Chapter 5

Approaches to optimising feeding regime in African catfish, *Clarias gariepinus* (Burchell, 1822) with various concentrations of dietary protein and energy

5.1 INTRODUCTION

Since feeding regime affects nutrient requirements, knowledge of optimum feeding regime is considered a prerequisite to determination of nutrient requirements (Tacon and Cowey, 1985; Talbot, 1985). Feeding regimes (e.g. ration size, frequency, duration of a meal etc.) are also reported to influence fish growth, feed conversion and body composition (Reinitz, 1983; Li and Lovell, 1992; Munsiri and Lovell, 1993; Arzel *et al.*, 1998; McGoogan and Gatlin III, 2000).

Efficient use of feed is important in achieving profitable aquaculture. In fish an inverse relationship between optimal dietary protein level (as a percentage of the diet) and feeding regime (ration size) has been suggested (Ogino, 1980; Tacon and Cowey, 1985). The optimum protein level for growth has generally been found to be over 40% for African catfish, *Clarias gariepinus* and hybrid *Clarias* catfish and somewhat lower for walking catfish, *Clarias batrachus* (Khan and Jafri, 1990; Singh and Singh, 1992; Machiels and Henken, 1985; Degani *et al.*, 1989; Uys, 1989; Jantrarotai *et al.*, 1998). However, ration size was not always accurately determined or indicated and the possible inverse relationship between feeding level and optimal dietary protein levels (Tacon and Cowey, 1985) cannot be investigated. On the contrary, investigations into the manipulation of dietary protein levels and feeding regimes in African catfish, *Clarias gariepinus* have been limited and inconclusive.

Ogino (1980) reported a notable decrease in the dietary protein requirement of juvenile carp, *Cyprinus carpio* L., and rainbow trout, *Onchorynchus mykiss* (Walbaum) from 60-65% to 30-32% when the feeding level was increased from 2% to 4% body weight per day in each species. Li and Lovell (1992) demonstrated no increase in weight gain as dietary protein increased in 6% increments from 26 to 38% when channel catfish were fed to satiation;

however, weight gain increased as protein increased from 26 to 38% when fish were fed a restricted daily allowance. Such a difference has clear economic consequences in aquaculture.

In channel catfish, *Ictalurus punctatus*, comparable weight gain was attained through feeding diets with low protein at a high ration level or high protein at a low ration (Cacho *et al.*, 1990). Therefore, it can be suggested that feeding rate may be manipulated to produce the desired protein and energy intakes from various levels of dietary protein and energy. Previous studies (Chapter 3 and Chapter 4) have already investigated optimised protein to energy ratio and lipid to carbohydrate ratio respectively in the diet to meet the requirements for maximum growth of African catfish, *Clarias gariepinus*. Thus, there is a need to determine the interaction between dietary protein and feeding levels, and the economics of using high or low dietary protein levels offered at both appetite and restricted feeding regimes.

The aim of the present study was to determine the interactions between dietary protein level and feeding regime on growth, protein utilisation and body composition in African catfish, *Clarias gariepinus*. The metabolic effects of feeding regime on digestive enzyme activities and blood plasma metabolites together with histology of liver were also studied.

5.2 MATERIALS AND METHODS

5.2.1 Experimental System

The experimental system described in Section 2.1 and Figure 2.1 was used for an experiment to optimise the feeding regime of African catfish, *Clarias gariepinus* (Burchell, 1822).

5.2.2 Experimental Fish

African catfish, *Clarias gariepinus* as described in Section 2.2 was used as the test fish species in this study. Thirteen-week old (13.45 ± 0.05g) fingerlings were obtained from broodstock maintained at the Institute of Aquaculture, University of Stirling following the procedure detailed in Section 2.2. Fish were randomly assigned into groups of 20 fish and each group was placed in a 30-L tank as described in Section 2.1 and as shown in Figure 2.1.

5.2.3 Experimental Diets

Three experimental diets were formulated with increasing levels of protein: as diet 1, 2 and 3 containing 28, 35 and 40% crude protein respectively and gross energy level of 14.42, 17.71 and 20.08 kJ/g respectively. The diets contained a P/E ratio of 20-mg protein per kJ of GE and L/CHO ratio (g/g) of 0.40 fixed on the basis of results obtained from previous studies (Chapters 3 and 4). Composition of the experimental diets and their proximate analyses are shown in Table 5.1. All diets contained a good balance of essential amino acids on a percentage of protein basis and the amino acid composition of diet 2 is shown in Table 5.2 as an example. Diet formulation and preparation were as described in Sections 2.3.1 and 2.3.2.

5.2.4 Experimental Procedure

Acclimation and periodical weighing of fish were as described in Section 2.4.1. The experiment was conducted for 8-weeks using fish in three replicates per treatment. Faeces collection was as described in Section 2.4.4. Before commencement of the feeding trial, 10 fishe were randomly sacrificed with an overdose of benzocaine, and triplicate pooled samples were taken for determination of initial whole body composition. At the end of the experiment, all fish were weighed and counted and 6 fish from each tank were collected for determination of whole body composition, organosomatic indices as well as liver histology. The remaining fish were fed their respective diets for another 2 days. Two days after the final weighing, 8 fish from each thank were collected for determination of liver lipid, liver glycogen, digestive enzymes and blood plasma components.

5.2.4.1 Fish Feeding

Two weeks before the experiment a mini trial was conducted to investigate the maximum feeding rate (% body weight/day) of the diet containing the low level (28% CP) of protein. In this trial it was observed that fish accepted a maximum of 5% of their body weight daily.

In six treatments, two feeding regimes (restricted and appetite) were offered for each of the three diets (28, 35 and 40% dietary protein level). In restricted feeding, fish were offered decreasing fixed rate of 5.0, 4.1 and 3.5% of their body weight of diets 1, 2 and 3 respectively to provide approximately the same amount of protein and energy intake in all treatments daily. These fish were offered the dietary ration subdivided into three equal parts at 10:00, 14:00 and 18:00 h daily. Appetite feeding of fish was also performed three times daily and was achieved by giving a small quantity of feed every 2 to 3 minutes and allowing the fish to eat until they stopped (each total meal spanned about 20-minutes). Total feed intake was recorded daily for each treatment. To avoid loss of diet, food was offered taking great care by giving small amounts of food at a time to be sure that the fish ate all the diet.

5.2.5 Water Quality Management

Water quality management was as followed described in Section 2.5. All values were within optimum ranges for this species (Table 2.4, Section 2.1).

5.2.6 Experimental Analyses

5.2.6.1 Proximate Analyses

Proximate analyses (moisture, crude protein, crude lipid and ash) of whole fish carcass, feed ingredients and experimental diets were determined by the methods described in Sections 2.6.1.1, 2.6.1.2, 2.6.1.3 and 2.6.1.5. Crude fibre and gross energy contents of experimental diets were determined as described Sections 2.6.1.4 and 2.6.1.7.1, while chromic oxide contents in fish faeces and experimental diets were determined by the method described in Section 2.6.1.8. Fish within each group were pooled for carcass analysis. Final values for each group represented the arithmetic mean of three replicates, all samples were analysed in triplicate.

5.2.6.2 Growth and Feed Performance

Growth and feed performance, protein, energy and dry matter digestibility determination and organ indices were calculated according to the methods described in Sections 2.6.2.1.1, 2.6.2.1.2, 2.6.2.1.3, 2.6.2.1.4, 2.6.2.2, 2.6.2.3, 2.6.2.4 and 2.6.2.5.

