Chapter 4

The effect of dietary non-protein energy on growth and protein utilisation: Approaches to optimising dietary non-protein, lipid to carbohydrate ratio in African catfish *Clarias gariepinus* (Burchell, 1822)

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4.1 INTRODUCTION

Both carbohydrate and lipid in the diet are the important non-protein energy sources for fish and should be included in their diets at appropriate levels which maximize the use of dietary protein for growth. The amount of non-pritein energy sources that can be incorporated in fish diets is not fully understood. No dietary requirement for dietary carbohydrate has been demonstrated in fish; certain fish species exhibit reduced growth rates when fed carbohydrate-free diets (Wilson, 1994).

The dietary lipid to carbohydrate ratios affects not only the growth and body composition, but also liver composition, digestive enzyme activities and blood plasma components in various fishes (Jatrarotai *et.al.*, 1994; Shimeno *et. al.*, 1993; Bazaz and Keshavanath, 1993; El-Sayed and Garling, 1988; Mukhopadhyay, 1977; Garling and Wilson, 1977; Scherbina *et. al.*, 1976; Kawai and Ikeda, 1972). While it seems that most fishes utilise dietary lipid well (Cowey and Sargent, 1979). At high dietary levels, lipids may reduce fish growth, adversely affect body composition (Erfanullah and Jafri, 1998a; Hanley, 1991; El-Sayed and Garling, 1988; Garling and Wilson, 1977), as well as create problems associated with pelleting and development of rancidity in stored feed (Jauncey, 1982). On the other hand, carbohydrate utilisation is much more variable and probably is related to natural feeding habits, and incorporation of this nutrient may add beneficial effects to the pelleting quality of the diet and to fish growth (Wilson, 1994; NRC, 1993). Excessive dietary CHO in fish diet may also lead to fat deposition by stimulating the activities of lipogenic enzymes (Likimani and Wilson, 1982).

In isonitrogenous and isoenergetic diets (same P/E ratio), testing various lipid to carbohydrate ratios, varying net protein utilisation and protein efficiency ratio reflects the ability of fish to use these nutrients to spare protein. In general, net protein utilisation and protein efficiency ratio peaks at some level between extreme lipid and carbohydrate concentration, sometimes nearer the lipid or carbohydrate extreme. Thus, rainbow trout (Brauge *et. al*,. 1994), *Tilapia zilli* (El-sayed and Garling, 1988), and red drum, *sciaenops* (Serrano *et. al.*, 1992; Ellis and Reigh, 1991) utilise lipids better than carbohydrate, while *Oreochromis niloticus* (Shimeno *et. al.*, 1993) utilises carbohydrates better than lipids. Other investigators report peaks in protein utilisation and protein efficiency ratio at intermediate levels of carbohydrate, like in walking catfish *Clarias batrachus* (Erfanullah and Jafri, 1998a), hybrid *Clarias* catfish (Jantrarorai *et. al.*, 1994), channel catfish *Ictalurus punctatus* (Garling and Wilson, 1977) and Indian major carps (Erfanullah and Jafri, 1998b). The variability of results could reflect not only the different capabilities of fishes to utilise carbohydrate, but also the various ranges of lipid to carbohydrate ratios tested and the varying sources of these nutrients.

Information on nutritional studies in African catfish *Clarias gariepinus* seems limited and have been dealt mainly with dietary protein and energy requirements using semi purified diets (Degani *et. al.*, 1989; Uys 1989; Henken *et. al.*, 1986; Machiels and Henken, 1985). Until now, lipid and carbohydrate utilisation has not been studied, although *Clarias gariepinus* is reported to be omnivorous (Babiker, 1984), might utilise carbohydrate well. In fact, investigate corncerning the nanipulation of dietary lipid to CHO ratios on growth, body composition, digestive enzyme activities, plasma metabolites and histopathology of African catfish *Clarias gariepinus* have been limited.

Optimising protein to energy ratios experiment (chapter 3) showed that African catfsh, *Clarias gariepinus* attained maximum growth when fed diets containing adequate protein and energy levels at a protein to energy ratio (P/E ratio) of 20.00 mg protein per kJ of GE. Therefore, it was decided to investigate the utilisation of dietary lipids and carbohydrates on growth and more depth investigation of the biochemical composition of vital body organs of *Clarias gariepinus* at the above indicated P/E ratio. The achieved performance parameters would better elucidate the utilisation of dietary non-protein energy sources as well as dietary protein and facilitate decisions regarding the balanced dietary profile of a cost-effective diet.

The objectives of this study were to determine the effects of lipid to carbohydrate ratios and its influence on growth, protein utilisation and body composition leading to the optimisation of dietary lipid to carbohydrate ratio in African catfish *Clarias gariepinus*. To get more information on the metabolic effect of dietary lipid to carbohydrate ratios on digestive enzymes activities and plasma metabolites associate with histopathology of liver, and therefore, these aspect were also studied.

4.2 MATERIALS AND METHODS

4.2.1 Experimental System

The experimental system described in section 2.1 and Figure 2.1 was used to conduct an experiment to optimise the dietary lipid to carbohydrate ratio for African catfish *Clarias gariepinus* (Burchell,1822)

4.2.2 Experimental Fish

African catfish *Clarias gariepinus* as described in section 2.2 was used as the species in this study. Twelve week (12.32 ± 0.04 g) 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 an individual 30 L cylindrical tank as described in section 2.1 and as shown in Figure 2.1.

4.2.3 Experimental Diets

Five experimental iso-nitrogenous (40% CP) and iso-energetic 20 kJ/g GE diets were formulated containing P/E ratio 20 mg protein kJ/g of GE based on results from previous studies (Chapter 3) and to meet determined requirements for maximum growth of this species. The non-protein energy percentage was adjusted by varying the ratios of lipid and carbohydrate in the diets. So, that the lipid to carbohydrate ratios (L/CHO, g/g) ranged from 1.36 to 0.29 or carbohydrate to lipid (CHO/L ratios, g/g) ranged from 0.74 to 3.42, corresponding to L/CHO energy ratios ranged from 4.00 to 0.67. The diets containing iso-nitrogenous crude protein 40% was fixed rather than 43% (based on the results of previous experiment) due to limited space with the ingredients in the formulation. Crude protein level (40% CP) in the experimental diets was also fixed to meet the requirement of *Clarias* catfish (Jantrarotai *et al.*, 1996; Degani *et al.,* 1989; Machiels and Henken, 1985; Khan and Jafri, 1990*)*. Diets are referred to by two numbers separated by a '/', the first number being the dietary percentage energy from dietary lipid, the second number the dietary percentage energy from dietary carbohydrate. Composition of the experimental diets and their proximate analysis are shown in Table 4.1. All diets were adequate in essential amino acids on a percentage-of-protein basis. The composition of the amino acids of diet 3 is shown in Table 4.2. Diet formulation and preparation were as described in section 2.3.1 and 2.3.2.

4.2.4 Experimental Practices

Fish were acclimated to the experimental system using commercial trout diet (Trouw Fry 02, crumble 1.00 to 1.50 mm) diet for two weeks before the start of the experiment. Acclimation and periodical weighing were as described in section 2.4.1. Fish were offered 5% of their body weight per day to provide approximately the same amount of protein and energy of all treatments and the stipulated diet sub-divided into three equal feeds at 10:00, 14:00 and 18:00 daily. Food was offered taking care provide small amounts of food at a time, to be sure that the fish ate all of the diet offered. The experiments was conducted for 8 weeks and fish within three replicates per treatment. Faeces collection was as described in section 2.4.4.

Before commencement of the feeding trial, 10-12 fishes from the acclimated lots 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 on the day, 6 fish from each tank were collected for determination of whole body composition, organ indices as well as histopathology of liver. 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, blood plasma component.

4.2.5 Water Quality Management

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

4.2.6 Experimental Analyses:

4.2.6.1 Proximate Analyses

Proximate analysis (moisture, crude protein, crude lipid and ash) of carcass, feed ingredients and experimental diets were determined as 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 content of experimental diets were determined as described sections 2.6.1.4 and 2.6.1.7.1, while chromic oxide levels in fish faeces and experimental diets were determined as section 2.6.1.8. Fish within each group were pooled for carcass analysis. Final values for each group represent the arithmetic mean of three replicates, all samples were analysed in triplicate.

