. The trials were run concurrently in Systems A (2 subgroups, 11 weeks old fish), System B (four week old fish, subgroups "a") and System C (four week old fish, subgroups "b"), with each trial lasting 21 days. There were 60 fish per container (or subgroup) for both age groups.

Results

The resulting growth rates and calculated performance parameters are listed in Table 6.11 and presented graphically in Figures 6.13 to 6.16. Growth rates were, again, positive for all groups of fish concerned, with the main trend being an increase in growth rate resulting from either higher energy levels or higher protein levels. Mortalities were negligible (<3%). Two different Anova models were computed for the results (Table 6.12). The usual one-way, two-level, nested Anova revealed that there was a highly significant ($P< 0.001$) added variance component among treatments (groups) with both ages (Table 6.12), and especially with the 11 week old fish ($P<<0.001$). Since there was was no evidence of a significant variance among replicate treatments (subgroups), the replicate means were subsequently pooled and a two-way Anova (also Table 6.12) was computed in accordance with the factorial design of the experiment. The two way Anova revealed that there was a highly significant ($P< 0.001$) added variance component caused by the interaction of protein and energy levels with the older age group. The younger age group was, however, characterized by extreme differentiality in growth rates within subgroups and this caused the biggest variance component in the Anova tables. There was no evidence of a significant interaction between protein and energy levels with the younger age group. Due to the wide 95% confidence intervals around the mean final weights of the younger age groups (Figure 6.13), significant differences among specific diets did not emerge.

Table 6.11
Growth and feed utilization of Clarias gariepinus fed on diets with different combinations of protein and energy content in a 3x3 factorial arrangement.

| Group No. | Protein content of diet % | DE of diet kJ/g | Mean initial weight g | Mean final length | (SD) | Mean final weight g | (SD) | Weight increase g | CF 100*wt/len3 | SGR % body wt/d | Food fed g | FCR | PER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial age 4 weeks (n=60) | | | | | | | | | | | | | |
| 1a | 38 | 12.5 | 0.96 | 104.1 | 8.2 | 9.1 | 2.6 | 9.1 | 0.81 | 10.7 | 7.2 | 0.79 | 3.34 |
| 1b | 38 | 12.5 | | 104.8 | 7.2 | 9.3 | 2.6 | 9.3 | 0.81 | 10.8 | 7.2 | 0.78 | 3.39 |
| 2a | 38 | 14.6 | | 104.9 | 8.9 | 10.0 | 3.1 | 10.0 | 0.88 | 11.1 | 7.7 | 0.77 | 3.41 |
| 2b | 38 | 14.6 | | 106.9 | 10.3 | 10.7 | 3.7 | 10.7 | 0.88 | 11.5 | 7.7 | 0.72 | 3.67 |
| 3a | 38 | 16.2 | | 106.0 | 7.7 | 10.5 | 2.9 | 10.5 | 0.88 | 11.4 | 7.7 | 0.74 | 3.58 |
| 3b | 38 | 16.2 | | 107.5 | 7.6 | 11.3 | 2.9 | 11.3 | 0.91 | 11.7 | 7.7 | 0.68 | 3.86 |
| 4a | 43 | 12.9 | | 107.8 | 8.1 | 11.0 | 2.2 | 11.0 | 0.88 | 11.6 | 7.7 | 0.70 | 3.32 |
| 4b | 43 | 12.9 | | 107.7 | 9.4 | 10.8 | 2.3 | 10.8 | 0.86 | 11.5 | 7.7 | 0.71 | 3.26 |
| 5a | 43 | 14.7 | | 108.0 | 8.0 | 12.0 | 2.5 | 12.0 | 0.96 | 12.0 | 7.8 | 0.65 | 3.59 |
| 5b | 43 | 14.7 | | 107.4 | 9.1 | 11.1 | 2.7 | 11.1 | 0.89 | 11.6 | 7.8 | 0.70 | 3.30 |
| 6a | 43 | 16.3 | | 108.2 | 9.3 | 12.0 | 3.4 | 12.0 | 0.95 | 12.0 | 7.9 | 0.66 | 3.54 |
| 6b | 43 | 16.3 | | 109.2 | 9.0 | 13.4 | 3.3 | 13.4 | 1.03 | 12.6 | 7.9 | 0.59 | 3.95 |
| 7a | 48 | 13.3 | | 110.2 | 8.6 | 12.7 | 3.1 | 12.7 | 0.95 | 12.3 | 7.9 | 0.62 | 3.36 |
| 7b | 48 | 13.3 | | 108.6 | 8.8 | 11.4 | 3.0 | 11.4 | 0.89 | 11.8 | 7.9 | 0.69 | 3.02 |
| 8a | 48 | 14.8 | | 111.2 | 7.4 | 13.6 | 2.7 | 13.6 | 0.99 | 12.6 | 7.9 | 0.58 | 3.58 |
| 8b | 48 | 14.8 | | 112.5 | 6.9 | 14.8 | 3.2 | 14.8 | 1.04 | 13.0 | 7.9 | 0.53 | 3.90 |
| 9a | 48 | 16.8 | | 111.0 | 8.6 | 13.9 | 3.5 | 13.9 | 1.02 | 12.7 | 7.8 | 0.56 | 3.71 |
| 9b | 48 | 16.8 | | 112.1 | 10.7 | 15.1 | 4.0 | 15.1 | 1.07 | 13.1 | 7.8 | 0.52 | 4.02 |
| 10a | 38 | (control) | | 106.7 | 9.4 | 10.6 | 3.0 | 10.6 | 0.87 | 11.4 | 7.8 | 0.74 | 3.58 |
| 10b | 38 | (control) | | 107.1 | 9.6 | 10.2 | 3.4 | 10.2 | 0.83 | 11.3 | 7.8 | 0.76 | 3.45 |
| Initial age 11 weeks (n=60) | | | | | | | | | | | | | |
| 1a | 38 | 12.5 | 62.3 | 249.7 | 9.2 | 112.7 | 10.2 | 50.4 | 0.72 | 2.8 | 108.2 | 2.15 | 1.23 |
| 1b | 38 | 12.5 | | 249.5 | 8.9 | 118.1 | 6.1 | 55.8 | 0.76 | 3.0 | 108.2 | 1.94 | 1.36 |
| 2a | 38 | 14.6 | | 264.1 | 8.8 | 142.8 | 14.1 | 80.5 | 0.77 | 3.9 | 113.3 | 1.41 | 1.87 |
| 2b | 38 | 14.6 | | 263.6 | 8.9 | 143.5 | 7.8 | 81.2 | 0.78 | 4.0 | 113.3 | 1.40 | 1.89 |
| 3a | 38 | 16.2 | | 267.7 | 9.0 | 157.0 | 9.5 | 94.7 | 0.82 | 4.4 | 115.0 | 1.21 | 2.17 |
| 3b | 38 | 16.2 | | 269.6 | 8.6 | 157.5 | 7.4 | 95.2 | 0.80 | 4.4 | 115.0 | 1.21 | 2.18 |
| 4a | 43 | 12.9 | | 274.6 | 13.1 | 149.3 | 18.0 | 87.0 | 0.72 | 4.2 | 123.9 | 1.42 | 1.83 |
| 4b | 43 | 12.9 | | 276.8 | 11.9 | 151.4 | 15.6 | 89.1 | 0.71 | 4.2 | 123.9 | 1.39 | 1.87 |
| 5a | 43 | 14.7 | | 282.4 | 10.0 | 180.0 | 16.3 | 117.7 | 0.80 | 5.1 | 127.2 | 1.08 | 2.15 |
| 5b | 43 | 14.7 | | 281.2 | 10.5 | 185.9 | 19.3 | 123.6 | 0.84 | 5.2 | 127.2 | 1.03 | 2.26 |
| 6a | 43 | 16.3 | | 288.7 | 9.5 | 192.6 | 14.6 | 130.3 | 0.80 | 5.4 | 124.6 | 0.96 | 2.43 |
| 6b | 43 | 16.3 | | 288.5 | 10.5 | 196.3 | 17.6 | 134.0 | 0.82 | 5.5 | 124.6 | 0.93 | 2.50 |
| 7a | 48 | 13.3 | | 278.2 | 11.7 | 167.6 | 16.0 | 105.3 | 0.78 | 4.7 | 123.8 | 1.18 | 1.77 |
| 7b | 48 | 13.3 | | 281.1 | 9.7 | 174.4 | 10.6 | 112.1 | 0.79 | 4.9 | 123.8 | 1.10 | 1.89 |
| 8a | 48 | 14.8 | | 274.7 | 13.6 | 175.2 | 16.6 | 112.9 | 0.85 | 4.9 | 123.8 | 1.10 | 1.90 |
| 8b | 48 | 14.8 | | 272.8 | 13.7 | 175.9 | 14.0 | 113.6 | 0.87 | 4.9 | 123.8 | 1.09 | 1.91 |
| 9a | 48 | 16.8 | | 277.3 | 9.8 | 181.1 | 10.3 | 118.8 | 0.85 | 5.1 | 124.9 | 1.05 | 1.98 |
| 9b | 48 | 16.8 | | 279.0 | 9.3 | 188.2 | 8.3 | 125.9 | 0.87 | 5.3 | 124.9 | 0.99 | 2.10 |
| 10a | 38 | (control) | | 253.1 | 11.6 | 122.8 | 12.7 | 60.5 | 0.76 | 3.2 | 82.7 | 1.37 | 1.93 |
| 10b | 38 | (control) | | 254.7 | 13.2 | 124.5 | 10.3 | 62.2 | 0.75 | 3.3 | 82.7 | 1.33 | 1.98 |

Note: The group number postscripts (a,b) denote replicate treatments for the same diet .

Table 6.12
Anova tables showing the sigificance of treatment effects on the final weights of fish fed on different combinations of dietary protein and energy levels in a 3x3 factorial arrangement (each age group analyzed independently).