Table 5.1 Formulation and composition of the experimental diets and proximate analysis (% dry weight basis)

	Diet number (Designation: % protein)		
Diet no.	1	2	3
(% protein)	(28%)	(35%)	(40%)
Ingredients:			
Fish meal (Herring type)[1]	25.00	30.00	40.00
Soybean meal (Dehulled solvent extract)[2]	15.00	20.00	17.30
Wheat flour (Whole wheat)[3]	10.00	6.50	6.50
Fish oil	3.50	4.28	4.89
Corn oil	3.50	4.28	4.89
Vitamin premix[4]	1.00	1.00	1.00
Mineral premix[5]	1.00	1.00	1.00
Chromic oxide ($Cr_2 O_3$)	0.50	0.50	0.50
Carboxymethyl cellulose (Binder)[6]	2.00	2.00	2.00
Corn Starch	8.50	14.35	19.70
$\propto$- Cellulose	30.00	16.08	2.22
Proximate composition:			
% as fed:			
Moisture	6.90	9.76	6.52
% Dry wt. basis:			
Crude Protein	28.15	35.08	40.23
Crude fat	9.53	11.68	13.38
Ash	6.93	7.96	9.30
Fibre	30.72	16.98	3.72
NFE[7]	24.67	28.30	33.37
$Cr_2 O_3$	0.47	0.49	0.50
Lipid / CHO ratio (g/g)[8]	0.39	0.41	0.40
GE (kJ/g)[9]	14.42	17.71	20.08
P/E ratio[10]	19.52	19.71	20.03

Proximate Analysis (% dry weight basis):

1. Moisture: 9.52; Crude protein: 74.67; Crude fat: 8.12; Fibre: 0.46; Ash: 15.98.
2. Moisture: 13.47; Crude protein: 54.35; Crude fat: 1.41; Fibre: 4.71; Ash: 8.31.
3. Moisture: 13.33; Crude protein: 12.07; Crude fat: 1.28; Fibre: 3.05; Ash: 2.25.
4. As listed in Table 2.2, Section 2.3.1
5. As listed in Table 2.3, Section 2.3.1
6. Carboxy methyl cellulose – Sodium salt, high viscosity
7. NFE = Nitrogen free extractives, calculated as 100 – (% Protein + % Lipid + % Ash + % Fibre)
8. Lipid/CHO ratio (g/g) = % wt. in lipid / % wt. in CHO
9. GE = Gross energy content
10. P/ GE ratio = Protein to energy ratio in mg protein/ kJ of GE

Table 5.2 Essential amino acid composition (EAA, g/100g protein) of experimental diet 2 and EAA requirements of African catfish, *Clarias gariepinus*

Essential amino acids:	Diet 2	EAA requirements [a]
Arginine	4.48	4.30
Histidine	1.71	1.50
Isoleucine	3.01	2.60
Leucine	5.44	3.50
Lysine	4.75	5.00
Methionine[b]	0.97	2.30
Phenylalanine[c]	3.16	5.00
Threonine	3.10	2.00
Valine	3.48	3.00

[a] Requirement of a related species channel catfish (NRC, 1993)

[b] In the absence of dietary cystine (NRC, 1993)

[c] Dietary protein contained 1.61 percent tyrosine. With 0.6 percent tyrosine the phenylalanine requirement was 2.0 percent of the dietary protein (NRC, 1993)

5.2.6.3 Liver Lipid and Liver Glycogen Determination

Liver lipid and liver glycogen were estimated as described in Sections 2.7 and 2.8.

5.2.6.4 Digestive Enzyme Assays

Digestive enzyme (protease, lipase and α-amylase) assays were as described in Section 2.10.

5.2.6.5 Blood Plasma Assays

Plasma assays were performed as described in Section 2.11.3.

5.2.6.6 Amino acid Analysis

Amino acid analysis was carried out by the method described in Section 2.12.

5.2.6.7 Histological Analysis

Histological analyses of fish liver were performed as described in Section 2.9.

5.2.6.8 Statistical Analysis

Data in this study were analysed by analysis of variance (ANOVA) using Mini Tab statistical software for Windows (release 12, 1998). Comparison among treatment means was carried out by two-way analysis of variance followed by a Benferroni test employed in evaluating a significance level of 0.05. Standard deviation (± SD) was calculated to identify the range of means. Treatment mean differences were tested between adjacent feeding regimes at each protein level. Percentage data were arc-sine transformed (Zar, 1948) prior to ANOVA and reversed afterward.

5.3 RESULTS

5.3.1 Growth, Survival and Feed Performance

No mortality nor external clinical symptoms occurred in any treatment during the period of this experiment. Absolute growth of *Clarias gariepinus* during the experimental period is shown in Figure 5.1 and the growth and feed response parameters are summarised in Table 5.3 and graphically in Figure 5.2. In general, growth performance increased significantly ($P < 0.05$) with increase in protein of the diets for both feeding regimes. Weight gain for both restricted and appetite feeding increased ($P < 0.05$) with increasing protein percentage and the 28% protein diet resulted in significantly ($P < 0.05$) lower weight gain. Highest weight gain was observed for fish consuming 40% protein with no significant differences ($P > 0.05$) between the 35% and 40% protein diets. Fish under appetite feeding tended to have greater weight gains ($P > 0.05$) than restricted fed fish at the same protein level. The greatest improvement in weight gain under appetite feeding was when dietary protein percentage rose from 28 to 35%.

A similar response was also observed in the case of specific growth rate (SGR) where increasing protein percentage, above that of 28%, resulted in significantly ($P < 0.05$) increased SGR (%) under both feeding regimes. It is interesting that no significant variation ($P > 0.05$) was found in SGR values for any diets in either appetite feeding and restricted feeding (Table 5.3).

Feed conversion efficiency (FCE) of fish under restricted feeding was significantly ($P < 0.05$) greater for the 40% protein diet than for either of the 28 or 35% protein diets (Table 5.3). Under appetite feeding FCE increased significantly ($P < 0.05$) as dietary protein percentage increased and the maximum value was observed in fish consuming 40% protein diet but this was not significantly different ($P > 0.05$) from that for the 35% protein diet. Appetite feeding resulted in insignificantly better ($P > 0.05$) FCE than feeding the same protein diet under a restricted regime. There were no significant differences ($P > 0.05$) in FCE in either restricted or appetite feeding for a particular diet (Table 5.3).

The coefficient of variation (CV) of final weight under restricted feeding was found to be lowest for the 35% protein diet. Under appetite feeding, CV of final weight had a tendency towards higher values with increasing protein level in the diet. Feeding the 35% protein to appetite produce a low CV in final weight.

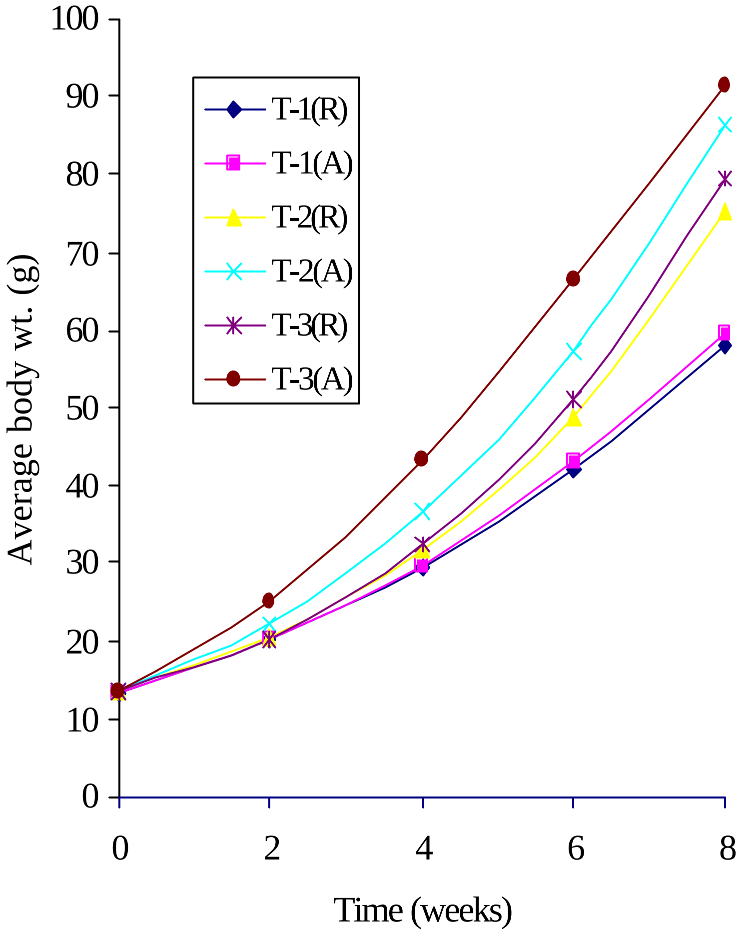

Figure 5.1 The mean fortnightly growth response of African catfish, *Clarias gariepinus* maintained on various dietary protein levels and two feeding regimes over 8 weeks.

