THE PROTEIN SPARING EFFECTS OF LIPID IN AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL, 1822)

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ABSTRACT
A feeding trial was conducted to determine the protein sparing effects of three lipid sources (Fish oil, Palm oil and Groundnut oil) in Clarias gariepinus. 150 fingerlings of mean weight 2.50 ±0.04 were allotted at random in a group of 15 fish per tank in triplicate. They were fed nine experimental diets and a commercial reference diet for 8 weeks. The experimental diets contained three levels of three sources of lipid and three levels of crude protein. There were significant differences ($p<0.05$) in the growth parameters measured. The groundnut oil spared most the protein at highest level of lipid inclusion (20%) followed by palm oil (15%) while the fish oil least spared the protein.

KEY WORDS: Lipid sources, protein sparing, *Clarias gariepinus*

INTRODUCTION
The dietary protein utilisation using lipids as non-protein source might create a protein-sparing effect and can minimise the use of more costly protein as an energy source (Watanabe, 1982; Daniels and Robinson, 1986; De Silva *et al.*, 1991; Van der Meer *et al.*, 1997; Jantrarotai *et al.*, 1998; Company *et al.*, 1999). At high dietary levels, lipids may reduce fish growth, and adversely affect body composition (El-Sayed and Garling, 1988; Erfanullah and Jafri, 1998; Hanley, 1991; Bell *et al.*, 2004). To reduce feeding costs in aquaculture, approaches to reducing dietary protein levels or improving protein utilisation have been studied extensively however most studies have concentrated on increasing dietary energy levels, or lowering the protein to energy ratio, to reduce the amount of protein in fish diets and have been confined mainly to studies of growth performance such as in salmon, trout, channel catfish, red drum, tilapia, hybrid *Clarias* catfish, snakehead and carp (Reis *et al.*,1989; Hassan *et al.*,1995; Samantaray and Mohanty,1997; Jantrarotai *et al.*,1998). In general, dietary protein requirement seem to be of the order of 40% for *Clarias gariepinus* and hybrid *Clarias* species and somewhat lower for *Clarias batrachus* (Machiels and Henken, 1985; Degani *et al.*, 1989; Khan and Jafri,1990;Singh and Singh,1992; Jantrarotai *et al.*, 1996, 1998).

The objective of the present study is to investigate sparing effects of dietary lipids on the growth of the African catfish, *Clarias gariepinus*.

MATERIALS AND METHODS
Experimental System
The study was conducted in a recycling water system of the Department of Water Resources, Aquaculture and Fisheries Technology of School of Agriculture and Agricultural Technology, Federal University of Technology, Minna. Fingerlings of *Clarias gariepinus* of mean weight (2.50±0.04g) obtained from the hatchery unit of the Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology, Minna were used for the study. The fishes were randomly assigned into groups of 15 fishes per tank and each group was placed in an individual 50litres cylindrical tank as described. Water quality parameters were monitored throughout the period of study:Temperature:24.00 - 25.60°C pH:6.5-7.5,Conductivity(μ/cm)x10-2:74.12

-90.34, Dissolve oxygen(mg/L):4.50-5.60±3.00, Ammonia nitrogen (mg/L): 0.07-0.36±0.05, Nitrate nitrogen (mg/L):0.38-6.07±250.00, Nitrite nitrogen (mg/L):0.02-0.24±0.25.

Experimental Diets

Nine experimental diets and one commercial reference diet [(CRD)- Coppens Catfish feed from Netherland)] were used for the feeding trial. The experimental diets were formulated using equational method of two unknowns. The diets were formulated to contain three levels of protein (P) and three levels of lipid (L) ratios (40P: 10L, 35P: 15L and 30P: 20L %), using three lipid sources; Fish oil, Palm oil and Groundnut oil and Fishmeal as protein source. The experimental design 3x3 factorial of complete randomized design (CRD). The table of formulation and its proximate analysis is shown in Table 1. The fishes were fed twice daily between the hours 10.00 and 16.00 to apparent satiation for 8 weeks.

