



Protein – Sparing Activity of Lipid and Carbohydrate in the Giant African Mudfish, *H. longifilis* Diets

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ABSTRACT: In two different experiments on lipid and carbohydrate requirement, *H. longifilis* was fed diets containing 13.68% to 24.66% of lipid, 17.00% to 20.86% of carbohydrates for 56 days. There was positive response in terms of growth of *H. longifilis* in the various diets fed in the experiments. There was significant variation ($P < 0.05$) in the specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU) and percentage survival (PS) in the fish fed varying lipid levels. As for the fish fed varying levels of carbohydrates, there was no significant variation ($P > 0.05$) in the SGR and FCR but there was significant variation ($P < 0.05$) in the PER, ANPU and PS. The protein/lipid ratio was 1.58 while the protein/carbohydrate ratio was 2.22. These ratios are very important in the formulation of *H. longifilis* feed for optimal growth of fish in that protein is spared for growth while the lipid and carbohydrate portions of the diet are used for energy production. @JASEM

In formulating diets for fish, it is pertinent to meet the entire nutrient requirement for its optimal growth. Inadequate protein in fish diet results in rapid reduction or assertion of growth, or loss of weight due to the withdrawing of protein from less vital tissues to maintain the functions of more vital ones (Wilson, 1984; Lovell, 1980a). On the other hand too much protein in the diet is broken down to produce energy (Wilson, 1984). There have been a few studies on the protein requirement of *H. longifilis* (Eyo, 1995 and Ovie 2003), the hybrid of *H. longifilis* and *C. garipinus* (Olufeagba, 1999). Fagbenro, et al. (1992) and Dada, et al. (2001) also reported the protein requirement for *H. bidorsalis*. Where all other nutrients are not provided in their appropriate levels, protein in the diet becomes an alternative source of energy. Protein is responsible for a large part of artificial fish feed cost. The expensive protein fraction should therefore be optimally utilised for growth rather than for maintenance of fish (Shiau and Lin 1993). NRC (1983), shows that diets formulated for fish are done considering the minimum protein requirement for optimal growth. The combination of other nutrients such as carbohydrates and lipid in the

diet therefore spares protein for growth. According to Ovie (2003), the lipid requirement of *H. longifilis* is 23.23% while the carbohydrate requirement is 19.5%. Shimeno, et al. (1978) reported poor growth and food efficiency in fish fed diets containing 10 to 20% digestible carbohydrate when compared to the control fish receiving no carbohydrate. According to Erfnullah and Jafri (1995), carbohydrates in fish diets limit as far as possible the catabolism of other dietary nutrients for energy. Khan (1991) reported that common carp had the maximum gain of 65% in live weight up to 30% dietary carbohydrate intake and thereafter a significant ($P = 0.01$; t-test) fall in weight was noted. Carbohydrates are the least expensive forms of dietary energy for humans and domestic animals but the utilisation by fish varies and is lower than domestic animals. Lipids are known to be vehicles for absorption of fat-soluble vitamins. They also exhibit many hormonal activities (NRC 1983). Tilapia species utilise complex carbohydrates such as dextrin or starch for growth more readily than simple sugars such as glucose (Tung and Shiau, 1991; Shiau and Lin 1993).

Knowledge of the optimal level of protein and the protein-sparing effects of non-protein nutrients such as lipids or carbohydrates can be used effectively in reducing feed costs (Shiau and Lin 1993). Adequate energy in the form of dietary lipid spares the protein to perform its function, i.e. build-up of tissues (Parazo 1990). According to Page and Andrew (1973), a high level of energy may produce fatty fish and decrease food consumption. El-Dahhar and Lovell (1995), stated that the optimum protein/digestible energy ratio is economically important for fish producers to produce maximum amounts of fish at minimal cost. This study reports on the ratios of protein/lipid and protein/carbohydrate in the formulation of *H. longifilis* diet that spares protein for growth.