4.2.6.2 Growth and Feed Performance

Growth and feed performance, nutrient, 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 4.1 Formulation and composition of the experimental diets and proximate analysis (percentage dry weight)

	Diet number (Designation: Lipid /Carbohydrate, % energy)				
Diet no.	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Ingredients:					
Fishmeal (Herring type)[1]	35.00	35.00	35.00	35.00	35.00
Soybean meal (Dehulled, solvent extract)[2]	23.50	23.50	23.50	23.50	23.50
Wheat flour (Whole wheat)[3]	6.30	6.30	6.30	6.30	6.30
Fish oil	8.90	7.58	6.23	4.89	3.55
Corn oil	8.90	7.58	6.23	4.89	3.55
Vitamin premix[4]	1.00	1.00	1.00	1.00	1.00
Mineral premix[5]	1.00	1.00	1.00	1.00	1.00
Chromic oxide (Cr_2O_3)	0.50	0.50	0.50	0.50	0.50
Carboxymethyl Cellulose(Binder)[6]	2.00	2.00	2.00	2.00	2.00
Corn starch	00.00	6.13	12.27	18.40	23.60
α- Cellulose	12.90	9.42	5.98	2.52	00.00
Proximate composition:					
% as fed:					
Moisture **% Dry wt. basis:**	2.93	5.20	5.29	7.66	6.48
Crude Protein	39.77	39.89	40.20	40.19	40.32
Crude fat	20.88	18.89	16.17	13.52	11.02
Ash	9.95	9.42	9.66	9.15	9.13
Fiber	14.01	10.52	7.09	3.72	1.85
NFE[7]	15.39	21.28	26.88	33.42	37.68
Cr_2O_3	0.50	0.54	0.51	0.53	0.53
CHO / Lipid ratio (g/g)[8]	0.74	1.13	1.66	2.47	3.42
Lipid / CHO ratio (g/g)[9]	1.36	0.89	0.60	0.40	0.29
Lipid/CHO energy ratio[10]	4.00	2.33	1.50	1.00	0.67
GE(KJ/g)[11]	20.39	20.22	20.02	19.97	19.88
P/GE ratio[12]	19.50	19.73	20.08	20.14	20.28

Proximate analysis (%dry weight basis):

1. Moisture: 7.16 ; Crude protein: 75.49 ; Crude fat: 8.43 ; Fibre: 0.69; Ash: 16.08.
2. Moisture: 13.55 ; Crude protein: 53.71 ; Crude fat: 2.25; Fibre: 3.88 ; Ash: 7.79.
3. Moisture: 13.01; Crude protein: 15.84 ; Crude fat: 1.82 ; Fibre: 2.27 ; Ash: 1.85.
4. As listed in Table 2.1, section 2.3.1
5. As listed in Table 2.2, 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. CHO/L ratio (g/g) = % wt. in CHO / % wt. in lipid
9. Lipid/CHO ratio (g/g) = % wt. in lipid / % wt. in CHO
10. Lipid/CHO energy ratio = % energy from lipid / % energy from CHO (calculated)
11. GE = Gross energy content
12. P/ GE = protein to energy ratio in mg protein/ kJ of GE

Table 4.2 Essential amino acid compositions (EAA, g/100g protein) of experimental diet 3, and EAA requirements of *Clarias gariepinus*

Essential amino acids:	Diet 3	EAA requirements [a]
Arginine	4.60	4.30
Histidine	1.87	1.50
Isoleucine	2.87	2.60
Leucine	5.45	3.50
Lysine	4.82	5.00
Methionine[b]	1.09	2.30
Phenylalanine[c]	3.07	5.00
Threonine	3.08	2.00
Valine	3.35	3.00

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

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

[c] Diet contained 1.76 percent tyrosine. With 0.6 percent tyrosine in the diet, phenylalanine requirement was 2.0 percent of protein (NRC, 1993)

4.2.6.3 Liver Lipid and Liver Glycogen Determination

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

4.2.6.4 Digestive Enzymes Assay

Digestive enzymes (proteas and lipase and α-amylase) assays were as described in section 2.10.

4.2.6.5 Blood Plasma Assay

Plasma assays were performed as described in section 2.11.3

4.2.6.6 Amino acid Analysis

Amino acid analysis was carried out as described in section 2.12.

4.2.6.7 Histopathological Analysis

Histopathological analyses of fish liver and intestine were performed as described in section 2.9.

4.2.6.8 Statistical Analysis

Statistical analyses were carried out as described in section 2.13.

4.3 RESULTS

4.3.1 Growth, Survival and Feed Performance

No mortality nor external clinical symptoms occured in any treatment during the period of this study. It appears that coefficient of variance (CV, %) of final weight of diet 4, and diet 5 were lower values, but no significant differences ($P > 0.05$) were found between treatments.

Growth and feed performance are shown in Table 4.3 and graphically in Figure 4.2. Weight gain significantly increased ($P < 0.05$) in response to higher dietary carbohyrate levels, but the higher dietary lipid levels trended in reduced weight gain. Fish fed either the lowest (0.29) or the highest (1.36) L/CHO ratio (g/g) trended to produce significantly lower ($P < 0.05$) growth and food conversion efficiency. Interesting growth rate was observed on diet 4, containing L/CHO (g/g) rario 0.40 (13.52 / 33.52 = %L / % CHO) which was sharply increased after 6th week of this study (Figure 4.1), followed by diet 5.

There was a trended of increasing growth performance with increasing inclusion of dietary carbohydrate with concomitant reduction dietary lipid level (decreasing L/CHO energy ratio) on the basias of percent weight gain, and specific weight gain (SGR). This trend was not maintained above dietary carbohydrate and lipid level 33.52% and 13.52% respectively of diet 4, containing L/CHO ratio 0.40 g/g, which produced best growth performance. Diet 1, containing highest dietary lipid and lowest dietary CHO gave the poorest results, being statistically different ($P < 0.05$) between diet 4 and diet 1.

Food conversion efficiency (FCE), values were showed a trend towards higher as the dietary carbohydrate level increased with concomitant reduction dietary lipid level and being highest

($P < 0.05$) for diet 4, containing (L / CHO ratio 0.40 g/g) and lowest for diet 1. No significant differences ($P > 0.05$) were observed berween the diets 1, 2 and 3 but diet 1 is significantly different ($P < 0.05$) from diets 4 and 5 (Table 4.3).

4.3.2 Nutrient and Energy Utilisation

Protein utilisation efficiency, measured in term of protein efficiency ratio (PER) and apparent net protein utilisation (ANPU), are summarised in Table 4.3 and Figure 4.2. PER was increased significantly ($P < 0.05$) up to maximum in diet 4 (L/CHO ratio g/g, 0.40) with decreasing dietary L/CHO ratios beyond which they showed decrease value of diet 5. No significant differences ($P > 0.05$) were found between the diets 3, 4 and 5 but diet 4 is significantly different with the diets 1 and 2 (Table 4.3).

The ANPU was significantly affected ($P < 0.05$) by decreasing L/CHO ratios (increasing CHO/L ratios) similarly to PER value. The highest value was produced by the diet 4, beyond which they showed a rapid decrease of diet 5. No significant differences ($P < 0.05$) were observed between diet 4 and diet 5 while diet 4 was significantly ($P < 0.05$) differences with the diets 1, 2 and 3 (Table 4.3). The ANLU was also significantly ($P < 0.05$) increased with increasing CHO/L ratios (decreasing L/CHO ratios) and highest value was observed in diet 5, containing highest CHO and lowest lipid level. No significant differences ($P > 0.05$) were found between diet 4 and diet 5.

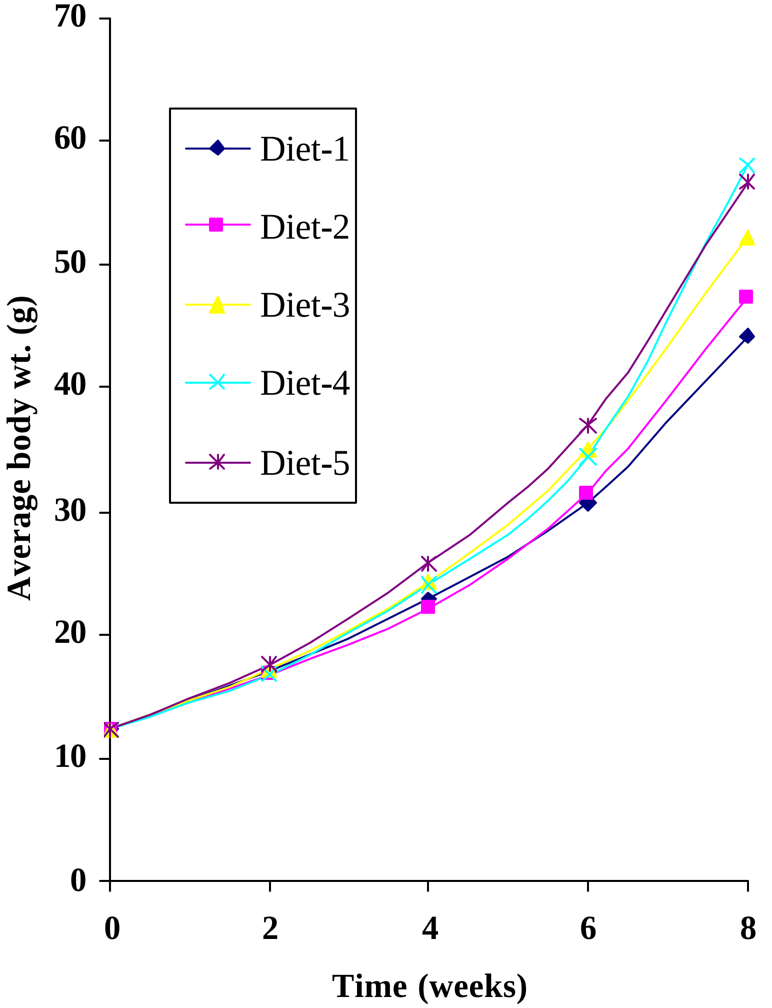

Fig. 4.1 The mean fortnightly growth response of African catfish *Clarias gariepinus* maintained on the five experimental diets on lipid to carbohydrate ratios over 8 weeks.