Experimental Analysis and Growth Parameters

Proximate analysis for moisture, crude protein, crude lipid and ash of carcass, feed ingredients and experimental diets were determined according to the methods of AOAC (1999). Final values for each group represent the arithmetic mean of the triplicates. Feed intake was monitored to measure average feed intake and their effects on growth. The growth and nutrient utilization parameters measured include weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), Apparent Net Protein Utilization (ANPU). The growth parameters were computed as stated below;

Mean weight gain = Mean final weight - mean initial weight

Specific Growth Rate (SGR) =

$$\frac{\text{Log } W_2 - \text{Log } W_1 \text{ X } 100}{T_2 - T_1}$$

Where, W_2 and W_1 represent - final and initial weight,

T_2 and T_1 represent - final and initial time

Feed conversion ratio - Feed fed on dry matter/fish live weight gain.

Protein efficiency ratio (PER) = Mean weight gain per protein fed

Protein intake (g) = Feed intake x crude protein of feed.

Apparent net protein utilization (ANPU %) = (P2 - P1) / Total protein consumed (g) x 100

Where, P1 is the protein in fish carcass (g) at the beginning of the study and P2 is the protein in fish carcass (g) at the end of the study.

Statistical Analysis

The experimental design was factorial and the data was subjected to one way analysis of variance to test their significant levels at 5% probability, the means were separated using Tukey's method and the regression coefficients were analysed using Minitab Release 14 while the graphs were drawn using the Microsoft excel window 2007.

RESULTS

Table 1 show the formulation and proximate analysis of feedstuffs used for the study. Table 2 shows the results of nutrient utilization. Mortality was recorded however, there were no pathological symptoms during the feeding trial. The fish oil based diets showed significance differences ($p<0.05$) in the mean weight gain (MWG) and specific growth rate (SGR) for the treatments, but significantly high ($p<0.05$) for 10: 40 (1.27g, 0.72 %). There were significant differences ($p<0.05$) in the feed conversion ratios (FCR) for all the treatments, which was lowest for 10:40. The groundnut oil based diets indicated significant differences ($p<0.05$) for all the treatments in the MWG and SGR, which were highest for 20: 30 L/P ratio (5.57g, 2.09 % respectively). There were significant differences ($p<0.05$) in FCR values for all the treatments, which was lowest for 20:30 L/P ratio (2.85). There were significant differences ($p<0.05$) in the MWG and SGR values for all the palm oil based diets but was highest for 15:35 L/P ratio (5.33g and 2.075 respectively). The FCR values showed no significant differences ($p>0.05$) between diets 10:40 and 15:35 L/P ratios, both of which were significantly lower than 20:30 L/P ratio.

The regression co-efficient for the palm oil based diets (Figure 1) indicated a moderately strong and significantly ($p<0.05$) positive relationship between the weight gain and the crude protein levels ($x = 1.19 + 0.06y$). The groundnut oil based diets showed weak negative significant ($p<0.05$) relationship between the weight gain and the dietary groundnut oil levels ($x = 3.14 - 0.259y$, $r^2 = 0.24$) and positive relationship with crude protein levels in the diets ($x=2.07 + 0.0191y$; $r^2=0.25$; $p<0.05$) (Figures 2 a, b). While, for the fish oil based diets, a weak positive significant ($p<0.05$) relationship was observed between the weight gain and the dietary fish oil levels in the diets ($x = -4.77 + 0.64 y$; $r^2 = 0.14$) and negative relationship with crude protein levels ($x = 25.5 - 0.59y$; $r^2=0.18$; $p<0.05$) (Figures 3a, b).

Nutrient Utilization

The protein efficiency ratio (PER) for fish oil based diets showed insignificant differences ($p>0.05$) between diets 15:35 and 20:30, which was highest for 10:40 (3.62). The apparent net protein utilization (ANPU) showed no significant differences for all the treatments but was highest for 10: 40 (73.83 %). However, the percentage survival was significant ($p<0.05$) for all the treatments but was highest for 20:30 L/P ratio.

