

## **Chapter 7**

### **General Discussion and Conclusions**

## 7.1 INTRODUCTION

The present research program was designed to further our understanding of nutritional requirements with respect to the relationship between dietary protein and energy levels and growth, body composition, digestive enzymes (protease, lipase and  $\alpha$ -amylase), blood plasma components (glucose, triglycerides, cholesterol and amino acid levels) and histology of intestine and liver of African catfish, *Clarias gariepinus*. The accumulated data and insights gained during this study may allow formulation of cost-effective feeds and optimisation of feeding regimes. Studies were conducted over two years with feeding and growth trials in closed recirculating systems in a controlled environment using fishmeal and soybean meal based diets.

Recognising the importance of protein, lipid and carbohydrate nutrition in fish culture, from a biological and economical point of view, the present thesis focussed on optimising the conversion of dietary protein, lipid and carbohydrate into fish body protein. The work presented here attempts to address several aspects of the nutrition of African catfish, *Clarias gariepinus*. Based on the results obtained in the present thesis, it is clear that manipulation and optimisation of dietary protein and energy intakes are important factors with respect to growth performance, body composition, digestibility, digestive enzyme activities and blood plasma components in African catfish, *Clarias gariepinus*.

## 7.2 DISCUSSION

The results from the first study (Chapter 3) showed that addition of dietary energy at different protein levels resulted in increases in growth and feed performance with respect to protein and energy utilisation. The highest energy level in the high protein diet led to a reduction in performance. The lowest growth rate, feed and protein utilisation efficiency were observed at

the lowest dietary energy level for both protein levels indicating that protein might be used for energy rather than growth. The possibility of increasing protein deposition by adding energy as lipid might be limited in high protein diets.

Higher lipid deposition in whole body and liver was observed with increasing dietary energy at both protein levels and higher deposition was found for lower protein diets (Section 3.3.4). Lower protein diets contained more carbohydrates and it is this that may have lead to increased lipid deposition. Higher carbohydrate contents in lower protein diets produced increased liver glycogen and blood plasma glucose accumulation, suggesting an inability of *C. gariepinus* to utilise dietary carbohydrate levels above 35% of the diet. VSI and HSI did not vary significantly ( $P < 0.05$ ) among test groups but there was a trend toward lower VSI and HSI in higher protein diets. No consistent trends were observed in protease and lipase activities of intestine and liver, or plasma free amino acid levels with changes in dietary lipid or protein level (Sections 3.2.5 and 3.2.6). This study suggested that the best performing diet for *C. gariepinus* contained an intermediate energy level of 21.18 kJ/g of GE, a crude protein level of 43%, a crude lipid level of 8.23% and a carbohydrate level of 35% resulting in an optimum P/E ratio of 20.54 mg protein / kJ of GE.

The appropriate levels of dietary non-protein, lipid and carbohydrate energy sources are also important in influencing growth performance and efficiency of feed and protein utilisation. Therefore, the second study (Chapter 4) attempted to determine the optimum lipid to carbohydrate level for *C. gariepinus*. Diets containing a P/E ratio 20 mg protein kJ/g of GE, a crude protein 40% and iso-energetic 20 kJ/g of GE were selected based on results from the previous study (Chapter 3). The diets contained 40% crude protein rather than 43% (based on

the results of the previous study) due to limited ‘space’ in the feed formulation with the fish meal and soybean meal based diets.

The results from the second experiment on optimising dietary lipid to carbohydrate ratio (Chapter 4), showed that dietary carbohydrate energy level increase (up to 50%) with concomitant reduction in dietary lipid energy level resulted in a trend towards higher growth and feed performance (protein, lipid and energy utilisation, and digestibility). These results suggest that *C. gariepinus* can utilise dietary carbohydrate for energy.

An increase in dietary carbohydrate with a concomitant reduction in dietary lipid resulted in reduced body lipid and liver lipid deposition in both viscera and non-visceral tissues whilst HSI tends to increase (Section 4.3.4). Since the liver is the main site of lipogenesis in fish, this probably reflected an increased level of lipogenesis fuelled by dietary carbohydrate. This is also supported by the fact that apparent lipid utilisation increased to > 100% in fish fed the high carbohydrate diet. The trend towards higher liver glycogen deposition and lower plasma glucose levels in fish fed increasing lipid, lower CHO, could be because increasing dietary lipid provided excess energy that was deposited as glycogen. In this species dietary carbohydrates are employed as an immediate energy-yielding nutrient and liver glycogen is likely to be of gluconeogenic origin from amino acid precursors. Such an interpretation is also supported by carcass composition data, higher  $\alpha$ -amylase activity and improved growth and protein utilisation of fish fed the higher carbohydrate-lower lipid diets rather than the lower carbohydrate-higher lipid diets.

No significant effects on digestive enzymes, protease and lipase, in intestine and liver were observed in response to different dietary lipid and carbohydrate levels while intestinal  $\alpha$ -

amylase activity increased at higher dietary carbohydrate levels (Section 4.3.5). This increased  $\alpha$ -amylase activity might be due to induction of synthesis or overall improved growth and protein utilisation. No significant effects were found on triglycerides (TG), cholesterol and amino acid levels in plasma in response to varying levels of dietary lipid and carbohydrate. This study suggested that the optimum dietary lipid to carbohydrate ratio for *C. gariepinus* was 2.47 (g/g) with crude lipid 13% and carbohydrate 33.42%.