ONE-WAY TWO-LEVEL NESTED ANOVA

| Source of variation | Variance component as % | df | SS | MS | Fs | Significance |
|---|---|---|---|---|---|---|
| Initial age 4 weeks | | | | | | |
| Among groups (diets) | 21.8 | 9 | 1570.368 | 174.485 | 18.372 | P<0.001 |
| Among subgrps.(replicates) | 1.1 | 10 | 134.1166 | 13.4116 | 1.4121 | not significant |
| Within subgroups | 77.1 | 580 | 5508.433 | 9.49729 | | |
| Total | | 599 | 7212.918 | | | |
| Initial age 11 weeks | | | | | | |
| Among groups (diets) | 79.00 | 9 | 382768.1 | 42529.7 | 230.76 | P<<0.001 |
| Among subgrps.(replicates) | 0.33 | 10 | 2737.683 | 273.768 | 1.4854 | not significant |
| Within subgroups | 20.67 | 580 | 106892.8 | 184.297 | | |
| Total | 100.00 | 599 | 492398.6 | | | |

TWO-WAY ANOVA (replicates pooled)

| Source of variation | Variance component as % | df | SS | MS | Fs | Significance |
|---|---|---|---|---|---|---|
| Initial age 4 weeks | | | | | | |
| A (DE in diets) | 13.66 | 2 | 361.89 | 180.95 | 19.15 | P<0.001 |
| B (dietary protein) | 20.33 | 2 | 1066.56 | 533.28 | 56.43 | P<0.001 |
| AxB (Interaction) | 0.06 | 4 | 35.80 | 8.95 | 0.95 | not significant |
| Within treatments | 65.95 | 531 | 5017.97 | 9.45 | | |
| Total | 100 | 539 | 6482.21 | | | |
| Initial age 11 weeks | | | | | | |
| A(cols;lipid) | 21.24 | 2 | 102264.6 | 51132.3 | 265.55 | P<0.001 |
| B(rows;protein) | 48.88 | 2 | 172840.9 | 86420.4 | 448.82 | P<0.001 |
| AxB (Interaction) | 9.02 | 4 | 20749.49 | 5187.37 | 26.940 | P<0.001 |
| Within subgroups | 20.86 | 531 | 102242.5 | 192.547 | | |
| Total | 100 | 539 | 398097.6 | | | |

df= degrees of freedom SS= sums of squares
MS= mean squares Fs= variance ratio

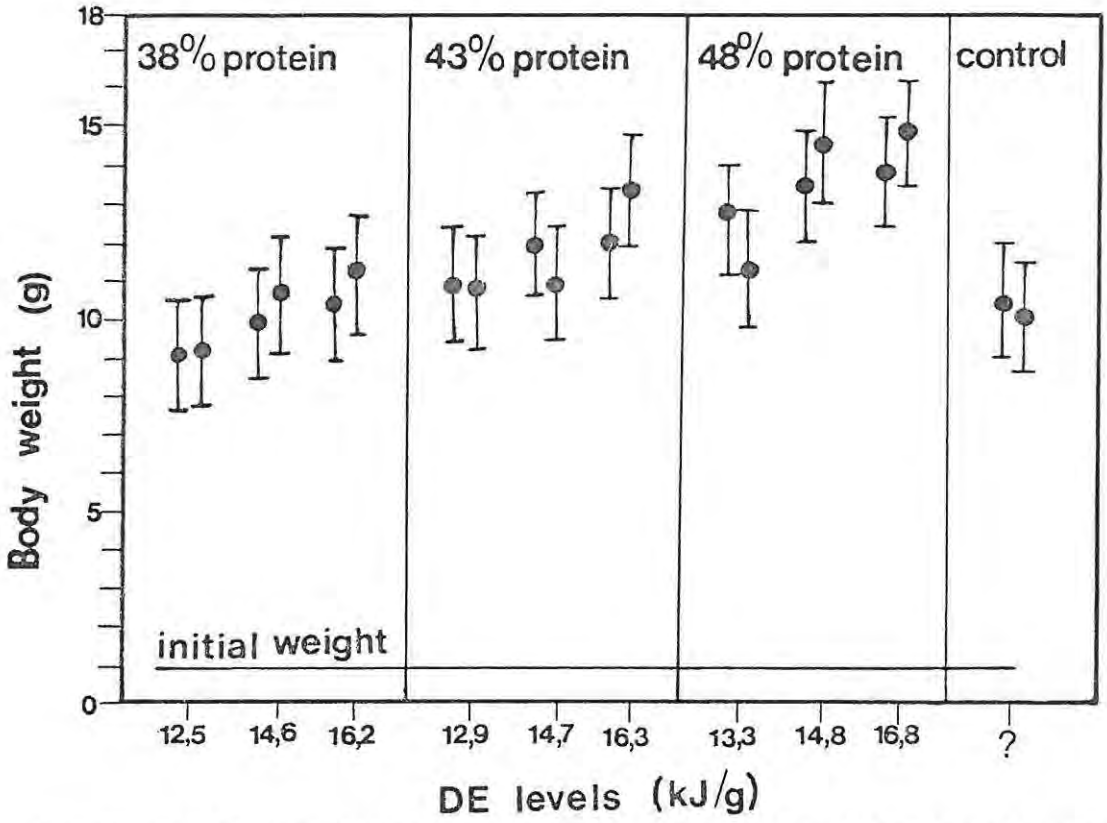


Figure 6.13. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on different combinations of dietary protein and energy levels for 21 days. Initial age 4 weeks. Means and comparison intervals of replicate treatments are shown separately.

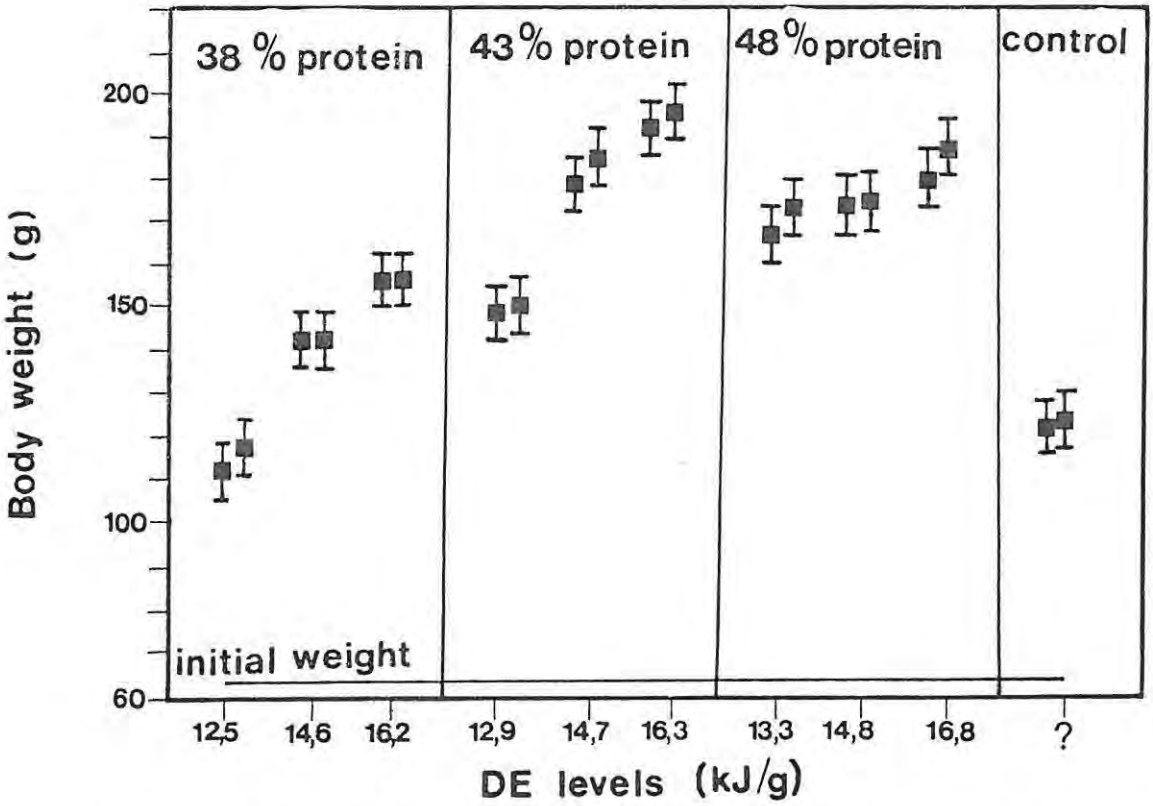


Figure 6.14. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on different combinations of dietary protein and energy levels for 21 days. Initial age 11 weeks. Means and comparison intervals of replicate treatments are shown separately.

In the older age group, the biggest source of variance stemmed from the treatment effects, and the variance within subgroups accounted for only slightly more than 20% of the total variance components. Protein levels were the biggest source of variance (48.9%) while the variance component caused by energy was lower at 21.2%. Although the results from both the age groups showed very much the same pattern (compare Figures 6.13 and 6.14), only the results from the older age group could bear further meaningful parametric analysis:

The "protein sparing effect" can be seen clearly in Figure 6.14, where the highest dietary energy levels allowed lower dietary protein levels to yield similar or better growth results. Figure 6.15 illustrates another perspective of the same results shown in Figure 6.14. An even better illustration of the "protein sparing effect" can be seen in Figure 6.16 in which it is evident that protein efficiency ratios were improved drastically by the higher dietary energy levels. It is also evident that feed conversion ratios were much improved by high energy levels at low protein levels. However, energy levels did not significantly affect either protein efficiency ratios or feed conversion ratios much at the highest protein levels (also Figure 6.16).

Discussion

Both in terms of maximal growth rate and maximal protein efficiency ratio, the best results were obtained with a dietary protein content of 43% combined with a calculated DE level of 16.3 kJ/g. This translates to a protein - energy ratio (P/DE) of 26.4 mg protein/kJ or an energy-protein ratio (DE/P) of 38.0 kJ/g of protein.

In imperial terms the DE/P ratio is 9.1 kcal/g protein, which is in close agreement with the DE/P values of 9.6 and 9.7 determined for optimal growth in small channel catfish by Garling and Wilson

(1977) and Page and Andrews (1973) respectively. It is also in close agreement with the tentative values of 8 to 9 kcal/g recommended for fingerling channel catfish by Stickney and Lovell (1977).

Assuming that only a minor correction is needed to convert DE to ME the findings of this experiment can also be compared to the work done on C. gariepinus in the Netherlands: The P/DE level of 26.4 mg protein/kJ DE from this study compares favourably with the values of 25.4 and 34.7 mg/kJ ME determined at 24°C and 29°C respectively by Henken et al. (1986).