MATERIALS AND METHODS

Experiment 1

Six practical diets formulated from soyabean (*Glycine max*), groundnut cake (*Arachis hypogea*), fish meal (*Pellomula afzeliusi*) and guinea corn (*Sorghum species*) containing 17.00%, 17.14%, 17.65%,

18.13%, 20.29% and 20.86% carbohydrate levels (Table 1) were fed to the fish in triplicate. The experiment was conducted in a mini flow-through system consisting of 18 plastic troughs supplied with 26.4 litres of biologically filtered water from an impounded reservoir.

Table 1: Ingredients and proximate composition of diets containing varying levels of carbohydrates

Diets	I(%)	II(%)	III(%)	IV(%)	V(%)	VI(%)
Ingredients						
Soyabean	28.73	28.92	28.82	28.63	28.53	28.44
Groundnut cake	28.73	28.92	28.82	28.63	28.53	28.44
Fishmeal	28.73	28.92	28.82	28.63	28.53	28.44
Guinea corn	2.81	0.08	1.45	4.18	5.54	6.91
Premix (vitamin and mineral)	2.00	2.00	2.00	2.00	2.00	2.00
Binder (cassava starch)	9.00	11.16	11.54	7.93	6.87	5.77
Proximate composition of feed (% as fed)						
Moisture	4.52	4.32	4.26	4.73	3.65	3.67
Crude protein (fixed as formulated)	45.36	45.36	45.36	45.36	45.36	45.36
Crude lipid	27.92	26.32	26.32	27.92	25.00	25.00
Ash	5.60	6.86	6.41	6.16	5.70	5.11
Crude fibre	16.42	16.09	17.32	16.98	17.32	16.10
NFE	0.58	1.05	0.33	1.15	2.97	2.94
Protein/ carbohydrate ratio	2.73	2.65	2.57	2.87	2.24	2.18

Biomix Vitamin and Mineral Premix (mg)

Experiment 2

Six practical diets formulated from the same ingredients as experiment 1 containing 13.68%, 19.66%, 23.15%, 23.81%, 24.02%, 24.66% of lipid levels (Table 2) were also fed in triplicates at 5% body weight for 56 days. They were fed thrice daily (8.00, 12.00 and 17.00hrs) with a third of the day's ration at each feeding time. The experiment was conducted in 18 plastic troughs in a mini flow-through system described in experiment 1. The experimental fish were acclimatised in hapas immersed in 2m x 2m concrete tanks for 24hrs. The fish were weighed individually and stocked 15 fingerlings per trough. Sampling was carried out in

each experiment biweekly and the amount of diet fed was adjusted accordingly. At the end of the experiment the fish were weighed. At the beginning and end of the experiment carcass analysis of the fish was measured according to AOAC (1990). Data obtained during the experiment were subjected to one-way analysis of variance using Turkey's test (Steel and Torrie 1960). Multifactor analysis of variance was utilised to check the significance of factors and their interactions in experiment. A second order polynomial relation (solid curve line) of weight gain and dietary requirement was done to determine the requirement level.

Table 2: Ingredients and proximate composition of diets containing varying levels of lipids fed to *H. longifilis*

Diets	I (%)	II (%)	III (%)	IV (%)	V (%)	VI (%)
Ingredients						
Soyabean	17.70	21.31	34.52	28.91	40.12	45.73
Groundnut cake	17.70	21.31	34.52	28.91	40.12	45.73
Fishmeal	38.95	32.06	18.30	25.18	11.41	4.53
Guinea corn	21.65	17.32	8.67	13.00	4.34	0.01
Premix (vitamin and mineral)	2.00	2.00	2.00	2.00	2.00	2.00
Binder (cassava starch)	4.00	6.00	5.99	2.00	2.01	2.00
Proximate composition of feed (% as fed)						
Moisture	3.30	3.50	4.10	3.92	5.31	4.32
Protein (fixed as formulated)	45.36	45.36	45.36	45.36	45.36	45.36
Lipid	13.68	19.66	23.15	23.81	24.02	24.66
Ash	2.70	5.00	6.12	5.60	6.25	5.68
Crude fibre	11.42	14.09	14.91	15.49	16.38	15.11
Protein/ lipid ratio	3.32	2.31	1.96	1.96	1.74	1.84