The performance of a feed is not only dependent on its quality but also on appropriate feeding management. Good quality and nutritionally adequate feed can give poor performance unless proper feeding regimes (feeding type and feeding rate) are employed. Therefore an experiment (Chapter 5) was conducted to investigate feeding regimes in this species, based on data generated from the two previous experiments (Chapters 3 and 4).

The results of Chapter 5 show that higher dietary protein or energy levels resulted in increased growth and feed performance with respect to the higher protein and energy utilisation under both appetite and restricted feeding regimes. When nutrient density varied it resulted in significant differences in growth even though provision was made to ensure that all fish received approximately same amounts of dietary protein and energy. Appetite feeding tended to result in higher growth performance and protein utilisation than feeding the same diets under a restricted regime.

Feeding regime (appetite and restricted) had no effect on body composition or digestive enzyme activities of fish fed the experimental diets (Sections 5.3.4 and 5.3.5). Blood plasma components such as glucose, triglycerides (TG), cholesterol and amino acid levels did not

show any notable variation (Section 5.3.6). These results suggest that appetite feeding regimes on a diet containing as low as 35% protein are suitable for *Clarias gariepinus*.

Histological examination of intestines and liver (Section 3.3.4) and only liver (Sections 4.3.4 and 5.3.4) of *C. gariepinus* showed no abnormalities when fish were fed different experimental diets. Histological studies of the intestine and liver generally supported the other observations of the experiments. In the present experiments (Chapters 3, 4 and 5) digestive enzyme activities (excluding  $\alpha$ -amylase activity, Section 4.3.5) and plasma amino acid levels were not affected by diet.

Chapter 6 showed that alternating periods of restricted (maintenance requirement) and appetite feeding in African catfish, *C. gariepinus* resulted in partial growth recovery. *C. gariepinus* showing partial compensatory growth under alternating periods of feeding restricted or appetite rations also displayed better feed, protein, lipid and energy utilisation efficiency in comparison to fish fed to appetite throughout (Sections 6.3.1 and 6.3.2). The different restricted and subsequent appetite feeding schedules had no significant effect on body composition parameters. However, alternating periods of restricted and appetite feeding caused changes in moisture and lipid content and sometimes in protein content in whole body carcass, eviscerated carcass and viscera composition (Sections 6.3.3, 6.3.5 and 6.3.6). These studies suggest that a *C. gariepinus* feeding schedule of 3 days restricted followed by 4 days appetite would be a plausible way to maintain growth and reduce feed input cost.

### 7.3 CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of this thesis a list of conclusions and recommendations may be summarised as follows:

- (a) The optimum dietary protein to energy ratio (P/E ratio) found for African catfish, *C. gariepinus* was 20.54 mg protein / kJ of GE, for a diet containing crude protein over 40% and gross energy more than 20 kJ/g.
- (b) The optimum dietary non-protein, lipid to carbohydrate ratio (L/CHO, g/g) found for this species was 2.47, for a diet containing 13% dietary lipid and 33% dietary carbohydrate based on the above optimised P/E ratio.
- (c) Differences found between these results on P/E ratio and those of other authors are interpreted mainly as a consequence of the use of different techniques for analysing experimental data. In addition, the use of different feed ingredients, feeding rates, fish sizes and environmental conditions by other authors contributed to the explanation of different results.
- (d) The dietary protein requirement of *C. gariepinus* was over 40%, and this level of dietary protein could be reduced to 35% by applying an appetite feeding regime.
- (e) Lipid carcass deposition increased as dietary energy (as lipid) increased. Excess or reserve lipids are stored in both visceral and non-visceral tissues in *C. gariepinus*.
- (f) Dietary lipid and carbohydrate are well utilised by *C. gariepinus* as energy sources and they may spare protein for growth.

- (g) Dietary proteins, lipids and carbohydrates were well digested, underlining their importance as “tissue-yielding” and “energy yielding” nutrients for this species.
- (h) At varying dietary protein levels under a restricted feeding regime *C. gariepinus* displayed significant growth differences although provision was made to ensure that all fish received approximately the same amount of dietary protein and energy.
- (i) Alternating periods of restricted (maintenance requirement) and appetite feeding in *C. gariepinus* resulted in partial growth recovery. Feeding to 3 days restricted followed by 4 days appetite would be a plausible way to obtain good growth and reduce feed input cost for this species.

#### **7.4 SUGGESTIONS FOR FUTURE WORK IN THIS FIELD**

Further research with African catfish, *Clarias gariepinus* should investigate fundamental aspects of the physiology and biochemistry of dietary protein and energy utilisation. Well known biochemical pathways provide the theoretical basis for this. Whilst information on optimal acceptable limits of individual nutrients is vital, a better understanding of the fundamental aspects of the dynamics of physical and chemical interrelationships between nutrients in rations, the process of digestion, and functional compartmentation of nutrients, could further enhance culture practices.

Increased reliance on radioisotopic studies for monitoring directions and rates of vital metabolic pathways may be a useful approach towards the realisation of this goal.