(The treatments were, T-1 (R), T-1 (A); T-2 (R), T-2 (A) and T-3 (R), T-3 (A), where R and A refers respectively to the restricted and appetite feeding regime. The numerical value 1, 2 and 3 refers to the number of diet contained 28, 35 and 40% protein respectively)

Table: 5.3 Mean growth performance, feed and nutrient utilisation efficiency of *Clarias gariepinus* at various protein levels and two feeding regimes for 56 days.

	Treatment (% protein and feeding regime)						
Diet number: (% protein)	1 (28%)		2 (35%)		3 (40%)		
Feeding regime: **Parameters:**	**Restr.**	**Appet.**	**Restr.**	**Appet.**	**Restr.**	**Appet.**	± SEM
Initial body wt. (g)	13.40^{a} ± 0.16	13.40^{a} ± 0.04	13.46^{a} ± 0.06	13.46^{a} ± 0.06	13.43^{a} ± 0.12	13.52^{a} ± 0.02	0.02
Final body wt. (g)	57.69^{a} ± 6.37	59.54^{a} ± 3.73	75.32^{b} ± 1.08	86.28^{bc} ± 4.83	79.61^{bc} ± 4.31	91.35^{c} ± 1.17	3.16
CV (%) of final weight	39.32 ± 11.57	40.38 ± 3.68	31.08 ± 1.18	35.57 ± 8.70	36.74 ± 2.09	42.57 ± 8.60	1.87
Weight gain (%)	330.95^{a} ± 52.22	344.41^{a} ± 29.29	459.61^{b} ± 10.12	541.03^{bc} ± 35.55	492.94^{bc} ± 32.78	575.47^{c} ± 7.94	23.20
Specific growth rate (SGR, % day)	2.60^{a} ± 0.23	2.66^{a} ± 0.12	3.07^{b} ± 0.03	3.31^{b} ± 0.10	3.18^{b} ± 0.10	3.41^{b} ± 0.05	0.08
Food conversion efficiency (FCE)	0.83^{a} ± 0.05	0.85^{a} ± 0.03	1.04^{b} ± 0.02	1.12^{b} ± 0.05	1.27^{c} ± 0.05	1.16^{bc} ± 0.04	0.04
Protein efficiency ratio (PER)	2.95^{a} ± 0.17	3.03^{a} ± 0.09	2.95^{a} ± 0.05	3.18^{a} ± 0.15	3.15^{a} ± 0.13	2.88^{a} ± 0.11	0.04
Apparent net protein utilisation (ANPU, %)	51.12^{a} ± 4.12	55.51^{a} ± 4.30	51.85^{a} ± 0.82	57.34^{a} ± 2.34	55.12^{a} ± 2.91	54.01^{a} ± 3.56	0.82
Apparent net lipid utilisation (ANLU, %)	70.72^{a} ± 0.84	71.52^{a} ± 1.22	79.14^{a} ± 10.23	70.55^{a} ± 6.42	73.97^{a} ± 3.82	66.58^{a} ± 3.25	1.41
Apparent net energy utilisation (ANEU, %)	42.01^{a} ± 2.03	44.25^{a} ± 1.84	44.86^{a} ± 2.92	45.18^{a} ± 1.60	45.52^{a} ± 1.82	43.06^{a} ± 1.54	0.50
Protein digestibility (%)*	84.29	86.18	88.18	88.45	88.28	90.13	
Energy digestibility (%)*	67.25	69.24	71.74	76.42	79.49	82.77	
Dry matter digestibility (%)*	65.77	70.44	66.97	67.02	74.49	78.63	

Note: Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$) and values in the same row with same superscript are not significantly different ($P > 0.05$).

* No statistical analysis was possible as determinations were performed on pooled samples.

5.3.2 Apparent Protein and Energy Utilisation

Protein utilisation efficiency, measured in term of protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) is summarised in Table 5.3 and graphically in Figure 5.2. PERs under both restricted and appetite feedings were not significantly different ($P > 0.05$) with changing protein percentage in the diet. Apparent net protein utilisation (ANPU) under restricted and appetite feeding did not vary significantly ($P > 0.05$) with protein level in the diets, similarly to PER. Feeding to appetite tended to produce better ANPU than feeding the same diets under a restricted regime and this was observed when the protein level rose from 28 to 35%.

As shown in Table 5.3, apparent net lipid utilisation (ANLU) and apparent net energy utilisation (ANEU) under restricted and appetite feeding was not influenced significantly ($P > 0.05$) by different protein percentage in the diet. ALEU for restricted feeding tended to have better values than appetite rations with increasing protein level. Feeding to appetite produced a better ANPU than restricted feeding in the diet containing 28 and 35% dietary protein.