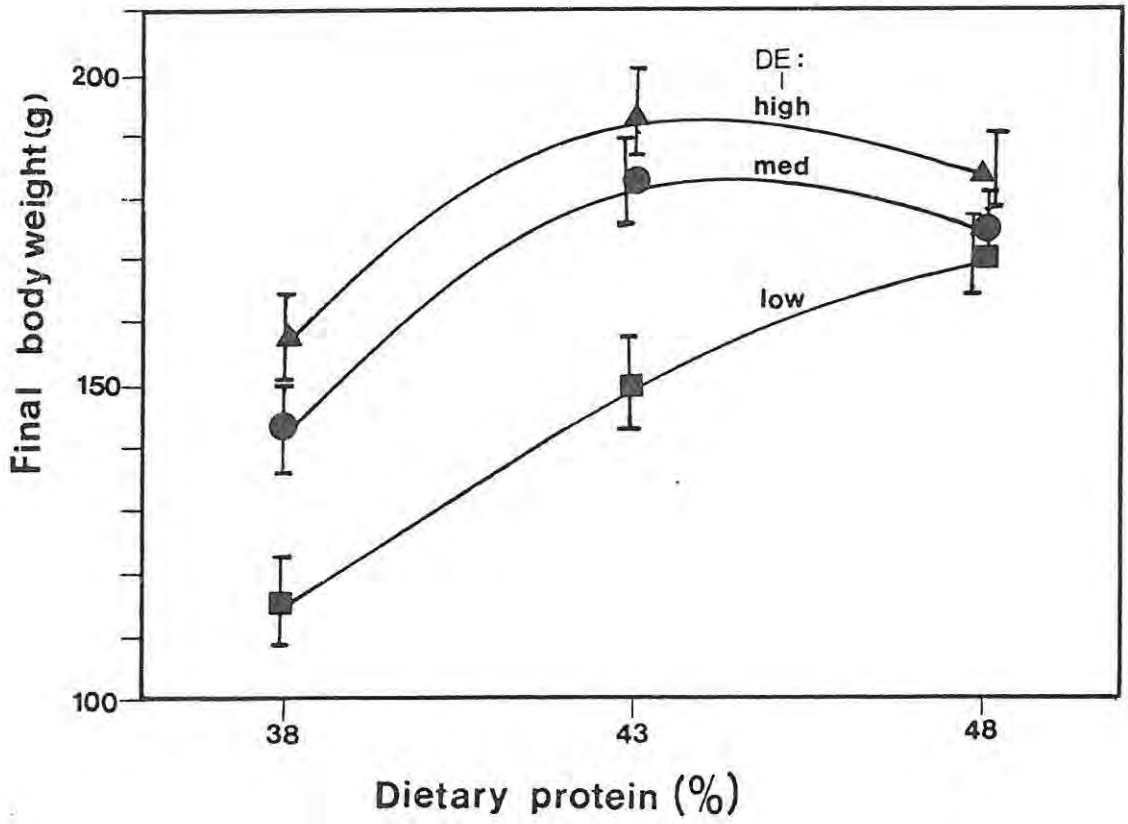


Figure 6.15. The effect of dietary protein level at three digestible energy levels on body weight in C. gariepinus sub-adults after 21 days. Initial age 11 weeks. Curves fitted by eye. Means of replicate treatments are pooled.

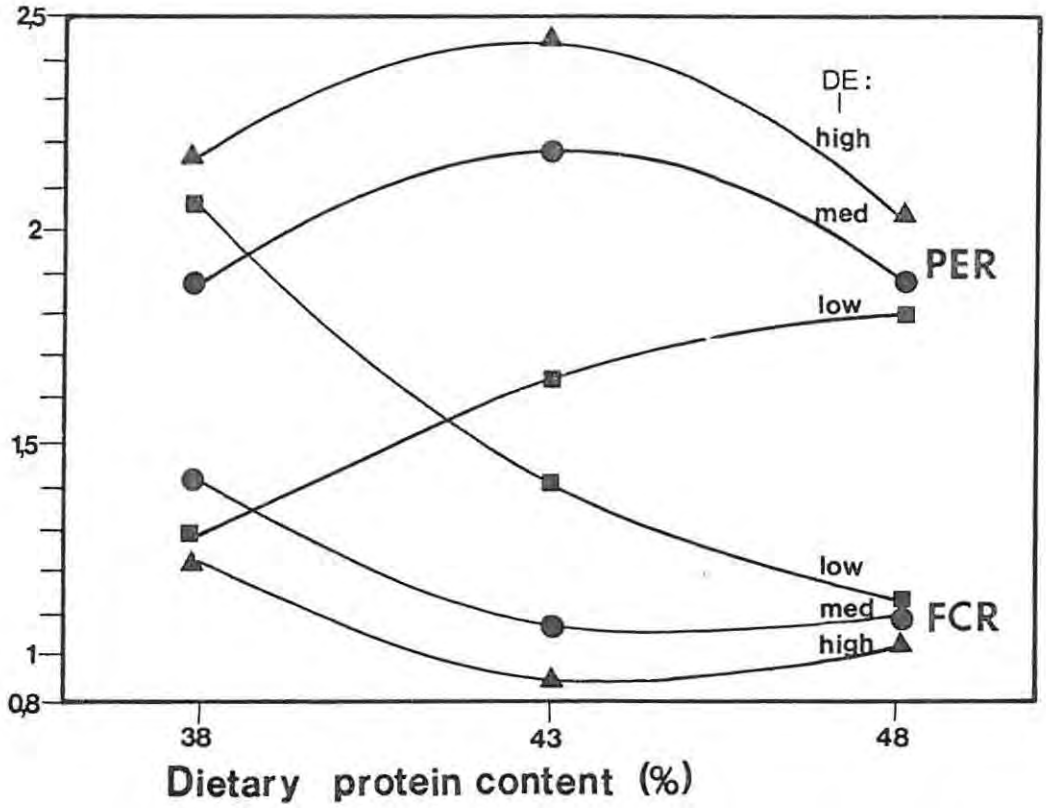


Figure 6.16. The effect of dietary protein level at three digestible energy levels on protein efficiency ratios and feed conversion ratios of C. gariepinus sub-adults after 21 days. Initial age 11 weeks. Curves fitted by eye. Means of replicate treatments are pooled.

EXPERIMENT 4

EFFECT OF VITAMIN AND MINERAL SUPPLEMENTATION

Materials and Methods

The test diet which proved to be the best in terms of growth, feed conversion ratio and protein efficiency ratio in the preceding experiment (diet no 6) was used as a basis for mixing six test diets with varying levels of vitamin and mineral premixes (Table 6.13).

Table 6.13
Formulation and calculated composition of the basic test diet to which different levels of vitamin and mineral premixtures (Px) were added.

| Ingredient | Basic diet | Diet No. | Vitamin Px added % | Mineral Px added % |
|---|---|---|---|---|
| Maize | 11.70 | 1 | 0 | 1 |
| Wheat Bran | 11.70 | 2 | 1 | 1 |
| Fish meal | 51.70 | 3 | 2 | 1 |
| Carcass meal | 5.00 | 4 | 1 | 0 |
| Blood meal | 5.00 | 5 | 1 | 2 |
| Fish acid oil | 3.90 | 6 | 1 | 4 |
| Molasses powder | 11.00 | | | |
| Total | 100 | | | |
| Crude protein % | 43.00 | | | |
| Crude fat % | 10.63 | | | |
| Crude fiber % | 2.66 | | | |
| N-free extract | 21.37 | | | |
| Moisture | 9.53 | | | |
| Ash % | 12.81 | | | |
| Total | 100.00 | | | |
| DE kJ/g * | 14.66 | | | |

* DE calculated on the basis of DE values of ingredients for Ictalurus punctatus (Lovell, 1984, Table 6.2)
Vitamin Px as per SRD H-440 without a-cellulose (Table 6.3)
Mineral Px as per SRD H-440 (Table 6.3)

Two separate trials were conducted concurrently in Systems A (2 subgroups, 10 week old fish) and System B (two subgroups, six week old fish), with each trial lasting 21 days. There were 50 fish per container (or subgroup) for both age groups.

## Results

The resulting growth rates and calculated performance parameters are listed in Table 6.14 and presented graphically in Figures 6.17 and 6.18.

Table 6.14
Growth and feed utilization of *Clarias gariepinus* fed for 21 days on diets with different levels of vitamins and minerals added.

| Group No. | Vitamin supplement % | Mineral supplement % | Mean initial weight g | Mean final length mm | (SD) | Mean final weight g | (SD) | Weight increase g | CF 100*wt/len3 | SGR % body wt/d | Food fed g | FCR | PER |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Initial age 6 weeks (n=50) | | | | | | | | | | | | | |
| 1a | 0 | 1 | 7.1 | 165.0 | 6.0 | 33.6 | 3.8 | 26.5 | 0.75 | 7.4 | 25.0 | 0.94 | 2.53 |
| 1b | 0 | 1 | | 166.5 | 8.3 | 34.5 | 4.5 | 27.4 | 0.75 | 7.5 | 25.0 | 0.91 | 2.61 |
| 2a | 1 | 1 | | 166.4 | 7.2 | 35.0 | 4.5 | 27.9 | 0.76 | 7.6 | 25.0 | 0.89 | 2.66 |
| 2b | 1 | 1 | | 167.2 | 7.3 | 35.5 | 4.3 | 28.4 | 0.76 | 7.7 | 25.0 | 0.88 | 2.70 |
| 3a | 2 | 1 | | 164.5 | 4.7 | 34.1 | 4.0 | 27.0 | 0.77 | 7.5 | 25.0 | 0.93 | 2.57 |
| 3b | 2 | 1 | | 169.9 | 5.1 | 37.8 | 4.0 | 30.7 | 0.77 | 8.0 | 25.0 | 0.82 | 2.92 |
| 4a | 1 | 0 | | 168.7 | 7.2 | 36.0 | 4.6 | 28.9 | 0.75 | 7.7 | 25.0 | 0.87 | 2.75 |
| 4b | 1 | 0 | | 170.2 | 7.3 | 37.8 | 5.2 | 30.7 | 0.77 | 8.0 | 25.0 | 0.82 | 2.92 |
| 5a | 1 | 2 | | 166.4 | 3.6 | 34.2 | 2.7 | 27.1 | 0.74 | 7.5 | 25.0 | 0.92 | 2.58 |
| 5b | 1 | 2 | | 166.6 | 6.2 | 34.7 | 4.2 | 27.6 | 0.75 | 7.6 | 25.0 | 0.90 | 2.63 |
| 6a | 1 | 4 | | 167.3 | 6.4 | 35.4 | 3.9 | 28.3 | 0.76 | 7.7 | 25.0 | 0.88 | 2.98 |
| 6b | 1 | 4 | | 169.1 | 5.4 | 35.6 | 4.1 | 28.5 | 0.74 | 7.7 | 25.0 | 0.88 | 3.00 |
| Initial age 10 weeks (n=50) | | | | | | | | | | | | | |
| 1a | 0 | 1 | 50.3 | 250.2 | 6.6 | 135.2 | 10.0 | 84.9 | 0.86 | 4.7 | 105.0 | 1.24 | 1.92 |
| 1b | 0 | 1 | | 252.7 | 5.9 | 141.8 | 16.8 | 91.5 | 0.88 | 4.9 | 105.0 | 1.15 | 2.07 |
| 2a | 1 | 1 | | 252.5 | 9.1 | 142.8 | 16.9 | 92.5 | 0.89 | 5.0 | 105.0 | 1.14 | 2.10 |
| 2b | 1 | 1 | | 253.7 | 8.8 | 142.4 | 11.6 | 92.1 | 0.87 | 5.0 | 105.0 | 1.14 | 2.09 |
| 3a | 2 | 1 | | 249.7 | 7.2 | 134.2 | 12.9 | 83.9 | 0.86 | 4.7 | 105.0 | 1.25 | 1.90 |
| 3b | 2 | 1 | | 258.0 | 7.5 | 148.6 | 11.5 | 98.3 | 0.87 | 5.2 | 105.0 | 1.07 | 2.23 |
| 4a | 1 | 0 | | 255.8 | 6.6 | 147.7 | 17.5 | 97.4 | 0.88 | 5.1 | 105.0 | 1.08 | 2.21 |
| 4b | 1 | 0 | | 258.3 | 10.1 | 148.7 | 17.6 | 98.4 | 0.86 | 5.2 | 105.0 | 1.07 | 2.23 |
| 5a | 1 | 2 | | 252.1 | 9.8 | 146.6 | 11.6 | 96.3 | 0.91 | 5.1 | 105.0 | 1.09 | 2.18 |
| 5b | 1 | 2 | | 252.8 | 9.6 | 142.3 | 10.0 | 92.0 | 0.88 | 5.0 | 105.0 | 1.14 | 2.09 |
| 6a | 1 | 4 | | 253.9 | 9.9 | 146.8 | 12.4 | 96.5 | 0.90 | 5.1 | 105.0 | 1.09 | 2.42 |
| 6b | 1 | 4 | | 256.7 | 8.2 | 147.9 | 12.7 | 97.6 | 0.87 | 5.1 | 105.0 | 1.08 | 2.45 |