Biomix Vitamin and Mineral Premix (mg)

RESULTS

There was significant variation ($P < 0.05$) in the specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER), and apparent net protein utilisation (ANPU) (Table 3).

Table 3: Growth parameters fed varying levels of lipids for 56 days

Diet	MIW(g)	MFW(g)	MWG (g)	SGR (%)	FCR	PER	ANPU(%)	PS
I (13.68%)	0.50±0.01 ^a	1.09±0.02 ^a	0.59±0.06	1.4±0.07 ^a	8.89±0.21 ^{abcd}	0.04±0.004 ^a	4.73±0.10 ^b	100±0.0 ^c
II (19.66%)	0.49±0.01 ^a	2.28±0.09 ^b	1.79±0.39	2.54±0.19 ^b	3.10±1.22 ^a	0.54±0.01 ^{ab}	3.45±0.10 ^c	53.33±5.88 ^b
III (23.15%)	0.05±0.01 ^a	2.47±0.25 ^b	1.97±0.74	3.09±0.13 ^b	8.75±2.44 ^{abcd}	0.067±0.004 ^{ab}	6.97±0.02 ^d	30.00±3.33 ^{ab}
IV (23.81%)	0.51±0.01 ^a	2.34±0.21 ^b	1.83±0.37	2.73±0.17 ^b	3.44±0.24 ^{ab}	0.48±0.01 ^a	7.94±0.02 ^d	40.00±0.0 ^{ab}
V (24.66%)	0.50±0.01 ^a	2.57±0.12 ^b	2.07±0.38	2.81±0.15 ^b	4.01±0.55 ^{abc}	0.083±0.01 ^b	6.47±0.10 ^d	55.56±0.0 ^b
VI (26.02%)	0.05±0.01 ^a	2.38±0.0 ^b	1.87±0.0	1.21±0.0 ^a	3.09±0.71 ^a	0.043±0.001 ^a	5.600.20 ^c	33.33±0.0 ^{ab}
±SEM	0.01±0.004	0.5±0.09	0.5±0.25	2.57±0.77	0.72±0.07	0.21±0.004	1.48±0.06	23.43±2.40

Figures in the same column having the same superscript are not significantly different ($P > 0.05$)

The fish fed 24.66% lipid had a higher weight gain than all others. In using the polynomial regression second order curve of weight gain against protein/lipid ratio the point at which dy/dx is equal to zero i.e. the protein/lipid ratio requirement was 1.58

(Fig. 1). There was no significant variation ($P > 0.05$) in the SGR and FCR of the fish fed the varying levels of carbohydrates. The PER and ANPU showed significant variation ($P < 0.05$) (Table 4).

Table 4: Growth parameters of *H. longifilis* fed varying levels of carbohydrates for 56 days