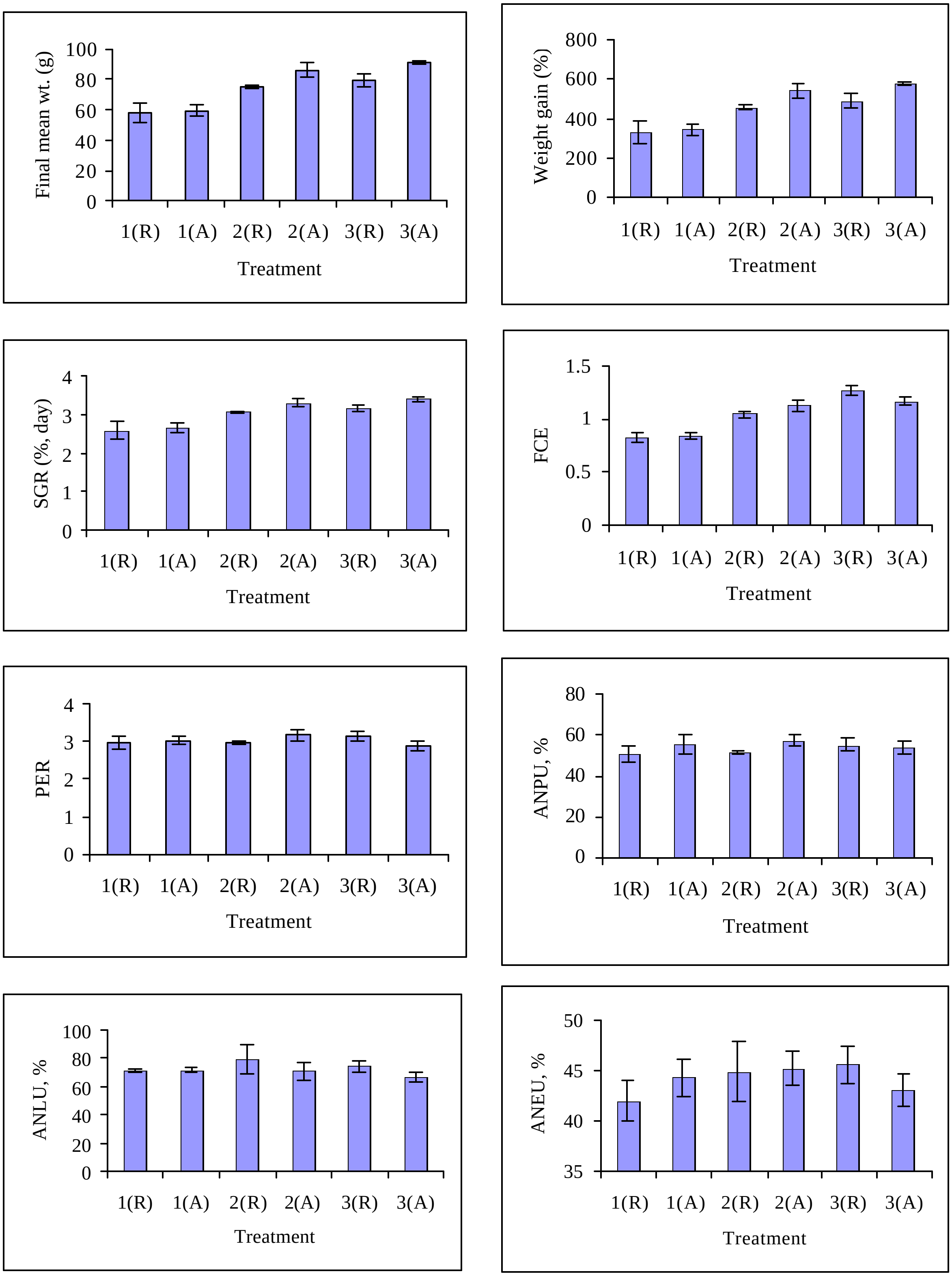

Figure 5.2 Mean growth performance, feed and protein utilisation efficiency of African catfish, *Clarias gariepinus* fed various levels of dietary protein with two feeding regimes (SGR = Specific growth rate; FCE = Food conversion efficiency; PER = Protein efficiency ratio; ANPU = Apparent net protein utilisation; ANLU = Apparent net lipid utilisation; ANEU = Apparent net energy utilisation).

Bars are means ± SD of three replicates. The treatments were, T-1 (R), T-1 (A); T-2 (R), T-2 (A) and T-3 (R), T-3 (A), where R and A refers respectively to the restricted and appetite feeding regime. The numerical value 1, 2 and 3 refers to the number of diet contained 28, 35 and 40% protein respectively.

5.3.3 Apparent Protein, Energy and Dry matter Digestibility

Apparent protein, energy and dry matter digestibility data are presented in Table 5.3. Apparent protein digestibilities were fairly high ranging from 84.29 to 90.13%. Protein digestibility for restricted and appetite feeding did not vary significantly ($P > 0.05$) but did increase slightly with increasing protein level in the diet.

Apparent energy digestibility ranged from 67.25 to 82.77%. For both feeding regimes, it increased with increasing dietary protein percentage. Apparent dry matter digestibility ranged from 65.77 (in restricted feeding, 28% dietary protein diet) to 78.63% (in appetite feeding, 40% protein diet).

5.3.4 Body Composition and Histology

Body composition data of final samples of fish from different treatments and initial condition are presented in Table 5.4. At the end of the experiment, in comparison to initial values, all the experimental groups exhibited higher percentage of protein, lipid and ash but a lower percentage moisture. In general, body composition under restricted and appetite feeding did not vary significantly ($P > 0.05$) with protein level in the diets. Fish fed different protein levels under restricted or appetite conditions showed no significant difference ($P > 0.05$) in body moisture content. Fish fed the lower protein diet (28% CP) under both feeding regimes showed no significant difference but had slightly lower body protein and body lipid than fish from the other treatments. The inverse relationship between body moisture and body lipid content was revealed from values for all treatments. Appetite feeding resulted in an increase in body lipid with increase in dietary protein. However, both feeding regimes tended (although statistically insignificant, $p > 0.05$) to have lower body ash content as protein level increased in the diets.

Liver lipid, liver glycogen and organ indices (VSI and HSI) of fish from different treatments at the end of the experiment are shown in Table 5.5. At the end of the experiment liver lipid, liver glycogen, VSI and HIS ranged from 5.42 to 6.15; 0.57 to 0.69; 6.96 to 11.77 and 0.98 to 1.35% (% wet weight) respectively. In general, liver lipid, liver glycogen and organ indices (HSI and VSI) from both feeding regimes were not significantly different ($P > 0.05$). Fish fed restricted or appetite regimes tended to have higher liver lipid contents when the protein percentage was raised from 28 to 40%.

Liver glycogen contents from both feeding regimes were not influenced by dietary protein level and did not show notable trends. Fish fed restricted and appetite regimes tended to have higher VSI value with increasing protein levels in the diets. In restricted feeding, VSI increased in fish fed both 35% and 40% dietary protein compared to fish fed 28% dietary protein, whereas it increased only in fish fed 40% dietary protein under appetite feeding. However, the changes were not significant ($P > 0.05$). In the case of HIS there was no definite pattern of changes of with either feeding regime or dietary protein level (Table 5.5).

Histological examination of livers showed no significant abnormalities among the treatment groups.

Table 5.4 Body composition (% wet weight basis) of African catfish, *Clarias gariepinus* at the start and end of the experiment

		Treatment (% protein and feeding regime)						
Diet number: (% protein)	Initial	1 (28%)		2 (35%)		3 (40%)		± SEM
Feeding regime:		Restr.	Appet.	Restr.	Appet.	Restr.	Appet.	
Parameters (%):								
Moisture	77.02	72.10 ± 0.42	71.40 ± 1.06	70.49 ± 1.77	71.30 ± 1.23	71.64 ± 0.67	70.99 ± 0.83	0.24
Crude Protein	16.01	17.05 ± 0.32	17.81 ± 0.99	17.29 ± 0.40	17.40 ± 0.73	17.22 ± 0.29	18.36 ± 0.58	0.16
Crude Lipid	3.81	7.39 ± 0.19	7.47 ± 0.10	8.03 ± 0.51	7.83 ± 0.49	7.99 ± 0.49	8.14 ± 0.41	0.19
Ash	3.16	3.47 ± 0.05	3.31 ± 0.25	3.27 ± 0.11	3.14 ± 0.29	3.15 ± 0.32	2.90 ± 0.10	0.06

Note: Liver lipid, VSI & HSI of d. f (5, 35) but liver glycogen d. f is (5,17)
Values are means ± SD of six replications for liver lipid, VSI and HSI but means ± SD of three replications for liver glycogen. No significant differences ($P > 0.05$) were detected.

Table 5.5 Liver lipid, liver glycogen (% wet weight) and organ indices (VSI and HSI) of African catfish, *Clarias gariepinus* under different treatments

	Treatment (% protein and feeding regime)						
Diet number: (% protein)	1 (28%)		2 (35%)		3 (40%)		± SEM
Feeding regime:	Restr.	Appet.	Restr.	Appet.	Restr.	Appet.	
Parameters (%):							
Liver lipid	5.88 ± 1.56	5.92 ± 2.25	5.59 ± 0.87	5.52 ± 1.96	5.92 ± 1.11	6.15 ± 1.50	0.28
Liver glycogen	0.66 ± 0.14	0.59 ± 0.12	0.58 ± 0.06	0.57 ± 0.06	0.69 ± 0.01	0.69 ± 0.02	0.02
VSI	6.96 ± 1.03	7.80 ± 2.44	8.96 ± 4.17	7.36 ± 1.38	8.23 ± 2.01	11.77 ± 4.36	0.52
HSI	1.35 ± 0.19	1.30 ± 0.35	1.33 ± 0.09	1.33 ± 0.10	1.05 ± 0.18	0.98 ± 0.24	0.04

Note: Liver lipid, VSI & HSI of d. f (5, 35) but liver glycogen d. f is (5,17)
Values are means ± SD of six replications for liver lipid, VSI and HSI but means ± SD of three replications for liver glycogen. No significant differences ($P > 0.05$) were detected.