Note: The group number postscripts (a,b) denote replicate treatments for the same diet .

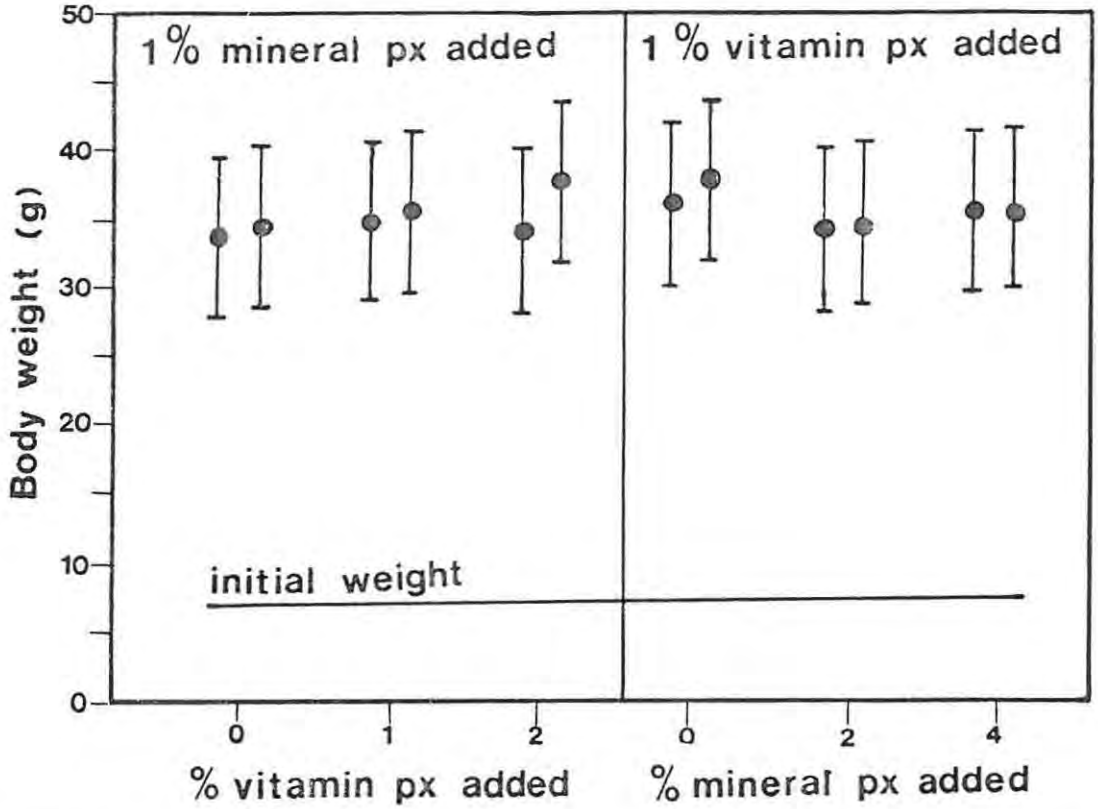


Figure 6.17. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on a diet with different levels of vitamin and mineral premix (px) supplementation. Initial age six weeks. Means and comparison intervals of replicate treatments are shown separately.

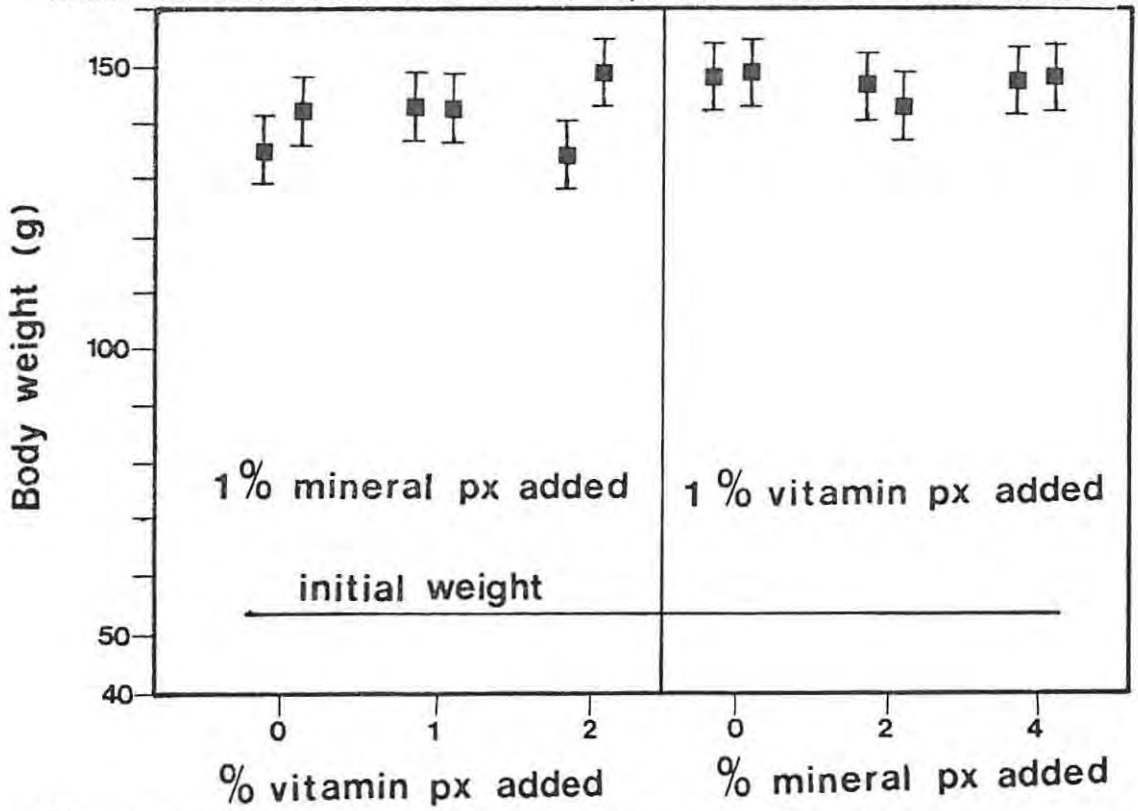


Figure 6.18. 95% comparison intervals by the T-method for the means of the final weights of C. gariepinus juveniles fed on a diet with different levels of vitamin and mineral premix (px) supplementation. Initial age 10 weeks. Means and comparison intervals of replicate treatments are shown separately.

Table 6.15
Anova tables showing the sigificance of treatment effects on the final weights of fish fed on a standard diet with different levels of vitamin and mineral supplementation (each age group analyzed independently).

| Source of variation | Variance component as % | df | SS | MS | Fs | Significance |
|---|---|---|---|---|---|---|
| Initial age 6 weeks | | | | | | |
| Among groups (diets) | 1.12 | 5 | 3479.03 | 695.81 | 1.18 | not significant |
| Among subgrps.(replicates) | 8.77 | 6 | 3528.38 | 588.06 | 3.53 | not significant |
| Within subgroups | 90.10 | 300 | 49971.46 | 166.57 | | |
| Total | 100.00 | 311 | 56978.87 | | | |
| Initial age 10 weeks | | | | | | |
| Among groups (diets) | 1.09 | 5 | 3549.99 | 710.00 | 1.20 | not significant |
| Among subgrps.(replicates) | 7.08 | 6 | 3536.85 | 589.47 | 3.01 | not significant |
| Within subgroups | 91.83 | 300 | 58847.62 | 196.16 | | |
| Total | 100.00 | 311 | 65934.45 | | | |

df= degrees of freedom SS= sums of squares
MS= mean squares Fs= variance ratio

The Anova (Table 6.15) revealed that there was no evidence of a significant added variance component among treatments. There were also no significant differences ($P<0.05$) between the mean final weights of any specific groups (Figures 6.17 and 6.18). None of the classic vitamin deficiency signs such as abnormal skin pigmentation or skin hemorrhages (NRC, 1977) were detected.

Discussion

It would seem that the ingredients used in the basic diet contained sufficient vitamins and minerals and/or that the duration of the experiments (21 days) was not long enough for dietary vitamin and mineral deficiencies to manifest in the fish. Since limited time and facilities did not allow for this experiment to be extended, no conclusions can yet be made as to the dietary vitamin and mineral requirements of *C. gariepinus*. It would seem advisable that future experiments of this nature be done with purified ingredients so as to prevent unknown quantities of vitamins and minerals from inadvertently being

incorporated into the diets. Until such time, it would be prudent to supplement the premixes as given in the SRD H-440 to commercial diets for C. gariepinus. Alternatively, the premix used in commercial diets for channel catfish (Robinson, 1984) should be used (Table 6.16).