Table: 4.3 Mean growth performance, feed and nutrient utilisation efficiency of *Clarias gariepinus* various dietary lipid to carbohydrate ratios for 56 days

	Diet number (Designation: Lipid / Carbohydrate, % energy)					
Parameters:	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)	±SEM
Initial body wt. (g)	12.28^{a} ±0.06	12.30^{a} ±0.06	12.37^{a} ±0.08	12.37^{a} ±0.08	12.32^{a} ±0.04	0.02
Final body wt. (g)	44.23^{a} ±4.61	47.41^{ab} ±2.41	52.16^{ab} ±4.77	58.02^{b} ±6.10	56.67^{ab} ±5.64	1.77
CV (%) of final weight	41.86 ±11.86	42.07 ±20.73	47.28 ±13.23	40.49 ±17.86	35.94 ±7.11	3.45
Weight gain (g)	31.95^{a} ±4.65	35.11^{ab} ±2.39	39.79^{ab} ±4.85	45.65^{b} ±6.10	44.35^{ab} ±5.66	1.76
Weight gain (%)	260.16^{a} ±38.44	285.36^{ab} ±19.21	321.93^{ab} ±41.34	369.16^{b} ±49.21	360.09^{ab} ±46.88	14.30
Specific growth rate (SGR) (% day)	2.28^{a} ±0.19	2.41^{ab} ±0.09	2.56^{ab} ±0.18	2.75^{b} ±0.18	2.72^{ab} ±0.18	0.06
Food conversion efficiency (FCE)	0.77^{a} ±0.02	0.87^{ac} ±0.07	0.93^{abc} ±0.11	1.13^{b} ±0.14	1.01^{bc} ±0.04	0.04
Protein efficiency ratio (PER)	1.93^{a} ±0.06	2.18^{a} ±0.16	2.32^{ab} ±0.28	2.81^{b} ±0.35	2.50^{ab} ±0.11	0.09
Apparent protein utilisation (ANPU,%)	34.58^{a} ±0.66	36.84^{ac} ±2.14	41.81^{ac} ±3.75	49.93^{b} ±3.66	44.49^{bc} ±3.34	1.60
Apparent lipid utilisation (APLU, %)	50.76^{a} ±5.11	72.65^{abc} ±4.57	87.06^{bcd} ±15.55	98.51^{cd} ±7.15	109.05^{d} ±14.73	5.92
Apparent energy utilisation (ANEU, %)	28.47^{a} ±0.97	32.42^{ab} ±0.05	35.95^{ab} ±4.66	40.11^{b} ±2.77	35.59^{ab} ±3.51	1.22
Protein digestibility (%)*	86.55	86.62	86.98	87.55	87.92	
Lipid digestibility (%)*	87.55	89.62	88.12	91.11	89.25	
Energy digestibility (%)*	68.53	70.95	71.00	74.83	81.26	
Dry matter digestibility (%)*	61.24	64.00	64.34	69.71	77.64	

Note: Values are means ± SD of three replications (d. f. 5, 14). Means in the same row having different superscripts are significantly different ($P < 0.05$) and value 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.

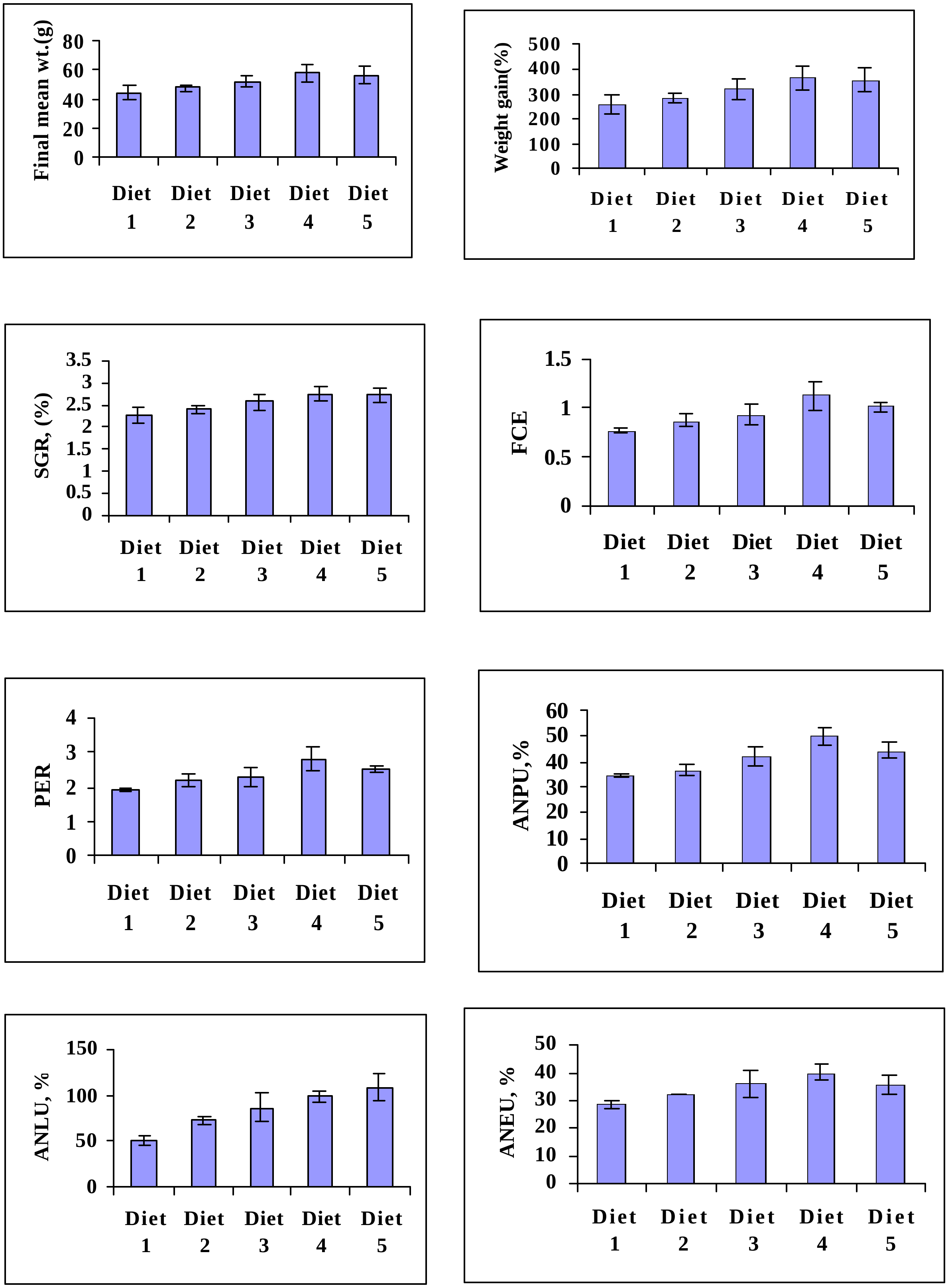

Figure 4.2 Growth and feed performance of *Clarias gariepinus* fed different levels of dietary lipid to carbohydrate ratios

(Bars are means ± SD of three replicates. Diets 1, 2, 3, 4 and 5 contained 1.36, 0.89, 0.60, 0.40 and 0.29 dietary lipid/ CHO ratios (g/g) respectively).

The apparent net energy utilisation (ANEU) was significantly ($P < 0.05$) increased with increasing dietary carbohydrate with concomitant reduction lipid level up to maximum in diet 4 (L/CHO ratio 0.40 g/g), followed by decreas. Diet 1, containing highest dietary lipid and lowest dietary CHO gave the poorest results, being statistically different ($P < 0.05$) between diet 4 and diet 1 (Table 4.3).

The data on percentage weight gain over the experimental period and apparent net protein utilisation were analysed using a polynomial regreesion analysis also described by Zeitoun et. al., (1976), in which a second order polynomial curve, represented by the equation $Y = B_0 + B_1 X + B_2 X^2$, is characterised by having a unique maximum point (Y_{max}) along its range. The value of X_{max} is that corresponds to Y_{max} is defined as an indication of the dietary L/CHO ratio that produces optimum growth or protein utilisation, and beyond which both parameters are depressed. In this case both figures suggest that maxima growth or protein utilisation was observed when the dietary CHO/L ratio (g/g) was 2.47 (L/CHO ratio g/g 0.40). This is is estimated to be the optimal L/CHO ratio for maximum growth or protein gain of *Clarias gariepinus* (Figure 4.3).

Diets containing P/E ratio of 20 mg protein / kj of GE, with increasing dietary carbohydrate to lipid ratio (g/g) produced significantly highest ($P < 0.05$) weight and protein utilisation, following a polynomial curve (Fig. 4.3). Weight gain and protein utilisation increased significantly at 33.42% carbohydrtae level, corresponding to CHO/L ratio g/g, reaching the maximum at 0.40 beyond which growth rate or protein utilisation found to decrease (Table 4.3 and Figgure 4.3).