5.3.5 Digestive Enzymes

Protease, lipase and amylase activities are presented in Table 5.6 and graphically in Figure 5.3. In general, protease, lipase and α-amylase activity under either restricted or appetite feeding did not change significantly ($P > 0.05$) in fish fed different dietary protein levels. However, comparatively higher protease activity was observed in appetite feeding fish (28 and 35% dietary protein) whereas this activity decreased at 40% dietary protein in both restricted and appetite feeding. Feeding to appetite again showed a tendency to produce higher protease activity than feeding the same dietary protein level under restricted feeding regime except in fish fed 40% protein.

α-amylase activity was not influenced by protein level or feeding regime. Intestinal lipase activity in fish fed the both rations increased with increasing protein in the diet (although they were not significantly different, $P > 0.05$).

Table 5.6 Protease, lipase and amylase activities in intestine and liver of African catfish, *Clarias gariepinus* at the end of the experiment

	Treatment (% protein and feeding regime)						
Diet number	1		2		3		±
(% protein)	(28%)		(35%)		(40%)		SEM
Feeding regime	Restr.	Appet.	Restr.	Appet.	Restr.	Appet.	
Enzymes:							
Intestinal Protease[1]	123.11 ± 5.82	130.38 ± 11.67	125.50 ± 6.81	132.39 ± 4.18	124.36 ± 6.97	117.75 ± 18.89	2.35
Intestinal lipase (EC 3.1.1.3)[2]	1.10 ± 0.17	1.37 ± 0.31	1.20 ± 0.10	1.37 ± 0.25	1.53 ± 0.15	1.83 ± 0.64	0.09
Intestinal ∝Amylase (EC 3.2.1.1)[3]	214.93 ± 29.41	209.43 ± 24.68	197.63 ± 31.93	173.96 ± 19.57	197.05 ± 31.19	188.29 ± 18.15	6.79
Liver protease[1]	107.85 ± 8.50	109.14 ± 11.83	110.43 ± 6.97	114.40 ± 2.80	114.06 ± 13.84	110.29 ± 7.89	1.94
Liver lipase (EC 3.1.1.3)[2]	1.33^{ab} ± 0.15	1.37^{ab} ± 0.45	0.97^{a} ± 0.23	2.07^{ab} ± 0.61	1.33^{ab} ± 0.21	2.23^{b} ± 0.57	0.13

Note:

1. Protease activity was expressed as the amount of protein (µg) digested by 0.5 ml of enzyme solution at pH 7.6 per min. at 30°C.
2. Lipase activity was expressed as the amount of fatty acids (Sigma / Tiez unit/L) liberated by 1ml of extracted enzyme solution per min. at 30°C.
3. ∝-amylase activity was expressed as the amount of maltose (µg) liberated by 200 µl of enzyme solution at pH 7.0 per min at 30°C.

Values are means ± SD of three replications. Means in the same row with no superscript are not significantly different ($P > 0.05$).

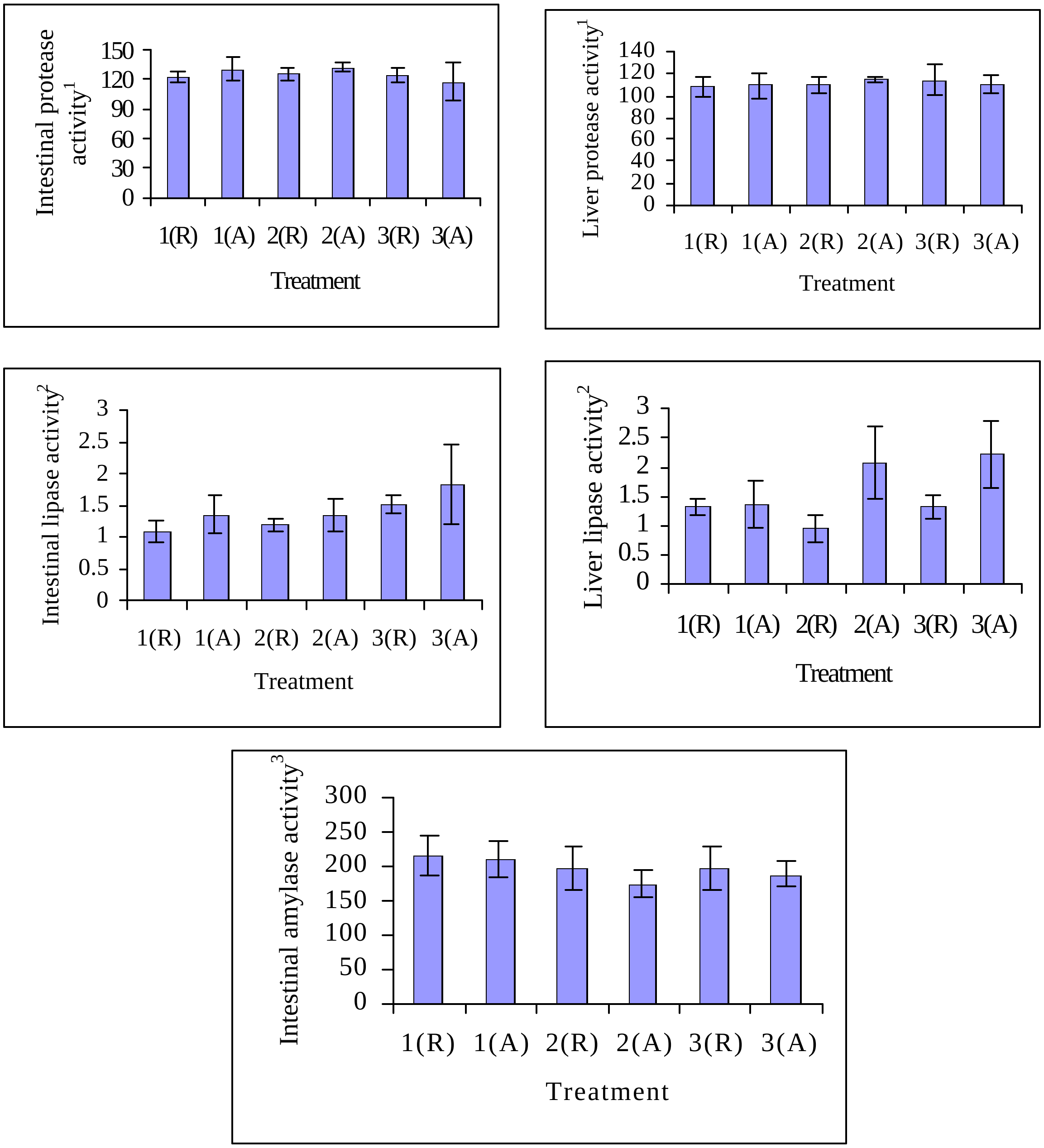

Figure 5.3 The protease, lipase and ∝-amylase activities of intestine and liver in *Clarias gariepinus* fed different treatments.

Bars are means ± SD of three replicates. The treatments were, T-1 (R), T-1 (A); T-2 (R), T-2 (A) and T-3 (R), T-3 (A), where R and A refers respectively to the restricted and appetite feeding regime. The numerical value 1, 2 and 3 refers to the number of diet contained 28, 35 and 40% protein respectively).

[1] Protein activity was expressed as the amount of protein (µg) digested by 0.5 ml of enzyme solution at pH 7.6 per min. at 30°C

[2] Lipase activity was expressed as the amount of fatty acids (Sigma / Tiez unit / L) liberated by 1 ml of extracted enzyme solution per min. at 30°C

[3] ∝-amylase activity was expressed as the amount of maltase (µg) liberated by 200 µl of enzyme solution at pH per min. at 30°C.