Similarly, the PER values for groundnut oil based diets were significantly different ($p<0.05$) for all the treatments but highest for 20:30 (5.52). However, there were no significant differences ($p>0.05$) between the 15:35 and 20:30 L/P ratios, which were significantly higher ($p<0.05$) than 10:40 L/P ratio diet. There were no significant differences ($p>0.05$) between the 10:40 and 15:35 L/P ratios both of which were significantly higher ($p<0.05$) than 20:30 L/P ratio.

The PER and ANPU values for the palm oil based diets indicated significant differences ($p<0.05$) for all the treatments which were highest for 15:35 L/P ratio (8.78, 63.33% respectively). The survival percentage was significantly different ($p<0.05$) for all the treatments but lowest for 20:30 L/P ratio (Table 2).

Body Composition

There were significant differences ($p<0.05$) in some cases in the body compositions fed different lipid (L) sources at different protein (P) ratios. The fish oil based diets showed no significant difference ($p>0.05$) in the crude protein body compositions between diets 15:35 and 20:30 L/P ratio both of which were significantly lower ($p<0.05$) than 10:40 L/P ratio. The body lipid showed insignificant difference ($p>0.05$) between diets 15:35 and 20:30 L/P ratios, which were significantly higher ($p<0.05$) than 10:40 L/P ratio.

For the groundnut oil based diets, there were no significant difference ($p>0.05$) in body crude protein contents between diets 10:40 and 15:35 L/P ratios, which were significantly lower ($p<0.05$) than diet containing 20:30 L/P ratio. There were no significant differences ($p>0.05$) for all the treatments for fat, ash and moisture contents. However, the body fat was lowest for 20:30 L/P, diet containing 10:40 gave lowest body ash while lowest moisture content was obtained for 15:35 L/P ratio.

The palm oil based diets also showed insignificant differences ($p>0.05$) between diets 10:40 and 20:30 both of which were significantly lower ($p<0.05$) than 15:35 in body crude protein contents. However, there were significant differences ($p < 0.05$) among all the treatments, with diet 20:30 gave the lowest body fat. As for body ash, there were insignificant differences ($p>0.05$) between diets 10:40 and 15: 35 both of which were significantly lower ($p<0.05$) than diet 20:30 L/P ratio. Moreover, there were significant differences ($p<0.05$) for the treatments in moisture contents, which was lowest for 15:35 (Table 3).

DISCUSSION

Of the three lipid sources fed *Clarias gariepinus*, fish oil (FO) poorly spared protein. The groundnut oil spared the protein at the highest level of lipid level (20 %) while the palm oil (PO) based diets spared protein at 15:35. The poor protein sparing of fish oil in this study can be attributed to high concentration of omega 3 fatty acids in the fish oil (linolenic-18:3n-3) which was reported to suppress growth (Aderolu *et al.*, 2009; Jauncey, 1998; NRC, 1993). Kanazawa *et al.* (1980) reported depressed growth in tilapia when fed diet supplemented with 18:3n-3 or n-3 HUFA. Ng *et al.* (2001) also reported slight depressed growth and feed efficiency ratio in hybrid red tilapia fed with cod liver oil that is high in concentration of n-3 fatty acid. The regression co-efficient revealed the trend in the lipid- protein sparing in which there is decrease in weight gain with increase in lipid level for all the lipid sources except for the groundnut oil. The palm oil based diets spared protein at L:P ratio of 15:35. This shows the ability of *Clarias gariepinus* to utilize palm oil in its diets, which is in agreement with the findings of Ng *et al.* (2003); Lim *et al.* (2001) and Ng *et al.* (2000) who reported significant difference in the growth performance of *Clarias gariepinus* fed palm oil based diets.

CONCLUSION

The lipid sparing effects of protein was achieved with groundnut oil and palm oil at 20% and 15% inclusion level, thereby lowering the crude protein inclusion levels to 30% and 35% respectively. These levels of crude protein were found adequate for the growth of catfish thereby reducing the cost of feed and ultimately the total cost of production.