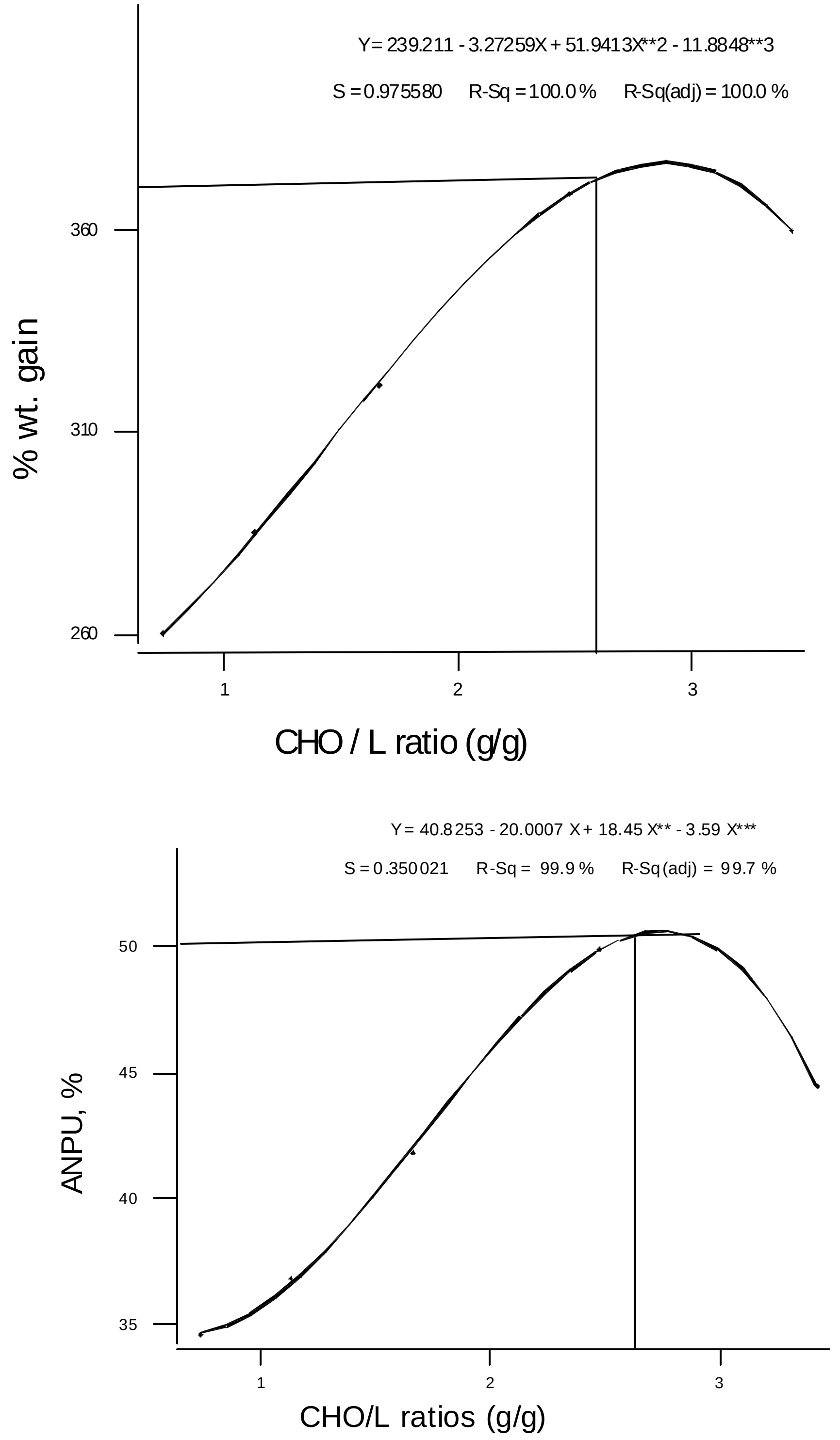

Figure 4.3 Dose response analysis (Polynomial regression, cubic): The polynomial relation of percent live weight gain and apparent net protein utilisation (ANPU) with dietary carbohydrate to lipid ratios (g/g) in *Clarias gariepinus*

4.3.3 Apparent Nutrients, Energy and dry matter digestibility

Apparent nutrients, energy and dry matter digestibility data are presented in Table 4.3. Apparent protein digestibility values were fairly high ranging from 85.48% to 87.92%. Diet 4 and diet 5 were showed the almost same value. The highest protein digestibility 87.95% was found in diet 5, containing highest dietary CHO level (L/CHO ratio of 0.29 g/g). The apparent lipid digestibility values were also fairly high and ranged from 87.55% to 91.11%, diet 4 showed the highest and the diet 1 showed the lowest value.

As shown in Table 4.3, apparent energy digestibility of the experimental diets ranged from 68.53% to 81.26% and diet 5 showed the highest value followed by diet 4. Apparent dry matter digestibility for different dietary treatments ranged from 61.24% to 77.64% and diet 5 was also showed highest, followed by diet 4. In general, energy and dry matter digestibility increased with increasing CHO/L ratios or decreasing L/CHO ratios.

4.3.4 Body Composition and Histopathology

Body composition data are presented in Table 4.4. At the end of the experiment, compare to the initial values, all the experimental groups exhibited a higher percentage of protein and ash and lower percentage of moisture and lipid content. There was an inverse relationship between body moisture and lipid content. Fish fed diets with higher inclusion level of dietary carbohydrate with concomitant lower dietary lipid (decreasing L/CHO ratio) had higher body moisture contents and lower body lipid contents. Fish fed diet 5 had highest moisture (71.99%) content whereas diet 2 showed lowest value but no significant ($P > 0.05$) differences between treatments.

Body protein content in different dietary groups ranged from 16.83% to 17.70% and were almost similar except diet 2 but no significant ($P > 0.05$) differences between treatments. Body ash content ranged from 3.03% to 3.34% and fish fed diet 1 had highest value with no significant ($P > 0.05$) differences between treatments. There was also an inverse relationship between ash and moisture content. Body lipid content ranged from 7.49% to 8.99% and lowest value was observed of diet 5, containing lowest dietary lipid. There was an overall trended of decreasing carcass lipid with decreasing inclusion level of dietary lipid (decreasing L/CHO ratio, g/g). The percent body lipid content positively correlated ($Y = 6.0 + 0.14 X$; $r = 0.68$; $P < 0.05$) to dietary lipid level.

Liver lipid, liver glycogen and organ indices (VSI and HSI) are shown in Table 4.5. There were trended to have significantly ($P > 0.05$) higher liver lipid content with increasing inclusion level of dietary lipid with concomitant decreasing dietary carbohydrate (increasing L/CHO ratios). Fish fed diet 1 was showed the highest ($P < 0.05$) and diet 5 was lowest ($P < 0.05$) value. Deposition of liver lipid showed a strong correlation ($Y = 0.69 + 0.29X$; $r = 0.70$; $P < 0.05$) with the dietary lipid level. Liver glycogen content ranged from 0.62% to 0.73% and highest value was observed of highest dietary lipid level. There was an overall trended of decreasing liver glycogen content with increasing inclusion levels of dietary carbohydrate with concomitant decreasing dietary lipid levels (decreasing L/CHO ratios, g/g). No significant ($P > 0.05$) differences between the treatments. Liver glycogen content was negatively correlated and showed inverse relationship ($Y = 0.80 – 0.005X$; $r = 0.69$; $P < 0.05$) to dietary carbohydrate.

Table 4.4 Body composition (% wet weight) of *Clarias gariepinus* at the start and end of the experiment

		Diet Number (Designation : Lipid / Carbohydrate, % energy)					
Percentage (%)	Initial	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)	±SEM
Moisture	71.64	70.58 ±0.18	71.05 ±1.23	70.20 ±0.28	71.80 ±0.87	71.99 ±1.04	0.26
Crude Protein	16.55	17.54 ±0.51	16.83 ±0.53	17.70 ±0.35	17.48 ±0.73	17.49 ±0.53	0.14
Crude Lipid	8.84	8.54^{ab} ±0.47	8.99^{b} ±0.85	8.76^{ab} ±0.41	7.64^{ab} ±0.30	7.49^{a} ±0.52	0.20
Ash	2.97	3.34 ±0.80	3.14 ±0.07	3.33 ±0.19	3.08 ±0.59	3.03 ±0.38	0.08

***Note:** Liver lipid, VSI & HSI of d. f (4, 29) but liver glycogen d. f is (4,14)
Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$) and value in the same row with no superscript are not significantly different ($P > 0.05$)

Table 4.5 Liver lipid, liver glycogen (% wet weight) and organ indices of *Clarias gariepinus*

	Diet Number (Designation : Lipid / Carbohydrate, % energy)					
Percentage (%)	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)	±SEM
Liver lipid	7.63^{b} ±2.59	5.01^{ab} ±0.98	5.48^{a} ±1.35	4.54^{a} ±1.69	4.12^{a} ±0.85	0.36
Liver glycogen	0.70 ±0.09	0.73 ±0.05	0.69 ±0.07	0.63 ±0.06	0.62 ±0.11	0.20
VSI	9.06 ±2.54	6.90 ±1.43	6.04 ±0.65	6.34 ±1.44	7.71 ±2.28	0.37
HSI	0.93 ±0.20	0.84 ±0.13	1.02 ±0.35	1.10 ±0.14	1.28 ±0.35	0.05

**Note: Liver lipid, VSI & HSI of d. f (4, 29) but liver glycogen d. f is (4,14)
Values are means ± SD of three replications. Means in the same row having different superscripts are significantly different ($P < 0.05$) and value in the same row with no superscript are not significantly different ($P > 0.05$)

As shown in Table 3.5, VSI and HSI values were impacted not significantly ($P > 0.05$) by dietary L/CHO ratios. The VSI ranged from 6.04% to 9.04% and highest value was observed of diet 1, containing highest dietary lipid. The HSI value was increased with increasing inclusion levels of dietary carbohydrate with concomitant reduction dietary lipid (decreasing L/CHO ratios, g/g). The highest value was observed at highest inclusion level of dietary carbohydrate of diet 5, containing lowest dietary lipid. No significant ($P > 0.05$) differences between treatments.

Results of histopathological examination the liver was observed that no significant abnormalities between the treatment groups. Fish fed the various levels of dietary L/CHO ratios displayed no changes, only normal cells were observed.

4.3.5 Digestive Enzyme

The protease, lipase and amylase activities are presented in Table 4.6 and Figure 4.3. Intestinal protease activity was not significantly affected ($P > 0.05$) with the inclusion levels of dietary lipid and carbohydrate level. Intestine protease activity was found to be highest in fish fed on diet 4 (L/CHO ratio, 0.40 g/g), containing 33.42% carbohydrate and 13.52% lipid level. In general, higher liver protease activity trend was observed not significantly ($P > 0.05$) on higher inclusion levels of dietary lipid (decreasing L/CHO ratios) and lowest value was found on diet 5, containing lowest lipid level.