5.3.6 Blood Plasma Components

Concentrations of blood plasma components such as glucose, triglycerides (TG) and cholesterol are shown in Table 5.7 and graphically in Figure 5.4. In general plasma glucose, TG and cholesterol contents showed no significant difference ($P > 0.05$). Fish fed to appetite had higher plasma glucose concentrations with increasing dietary protein level, this was not observed with restricted feeding. Plasma glucose in fish fed to appetite was positively correlated ($Y= - 20.56 + 4.53X$; $r = 0.83$; $P < 0.05$) with the dietary carbohydrate level.

Plasma triglycerides did not show any definite pattern of change due to feeding regime. However, the highest plasma TG value was found in fish fed 35% dietary protein under both appetite and restricted regimes. Similarly, cholesterol did not vary significantly with different feeding regime and values ranged from 185.23 mg/100 ml in fish fed 28% dietary protein to appetite level to 249.00 mg/ 100ml in fish fed 40% dietary protein to appetite level. In case of plasma cholesterol, values increased with increasing dietary lipid level in diet showing a positive correlation ($Y = 29.23 + 16.93X$; $r = 0.81$, $p > 0.05$)

Blood plasma amino acid levels of fish in the different treatments are shown in Table 5.8. In general after feeding, the plasma amino acid levels were not greatly influenced ($P < 0.05$) and did not show notable changes with diet or feeding regime.

Table 5.7 Blood plasma concentrations of glucose, triglycerides and cholesterol in African catfish, *Clarias gariepinus* at the end of the experiment

	Treatment (% protein and feeding regime)						
Diet number	1		2		3		± SEM
(% protein)	(28%)		(35%)		(40%)		
Feeding regime	Restr.	Appet.	Restr.	Appet.	Restr.	Appet.	
Components:							
Glucose (mg/100 ml)	98.41 ± 9.03	95.15 ± 22.41	111.88 ± 7.23	100.50 ± 12.70	106.19 ± 37.95	133.36 ± 57.90	6.79
Triglyceride (mg/100 ml)	170.47 ± 67.70	143.34 ± 38.97	226.63 ± 77.00	243.95 ± 126.61	189.53 ± 36.07	191.66 ± 84.79	18.50
Cholesterol (mg/100ml)	189.19 ± 23.00	185.23 ± 3.18	211.37 ± 19.26	239.19 ± 35.78	190.37 ± 29.58	249.00 ± 44.15	8.04

Note: Degree of freedom is (5, 17)

Values are means ± SD of three replicates. No significant differences ($P > 0.05$) were detected.

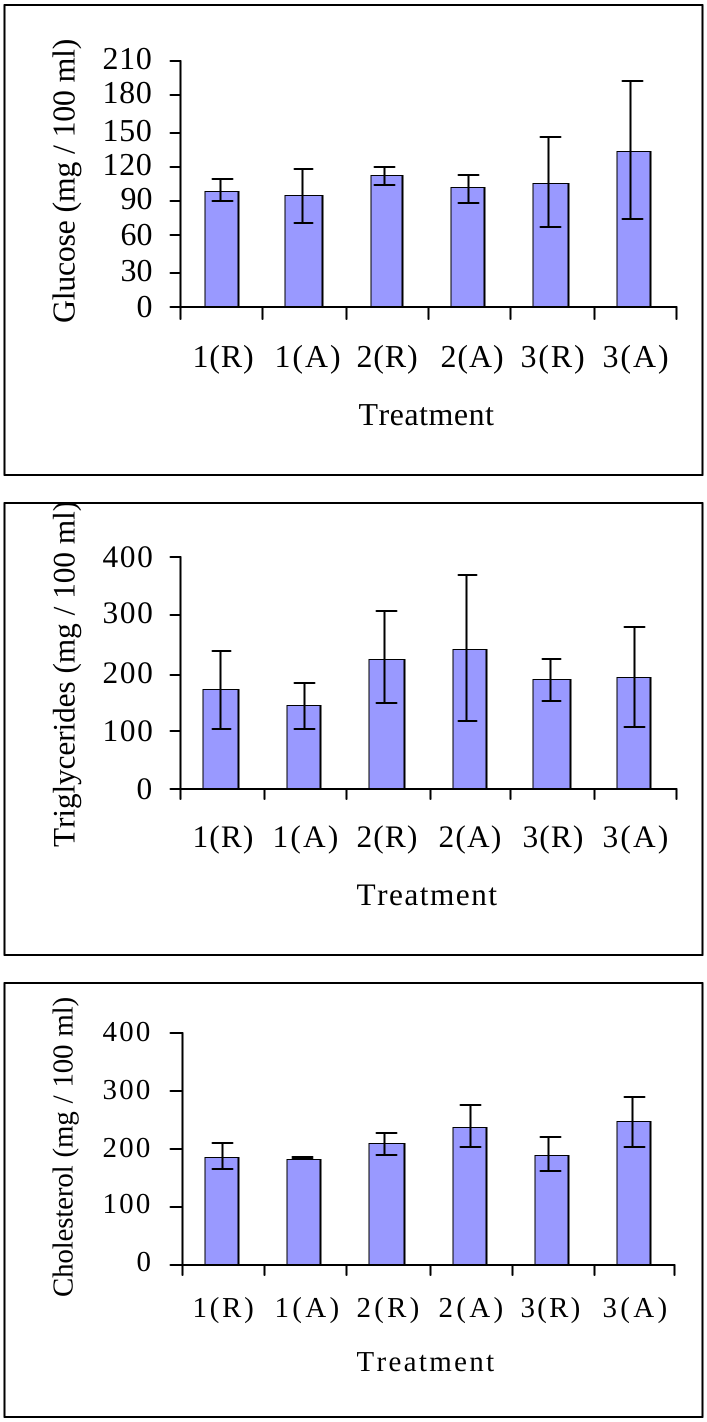

Figure 5.4 Blood plasma concentrations of glucose, triglycerides and cholesterol in *Clarias gariepinus* at the end of the experiment.

Bars are means ± SD of three replications. The treatments were, T-1 (R), T-1 (A); T-2 (R), T-2 (A) and T-3 (R), T-3 (A), where R and A refers respectively to the restricted and appetite feeding regime. The numerical value 1, 2 and 3 refers to the number of diet contained 28, 35 and 40% protein respectively.

Table 5.8 Plasma amino acid levels (µM / ml of blood plasma) in *Clarias gariepinus* at the end of the experiment