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Table 1: Formulation and its proximate analysis

	FISH OIL			GROUND NUT OIL			PALM OIL			CRD
Feedstuffs	Diet1	Diet2	Diet3	Diet4	Diet5	Diet6	Diet7	Diet8	Diet 9	Diet10
	10 L: 40P	15L:35P	20L:30P	10 L: 40P	15L:35P	20L:30P	10 L: 40P	15L:35P	20L:30P	
Fishmeal	40.73	29.70	16.95	40.73	29.7	16.95	0.73	29.70	16.95	
Fish oil	54.27	65.31	78.05	0.00	0.00	0.00	0.0	0.00	0.00	
G/Nut oil	0.00	0.00	0.00	54.27	65.31	78.05	0.00	0.00	0.00	
Palm oil	0.00	0.00	0.00	0.00	0.00	0.00	54.27	65.31	78.05	
V/Mineral	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	
Total	100	100.01	101	100	100.01	100	100	100.01	100	
Proximate	DIET1	DIET2	DIET3	DIET4	DIET5	DIET6	DIET7	DIET8	DIET9	DIET10
Crude protein	39.45	34.28	29.65	41.35	34.35	29.78	39.8	34.64	29.56	44.89
Crude fat	11.26	15.22	20.29	10.61	16.39	18.99	10.11	16.96	19.78	11.88
Crude fibre	8.51	5.42	4.81	8.60	6.76	5.32	8.71	7.65	6.43	5.62
Ash	18.25	17.96	16.37	17.23	15.32	14.10	19.30	8.23	16.21	10.12
NFE	21.41	15.58	26.10	21.17	23.89	28.90	20.76	29.98	26.79	18.26
Moisture	1.12	1.54	2.78	1.04	3.29	2.61	1.32	2.54	1.23	9.23
Total	99.69	100	100	99.8	100	102	100	100	99.61	100

Means on the same row carrying letters (s) with different superscript are significantly different from each other ($p<0.05$)

Table 1: **Formulation andits proximate analysis**

	FISH OIL			GROUND NUT OIL			PALM OIL			CRD
Feedstuffs	**Diet1 10 L: 40P**	**Diet2 15L:35P**	**Diet3 20L:30P**	**Diet4 10 L: 40P**	**Diet5 15L:35P**	**Diet6 20L:30P**	**Diet7 10 L: 40P**	**Diet8 15L:35P**	**Diet 9 20L:30P**	**Diet10**
Fishmeal	40.73	29.70	16.95	40.73	29.7	16.95	0.73	29.70	16.95	
Fish oil	54.27	65.31	78.05	0.00	0.00	0.00	0.0	0.00	0.00	
G/Nut oil	0.00	0.00	0.00	54.27	65.31	78.05	0.00	0.00	0.00	
Palm oil	0.00	0.00	0.00	0.00	0.00	0.00	54.27	65.31	78.05	
V/Mineral	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	
Total	**100**	**100.01**	**101**	**100**	**100.01**	**100**	**100**	**100.01**	**100**	
Proximate	DIET1	DIET2	DIET3	DIET4	DIET5	DIET6	DIET7	DIET8	DIET9	DIET10
Crude protein	39.45	34.28	29.65	41.35	34.35	29.78	39.8	34.64	29.56	44.89
Crude fat	11.26	15.22	20.29	10.61	16.39	18.99	10.11	16.96	19.78	11.88
Crude fibre	8.51	5.42	4.81	8.60	6.76	5.32	8.71	7.65	6.43	5.62
Ash	18.25	17.96	16.37	17.23	15.32	14.10	19.30	8.23	16.21	10.12
NFE	21.41	15.58	26.10	21.17	23.89	28.90	20.76	29.98	26.79	18.26
Moisture	1.12	1.54	2.78	1.04	3.29	2.61	1.32	2.54	1.23	9.23
Total	**99.69**	**100**	**100**	**99.8**	**100**	**102**	**100**	**100**	**99.61**	**100**

Means on the same row carrying letters (s) with different superscript are significantly different from each other ($p<0.05$)

Tabl e 2: Mean growth parameters for *Clarias gariepinus* fed with various lipid sources