Table 6.16
Vitamin premix levels used in commercial diets for Ictalurus punctatus (Robinson, 1984).

| | |
|---|---|
| Thiamin | 11 g |
| Riboflavine | 13 g |
| Pyridoxine | 11 g |
| Pantothenic acid | 35 g |
| Nicotinic acid | 88 g |
| Folic acid | 2.2 g |
| B12 | 0.09 g |
| Choline | 550 g |
| Ascorbic acid | 350 g |
| A | 4400 IU x 1000 |
| D | 2200 IU x 1000 |
| E | 55 IU x 1000 |
| K | 11 IU x 1000 |

Note: Make up to 2kg with a filler such as maize meal. One kg of this mixture is sufficient for 1000kg of pelletized feed or 500kg of extrusion processed feed.

CONCLUSIONS

The results of the feeding trials substantiate the findings of the previous chapters, namely that C. gariepinus has a relatively high dietary protein requirement, but that it is an efficient convertor of food. It can be said with certainty that the dietary protein requirement of this animal is in excess of 40%. This statement is also borne out by the findings of Machiels and Henken (1985) and Henken et al. (1985).

Based on the growth and feed uitilization results of the feeding trials, it is recommended that commercial diets for C. gariepinus should contain 40-42% protein and 10-12% lipid. The digestible energy content of their diet (based on digestibilities of feed ingredients in Table 6.2), should be in the order of 14-16 kJ/g, but this should be adjusted accordingly with the protein content to result in a protein-to-energy ratio of about 26-29mg protein per kJ of digestible energy. These levels are the most important dietary aspects pertaining to commercial feed formulation since they virtually determine the cost of the feed. The above recommendations are made with the assumption that a well balanced protein source such as fish meal will be used.

Dietary requirements are evidently the same for juveniles (4 weeks old) and sub-adults, except that younger fish have a much higher relative growth rate, and therefore require higher relative feeding levels. Feed conversion ratios are also more efficient in younger fish. This is in agreement with Hogendoorn et al. (1983) who have shown that body weight and temperature drastically influence feed consumption and growth rate in C. gariepinus.

# CHAPTER 7
# LEAST COST FEED FORMULATION

## INTRODUCTION

As a final part of this thesis, an evaluation of practical feed formulations is presented. It was not the intent to compare growth responses elicited by the different feed formulations, but merely to gauge their practical and economic feasibility *per se*.

To compound a feed from a number of ingredients in such a fashion that it complies with certain nutritional requirements as economically as possible, is a relatively complex mathematical problem. It is ironic that the algorithm commonly used to solve such problems is called the "Simplex" method for linear programming. Computerized linear programming has gained widespread acceptance as a means of solving least cost diet problems in the animal feed industry (Chow *et al.*, 1980; Blake, 1987). Various commercial software packages which employ linear programming techniques are available for use with personal computers and larger hardware configurations. In the course of this study, a customized and user friendly computer program was developed with which to formulate feeds by linear programming. The Appendix to this thesis includes a listing of the program in BASIC, as well as a user manual for its application.

## METHODS AND MATERIALS

A database on available feed ingredients was compiled, noting for each ingredient its price, crude protein content, lipid content, and digestible energy. During the course of conducting the feeding trials described in Chapter 6, and as information regarding the nutritional requirements of the fish became available, tentative practical diets were formulated with the aid of the computerized least-cost feed formulation program mentioned above. The diets were applied in two sets of empirical

experiments; one being a long-term (10 month) rearing trial of a single batch of catfish from juveniles (1g) up to marketable size adults (1000g), and the other being short-term (21 day) feeding trials with several batches of fish, mainly to evaluate the efficiency of low cost diets. In both cases, the protein and energy requirements were initially set too low, but were corrected as the findings of the feeding trials described in Chapter 6 became available.

The short term trials were run in the 2 and 3m dia. circular tanks (System A, Fig 2.3), while the long term trial was run in earthen ponds similar to those shown Figure 2.3. Batches of 50 - 100 fish of various ages were used in the short term trials. Initial and final body weights were recorded, as well as the weight of feed fed (satiation feeding two to three times daily).

In the long term feeding trial, 6000 juveniles with a mean individual weight of approximately one gram, were initially stocked into a 1000 m2 earthern pond. They were fed three times daily on the then current least-cost diet. Satiation feeding could not be observed due to high pond turbidity and the size of the pond. They were, therefore, fed at levels calculated on the basis of a size and temperature related feeding schedule proposed by Hogendoorn et al. (1983). After 3 months the fish were size sorted and transferred to two 600m2 earthern pond, in which they were reared to market size. At the end of 10 months, mean body weight was determined by sampling, and the total harvest was weighed.

For both the short- and long term trials, overall feeding costs, feed conversion ratios, weight increase and market values were calculated.

RESULTS

The series of least cost diets which were formulated for the short term feeding trial and the results are given in Table 7.1. It is evident that at the prevailing ingredient prices, the feed cost to produce 1kg (live weight) of catfish were highly economical (59-71c/kg at a selling price of R2.00 - R2.20/kg; and later, R1.18-R1.22/kg at a selling price of R3.00/kg). It was also demonstrated that unconventional ingredients (e.g. tomato waste) could make a large contribution to the economic efficiency of catfish feed.

The interesting fact that emerged was that the pursuit of 100% vegetable diets seemed pointless at the prevailing feed ingredient prices. Carcass meal and poultry-by-product meal (Pbpm) were cheaper on a price per unit protein and energy basis than any conventional vegetable ingredient. Pbpm qualified as the best nutritional value-for-money ingredient, but due to its poor pelletability, it could only be incorporated at a maximum level of 10%.

The results of the long term feeding trial were briefly as follows: By using available conventional and unconventional feed ingredients over the 10 month trial period, the average feed cost was R464/ton (46.4c/kg). After a 10 month growing period, the following overall production figures were achieved:

-Mortality : 18.3%
-Weight increase: 5282 kg (live weight).
-Mean individual weight 1078g
-Total feed consumed : 7000 kg
-Feed conversion ratio: 1.33
-Feed cost R3248 (total) or 61.7c/kg
-Additional operating costs :± R3200 (total) or 61c/kg.
-Total production cost : R6448 or R1.22/kg
-Market value : R10 564 (@ R2.00/kg live weight)
-Production: 44ton/ha /10 months.
-Pond area and depth: 2 ponds of 600m2 each by 1m deep.

Table 7.1
Examples of least cost diets for Clarias gariepinus with resulting food conversion ratios (FCR) and cost efficiencies.

| DIET NO: | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Maize % | | 30 | 10.5 | | 18 | | |
| Wheat% | | | | 14 | 18 | | |
| Cotton oil cake % | | | 25 | | | | |
| Soya oil cake % | 10 | | 10 | 10 | | 30 | 36.8 |
| Fishmeal % | 24.7 | 10 | 20 | 10 | 43.5 | 28.2 | 21.2 |
| Poultry by product% | 10 | 10 | 9 | 10 | | | |
| Carcass meal % | 10.5 | 39.5 | | 22.7 | 10 | 25 | 30 |
| Lucern meal % | 30 | | | | | | |
| Tomato waste % | | | 8 | 20 | | | |
| Fish acid oil % | 6.8 | 2.5 | 7.5 | 3.3 | 2.5 | 0.7 | |
| Molasses powder % | 8 | 8 | 10 | 10 | 8 | 10 | 10 |
| Crude protein % | 39.2 | 38.2 | 39.4 | 38.7 | 40.6 | 42 | 42 |
| Total lipid % | 13.5 | 11.7 | 14.2 | 12.5 | 9 | 13 | 13 |
| D.E. (kJ/g)* | 13.1 | 12.8 | 14.1 | 13.2 | 13.1 | 14.8 | 14.9 |
| Price/ton (R) | 655 | 569 | 603 | 531 | 722 | 982 | 899 |
| FCR | 1.05 | 1.25 | 1.13 | 1.12 | 0.98 | 1.2 | 1.36 |
| Feed cost/kg fish | 0.69 | 0.71 | 0.68 | 0.59 | 0.71 | 1.18 | 1.22 |
| Fish selling price (R) | 2.00 | 2.00 | 2.00 | 2.00 | 2.20 | 3.00 | 3.00 |

* Calculated on the basis of DE values for channel catfish (Lovell, 1984).

DISCUSSION

The empirical results in Table 7.1 suggest that fishmeal remains an important source of well balanced and digestible protein. The best feed conversion ratios (but not necessarily best economic returns) were obtained with diets containing high fishmeal levels. This is generally the case with commercial fish feeds for many cultured species, unless appropriate amino acid supplementations (usually methionine and lysine) are made to compensate for imbalances when low fishmeal diets are formulated (Ketola, 1982; Randall Robinette, 1984).

An important principle in commercial fish feed formulation was also demonstrated: Economic consideration often come into conflict with nutritional considerations (see Crampton, 1985).

A small concession in feed quality, may often result in a considerable saving in the cost of the feed. If growth rate and/or feed conversion are affected only marginally by such a concession it is, in most cases, justifiable. Compare, for instance, diets number 6 and 7 in Table 7.1: Diet number 7, due to its lower fish meal content, costs 8% less than diet number 6. Although diet 6 resulted in a better feed conversion ratio, the cost to rear one kg of fish was essentially the same. A fish farmer purchasing feed might perceive diet 7 to be the better option. The same applies to diets 4 and 5, where the nutritionally inferior diet four yields better economic returns than diet 5.

A prudent principle by which to make nutritional concessions under economic pressures, would be to make such concession only if there is a small difference between prevailing feed price and fish price. Where the fish price is much higher (five times or higher) than the feed price, it would be best to give nutritional consideration priority, because high growth rate and survival is then much more important than the cost of the feed.

# CHAPTER 8

## CONCLUDING DISCUSSION

The objectives of this study have been met in that the nutritional requirements of C. gariepinus have been elucidated and that information pertaining to the formulation of commercial feeds for the species has been provided. The results obtained under controlled conditions (Chapters 5-7) largely complement the field observations on the fishes' feeding biology (Chapter 3) and the morphological basis of its feeding and digestive physiology (Chapter 4). The broad tolerances this animal displays for environmental factors, and its euryphagic habits were reflected in the high growth rate, survival rate and efficient feed conversion under controlled conditions.

Both the field and laboratory observations substantiate the notion that C. gariepinus may be characterized as being an efficient opportunist and survivor, being well equipped to exploit whatever resources are available under favourable or adverse conditions. It cannot be disputed that this fish is a prime candidate for the development of an aquaculture industry in South Africa, as well as in other countries where markets for its meat can be developed.

This project has demonstrated that intensive culture of C. gariepinus is economically feasible. All animal husbandry ventures are, of course, at the mercy of feed prices on the one hand, and market demand for the product on the other. An adverse shift in either of these factors might render the venture uneconomical overnight. However, with the efficiency by which this fish converts feed, and given the high quality of its meat, it would not easily be ousted from the marketplace by other animal husbandry products.