	Treatments (% Protein and feeding regime)						
Diet number	1		2		3		±
(% protein)	(28%)		(35%)		(40%)		SEM
Feeding regime	Restr.	Appet.	Restr.	Appet.	Restr.	Appet.	
Essential amino acids:							
Arginine	0.11 ± 0.04	0.12 ± 0.07	0.14 ± 0.05	0.09 ± 0.03	0.12 ± 0.03	0.13 ± 0.03	0.01
Histidine	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.00
Isoleucine	0.11 ± 0.02	0.12 ± 0.05	0.13 ± 0.03	0.12 ± 0.04	0.11 ± 0.02	0.13 ± 0.01	0.01
Leucine	0.17 ± 0.04	0.17 ± 0.06	0.19 ± 0.04	0.18 ± 0.04	0.18 ± 0.04	0.19 ± 0.01	0.01
Lysine	0.25 ± 0.07	0.26 ± 0.11	0.24 ± 0.06	0.19 ± 0.05	0.24 ± 0.05	0.27 ± 0.05	0.01
Methionine	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.00
Phenylalanine	0.07 ± 0.01	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.00
Threonine	0.46 ± 0.12	0.45 ± 0.16	0.46 ± 0.08	0.42 ± 0.15	0.40 ± 0.07	0.49 ± 0.12	0.03
Valine	0.14 ± 0.04	0.15 ± 0.03	0.15 ± 0.03	0.15 ± 0.03	0.15 ± 0.03	0.16 ± 0.02	0.01
Non-essential amino acids:							
Alanine	0.55 ± 0.14	0.53 ± 0.16	0.48 ± 0.07	0.46 ± 0.15	0.42 ± 0.09	0.56 ± 0.08	0.02
Aspartic acid	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.04	0.00
Cystine	0.04 ± 0.01	0.07 ± 0.05	0.06 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.03	0.01
Glutamic acid	0.18 ± 0.06	0.16 ± 0.08	0.14 ± 0.05	0.12 ± 0.04	0.12 ± 0.04	0.18 ± 0.04	0.01
Glycine	0.25 ± 0.04	0.26 ± 0.06	0.29 ± 0.08	0.25 ± 0.06	0.28 ± 0.07	0.28 ± 0.02	0.01
Proline	0.15 ± 0.03	0.20 ± 0.05	0.17 ± 0.02	0.17 ± 0.06	0.18 ± 0.07	0.22 ± 0.03	0.01
Serine	0.11 ± 0.04	0.12 ± 0.04	0.14 ± 0.05	0.09 ± 0.01	0.11 ± 0.01	0.12 ± 0.02	0.01
Tyrosine	0.06 ± 0.01	0.05 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.00

Note: Tryptophan, is an essential amino acids, not measured by the technique.

Values are means ± SD of three replications and values in the same row are not significantly different ($P > 0.05$).

5.4 DISCUSSION

5.4.1 Growth Performance and Feed Efficiency

The results in this study suggest that there was no significant interaction between dietary protein level and feeding regime on growth rates or feed and protein efficiency. Tacon and Cowey (1985) suggested that by increasing the feeding rate of fishes, protein requirement as a proportion of the diet is reduced because fish are able to compensate for reduced dietary protein levels by consuming more diet. However, such an effect was not seen in this study where growth rate increased with increasing dietary protein level in restricted fed fish that all received the same protein and energy intakes. Fish fed diets with low protein and energy levels were unable to compensate by increased intake to achieve comparable growth to fish fed high protein and energy levels. This is in agreement with studies reported on red drum (McGoogan and Gatlin III, 2000) and rainbow trout (Alanara, 1994).

Fish fed the lowest protein (28%) diet under either restricted or appetite feeding had significantly lower weight gains and SGR than fish fed higher protein levels. It is possible that fish fed this low protein and energy diet either could not ingest the bulk required and could not adequately process / digest it. Similar observations have been reported on red drum (McGoogan and Gatlin III, 2000). They concluded that red drum fed the low protein and energy diet could not physically ingest all of the ration and thereby actually had reduced nutrient and energy intake.

With both feeding regimes the lowest-protein (28%) diet resulted in similar weight gain and SGR. This is probably due to the same amount of feed (5% of their body weight per day) being consumed by the fish. A pre-experimental feeding trial with a diet containing 28% crude protein showed that fish could accept 5% body weight at their satiation level. Fish fed on higher protein diets (35 to 40% CP) under appetite feeding produced greater weight gain and SGR than fish fed the same diets on a restricted regime. This could be due to differences in actual feed intake between fish using these two feeding regimes. Johansen and Jobling (1998) observed that Atlantic salmon fed to satiation consumed more and grew faster than those fed on restricted rations. Martinez-Palacios *et al.,* (1996) determined that the predicted protein requirements for maximum weight gain of the Mexican cichilid is decidedly different when feeding to satiation vs. feeding at a fixed rate. Munsiri and Lovell (1993) demonstrated that weight gain under both satiation and restricted feeding increased linearly when channel catfish were fed the same diet with increasing protein quality. They also stated that feeding to satiation resulted in higher weight gain than feeding the same quality protein on restricted feeding regime. On the contrary, Li and Lovell (1992) showed a linear increase in weight gain with increasing dietary protein level in the diets when channel catfish were fed on a restricted feeding regime but not under satiation feeding. They concluded that channel catfish could meet their protein requirements from a relatively low-protein diet when fed an amino acid balanced diet to satiation, while high-protein diets will allow maximum growth for restricted feeding.

The improved feed conversion efficiency (FCE) in fish fed either restricted or appetite ration and higher protein diets confirms the general observation that there is direct relationship between dietary protein level and FCE. However, increasing protein and energy density does seem to be an effective dietary manipulation under conditions of reduced consumption to ensure that requirements for maximal growth are met resulting in better FCE (McGoogan and Gatlin III, 2000; Jirsa *et al.,* 1997).

Different coefficients of variation (CV) in final weight were observed in *Clarias gariepinus* during the present study (Table 5.3). The lowest CV in final weight was found in fish fed the 35% protein diet under both feeding regimes. It appears that fish fed a 35% protein diet to appetite showed highest growth rates and better feed efficiency suggesting that this is the optimum feeding regime for *Clarias gariepinus* under these experimental conditions.

5.4.2 Protein and Energy Utilisation

Fish fed either the restricted or appetite ration at the different protein levels did not show any notable effects on PER or ANPU. Many studies report decreasing efficiency of protein utilisation (PER, ANPU) with increasing dietary protein level in isoenergetic diets (Hassan *et al.*, 1995; Samantaray and Mohanty, 1997; Jantrarotai *et al.*, 1998). In such experiments P: E ratio increases with increasing dietary protein level. This did not happen here. Similarly, ANLU and ANEU were not greatly influenced by diet or feeding regime. This is probably because diets in both feeding regimes contained similar protein to energy ratios and lipid to CHO ratios. ANLU values tended to be better at restricted feeding than appetite rations in the higher protein diets.

5.4.3 Protein, Energy and Dry matter Digestibility

Protein, energy and dry matter digestibility were found to be slightly higher with increasing protein level and feeding to appetite resulted in comparatively better values than feeding the same diet at a restricted level. This is probably because fish fed the restricted regime were feeding at rates calculated to supply the same amounts of protein and energy. When fish ingesting similar amounts of protein from different diets are compared, those fed the lowest protein diet (28% CP) had received higher absolute amounts of food. The higher amount of feed in the digestive tract may cause more rapid gut evacuation resulting in lower digestibility

(Henken *et al.*, 1985). Similarly under restricted feeding, a decrease in protein and energy digestibility with increased ration level has been reported in some studies for gilthead sea bream (Fernandez *et al.*, 1998), and rainbow trout (Bergot and Breque, 1983).

In the present study, protein digestibility under appetite feeding was found to be slightly higher in diets containing higher protein levels. This could be because less wheat flour and more fishmeal are present in higher-protein diets, as the fish protein was more digestible than the wheat protein. Several investigators have reported the same observations where protein digestibility increased in proportion to increase in dietary protein (Machiels and Henken, 1985; Santinha *et al.*, 1996). Energy and dry matter digestibility were lower in fish fed the diets containing lower protein levels under both feeding regimes. This might be due to a greater inclusion of wheat flour with a relatively higher level of fibre and consequently poor digestion of the carbohydrate portion of this dietary component (Bergot and Breque, 1983; Valente *et al.*, 1998).

5.4.4 Body Composition and Histology

Body composition of fish was not significantly influenced by feeding regime or dietary protein level. This is possibly due to fact that fish were fed diets containing the same protein to energy and lipid to CHO ratios, therefore nutrient and energy intakes were balanced. Fish fed the restricted ration and the lowest protein diet (28% CP) tended to have lower body protein and lower body lipid deposition than fish fed the other diets. In this case, it is likely that fish could not utilise the high physical volume of diet. Similarly, feeding this diet to appetite resulted in the same food intake and thus the same resultant lower body protein and body lipid deposition.