Table 4.6 Proteas, lipase and amylase activities of intestine and liver in *Clarias gariepinus* at the end of the experiment

	Diet Number (Designation : Lipid / Carbohydrate, % energy)					
Enzymes:	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)	±SEM
Intestinal Protease[1]	130.81 ±29.34	127.22 ±18.15	130.09 ±8.61	134.09 ±13.59	126.36 ±6.05	3.84
Intestinal lipase (EC 3.1.1.3)[2]	1.88 ±1.13	1.43 ±0.83	1.47 ±0.53	1.30 ±0.59	0.78 ±0.15	0.19
Intestinal ∝Amylase (EC 3.2.1.1)[3]	152.78^{a} ±19.78	191.23^{a} ±29.19	205.00^{ab} ±9.26	293.76^{b} ±58.16	220.46^{ab} ± 45.51	14.80
Liver protease[1]	113.30 ±12.41	123.06 ±20.67	112.44 ±14.28	103.97 ±17.92	102.11 ±17.17	4.17
Liver lipase (EC 3.1.1.3)[2]	2.02 ±0.23	2.02 ±0.14	1.95 ±0.09	1.53 ±0.51	1.87 ±0.66	0.10

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^{0} 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^{0} 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^{0} C.

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

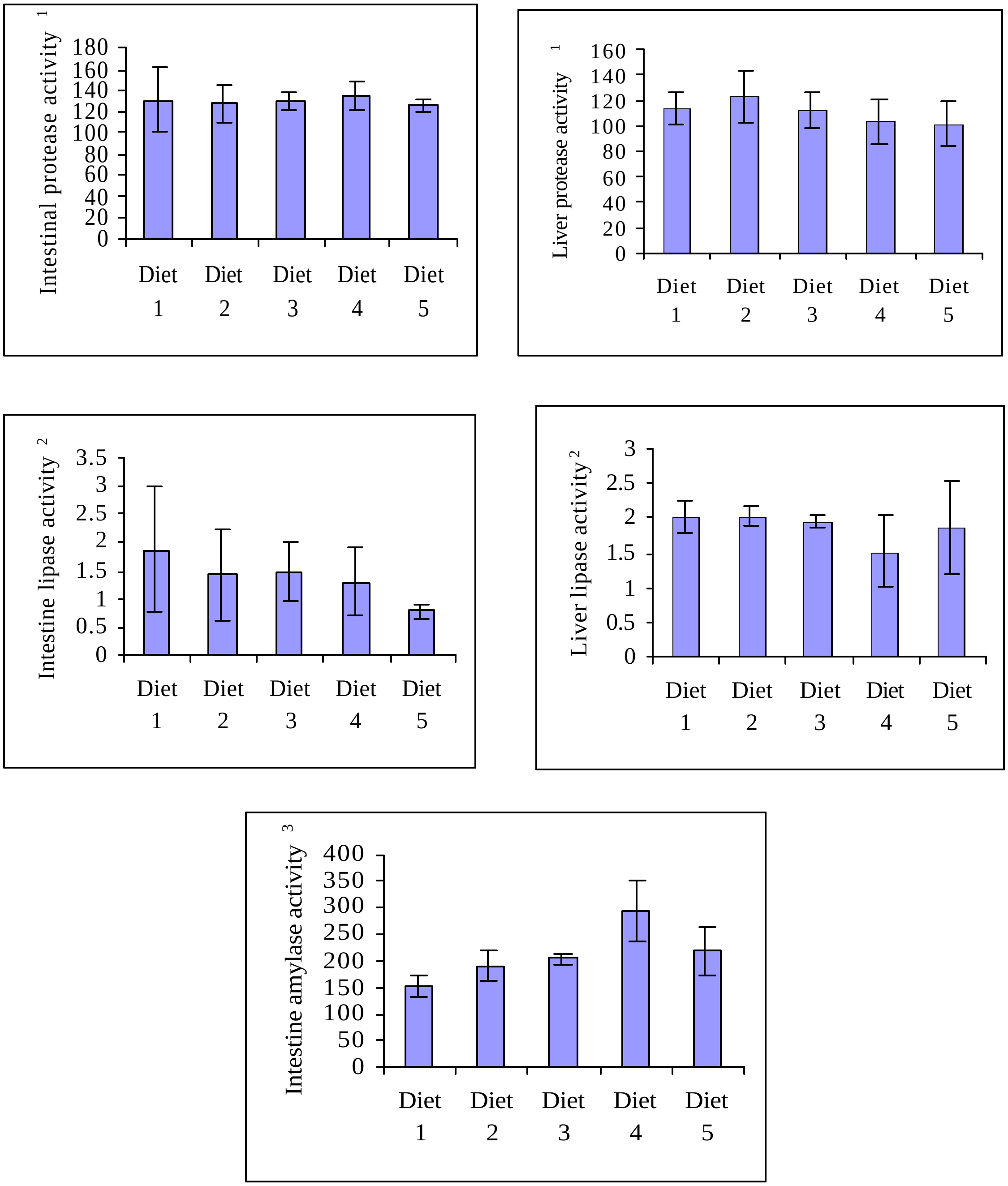

Figure 4.4 The protease, lipase and amylase activities of intestine and liver in *Clarias gariepinus* fed different dietary lipid to carbohydrate ratios.

(Bars are means ± SD of three replications. Diets 1, 2, 3, 4, and 5 contained 1.36, 0.89, 0.60, 0.40 and 0.29 lipid to CHO ratios 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 30^0 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 30^0 C

[3] ∝-amylase activity was expressed as the amount of maltase (µg) liberated by 200 µl of enzyme solution at pH per min. at 30^0 C

Intestinal α-amylase activity was significantly ($P < 0.05$) influenced with the inclusion levels of dietary carbohydrate and lipid level. Amylase activities were increased with increasing dietary carbohydrate levels with concomitant reduction lipid levels (decreasing L/CHO ratios). Highest ($P < 0.05$) amylase activity was observed in fish fed diet 4, containing L/CHO ratio 0.40 and no significant ($P > 0.05$) differences with higher CHO diets 4 and 5. Intestine and liver lipase activity were decreased insignificantly ($P > 0.05$) with decreasing dietary lipid with concomitant increment dietary carbohydrate level (decreasing L/CHO ratios). Lipase activities of both tissues were found to be highest in fish fed on diet 1 (on highest L/CHO ratio) containing highest dietary lipid and lowest CHO level. However, protease and lipase activity was found to be higher in the intestine than in the liver.

4.3.6 Blood Plasma Component

Concentration of plasma components such as glucose, triglycerides (TAG) and cholesrerol are shown in Table 4.7 and Figure 4.5. Plasma glucose concentration was increased significantly ($P < 0.05$) with increasing inclusion levels of dietary carbohydrate with concomitant reduction dietary lipid. Highest glucose concentration was observed on diet 5, containing highest dietary carbohydrate level (L/CHO ratio, 0.29 g/g) but significant ($P < 0.05$) differences with other treatments. Glucose concentration was positively correlated ($Y = 55.0 + 1.21X$; $r = 0.50$; $P < 0.05$) to the dietary carbohydrate level but negatively correlative ($Y = 115 - 2.91X$; $r = 0.57$; $P < 0.05$) to the dietary lipid level.

Concentration of triglycerides increased insignificantly ($P > 0.05$) with increasing dietary lipid level with concomitant to increment dietary carbohydrate level (decreasing L/CHO ratios). Highest concentration value was observed on diet 1, containing highest inclusion level of dietary lipid. Cholesterol concentration increased not significantly ($P > 0.05$) with increasing inclusion levels of dietary carbohydrate with concomitant to reduction dietary lipid level. Highest concentration of cholesterol was observed on diet 5, containing highest dietary carbohydrate (L/CHO ratio, 0.29 g/g). Cholesterol concentration positively correlated ($Y = 295 + 3.97X$: $r = 0.80$; $P < 0.05$) to the dietary carbohydrate level.

Table 4.7 Blood plasma concentration of glucose, triglycerides and cholesterol of *Clarias gariepinus* at the end of the experiment

	Diet Number (Designation : Lipid / Carbohydrate, % energy)					
Components:	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)	±SEM
Glucose (mg/100 ml)	58.28^a ±11.33	58.84^a ±8.09	67.77^a ±6.85	59.30^a ±8.47	94.44^b ±11.81	4.23
Triglyceride (mg/100 ml)	173.12 ±60.55	164.59 ±56.54	158.45 ±34.30	139.51 ±55.57	154.10 ±47.61	11.70
Cholesterol (mg/100ml)	201.14 ±75.53	231.33 ±21.37	236.97 ±45.26	239.41 ±54.23	248.18 ±26.48	11.50

*Note: Values are means ± SD of three replications. Means in the same row having different Superscripts are significantly different ($P < 0.05$) and value in the same row with no Superscript are not significantly different ($P > 0.05$)