Parameters	Fish oil			Groundnut oil			Palm oil			CRD	SD
	10L :40P	15L : 35P	20L: 30P	10L : 40P	15L : 35P	20L : 30P	10L : 40P	15L : 35P	20L : 30P		±
Initial Body Wt. (g)	2.58^{a} ±0.26	2.52^{a} ±0.26	2.54^{a} ±0.33	2.57^{a} ±0.24	2.52^{a} ±0.53	2.51^{a} ±0.21	2.52^{a} ±0.42	2.54^{a} ±0.20	2.37^{a} ±0.42	2.27^{b} ±0.07	0.30
Final Body Wt. (g)	3.85^{c} ±0.68	3.04^{bc} ±0.78	2.73^{bc} ±0.42	2.66^{bc} ±0.30	2.76^{bc} ±0.25	8.08^{a} ±8.72	5.19^{b} ±2.10	8.08^{a} ±7.91	2.73^{bc} ±0.80	2.99^{bc} ±0.41	3.81
Weight gain (g)	1.27^{c} ±0.93	0.52^{d} ±0.54	0.19^{cd} ±0.10	0.90^{d} ±0.06	0.23^{cd} ±0.35	5.57^{a} ±8.56	2.68^{b} ±2.43	5.53^{a} ±7.94	0.36^{cd} ±0.61	0.72^{d} ±0.48	3.80
SGR(%)	0.72^{c} ±0.48	0.34^{bc} ±0.52	0.15^{b} ±0.06	0.06^{b} ±0.04	0.13^{bc} ±0.05	2.09^{a} ±1.73	1.29^{ac} ±0.91	2.07^{a} ±1.68	0.25^{bc} ±0.03	0.49^{bc} ±0.29	0.90
FCR	1.21^{d} ±0.45	2.97^{c} ^{c}±1.78	3.64^{b} ±1.01	6.56^{a} ±0.00	4.61^{b} ±1.06	2.85^{c} ±2.27	1.00^{d} ±0.71	1.29^{d} ±1.16	2.45^{c} ±0.49	4.24^{b} ±2.72	1.58
PER	3.62^{c} ±1.26	2.37^{d} ±1.31	2.17^{d} ±3.21	0.32^{f} ±0.02	0.85^{c} ±0.19	5.52^{b} ±8.48	5.05^{b} ±4.58	8.78^{a} ±12.61	1.40^{de} ±0.28	0.61^{c} ±0.41	5.43
ANPU(%)	73.83^{a} ±0.01	9.17^{f} ±0.01	16.15^{e} ±0.01	17.75^{b} ±0.01	44.02^{c} ±0.00	44.02^{c} ±0.1	25.00d^{d} ±0.01	63.33^{b} ±0.01	15.73^{e} ±0.58	64.34^{b} ±0.05	0.18
Survival (%)	66.67^{b} ±20.82	73.33^{ab} ±5.77	80.00^{a} ±17.32	76.67^{ab} ±11.55	73.33^{ab} ±11.55	56.67^{c} ±40.41	40.00^{e} ±30.00	43.33^{d} ±28.87	10.00^{f} ±0.00	76.67^{a} ±5.77	21.06

Means on the same row carrying letter (s) with different superscript are significantly different from each other ($p<0.05$)

Table 3: Body composition for *Clarias gariepinus* fed different Lipid -Protein ratios for 8 weeks