In conclusion, it can safely be stated that the technology for the commercial production of C. gariepinus has reached a stage where no further impediments prevent the industry from developing. The development of this technology has been extremely rapid due to the rational and committed research from which it has proceeded. The large number of research papers on C. gariepinus culture published in recent years by many workers based in several countries, reflect the tremendous interest this animal has generated amongst academics and entrepreneurs alike. Although a sound information base now exists for commercial application of Clarias culture, there still exists a vast potential for improving and refining the technology for large scale and intensified production of this fish.

Further research into least cost formulations as well as fundamental nutritional issues should be continued for as long as commercial C. gariepinus culture is a reality. Of immediate importance in this field, are digestible energy determinations of conventional as well as unconventional feed ingredients. Also, dietary mineral and vitamin requirements of C. gariepinus need to be further researched.

Although valuable work on the effect of temperature on the nutritional requirements of C. gariepinus has been published by Hogendoorn et al. (1983) and Henken et al. (1986), this avenue of research needs to be further investigated in order to provide feeding schedules and diet formulations for winter months as well as colder geographical regions.

The potential of hitherto unacceptable feed ingredients (from an aesthetic or health point of view), such as abattoir wastes, and activated sludge from domestic sewage and other sources should be investigated. C. gariepinus could well be used to convert waste products such as these into a marketable product. Also, the South African practise of legal restrictions surrounding the use of unregistered feed ingredients should be urgently reconsidered.

In the non-nutritional field, perhaps the most important aspects which need further investigation, are optimal economic stocking densities and environmental requirements, with special attention given to high-density culture techniques. There is forever economic pressure to intensify agricultural ventures. If catfish culture is to compete for agricultural resources (space, water, feeds) with other farming sectors, optimal resource use will become even more important.

# APPENDIX

## LINEAR PROGRAMMING FOR LEAST COST FEED FORMULATION

Linear programming has gained widespread acceptance as a means of solving least cost diet problems in the animal feed industry (Chow, Rumsey and Waldroup, 1980; Blake, 1987). Various commercial software packages for this purpose are available for use with personal computers and larger hardware configurations, but these are expensive.

The program described in this section was developed in order to provide a system for solving diet formulation problems without having to purchase any expensive software. The program is written in BASIC and can be run on an IBM* compatible personal computer by means of a BASIC interpreter such as IBM Advanced Basic (BASICA**) or GWBASIC**.

The Simplex Method for linear programming with the "upper bounding" technique was originally developed by Dantzig (1955). The algorithm used in this work is based on the Revised Simplex Method with consideration of explicit bounds on variables (Chvatal, 1983). Modifications concerning the determination of the initial feasible solution were developed by the author and M. Frick (Dept. Mathematics, UNISA) . It is not the intent of this contribution to describe the algorithm in full but rather to clarify the application of the program.

The correct functioning of the program was verified by solving diet problems with known optimal solutions from Chvatal (1983), and Blake (1987). It was further verified by testing it against a program developed by Ho (1987).

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NOTES ON USING THE PROGRAM

An IBM or -compatible Personal computer with an MSDOS* (or compatible) system is required. The user must also have access to a BASIC interpreter such as BASICA or GWBASIC. The two files "DT1.BAS" and "DT2.BAS" provided on the enclosed floppy disk, must be copied unto a disk which has a resident system (such as MSDOS) as well as a BASIC interpreter. It is important that the BASIC interpreter and the two program files reside in the same directory. To initiate the program, the user has to make the directory containing these files the current directory. Once this is done, type the name of the BASIC interpreter, followed by "DT1". For example, type: GWBASIC DT1. This will activate the data input routine, which allows the user to either type in the feed ingredient data, or to load a previously saved datafile. Two such datafiles are also provided on the supplied disk (CATMIX.DT and SAMPLE.DT). The data input routine works like a spreadsheet, and the command menu is activated by pressing the "/" -key.

The following hypothetical problem serves to demonstrate the procedure of least cost formulation:
Four ingredients are available and their respective costs and nutritional analyses are known. With these a least cost diet with minimum 38% protein, 12% fat and 11kJ/g digestible energy (DE) is to be formulated. It is known from experience that at least 10% fish meal should be included and that the pellet press cannot tolerate more than 12% fish oil in the mixture. Therefore a lower bound of 10% is allocated to fish meal and an upper bound of 12% is allocated to fish acid oil. All the information required by the program is summarized in Table A1. Notice that unspecified lower bounds must be chosen as 0 and unspecified upper bounds are chosen arbitrarily as 100%. Upper bounds must

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be greater than lower bounds. Upon initiation of the program (dt1.bas), the user is presented with a "data input form" which has the structure of Table A1. The input form facilitates ease of data input and management. A maximum of 18 ingredients and seven nutrient analyses (e.g. protein, fat, DE) are allowed. The values are entered directly into the form by using the arrow keys to move from one entry "cell" to the other, much like using a spreadsheet program. The command menu is activated by the "/" key and the user can save or load data forms, enter ingredient names, edit analyses headings, run the solving routine, or exit to system by typing the first letter of the appropriate command which appears in the menu.

When the Run option is selected from the command menu, program execution branches to the solving routine (dt2.bas) and the current dataset in the input form is regarded as the problem. If a feasible solution is not found, the user has imposed constraints which are too rigorous. The constraints must be slackened by either increasing the upper bounds of the bounded ingredients or by reducing some of the minimum nutritional requirements. If this cannot be done, more, or alternative ingredients must be found.

TABLE A1.
Example of a least cost feed formulation problem.

| Ingredient | Cost (R/ton) | Bounds (%) Lower | Upper | Protein (%) | Fat (%) | DE (kJ/g) |
|---|---|---|---|---|---|---|
| Fish meal | 900 | 10 | 100 | 60 | 10 | 15.4 |
| Soy oil cake | 540 | 0 | 100 | 48 | 1 | 7.9 |
| Maize | 200 | 0 | 100 | 9 | 4 | 9.3 |
| Fish acid oil | 600 | 0 | 12 | 0.1 | 98 | 35.2 |
| Minimum requirements: (constraints) | | | | 38 | 12 | 11 |

If a feasible solution is found, the program subsequently passes through a number of iterations of the Simplex Method until an optimal solution is found and displayed (Table A2). The ratios at which the ingredients should be mixed are displayed as percentages and the cost of the formulation is displayed. A sensitivity analysis is also shown (Table A3). At this stage the user is presented with another command menu. The appropriate command can be selected by typing the first letter of the command. The command options allow the user to print the the solution, return to the data input form or to exit to system.

TABLE A2.
Solution to diet problem (program output).

| Ingredient | Quantity (%) | Cost (R/ton) |
|---|---|---|
| Fish meal | 10% | |
| Soy oil cake | 63.5 | |
| Maize | 16.6 | |
| Fish acid oil | 9.9 | |
| Total | 100 | 543.86 |

DISCUSSION

Since the program involves long and elaborate computations, rounding errors might accumulate to cause small inaccuracies in the results. These are usually small enough to be disregarded. The program output includes a "sensitivity analysis" which provides useful information on the cost of nutrients (as opposed to the cost of ingredients which is already known). In Table A3, for example, protein is shown to have a a sensitivity or unit cost of 6.18. This means that if the minimum requirement (constraint) for protein is reduced from 38 to 37%, the diet cost will be reduced by R6.18. This allows the nutritionist to make

decisions regarding nutritionally sub-optimal diets. Assuming that it is known to what extent the growth of the fish will be affected by a specific reduction in the nutritional value of the diet, one can now weigh the advantage of cost saving on the diet against the expected reduction in growth rate and food conversion efficiency. This facilitates true least costing, which is, in fact, least costing of the fish rather than simply least costing of the diet.

TABLE A3
Sensitivity analysis

| Nutrient | Requirement | Amount in diet | Excess | Unit cost |
|---|---|---|---|---|
| Protein (%) | 38 | 38 | 0 | 6.18 |
| Fat(%) | 12 | 12 | 0 | 3.67 |
| D.E. (kJ/g) | 11 | 11.58 | .58 | 0 |

The limitations to linear programming as a means of fish feed formulation are governed only by the accuracy of the input data. It is, of course, important that the dietary requirements of the fish be well understood and that the constraints imposed by processing, anti-nutritional factors and storage problems be considered when choosing bounds and constraints for the input data.

An experienced fish nutritionist has a certain amount of intuitive knowledge regarding feed formulation which might be difficult to express in terms of numbers. The program given here, due to the relative ease of data management, allows the user to experiment with different numerical constraints, which makes it a valuable tool for further development of intuitive skills. Optimal solutions to diet problems are often surprising, especially when several ingredient are discarded altogether.

This forces us to examine our prejudices and perhaps discover that reasons for using certain ingredients must be re-evaluated in terms of numerical constraints.