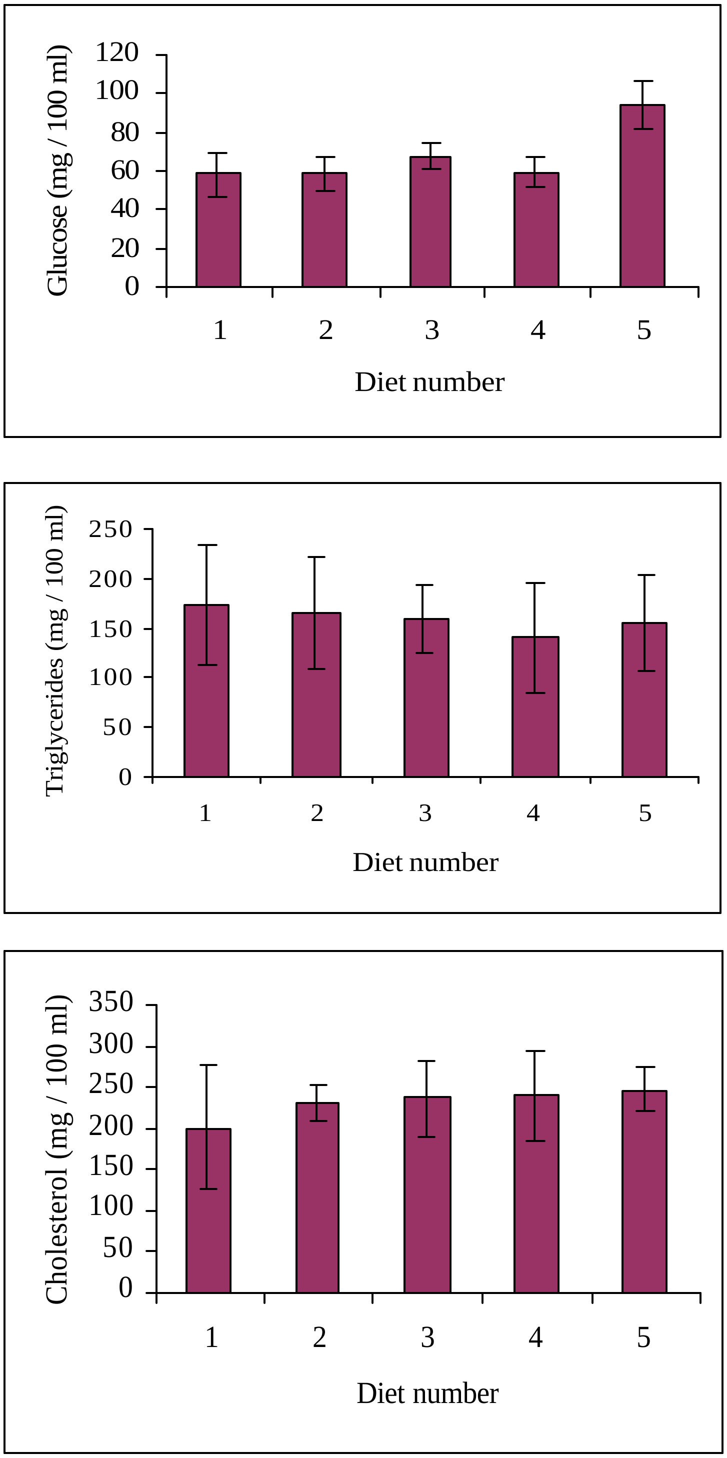

Figure 4.5 Blood plasma concentration of glucose, triglycerides and cholesterol in *Clarias gariepinus* fed various dietary lipid to carbohydrate ratios

(Bars are means ± SD of three replications. Diets 1, 2, 3, 4, and 5 contained 1.36, 0.89, 0.60, 0.40 and 0.29 lipid to CHO ratios (g/g) respectively)

Plasma free amino acid levels are presented in Table 4.8. Most of the essential amino acids, were increased not significantly (P > 0.05) with increasing dietary carbohydrate with concomitant reduction dietary lipid and maximum value was found of diet 4 (L/CHO ratio of 0.40 g/g), followed by sharply declined of diet 5. All non-essential amino acids increased not significantly (P > 0.05) with increasing inclusion levels of dietary carbohydrate with concomitant reduction dietary lipid (decreasing L/CHO ratio). Maximum value was showed of diet 4 (L/CHO ratio 0.40), followed by declined on diet 5, containing highest dietary carbohydrate level.

Table 4.8 Plasma amino acid levels (μM / ml of blood plasma) in *Clarias gariepinus* at the end of the experiment

Diet Number (Designation : Lipid / Carbohydrate, % energy)						
Amino acids:	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)	±SEM
Essential amino acids:						
Arginine	0.112 ± 0.017	0.123 ± 0.016	0.091 ± 0.016	0.119 ± 0.063	0.077 ± 0.023	0.008
Histidine	0.045 ± 0.011	0.065 ± 0.012	0.051 ± 0.005	0.059 ± 0.014	0.050 ± 0.002	0.003
Isoleucine	0.106 ± 0.006	0.108 ± 0.016	0.101 ± 0.012	0.119 ± 0.011	0.090 ± 0.020	0.004
Leucine	0.188 ± 0.018	0.216 ± 0.022	0.186 ± 0.024	0.213 ± 0.035	0.185 ± 0.030	0.007
Lysine	0.309 ± 0.039	0.343 ± 0.034	0.319 ± 0.029	0.300 ± 0.078	0.249 ± 0.059	0.014
Methionine	0.026 ± 0.002	0.027 ± 0.004	0.027 ± 0.002	0.031 ± 0.002	0.026 ± 0.003	0.001
Phenylalanine	0.064 ± 0.022	0.077 ± 0.009	0.056 ± 0.010	0.062 ± 0.013	0.067 ± 0.016	0.004
Threonine	0.630*	0.567*	0.410*	**	**	
Valine	0.192 ± 0.021	0.197 ± 0.017	0.181 ± 0.025	0.210 ± 0.019	0.179 0.032	0.006
Non-essential amino acids:						
Alanine	0.268 ± 0.046	0.294 ± 0.059	0.307 ± 0.032	0.360 ± 0.043	0.269 ± 0.047	0.013
Aspartic acid	0.031 ± 0.013	0.035 ± 0.003	0.034 ± 0.001	0.052 ± 0.034	0.027 ± 0.004	0.004
Cystine	0.088 ± 0.012	0.081 ± 0.020	0.076 ± 0.017	0.089 ± 0.027	0.065 ± 0.025	0.005
Glutamic acid	0.098 ± 0.062	0.097 ± 0.036	0.088 ± 0.026	0.127 ± 0.029	0.073 ± 0.022	0.010
Glycine	0.172 ± 0.050	0.238 ± 0.072	0.171 ± 0.006	0.243 ± 0.012	0.255 ± 0.013	0.012
Proline	0.098 ± 0.008	0.115 ± 0.050	0.131 ± 0.025	0.149 ± 0.008	0.132 ± 0.025	0.008
Serine	0.250*	0.237*	0.270*	**	**	
Tyrosine	0.043 ± 0.005	0.038 ± 0.005	0.028 ± 0.003	0.047 ± 0.018	0.033 ± 0.012	0.003

* No data in all replicates as peak and not differentiated in the chromatogram.

Values are means ± SD of three replications and values in the same row are not

** No data in one/two replicates, as peak not differentiated in the chromatogram

significantly different ($P > 0.05$).

*** Tryptophan, an essential amino acid, not measured by the technique.

4.4 DISCUSSION

4.4.1 Growth Performance and Feed efficiency

Differential coefficient of variation (CV, %) of final body weight were observed in *Clarias gariepinus* during the present study. Diet 4, containing L/CHO ratio of 0.40 g/g) and diet 5, containing L/CHO ratio of 0.29 g/g had lower CV (%) and resulted in higher growth performance, feed and protein utilisation.

The results clearly demonstrate that the growth rate and feed conversion efficiency are influenced by lipid to carbohydrate ratios. Reduction in dietary lipid level from 20.88% to 13.52%, with concomitant increase in carbohydrate level from 15.39% to 33.42%, corresponding to L/CHO energy ratios of 1.36 to 0.40 g/g (CHO/L ratios of 0.74 to 2.47 g/g), significantly improved the growth performances and food conversion efficiency. Investigation in walking catfish *Clarias batrachus* diets containing 40% CP and 3.46 kcal/g.(ME), with increament of carbohydrate level from 0.44 to 27.28%, with concomitant reduction in lipid level from 19.95% to 8.07%, corresponding to CHO/L ratios (g/g) ranging from 0.02 to 3.38, has been reported to produce fish with significantly improved growth performance and feed utilisation (Erfanullah and jafri, 1998a).

Similarly, Jantrarotai *et. al*., (1994) reported in hybride *Claris* catfish diets containing 33% CP and 281kcal/100g (DE), with increament of carbohydrate level from 30.45% to 49.68%, with concomitant reduction in lipid level from 11.98% to 4.42%, corresponding to CHO/L ratios ranging from 2.54 to 11.24 produce fish improved weight gain, SGR, and food conversion efficiency. Other studies, in channel catfish diets containing 24% CP and 275 kcal estimated available energy / 100g, with CHO/L (g/g) ratios ranging from 0.45 to 4.5, have been reported to produce fish with insignificantly increased in weight gain (Galing and Wilson, 1977).

In this study, the maximum growth and best feed utilisation observed in fish fed 33.42% carbohydrate and 13.52% lipid (L/CHO ratio, 0.40 g/g), corresponding to L/CHO energy ratio of 1 in the diet 4. Moreover, increasing carbohydrate in the diet to 37.68% resulted in a decrease in growth and food conversion efficiency. Improved growth performance express in terms of percent weight gain, specific growth rate (SGR,) and feed conversion efficiencies (FCE), fish fed diets containing higher dietary carbohydrate with lower dietary lipid levels have also been reported in walking catfish *Clarias batrachus* (Erfanullah and Jafri, 1998a), Indian major carps (Erfanullah and Jafri, 1998b), hybrid *Clarias* catfish (Jantrarotai *et. al.*, 1994), channel catfish (Garling and Wilson, 1977) and tambaqui *Colossoma macropomum* (Gunther, 1996).

Reduced growth rate and feed conversion efficiency in fish fed the highest-lipid lowest-carbohydrate diet (CHO/L ratio 0.74, corresponding to CHO/L energy ratio 4.0). This could be the result of reduced feed consumption by the fish due to high dietary lipid because of excessive food energy, thereby preventing the intake of necessary amounts of protein and other nutrients required for maximum growth. Excess dietary lipids are known to affect normal metabolic functions adversely and also affect nutritional balance (Chuntapa *et. al.*, 1999). Since this diet also contained a high level of indigestible fibre in the form of ∝-cellulose, less efficient absorption and reduced availability of other nutrients seems understandable. Although, surprisingly African catfish fish *Clarias gariepinus* appear to be less tolerant to high lipid intake than walking catfish *Clarias batrachus* (Erfanullah and Jafri, 1998a) and hybrid *Clarias* catfish (Jantrarotai *et. al.*, 1994).