		Fish oil			Groundnut Oil			Palm Oil				
Proximate Analysis (%)	Initial	10L:40P	15L:35P	20L:30P	10L:40P	15L:35P	20L:30P	10L:40P	15L:35P	20L:30P	CRD	SD ±
Crude protein	54.45[d] ±0.01	65.45[b] ±0.01	55.64[d] ±0.01	54.97[d] ±0.01	57.75[c] ±0.01	57.77[c] ±0.01	60.25[b] ±0.01	54.50[d] ±0.01	65.52[b] ±0.01	54.96[d] ±0.01	77.15[a] ±0.01	0.01
Crude fat	8.65[g] ±0.01	15.49[ef] ±0.21	22.00[d] ±0.01	20.88[d] ±0.01	20.04[e] ±0.01	24.56[c] ±0.01	17.00[f] ±0.01	30.08[a] ±0.01	26.55[b] ±0.01	25.05[c] ±0.01	6.29[h] ±0.01	0.01
Crude fibre	9.20[b] ±0.01	5.45[e] ±0.01	5.49[e] ±0.01	6.38[d] ±0.01	4.78[e] ±0.01	5.47[e] ±0.01	7.00[c] ±0.01	3.19[g] ±0.02	3.20[g] ±0.01	11.81[a] ±0.01	4.01[f] ±0.01	0.01
Ash	9.00[a] ±0.01	5.76[c] ±0.01	5.61[c] ±0.01	5.41[bc] ±0.01	6.98[b] ±0.01	7.00[b] ±0.01	8.29[a] ±0.01	3.53[d] ±0.02	3.71[d] ±0.01	4.80[bc] ±0.01	3.70[d] ±0.01	0.01
Moisture	15.25[a] ±0.01	6.99[d] ±0.01	9.86[b] ±0.01	8.10[c] ±0.01	8.33[c] ±0.01	5.08[de] ±0.01	6.55[cd] ±0.01	7.35[d] ±0.01	5.37[de] ±0.01	5.88[e] ±0.01	6.03[e] ±0.01	0.01

Means on the same row carrying letters (s) with different superscripts are significantly different from each other ($p<0.05$)

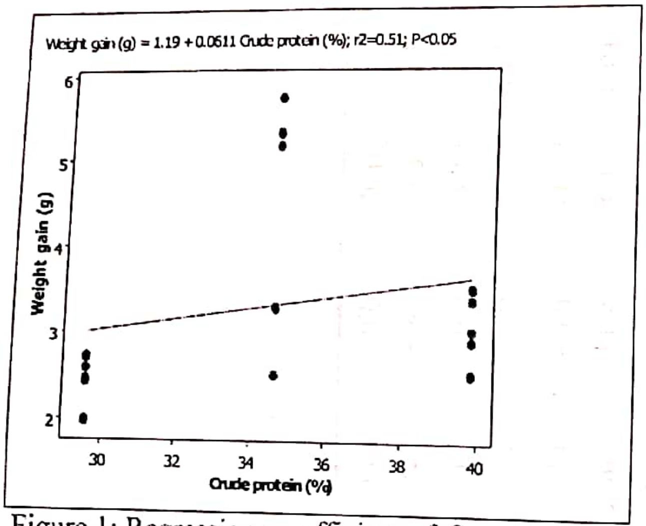

Figure 1: Regression co-efficient of *C. gariepinus* fed different levels of Palm oil/Protein ratios for 8 weeks

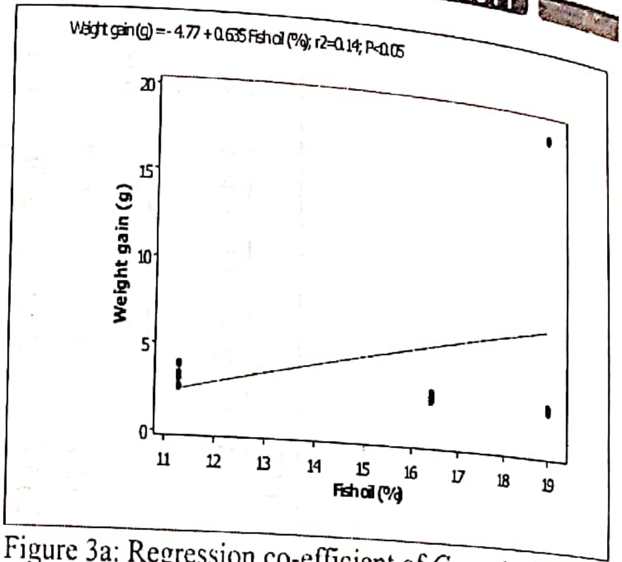

Figure 3a: Regression co-efficient of *C. gariepinus* fed different of Fish oil/protein based diets for 8 weeks