However, there was a trend towards higher body protein and body lipid and lower body moisture and body ash deposition with increasing dietary protein level. These differences in body composition were attributed to differences in the amounts of protein and energy ingested by fish from all rations under both feeding regimes. Feeding to appetite a 35% protein diet, as compared with the other treatments, produced lower body lipid and improved protein utilisation without growth retardation. This result agrees with the findings of Li and Lovell (1992), who reported an increase in muscle moisture content, while muscle lipid content decreased, with increasing protein level in the diets when channel catfish were fed on both restricted and appetite feeding regimes. Similarly, Munsiri and Lovell (1993) demonstrated that body moisture content increased while body lipid content decreased under both restricted and satiation feeding regimes when channel catfish were fed the same diet with increasing protein quality. Love (1980) also reported that depletion of body lipid results in an increase in the water content of the muscle and Sargent (1976) explained this inverse relationship as a result of lipid mobilisation. Comparable results on body composition of African catfish, *Clarias gariepinus* under different feeding regimes using various dietary protein levels are currently lacking.

Liver lipid and liver glycogen under each feeding regime did not vary significantly with dietary protein level. This is because all fish received diets with the same protein to energy and lipid to CHO ratio. However, comparatively higher liver lipid levels were observed with appetite feeding of the diet containing the highest-protein level. This was probably due to the grater amount of lipid and CHO ingested under appetite feeding (Garling and Wilson, 1977; Shimeno *et al.*, 1993).

Similarly to liver lipid and liver glycogen, VSI and HSI under both feeding regimes were not influenced by dietary protein level. With restricted feeding VSI tended to increase with an increase in protein level and decreasing feed rate. Similar observations have been reported for red drum (McGoogan and Gatlin III, 2000). Under the appetite feeding regime a higher VSI in the diet containing higher protein level was seen. This higher VSI can be related to the influence of dietary lipid (Ramachandran Nair and Gopukumar, 1981; Hanley, 1991).

Histological examination of livers revealed no effects of feeding regime or diet. This is probably because all fish received diets with the same protein to energy ratio and lipid to CHO ratio. Comparable results in published literature on varying dietary protein level under restricted or appetite-feeding regime are currently lacking. However, Hepher (1988) reported that other experiments in which very high levels of dietary lipid were fed to fish without any growth depression or pathological effect. Refstie and Austreng (1981) fed rainbow trout with a diet containing 41.6% nitrogen-free extracts for 282 days with no apparent pathological signs, but this diet reduced growth and produced larger livers than diets lower in carbohydrates. Similarly, Garling and Wilson (1977) reported in channel catfish that excessive dietary lipid and carbohydrate (various CHO/L ratios) did not produce any cell damage or glycogen deposition in cellular vacuoles.

5.4.5 Digestive Enzymes

The results show no clear effect of either feeding regime or diet. This is probably largely due to the fact that fish fed diets containing the same protein to energy ratio and lipid to CHO ratio on both feeding regimes. Several investigators reported that protease activity is influenced by dietary protein in fish (Kawai and Ikeda, 1972; Mukhopadhyay *et al.*, 1978; Knauer *et al.*, 1996; Gangadhara *et al.*, 1997). Gangadhara *et al.*, (1997) observed higher levels of intestinal protease activity when the dietary protein level was raised from 25 to 30% at an intermediate level of dietary lipid when rohu, *Labeo rohita* were fed on a fixed feeding rate of 5% of their body weight per day. However, the present investigation suggests that protease activity in intestine and liver was slightly higher in fish fed to appetite up to 35% dietary protein level.

Intestine and liver lipase activity was very slightly increased with increasing dietary protein level under both feeding regimes. However, there is no clear relationship between growth with enzyme activities in the present study. Variation in enzyme activity might be related to the amount of lipid and protein ingested or duration of retention of feed in the digestive tract which in turn depends on the fibre content and physical consistency of the diet (Venkatesh *et al.*, 1986). Comparable results on protease and lipase activities in African catfish, *Clarias gariepinus* are currently lacking. It seems to be very difficult to define the effect of food on protease and lipase activities and to compare the results of different investigators, especially because it is necessary to know the time that tissue samples were taken after a meal, (this is often not given) for meaningful comparison.

5.4.6 Blood Plasma Components

Blood plasma component measurements (plasma glucose, triglycerides and cholesterol) were not greatly influenced by diet or feeding regime (Table 5.7). Fish were fed diets containing similar protein to energy and lipid to CHO ratios and nutrient and energy intakes were similar.

Under both feeding regimes fish fed the low protein diet (28% CP) exhibited comparatively lower plasma glucose concentration. This may be because this feed contained the least carbohydrate (as NFE, Table 5.1). Shimeno *et al.*, (1997) and Shikata *et al.*, (1993) reported that plasma glucose level increased with increasing feeding rate in a diet for common carp.

The results of this experiment are also similar to be the results of the previous study (Chapter 4) that plasma glucose levels in *Clarias gariepinus* clearly increased with increasing dietary carbohydrate levels in diets containing a with fixed protein to energy ratio. Similarly, Shimeno *et al.*, (1996) reported that plasma glucose concentration increased in yellowtail fed diets containing fixed dietary protein with increasing inclusion level of dietary CHO, with concomitant reduction of dietary lipid, at a fixed feeding rate.

Slightly lower plasma triglycerides (TG) and plasma cholesterol levels were also observed in fish fed the lower protein diet (28% CP) under both feeding regimes. This may be due to reduced dietary lipid intakes as fish could not properly utilise all the ration of this low protein diet (28% CP). Similarly, explanations may be advanced for fish fed the lower protein diet (28% CP) to appetite. Shikata *et al.*, (1993) and Shimeno *et al.*, (1997) found that serum TG and serum cholesterol levels increased with increasing feeding rate in common carp. Shimeno *et al.*, (1995a,b) also reported that serum TG and plasma cholesterol level increased in common carp fed isoenergetic diets containing increasing dietary lipid level, with concomitant reduction of protein level at a constant feeding rate. Shimeno *et al.*, (1993) reported that plasma triglyceride levels greatly increased when tilapia, *Oreochromis niloticus* were fed diets containing fixed protein with increasing dietary lipid, and concomitant reduction of dietary CHO level at a constant fixed feeding rate.

Data obtained (Table 5.8) from this study indicate that most essential and non-essential amino acid levels in plasma were not influenced by dietary protein level or feeding regime. This is possibly due to fact that fish were fed diets containing the same protein to energy and lipid to CHO ratios, therefore nutrient and energy intakes were similar. Similarly, Wilson *et al.*, (1985) reported that the serum amino acid levels of channel catfish fed diets containing various protein level with fixed protein to energy ratio, did not exhibit marked differences in the plasma amino acid levels.

In contrast, several reports have shown that free amino acid levels in plasma and other tissues were increased by increasing dietary protein levels such as in rainbow trout (Yokoyama *et al.*, 1994; Yamamoto *et al.*, 2000;), masu salmon (Ogata and Murai, 1994) and European eel (Ogata *et al.*, 1985). This did not happen here. Yamamoto *et al.*, (2000) concluded that not only dietary protein level but also dietary lipid levels influenced plasma amino acid levels in rainbow trout. In addition, Ogata *et al.*, (1985) concluded that the whole body free essential amino acid levels of European eel were strongly correlated with protein intake.

In conclusion, fish fed the 35 to 40% protein diets to appetite displayed higher weight gain, SGR and FCE, while protein and energy utilisation were improved when the protein level was below 40% in the diet. Moreover, on the basis of economics, a lower protein level diet giving the same performance should be preferred. The results show that under appetite feeding a 35% protein diet lowers the body fat and liver fat and improves protein utilisation without growth retardation. This indicates that appetite-feeding of a diet containing 35% protein is suitable for African catfish, *Clarias gariepinus* under these conditions.