Depressed growth rate and poor feed conversion efficiencies obtained a lowest lipid-highest carbohydrate of diet 5, containing higher CHO level than the optimum (>33.42%), corresponding to L/CHO energy ratio = 0.67, point to the fact that this fish is unable to handle higher CHO level in the diet. This might be excess dietary carbohydrate are to cause degenerative change in the digestive gland, resulting in poor growth (Pascual, Coloso & Tamse 1983).

4.4.2 Nutrient and Energy utilisation

Increased protein utilisation, measured in terms of PER and ANPU, were as increasing dietary carbohydrate, with concomitant reduction dietary lipid levels for fish growth up to L/CHO ratio 0.40 g/g. The highest PER, ANPU and ANEU were noted in fish fed diet 4, having maximum weight gain and feed utilisation. Fish fed diets containing either higher lipid with lower carbohydrate or lowest lipid with highest carbohydrate significantly reduced PER, ANPU and ANEU. Erfanullah and Jafri (1998a) have been observed similar results in terms of PER, ANPU and ANEU in the diet of walking catfish *Clarias batrachus* to those reported in the present study. They concluded that fish fed diets with similar energy content, supplied by different energy sources, result in differential utilisation of lipid and carbohydrate energy. The ANEU value decreased in fish fed lowest lipid and highest carbohydrate diet may be attributed to a high energy loss as a heat (heat increment) resulting from high dietary carbohydrate intake (Lovell, 1989). Similar results on reduce PER, ANPU and ANEU values in fish fed diets containing either higher lipid with lower carbohydrate or lowest lipid with highest carbohydrate diets were reported on Indian major carps (Erfanullah and Jafri, 1998b), hybrid *Clarias* catfish (Jantrarotai *et. al.,* 1994), channel catfish (Garling and Wilson, 1977) and tambaqui *Colossoma macropomum* (Gunther, 1996).

Since fish fed the diets were isonitrogenous and isoenergetic, the increased in PER, ANPU, ANLU and ANEU with increasing dietary carbohydrate (corresponding to increasing CHO/L ratios) could be attributed to the relative amount non-protein energy sources. This may indicate that *Clarias gariepinus*, despite being omnivorous, can utilise dietary carbohydrate more efficiently than lipid. It is difficult to determine whether *Clarias gariepinus* utilised lipid (ANLU, %) less efficiently than carbohydrate. Because of isocaloric conditions, higher lipid levels could be attained only by increasing the fibre content of the diet. There are several reports indicating that high dietary fibre reduces the utilisation of other nutrients (Anderson *et. al.*, 1984; Hilton *et. al.,* 1983), but this view has been challenged (Jantraotai *et. al.*, 1994). Thus, it remains unclear if reduced growth of fish fed high lipid diets was due to inefficient lipid utilisation by fish, as compared with carbohydrate utilisation, or to deleterious effects of the high dietary fibre or lipid level.

It may be stated that on the basis the best weight gain, feed conversion efficiencies, nutrient and energy utilisation was achieved on diet 4, containing 33.42% carbohydrate and 13% lipid. This diet presumably the optimum / most appropriate CHO/L ratio g/g, 2.47 (corresponding to L/CHO energy ratio = 1) through polynomial regression curve (cubic) Fig. 4.3. Zeitoun *et. al.*, (1976) have recommended the use of polynomial regression analysis as a mean of approximating the relationship of weight gain and essential nutrient intake as indicated by these workers, the value corresponding to maximal gain by polynomial cubic regression is defined as the maximum concentration of dietary nutrient that produce optimal growth, and beyond which growth is depressed.

In the present study, the relationship between growth rate over the experimental period and net protein utilisation with dietary CHO/L ratio g/g are best expressed statistically by a second polynomial cubic curve (Fig. 4.3). The maximum of the curves were obtained at CHO/L ratio g/g, below 3, i. e of 2.6 (corresponding to L/CHO energy ratio = 1).

4.4.3 Nutrients, Energy and Dry matter Digestibility

Apparaent protein digestibility (APD) values were observed to be higher in diets with higher dietary carbohydrate levels. Decrease in protein digestibility as a result of decreasing dietary CHO level beyond 33.42%. The APD data of privious experiment (diets containing same P/E raios of chapter 3) of this species showed that the APD values decreased with increasing dietary CHO level beyond 35%. However, higher APD valus in this study was not significantly affected by the inclusion level of dietary lipid and carbohydrate. Although, the protein digestibility increased in this study as increasing CHO levels of the diets. It indicates that *Clarias gariepinus* could be needed inclusion level of 33% dietary CHO and beyond this certain percentage of dietary carbohydrate resulting poor protein digestibility values as well as growth performance. Between the two non-protein energy sources, Jantraotai *et. al.,* (1994) demonstrated that based on growth, hybrid *Clarias* catfish utilise carbohydrate more efficiently than lipid as an energy source when sufficient dietary protein was available. The effect of lipid and carbohydrate in the digestibility of protein in this study is consistent with this observation.

The apparent lipid digestibility (ALD) of the experimental diets in *Clarias gariepinus* was high as reported for fish (NRC, 1993). Apparent lipid digestibility increased as decreasing inclusion levels of dietary lipid, with concomitant increament of dietary carbohydrate levels. Two assumptions might be suggested to explain the low lipid digestibility in higher dietary lipid levels. Firstly, the African catfish has a lower ability to digest fat (Degani and Revach, 1991) and secondly, the higher levels of dietary fibre level may also have inhibited lipid digestibility (Kuzinger *et. al*., 1986). In general, nutrient digestibility (APD and ALD) were not significantly affected on sufficient protein level (40% CP) when the non-protein dietary inclusion levels of lipid and CHO ranging from 11 to 13% and 33 to 37% respectively.

The apparent energy and dry matter digestibility showed that the increased values as increasing dietary CHO level and highest value was found on highest dietary CHO level. The energy and dry matter digestibility values trends to increase with the increasing level of dietary CHO, with concomitant to reduction inclusion level of dietary lipid. However, the ADC value of energy and dry matter increased as increasing dietary CHO whereas dietary fibre level decreasing. This could be attribute to the relatively high levels of fibre of the same protein and energy diets, indicating poor digestion of the carbohydrate portion of the diets in *Clarias gariepinus* (Santinha *et. al*., 1996).

Apparently, it is difficult to compare values obtained in different experiments because the degree of digestion of a specific diet may very with dietary ingredients, their chemical composition and inclusion level (Jobling, 1994; De Silva and Anderson, 1995). Further, factor such as water temperature, feeding rate and animal model also affect feed digestibility (Jobling, 1994; De Silva and Anderson, 1995). Several of these factors, alone or in combination, may have contributed to the relatively low energy and dry matter digestibility reported in the study.

4.4.4 Body Composition and Histopathology

Data obtained on whole-body composition in this study indicated significant influences between dietary treatments. These influences in body composition were attributed to differences in inclusion level of lipid and carbohydrate in the diets. Increases in moisture levels in fish carcass fed higher carbohydrate diets was accompanied by concomitant decreases in lipid levels. Body lipid and body moisture are interchangeable, confirms with the results on walking Catfish *Clarias batrachus* (Erfanullah and Jafri, 1998a), Indian major carp (Erfanullah and Jafri, 1998b), red drum (Ellis and Reigh, 1991) and channel catfish (Garling and Wilson, 1977). Love (1980) reported that depletion of body lipid results in an increase in the moisture content of the muscle and Sargent (1976) explained this inverse relationship as a result of lipid mobilisation.

Body protein and body ash did not show significant difference and remained almost contant. The different CHO/L ratios of the experimental diets had no significant effect on body protein and ash content of the whole body of *Clarias gariepinus*. Similar results have been repoted in walking catfish *Clarias batrachus* (Erfanullah and Jafri, 1998a) and hybrid *Clarias* catfish (Jatrarotai *et .al.*, 1994).

Increased in body lipid with increasing dietary lipid (corresponding to increasing CHO/L ratio, g/g) and the positive correlation noted between dietary lipid level and body lipid content. The inverse relationship between dietary CHO and whole body lipid content was interesting, since increased CHO failed to produced undesirable fat accumulation in the body of the fish. The observation seems in agreement with the results reported on walking catfish *Clarias batrachus* (Erfanullah and Jafri, 1998a), hybrid *Clarias* catfish (Jatrarotai *et.al.,* 1994), channel catfish

(Garling and Wilson, 1977), tilapia (El-Sayed and Garling, 1988; Hanley, 1991); hybrid strip bass (Nematipour *et.al.*, 1992) and Indian major carps (Erfanullah and Jafri, 1998b).

The differences in liver lipid, liver glycogen, viscerosomatic index (VSI) and hepatosomatic index (HSI) of *Clarias gariepinus* fed various CHO/L ratios were obvious when the diets contained high carbohydrate and high lipid (diet 5 and 1, respectively). According to Anderson and Sargent (1981), the liver is the main site of fatty acid synthesis in salmonids. Fish feeding the high lipid diet tended to yield significantly a high liver lipid content and the positive correlation noted between dietary lipid level and liver lipid content. Results obtained in this study are similar to those reported from previous experiments with effect of carbohydrate to lipid ratios. Attempts by Jantrarotai *et. al*., (1994) on hybrid *Clarias* catfish reported that feeding high lipid diet tended to yield a high liver lipid content. Similar observations have been reported in tilapia (Shimeno *et. al*., 1993), hybrid striped bass (Nematipour *et.al.*, 1992), channel catfish (Garling and Wilson, 1977) and salmonids (Buhler and Halver, 1961) and were attributed to deposition of excess lipid, and increased glycogenic processes respectively. In contrast, Brauge *et. al.*, (1994) reported that a high dietary carbohydrate (high dietary CHO/L ratio) enhances liver lipid synthesis and deposition in liver of rainbow trout. The diet containing the highest level of lipid led to the lowest level of liver lipid, suggesting that lipid deposition from dietary lipid does not occur in liver but probably in adipose tissue.