PROGRAM LISTING

```
10 REM     LISTING FOR DATA MANAGEMENT PROGRAM  (DT1)
20 COLOR 7,0
30 DIM V(19,11),INGR$(19):KEY OFF:COMMON H$(),HH$(),V(),NM$,INGR$()
35 FOR I=1 TO 7:READ H$(I):NEXT :FOR I=1 TO 7:READ HH$(I):NEXT
40 ON ERROR GOTO 460:IF INGR$(1)="" THEN FOR I= 1 TO 18 :
   INGR$(I)=STR$(I):NEXT:ELSE GOTO 60
60 DATA PROT,FAT,D.E.,Ca,P,METH,LYS,%,%,kJ/g,%,%,%,%
70 CLS:LOCATE 1,31:COLOR 0,7:PRINT "DATA INPUT FORM"
80 LOCATE 1,60:PRINT NM$;:COLOR 7,0:PRINT SPACE$(7)
90 PRINT "INGR.              BOUNDS ":FOR I=1 TO 7:J=24+(I*7):
   LOCATE 2,J:PRINT H$(I):LOCATE 3,J:PRINT HH$(I):NEXT
100 LOCATE 3,1: PRINT "          COST   LOWER  UPPER"
110 I=0:FOR P=4 TO 22:I=I+1:J=0:LOCATE P,1:PRINT INGR$(I):
    FOR Q=9 TO 72 STEP 7:J=J+1:LOCATE P,Q:PRINT V(I,J):NEXT:NEXT
120 LOCATE 22,1:PRINT "   MINIMUM REQUIREMENTS:";:LOCATE 25,1:
    PRINT "(Type [/] to activate command menu)";
130 ON KEY(11) GOSUB 200:ON KEY(12) GOSUB 210:ON KEY(13) GOSUB 220:
    ON KEY(14) GOSUB 230:KEY(11) ON:KEY(12) ON:KEY(13) ON:
    KEY(14) ON
140 P=4:Q=9 :I=1:J=1:LOCATE 1,1:COLOR 0,7:PRINT "       "
150 LOCATE P,Q:COLOR 0,7:PRINT V(I,J):T$=""
160 V$=INKEY$: IF V$="" THEN GOTO 160 ELSE N=ASC(V$)
170 IF N=47 THEN GOTO 240 ELSE IF N=13 THEN 190 ELSE IF N<46 OR
    N>57 THEN BEEP:GOTO 160
180 T$=T$+V$:V(I,J)=VAL(T$):LOCATE 1,1:COLOR 0,7:PRINT V(I,J):
    GOTO 160
190 GOSUB 550 : GOTO 150
200 GOSUB 550:IF P=4 THEN BEEP:RETURN 150 :ELSE LOCATE P,Q:
    COLOR 7,0:PRINT V(I,J);" ":P=P-1 :I=I-1:RETURN 150
210 GOSUB 550:IF J=1 OR I=19 AND J=4 THEN BEEP:RETURN 150:
    ELSE LOCATE P,Q:COLOR 7,0:PRINT V(I,J);" ":Q=Q-7:J=J-1 :
    RETURN 150
220 GOSUB 550:IF J=10 AND I>17 THEN BEEP:RETURN 150 ELSE IF J=10
    THEN :LOCATE P,Q:COLOR 7,0:PRINT V(I,J);" ":J=1:I=I+1:Q=9:P=P+1:
    RETURN 150: ELSE LOCATE P,Q:COLOR 7,0:PRINT V(I,J);" ":Q=Q+7:
    J=J+1:RETURN 150
```

```
230 GOSUB 550:IF I=18 AND J<4 THEN BEEP:RETURN 150 ELSE IF I=19
    THEN BEEP:RETURN 150:ELSE LOCATE P,Q:COLOR 7,0:PRINT V(I,J);
    " ":I=I+1:P=P+1:RETURN 150
240 COLOR 7,0:LOCATE 25,1:PRINT SPACE$(30):COLOR 0,7:LOCATE 25,1:
    PRINT "S";:LOCATE 25,12:PRINT "L";:LOCATE 25,23:PRINT "E";:
    LOCATE 25,42:PRINT "N";:LOCATE 25,61:PRINT "R";:LOCATE 25,70:
    PRINT "Q";
250 COLOR 7,0:LOCATE 25,2:PRINT "ave data";:LOCATE 25,13:PRINT
    "oad data";:LOCATE 25,24:PRINT "dit headings";:LOCATE 25,43:
    PRINT "ame ingredients";:LOCATE 25,62:PRINT "UN";:
    LOCATE 25,71:PRINT "UIT";
260 C$=INKEY$:IF C$="" THEN 260:ELSE LOCATE 25,1:PRINT SPACE$(78);
270 IF C$="S" OR C$="s" THEN GOTO 310 ELSE IF C$="L" OR C$="l"
    THEN GOTO 360
280 IF C$="N" OR C$="n" THEN GOTO 480
290 IF C$="E" OR C$="e" THEN GOTO 400 ELSE IF C$="R" OR C$="r"
    THEN GOTO 530
295 IF C$="Q" OR C$="q" THEN SYSTEM
300 BEEP: GOTO 150
310 GOSUB 600:LOCATE 25,1: INPUT "SAVE FILE NAME";OFL$:
    IF RIGHT$(OFL$,3)=".DT" THEN 315 ELSE OFL$=OFL$+".DT"
315 OPEN "O",£1,OFL$:NM$=OFL$
320 LOCATE 1,60:COLOR 0,7:PRINT NM$;:COLOR 7,0:PRINT SPACE$(7)
330 LOCATE 25,1:PRINT "WRITING FILE : "; OFL$;SPACE$(10);
340 FOR I= 1 TO 19:FOR J=1 TO 11:PRINT £1,V(I,J):NEXT:NEXT:
    FOR I=1 TO 8:PRINT £1,H$(I):PRINT £1,HH$(I):NEXT:FOR I=1
    TO 18:PRINT £1,INGR$(I):NEXT :CLOSE
350 LOCATE 25,1:PRINT SPACE$(70):GOTO 70
360 GOSUB 600:LOCATE 25,1: INPUT "FILE TO LOAD  ";IFL$:IF
    RIGHT$(IFL$,3)=".DT" THEN 365 ELSE IFL$=IFL$+".DT"
365 OPEN "I",£1,IFL$:NM$=IFL$
370 LOCATE 25,1:PRINT "READING FILE : "; IFL$;SPACE$(10);
380 FOR I= 1 TO 19:FOR J=1 TO 11:INPUT £1,V(I,J):NEXT:NEXT:FOR I=1
    TO 8:INPUT £1,H$(I):INPUT £1,HH$(I):NEXT:FOR I=1 TO 18:
    INPUT £1,INGR$(I):NEXT :CLOSE
390 LOCATE 25,1:PRINT SPACE$(70):GOTO 70
400 CLS: PRINT "OLD HEADING     UNITS              NEW HEADING      UNIT
410 LOCATE 25,1:PRINT "ENTER NEW HEADING OR HIT RETURN FOR SAME":
    LOCATE 2,1
420 FOR I=1 TO 7 :PRINT H$(I):LOCATE I+1,17:PRINT HH$(I):NEXT
430 FOR I=1 TO 7:LOCATE I+1,40:INPUT "", HN$(I):IF HN$(I)<>""THEN
    H$(I)=HN$(I) ELSE LOCATE I+1,40:PRINT H$(I)
440 LOCATE I+1,50:INPUT "",HHN$(I):IF HHN$(I)<>"" THEN HH$(I)=HHN$(I)
    ELSE LOCATE I+1,50:PRINT HH$(I)
450 NEXT:GOTO 70
```

```
460 IF ERR = 53 THEN RESUME 610 ELSE BEEP:LOCATE 25,1:
    PRINT "ERROR!    TYPE ANY KEY TO CONTINUE":COLOR 7,0
470 V$=INKEY$:IF V$="" THEN 470 ELSE LOCATE 25,1:PRINT
    SPACE$(60):RESUME 70
480 CLS: PRINT "  INPUT NAMES OF INGREDIENTS (max 8 characters)":
    PRINT " Type RETURN for same and a zero to end input sequence":
    PRINT
490 FOR I=1 TO 18:PRINT INGR$(I);:LOCATE I+3,14:INPUT " ",INGR$
500 IF INGR$="0" THEN I=18 ELSE IF INGR$<>"" THEN INGR$(I)=INGR$
    ELSE LOCATE I+3,14:PRINT INGR$(I)
510 IF LEN(INGR$(I))>8 THEN INGR$(I)=LEFT$(INGR$(I),8) ELSE
    Z=LEN(INGR$(I)):INGR$(I)=INGR$(I)+SPACE$(8-Z)
520 NEXT:GOTO 70
530 CLS
540 CHAIN "DT2"
550 LOCATE 1,1:COLOR 0,7:PRINT SPACE$(6);:COLOR 7,0:PRINT
    SPACE$(8);:LOCATE P,Q:COLOR 0,7:PRINT V(I,J):RETURN
600 CLS : FILES "*.DT"
610 RETURN

10 REM  LISTING FOR LEAST COST FEED FORMULATION WITH LINEAR
   PROGRAMMING (DT2)       Developed by W. Uys & M. Frick,
   PO Box 408, HOEDSPRUIT 1380, South Africa.
20 CLS:LOCATE 8,30:PRINT "BUSY WITH:":LOCATE 10,30:PRINT
   "INITIALIZE"
30 FOR I=1 TO 18:IF V(I,1)>0 THEN N=I
40 NEXT :FOR J= 4 TO 11:IF V(19,J)>0 THEN M=J-2
50 NEXT:IF M>N THEN O=N:Q=M: ELSE O=M:Q=N
60 DIM VN(N),D(M),N(M,N),XB(M),XN(N),CB(M),RCB(M),CN(N),
   RCN(N),UB(M),UN(N),B(M,M),M(18),R(M),X(N),C(N),SV(N),F(M,M),
   BT(M,M),Z(Q),VB(M),RAT1(M),RAT2(M),RECXN(N),RECXB(M),A(M,N)
70 COMMON H$(),HH$(),V(),NM$,INGR$()
80 FOR I=1 TO N:RECXN(I)=I:NEXT:FOR I=1 TO M:RECXB(I)=-I:NEXT
90 FOR I=1 TO N: RCN(I)=V(I,1):C(I)=RCN(I):CN(I)=1:VN(I)=V(I,2):
   XN(I)=VN(I):UN(I)=V(I,3):IF UN(I)=VN(I) THEN BEEP:CLS:PRINT
  "EACH UPPER BOUND MUST BE GREATER THAN ITS CORRESPONDING
   LOWER BOUND":INPUT "PRESS ENTER TO CONTINUE",D:CHAIN "DT1",40
100 NEXT
110 FOR I=1 TO M-1:FOR J=1 TO N: N(I,J)=V(J,I+3):A(I,J)=N(I,J):
    IF N(I,J)=0 THEN N(I,J)=.001
115 NEXT:NEXT
120 FOR J=1 TO M-1:D(J)=V(19,J+3)*100:NEXT:FOR I=1 TO N:N(M,I)=1:
    NEXT :D(M)=100
130 LOCATE 10,30:PRINT "SLACK VARIABLES":GOSUB 230 'check if feasible
140 IF YN$="N" THEN 150 ELSE 210
```

```
150 YN$="Y":FOR I=1 TO N:SV(I)=0:NEXT:FOR I=1 TO M:FOR J=I TO N:
    SV(J)=SV(J)+(UN(J)*N(I,J)):NEXT :NEXT
160 FOR I=1 TO N:IF SV(I)>MAX THEN MAX=SV(I):SVI=I
170 NEXT:IF MAX=0 THEN BEEP:LOCATE 10,30:
    PRINT "THE CONSTRAINTS DO NOT ALLOW A FEASIBLE SOLUTION":
    GOTO 1050: ELSE XN(SVI)=UN(SVI)
180 GOSUB 230
190 IF YN$="N" THEN SV(SVI)=0:MAX=0:YN$="Y":GOTO 160
200 FOR I= 1 TO N:X(I)=XN(I):NEXT
210 FOR I=1 TO M:XB(I)=XB(I)-D(I):UB(I)=99999!:NEXT
220 FOR I=1 TO M:VB(I)=0:NEXT :GOTO 260
230 FOR I= 1 TO M:XB(I)=0:NEXT:FOR I=1 TO M: FOR J=1 TO N:
    XB(I)=XB(I)+(XN(J)*N(I,J)):NEXT
240 IF XB(I)<D(I) THEN YN$="N":I=M
250 NEXT:RETURN
260 FOR I=1 TO M:FOR J=1 TO M:IF I=J THEN B(I,J)=-1 ELSE B(I,J)=0
270 NEXT:NEXT:PHASE =1
280 P=P+1:LOCATE 10,30:PRINT "PRICING ";P;SPACE$(10)
290 LOCATE 9,30:PRINT "PHASE ";PHASE
300 FOR I=1 TO M:FOR J=1 TO M :BT(I,J)=B(I,J):F(I,J)=0:
    NEXT:F(I,I)=1:NEXT
310 FOR I=1 TO M :DIV=BT(I,I):IF DIV=0 THEN DIV=1E-10
320 FOR J=1 TO M: BT(I,J)=BT(I,J)/DIV:F(I,J)=F(I,J)/DIV
330 NEXT J: FOR J=1 TO M:IF I=J THEN 360
340 R=BT(J,I): FOR K=1 TO M:BT(J,K)=BT(J,K)-R*BT(I,K):
    F(J,K)=F(J,K)-R*F(I,K)
350 NEXT K
360 NEXT J
370 NEXT I
380 FOR I=1 TO Q:Z(I)=0:M(I)=0:NEXT
390 FOR K=1 TO M:FOR J=1 TO M:Z(J)=Z(J)+CB(K)*F(K,J):NEXT :NEXT
400 FOR K=1 TO M:FOR J=1 TO N:M(J)=M(J)+Z(K)*N(K,J):NEXT :NEXT
410 FOR I= 1 TO N: M(I)=CN(I)-M(I):NEXT:DELTA=0
420 FOR I=1 TO N: IF XN(I)=VN(I) AND M(I)<0 THEN COL=I:DELTA=1:I=N
430 NEXT :IF DELTA=1 THEN 470
440 FOR I=1 TO N: IF XN(I)=UN(I) AND M(I)>0 THEN COL=I:DELTA=-1:I=N
450 NEXT :IF DELTA =-1 THEN 470
460 IF PHASE=1 THEN GOTO 900 ELSE CLS:
    PRINT "OPTIMUM FOUND!     ";:FOR I=1000 TO 2000 STEP 200 :
    SOUND I,.3:NEXT:PRINT NM$ :GOTO 900
470 LOCATE 10,30:PRINT "RATIO TEST        "  :FOR I= 1 TO M:
    R(I)=0:RAT1(I)=9999:RAT2(I)=9999:NEXT:RAT1=9999:RAT2=9999
480 FOR K= 1 TO M:FOR I=1 TO M:R(I)=R(I)+F(I,K)*N(K,COL):NEXT:NEXT
490 IF DELTA=1 THEN 500 ELSE 560
500 FOR I=1 TO M: IF R(I)<=0 THEN 510
    ELSE RAT1(I)=(XB(I)-VB(I))/ABS(R(I))
```

```
510 NEXT
520 FOR I= 1 TO M:IF RAT1(I)<RAT1 THEN RAT1=RAT1(I)
530 NEXT :FOR I= 1 TO M:IF R(I)>=0 THEN 540 ELSE
    RAT2(I)=(UB(I)-XB(I))/ABS(R(I))
540 NEXT: FOR I=1 TO M:IF RAT2(I)<RAT2 THEN RAT2=RAT2(I)
550 NEXT:GOTO 610
560 FOR I=1 TO M: IF R(I)>=0 THEN 570 ELSE
    RAT1(I)=(XB(I)-VB(I))/ABS(R(I))
570 NEXT:FOR I= 1 TO M:IF RAT1(I)<RAT1 THEN RAT1=RAT1(I)
580 NEXT:FOR I= 1 TO M:IF R(I)<=0 THEN 590 ELSE
    RAT2(I)=(UB(I)-XB(I))/ABS(R(I))
590 NEXT: FOR I= 1 TO M:IF RAT2(I)<RAT2 THEN RAT2=RAT2(I)
600 NEXT
610 IF RAT1<RAT2 THEN RAT=RAT1 ELSE RAT=RAT2
620 IF UN(COL)<RAT THEN RAT=UN(COL)
630 LOCATE 10,30:PRINT "UPDATE          "
640 XN(COL)=XN(COL)+(RAT*DELTA):IF ABS(XN(COL))<.001
    THEN XN(COL)=0
650 IF ABS(XN(COL)-VN(COL))<.001 THEN XN(COL)=VN(COL)
660 IF ABS(XN(COL)-UN(COL))<.001 THEN XN(COL)=UN(COL)
670 FOR I=1 TO M: XB(I)=XB(I)-(RAT*DELTA*R(I)):
    IF ABS(XB(I))<.001 THEN XB(I)=0
680 NEXT
690 FOR I=1 TO M:IF ABS(XB(I)-VB(I))<.001 THEN XB(I)=VB(I)
700 IF ABS(XB(I)-UB(I))<.001 THEN XB(I)=UB(I)
710 NEXT
720 IF RAT=UN(COL) THEN SOUND 1000,.2: GOTO 280
730 LOCATE 10,30:PRINT "PIVOT      ":L=0:SOUND 2000,.2
740 IF DELTA=1 THEN 750 ELSE 790
750 FOR I=1 TO M: IF XB(I)=VB(I) AND R(I)>0 THEN L=I:I=M
760 NEXT:IF L>0 THEN GOTO 830
770 FOR I=1 TO M:IF XB(I)=UB(I) THEN L=I:I=M
780 NEXT :GOTO 830
790 FOR I=1 TO M: IF XB(I)=VB(I) AND R(I)<0 THEN L=I:I=M
800 NEXT:IF L>0 THEN GOTO 830
810 FOR I=1 TO M:IF XB(I)=UB(I) AND R(I)>0 THEN L=I:I=M
820 NEXT
830 IF L=0 THEN  PRINT "CAN'T FIND LEAVING VARIABLE -
    HIT RETURN TO DISPLAY SUB-OPTIMAL SOLUTION":
    INPUT " ", DUMMY:GOTO 900
840 FOR I=1 TO M:SWAP B(I,L),N(I,COL):NEXT
850 SWAP XB(L),XN(COL) :SWAP RECXB(L),RECXN(COL):SWAP RCB(L),RCN(COL)
860 SWAP CB(L),CN(COL):SWAP UB(L),UN(COL):SWAP VB(L),VN(COL):
    IF PHASE=2 THEN 280
870 FOR I=1 TO N:IF RECXN(I)=-M THEN XBM=XN(I)
880 NEXT: FOR I =1 TO M:IF RECXB(I)=-M THEN XBM=XB(I)
```

```
890 NEXT:IF ABS(XBM)<.01 THEN 1170 ELSE GOTO 280
900 TOT=0 :FOR I=1 TO N:FOR J=1 TO N
910 IF RECXN(J)=I THEN X(I)=XN(J)
920 NEXT:NEXT: FOR I=1 TO N:FOR J=1 TO M:
    IF RECXB(J)=I THEN X(I)=XB(J)
930 NEXT :NEXT:FOR I=1 TO N:TOT=TOT+X(I):NEXT:
    IF PHASE =1 THEN 1160
940 FOR I=1 TO M:FOR J=1 TO N:IF RECXN(J)=-I THEN SENSAL(I)=M(J)
950 NEXT :NEXT
960 PRINT :PRINT "RATIOS TO MIX AS FOLLOWS":
    PRINT "INGREDIENT          QUANT %"
970 FOR I=1 TO N:LOCATE I+5,1:PRINT INGR$(I):LOCATE I+5,18:
    PRINT X(I):NEXT:PRINT "TOTAL           = ";TOT
980 FOR I=1 TO N: COST=COST+C(I)*X(I):NEXT
990 LOCATE 4,42:PRINT "COST OF FORMULATION = ";COST/100
1000 FOR I=1 TO M:FOR J=1 TO N: AN(I)=AN(I)+(X(J)*A(I,J)):NEXT :NEXT
1010 FOR I= 1 TO M-1:LOCATE 5+I,42:PRINT H$(I);" = ";AN(I)/100;
     " ";HH$(I);:NEXT
1020 LOCATE 13,42:PRINT "SENSITIVITY ANALYSIS"
1030 LOCATE 14,42:PRINT "Nutrient          Unit cost"
1040 FOR I=1 TO M-1:LOCATE 14+I,42:PRINT H$(I):LOCATE 14+I,60:
     PRINT SENSAL(I):NEXT
1050 LOCATE 25,4:COLOR 0,7:PRINT "P              R              E";
1060 COLOR 7,0:LOCATE 25,5:PRINT "rint data    ";:
     LOCATE 25,19:PRINT "un again    ";:LOCATE 25,32:
     PRINT "xit to system";
1070 C$=INKEY$:IF C$="" THEN 1070 ELSE IF C$="p" OR C$="P"
     THEN 1090
1080 IF C$="R" OR C$="r" THEN 1140 ELSE IF C$="E" OR C$="e"
     THEN 1150 ELSE BEEP:GOTO 1070
1090 LOCATE 25,1:PRINT "GET PRINTER READY AND HIT RETURN";
     SPACE$(16);:INPUT " ",Q
1100 LPRINT SPACE$(15);NM$:LPRINT:
     LPRINT "RATIOS TO MIX AS FOLLOWS":LPRINT
1110 LPRINT "INGREDIENT          QUANT %":FOR I=1 TO N:
     LPRINT INGR$(I);SPACE$(10);X(I):NEXT
1120 LPRINT :LPRINT "COST OF FORMULATION = ";COST/100:LPRINT
1130 FOR I=1 TO M-1:LPRINT H$(I);" = ";AN(I)/100;" ";HH$(I):
     NEXT :LPRINT :LPRINT "SENSITIVITY ANALYSIS":
     LPRINT "NUTRIENT      UNIT COST":FOR I=1 TO M-1:
     LPRINT H$(I);"       ";SENSAL(I):NEXT:LOCATE 25,1:
     PRINT "     ";: GOTO 1050
1140 CHAIN "DT1",40
1150 SYSTEM
1160 IF TOT > 101 THEN CLS:BEEP: PRINT "NO FEASIBLE SOLUTION":
     INPUT "PRESS RETURN TO CONTINUE",D:CHAIN "DT1",40
```

```
1170 PHASE=2:FOR I=1 TO N:CN(I)=RCN(I):IF RECXN(I)=-M
     THEN UN(I)=.001
1180 NEXT:SOUND 3000,1:SOUND 0,2:SOUND 3000,1
1190 FOR I=1 TO M:CB(I)=RCB(I):IF RECXB(I)=-M THEN UB(I)=.001
1200 NEXT:GOTO 280
```

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