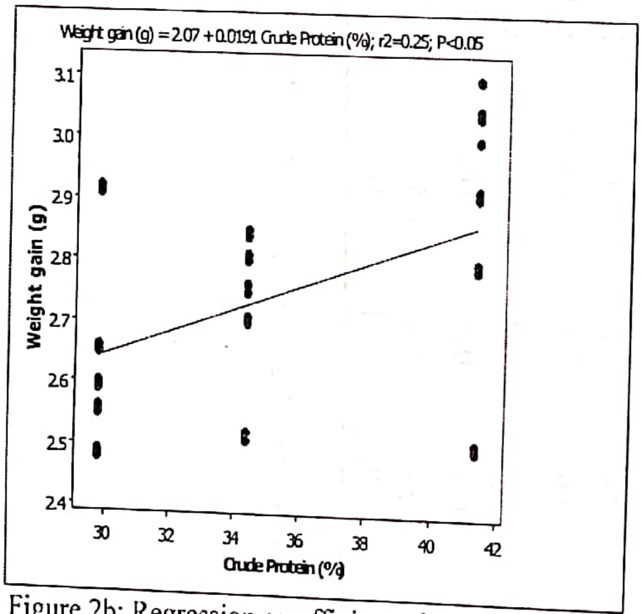

Figure 2b: Regression co-efficient of *C. gariepinus* fed Groundnut oil/protein ratio for 8 weeks

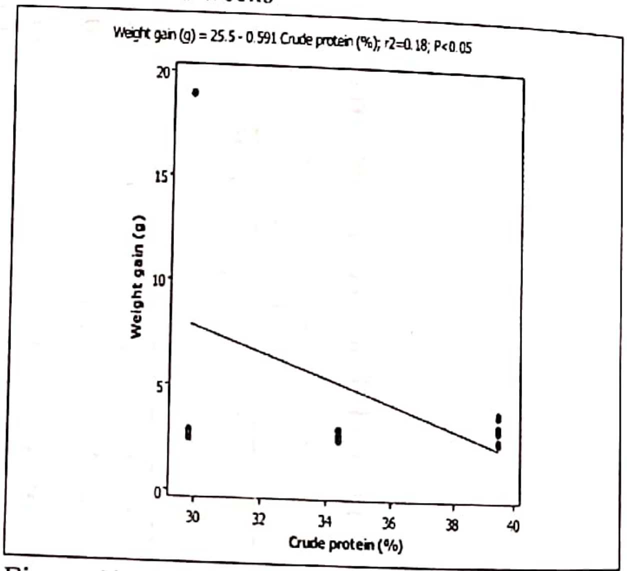

Figure 3b: Regression of *C. gariepinus* fed with Different levels of Fish oil/ Protein based diets for 8weeks

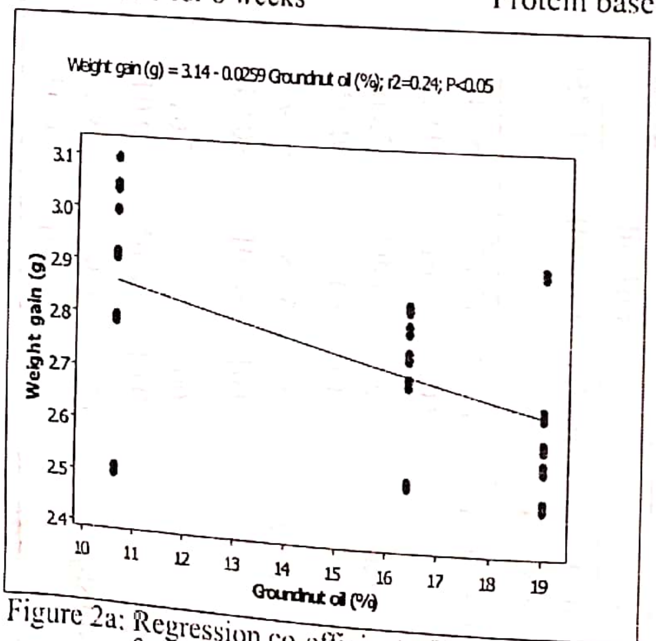

Figure 2a: Regression co-efficient of *C. gariepinus* fed different Groundnut oil/protein levels for 8 week