Diet/% lipid	MIW(g)	MFW(g)	MWG (g)	SGR(%)	FCR	PER	ANPU(%)	PS
I (17.00)	0.49±0.02 ^a	3.17±0.49 ^a	2.68±	3.53±0.23 ^a	1.60±0.17 ^a	0.053±0.01 ^b	4.43±0.02 ^c	86.76±1.73 ^b
II (17.14)	0.50±0.01 ^a	2.69±0.17 ^a	2.19±0.36	3.23±0.09 ^a	2.40±0.12 ^a	0.049±0.004 ^a	8.70±0.10 ^f	68.89±1.16 ^{ab}
III (17.65)	0.47±0.002 ^a	3.20±0.01 ^a	2.73±0.45	3.43±0.02 ^a	1.71±0.37 ^a	0.053±0.01 ^{ab}	5.87±0.01 ^d	68.89±4.51 ^{ab}
IV (18.13)	0.48±0.02 ^a	2.31±0.04 ^a	1.83±1.32	2.80±0.04 ^a	3.55±1.38 ^a	0.083±0.01 ^a	3.42±0.02 ^b	46.67±7.00 ^c
V (20.29)	0.49±0.02 ^a	2.49±0.36 ^a	2.00±1.17	3.52±0.41 ^a	1.70±0.15 ^a	0.043±0.001 ^a	2.390±0.2 ^c	95.56±1.15 ^c
VI (20.89)	0.49±0.02	3.29±0.18 ^a	2.8±0.26	3.35±0.10 ^a	2.37±0.33 ^a	0.048±0.01 ^{ab}	5.20±0.20	80.00±0.58 ^b
±SEM	0.009±0.004	0.38±0.17	0.38±0.44	0.25±0.44	0.68±0.44	0.013±0.004	2.01±0.07	15.60±2.31

Figures in the same column having the same superscript are not significantly different ($P > 0.05$)

The body lipid of fish fed varying levels of lipid increased over the initial and there was significant variation ($P < 0.05$) in the body lipid (Table 5). There was also an increase in body protein, with the fish fed 23.51% lipid having a higher body protein than others. There was also significant variation ($P < 0.05$) in the body protein and the body lipid (Table 5). The highest body protein was found in the fish that was

fed 17.00% carbohydrate. The fish fed 20.86% carbohydrate (Table 6) had the highest body lipid. In plotting weight gain of fish fed varying carbohydrate level against protein/carbohydrate ratio in diet using polynomial regression third order curve, the protein/carbohydrate ratio requirement was found to be 2.22 (Fig. 2).

Table 5: Carcass proximate composition of *H. longifilis* fed varying dietary lipid levels for 56 days

Diet/% lipid	Percentage proximate composition			
	Moisture	Crude protein	Lipid	Ash
Initial	79.10±0.058 ^f	10.45±0.058 ^a	7.80±0.58 ^{bc}	2.10±0.058 ^b
I (13.68)	78.06±0.006 ^e	11.3±0.058 ^b	9.30±0.058 ^{cd}	2.17±0.069 ^b
II (19.66)	77.22±0.058 ^{ab}	11.45±0.058 ^b	9.17±0.064 ^c	2.34±0.055 ^b
III (23.15)	77.86±0.052 ^{de}	13.32±0.127 ^c	7.78±0.12 ^{bc}	2.24±0.116 ^b
IV (23.81)	77.51±0.167 ^{cd}	12.70±0.58 ^c	7.81±0.064 ^d	2.94±0.006 ^c
V (24.66)	77.10±0.058 ^a	13.10±0.058	8.61±0.064 ^d	1.47±0.064 ^a
VI (26.02)	77.99±0.00 ^c	12.97±0.012 ^{cd}	7.02±0.007 ^a	1.64±0.12 ^a
±SEM	0.62±0.05	1.02±0.03	0.77±0.03	0.45±0.04

Figures in the same column with the same superscript are not significantly different ($P > 0.05$)

Table 6: Carcass proximate composition of *H. longifilis* fed varying dietary carbohydrates levels for 56 days

Diet/ carbohydrate	%	Percentage proximate composition			
		Moisture	Crude protein	Lipid	Ash
Initial		79.10±0.058 ^e	10.45±0.061 ^a	7.80±0.00 ^b	2.10±0.058 ^a
I (17.00)		75.13±0.058 ^a	14.15±0.035 ^f	8.66±0.064 ^c	2.11±0.064 ^a
II (17.14)		75.94±0.00 ^b	13.16±0.052 ^e	10.16±0.058 ^e	2.69±0.058 ^b