In the present study, low concentrations of liver glycogen without correlation to dietary carbohydrate were found. Liver glycogen content was insignificantly decreased with increasing inclusion levels of dietary carbohydrate, corresponding to increasing CHO/L ratios. Glycogen content of liver has generally been observed to increase with dietary carbohydrate

(Hilton and Atkinson, 1982; Garling and Wilson, 1977; Buhler and Halver, 1961). In contrast, liver glycogen content of red drum *Sciaenops ocellatus* (Serrano *et.al*., 1992) was not affected by dietary carbohydrate levels. Liver glycogen content increased in response to dietary lipid levels rather than dietary carbohydrate levels and negative relationship between dietary carbohydrate and liver glycogen levels. This study follows silimar observations reported for hybrid striped bass (Nematipour *et.al.,* 1992). A possibile explanation for these responses is that increasing dietary lipid provided excess energy that was deposite as glycogen as well as VSI.

The higher visceromatic index (VSI) value obtained with the treated fish can be related to the influence of dietary lipid level. Hanley (1991) and Ramachandra Nair and Gopukumar (1981) also reported increased visceral fat deposition in tilapia receiving lipid-rich diets. Fish feeding the high lipid diet, corresponding to high L/CHO ratio (g/g) tended to yield a high VSI. This finding is in agreement with earlier studies of hybrid striped bass (Nematipour *et.al.,* 1992) and rainbow trout *Oncorhynchus mykiss* (Watanabe, 1982; Takeuchi *et. al.,* 1978). Higher values of hepatosomatic index (HSI), noted in fish with higher levels of dietary carbohydrate (increasing CHO/L ratio), was not found to affect growth, conversion efficiencies and protein or energy utilisation. Brauge *et. al.,* (1994) reported that HIS values were higher the diets containing higher level of dietary CHO fed in rainbow trout. They concluded that absorbed CHO is not utilise to provide energy can be deposited in the liver both as glycogen and as lipid after conversion. Similar findings have been reported on young catfish *Heteropneustes fossilis* (Erfanullah and Jafri, 1998c) hybrid *Clarias* catfish (Jantrarotai *et. al*., 1994); rainbow trout (Hilton and Atkinson, 1982) and channel catfish (Garling and Wilson, 1977). The lower HSI values observed in fish fed higher levels of lipid may be attributed to fat mobilisation from liver to muscle as observed by Bazaz and Keshavanath (1993) and Lone and Matty (1980). The liver lipid and the body lipid data (Table 3.4 and Table 3.5) also support this.

Histopathological examination of liver indicated that various inclusion levels of high dietary lipid and carbohydrate (various L/CHO ratios) did not produce excessive parenchymal cell damage or lipid or glycogen deposition in cellular vacuoles. Histological examination of the liver generally supported the biochemical observations. Similarly, Garling and Wilson (1977) reported on channel catfish that excessive dietary lipid and carbohydrate (various CHO/L ratios) did not produce any cell damage or glycogen deposition in cellular vacuoles.

4.4.5 Digestive Enzyme

It would appear that the dietary L/CHO ratios in the present study also altered digestive enzyme profile. Scherbina *et. al.,* (1976) reported that protease activity tend to very depending on the type of diet and its protein content. The protease activity in the present study did not differ significantly by various L/CHO ratios at the same dietary protein level. Intestinal protease activity was observed to be highest on L/CHO ratio g/g 0.40. This indicated that the L/CHO ratio g/g 0.40, containing dietary carbohydrate 33.42 % with lipid 13.52% affected the digestive system in such a way that digestive and / or assimilative mechanisms were activated. The increased protease activity might be direct effect on intestine with enhance *de novo* synthesis of enzyme or might be an increase in growth rate of fish. Similar results to those observed in the present study were also reported by Dabrowski and Glogowski (1977) and Mukhopadhyay and Hajra (1986), while Bazaz and Keshavanath (1993) observed higher induction of intestinal protease activity when the diets were supplimented with higher inclusion level of lipid. Adaptive changes in the activity of protease enzyme in relation to the type of diet has been reported by Kawai and Ikeda (1972) and Scherbina *et. al.,* (1976) in common carp.

Fish fed on higher level of dieraty lipid (higher L/CHO ratios) also observed higher trend of liver protease activity. Similar results to those observed in the present study were also reported by Bazaz and Keshavanath (1993) who observed higher induction of liver protease activity when the diet of masheer, *Tor khudree* was supplemented with higher dietary lipid.

∝-amylase activity in African catfish was increased significantly correlated with the carbohydrate content of the diet. ∝-amylase activity was observed to be highest on L/CHO ratio g/g 0.40. This indicated that the L/CHO ratio g/g, containing dietary carbohydrate 33.42 % affected the digestive system in such a way that digestive and / or assimilative mechanisms were activated. This suggesting higher CHO turnover and active mobilisation, which is further evidence for protein sparing by dietary carbohydrate. The increased ∝-amylase activity might be direct effect on intestine with enhance *de novo* synthesis of enzyme or might be an increase in growth rate of fish. Similar results to those observed in the present study were also reported by on *Clarias batrachus* (Mukhopadhyay, 1977) and Common carp (Kawai and Ikeda, 1972)

Intestinal and liver lipase activity was observed higher trends in fish fed higher inclusion level of dietary lipid, corresponding to increasing CHO/L ratio, suggesting higher lipid turnover. Higher-lipid diets may also impair the ability of fish to digest and assimilate lipid. This also perhaps indicated that the supplementation of lipid in the diet had not favourable affect for the growth and well being of this fish species. Such adaptive change in the activity of lipase has been reported earlier on carp (Mukhopadhyay and Rout, 1996) and masheer, *Tor khudree*

(Bazaz and Keshavanath, 1993). Moreover, Protease and lipase activities were greater in the intestine than in liver, which is in agreement with the results observed by Bazaz and Keshavanath (1993).

4.4.6 Blood Plasma Component

Plasma glucose concentrations in *Clarias gariepinus* clearly increased significantly with increasing highest dietary carbohydrate content (decreasing L/CHO ratio) and positive correlation noted between dietary carbohydrate and plasma glucose level. This is in accordance with earlier studies with rainbow trout (Hilton *et. al.*, 1987), Atlantic salmon (Hemre *et. al.*, 1996). Shimeno *et. al.*, (1993) reported that plasma glucose concentration did not differ significantly in tilapia fed increasing inclusion level of dietary CHO, with concomitant reduction dietary lipid level. They concluded that tilapia have high ability to utilise effectively dietary carbohydrate. Brauge *et. al.*, (1994) reported that Plasma glucose level were related to the dietary carbohydrate of the diets and remained high 24 h after feeding the high carbohydrate diets in rainbow trout. The high plasma glucose concentration induced by high dietary levels of carbohydrate were initially though to be due to a lack of insulin secretion (Palmer and Ryman, 1972). Recent studies have clearly demonstrated that salmonids do have the ability to secrete insulin and to increase insulin secretion in response to high levels of dietary carbohydrate (Hilton *et. al.*, 1987). Comparable results on plasma glucose level with dietary carbohydrate content in African catfish are currently lacking. Therefore, the present study suggests that African catfish can tolerant up to 33.42% dietary carbohydrate beyond that sharply increased plasma glucose concentration.

Plasma triglycerides concentrations in African catfish were found to be higher trends in higher dietary lipid level (higher L/CHO ratios). Shimeno *et. al.*, (1993) reported results comparable to the results of the present study in which a increased plasma trigltcerides level significantly was observed in tilapia *Oreochromis niloticus* fed higher dietary lipid, with concomitant reduction dietary CHO level. They concluded that tilapia can not effectively adapt to high-lipid carbohydrate-low diets.

Plasma cholesterol concentrations were found to be higher trend in higher dietary CHO level, with concomitant reduction dietary lipid level. Similar regulatory pattern to dietary CHO were observed by Waagbo *et. al.*, (1994) in Atlantic salmon. They concluded that plasma cholesterol in the juveniles in freshwater seemed to be even more influenced by osmotic conditions, as judged from their mutual significant correlation. According to Hemre *et. al.*, (1995), high dietary levels of CHO as strach do not influence serum cholesterol in Atlantic salmon. The complex regulation of blood and extracellular fluid volume in fish was recently reviewd by Olson (1992).

In the present study, most of the essential and non-essential plasma amino acid levels trended to higher as the dietary CHO in the diet rises up to 33.42% beyond declined. Plasma amino acid levels are influenced by the dietary protein levels (Yamamoto *et. al.*, 2000; Yokoyama *et. al.*, 1994; Ogata and Murai, 1994). Yamamoto *et. al.*, (2000) also reported that not only dietary protein levels but also dietary lipid levels influenced plasma amino acids levels in rainbow trout. In channel catfish, the serum amino acid levels of fish fed diets with various protein to energy ratios were compared, and the effect of dietary energy (fat) level was found to be insignificant (Wilson *et. al.*, 1985). The increase in plasma amino acids levels in this study may have been caused by supply from higher certain dietary carbohydrate level. Carbohydrates may serve as precursors for the dispensable amino acids and nucleic acids, which are metabolic intermediates necessary for growth (NRC, 1993). Comparable results on plasma amino acid levels with dietary lipids and CHO are currently lacking. More research is need in this area particularly dietary CHO and plasma amino acid levels to clarify the point.

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