III (17.65)	76.19 ± 0.058 ^b	12.37 ± 0.058 ^d	11.00 ± 0.033 ^f	2.14 ± 0.052 ^g
IV (18.13)	76.68 ± 0.17 ^c	11.92 ± 0.00 ^e	7.10 ± 0.00 ^a	3.27 ± 0.052 ^h
V (20.29)	76.20 ± 0.058 ^b	11.46 ± 0.122 ^b	8.50 ± 0.058 ^d	2.62 ± 0.058 ^b
VI (20.86)	78.30 ± 0.058 ^d	11.75 ± 0.104 ^{bc}	9.20 ± 0.058 ^e	2.79 ± 0.058 ^b
±SEM	1.30 ± 0.05	1.11 ± 0.04	1.24 ± 0.03	0.41 ± 0.004

Figures with the same superscript in the same column are not significantly different ($P > 0.05$)

Polynomial regression equation for the second order solid curve

$Y = -245.82 + 314.63x - 99.65x^2$ $r^2 = 0.96$ developed from microsoft excel was used in computing 1.58 as the protein/lipid ratio requirement of *H. longifilis*.

Polynomial regression equation for the third order curve

$Y = 12.46 - 1.48x + 9.01x^2 - 1.59x^3$ $r^2 = 0.56$ developed from microsoft excel was used in computing 2.22 as the protein/carbohydrate ratio requirement of *H. longifilis*.

DISCUSSION

Shiau and Lin (1993) stated that only a proper protein to energy ratio could spare protein in feed. According to Phillips (1972) and Prather and Lovell (1973) when excess protein in relation to energy is fed, the excess protein is used as an energy source. High protein diets caused reduced growth rate of channel catfish fed to satiation due to the low feed consumption (Mangalik 1986; Li and Lovell 1992a). According to Li (1989) and Reis, et al. (1989) low - protein diets (24 to 26 %) provide channel catfish with sufficient nutrient for maximum growth when fed to satiation. However juvenile *O. mossambicus* increased with dietary protein concentration up to 38 to 40% and thereafter decreased with higher concentration of protein in the diet (Jauncey 1982). The inclusion of excess energy in diets is known to produce fatty fish, reduce feed consumption and inhibit proper utilisation of other feedstuffs (Nose and Arai 1972; Takeda, et al. 1975). The protein/ lipid ratio for *H. longifilis* was 1.58 while the protein/carbohydrate ratio was 2.22. These ratios were

all obtained at optimum protein level. This however, does not compare well with Fynn - Aikins, et al. (1992) who observed protein-sparing effect when sub-optimal level of protein was provided. According to Shiau and Lin (1993) the optimum level of dietary protein does not necessarily lead to the most economical production of fish due to high cost of the protein component of the diet. The fact that the ratios were obtained at optimum protein level shows that the fish will grow best when diets are prepared with the ratios in consideration. In *Penaeus monodon* culture, Shiau and Lin (1993) showed that the dietary protein content can be decreased from the optimum level of 40% to 30% and increasing the starch content in the diet from 20% to 30%. Body lipid was highest with *H. longifilis* fed the least lipid. All others did not show any particular trend in relationship with the lipid supplied. Body protein also did not have any known relationship with the lipid supplied in the diet

although the fish fed diet III, 23.51% lipid had the highest body protein. The fish fed the least quantity of carbohydrates also had the highest body protein as the level of carbohydrate increased. The body lipid had no specific relationship with the level of carbohydrate fed although the highest body lipid was observed with fish fed 17.65% carbohydrate. According to El-Dahhar and Lovell (1995) carcass protein of Mozambique tilapia was not affected by increasing level of dietary energy, dietary protein or P/DE ratio of the diets. This compares well with the relationship between body lipid level of P/L ratio experiment in this study. This study has proved that protein is spared for growth of *H. longifilis* when the ratios of protein/lipid and protein/carbohydrates in its diets are appropriately combined.

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