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Optimum dietary carbohydrate to lipid ratio in stinging catfish, *Heteropneustes fossilis* (Bloch, 1792)

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Abstract
A feeding trial of 8-weeks was conducted in a static indoor rearing system to investigate the optimum carbohydrate to lipid ratio (CHO:L ratio) in stinging catfish, *Heteropneustes fossilis*. Five isonitrogenous (35% crude protein) and isoenergetic (17.06 kJ g^{-1} gross energy (GE)) fish meal based diets with varying carbohydrate to lipid (CHO:L g/g) ratios of 0.60, 0.98, 1.53, 2.29 and 3.44 for diets 1–5, were tested, respectively. The diets containing a fixed protein to energy ratio (P:E ratio) of 20.50-mg protein kJ^{-1} GE were fed to triplicate groups of 40 fish (per 70-L tank). Fish were fed 5% of their body weight per day adjusted fortnightly. Diet 1, containing 10% carbohydrate and 17% lipids with a CHO:L ratio of 0.60 produced the poorest ($p<0.05$) growth rates, feed and protein efficiency. Increasing carbohydrate content in the diets to 26% concomitant with a reduction in lipid content to 11% with a CHO:L ration of 2.29 of diet 5 significantly improved ($p<0.05$) growth rates, feed and protein efficiency. But did not differ with diet 4, containing CHO:L ratio 2.29. A further increase in dietary carbohydrate up to 31% and a decrease in lipids levels to 9% with a CHO:L ratio ranging from 2.29 to 3.44 (diet 4-5) did not significantly improve the fish performance. Apparent net protein utilisation (ANPU) of fish fed diet 5 was higher ($p<0.05$) than for diets 1& 2 but did not differ from diets 3 & 4. Higher lipid deposition ($p<0.05$) in whole body was observed with decreasing dietary CHO:L ratios as increasing lipid levels. Whole body protein of fish fed varying CHO:L diets did not show any discernible changes among the dietary treatments. This study revealed that *H. fossilis* can perform equally well on diets containing carbohydrate ranging from 26 to 31%, with 9 to 11% lipid or at CHO:L g/g ratio of 2.29–3.44.

Key words: *Heteropneustes fossilis*, Carbohydrate to lipid ratio, Protein utilization

Introduction

Carbohydrate and lipid are the major non-protein energy sources in fish diets and should be included at appropriate levels to maximise use of dietary protein for growth. Compared to dietary lipid, carbohydrates are relatively inexpensive and a readily available source of energy to many fish species. In warm water fish, dietary carbohydrate utilisation is considerably high, and incorporation of this nutrient may add beneficial effects to the pelleting quality of the diet and to fish growth (NRC 1993, Wilson 1994). High levels of dietary lipid may create problems in the pelleting quality of the diet (Jauncey and Ross 1982) as well as, adversely affect the fish carcass/body composition (Hanley 1991). Any imbalance with respect to non-protein energy sources and/or their levels of inclusion may have a direct effect on growth, conversion efficiencies, nutrient retention, and body composition. In addition, the levels and forms of dietary non-protein energy that can be incorporated in fish feeds are not fully understood.

In isonitrogenous and isoenergetic diets (i.e. the same P/E ratio) testing various carbohydrate to lipid 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 peak at some point between extremes of lipid and carbohydrate concentration, sometimes nearer the lipid or carbohydrate extreme. Thus rainbow trout (Brauge *et al.* 1994), and *Tilapia zilli* (El-Sayed and Garling 1988) utilise lipids better than carbohydrate, while *Oreochromis niloticus* (Shimeno *et al.* 1993) and African catfish, *Clarias gariepinus* (Ali and Jauncey 2004) utilises carbohydrates better than lipids. Other investigators report peaks in protein utilisation and protein efficiency ratio at intermediate levels of carbohydrate such as in walking catfish, *Clarias batrachus* (Erfanullah and Jafri 1998), hybrid *Clarias* catfish (Jantrarotai *et al.* 1994) and channel catfish, *Ictalurus punctatus* (Garling and Wilson 1977). The variability of results could reflect not only the different capabilities of fishes to utilise carbohydrate, but also the various ranges of carbohydrate to lipid ratios tested and the varying sources of these nutrients.

The stinging catfish, *Heteropneustes fossilis* an air-breathing cat fish, is widely distributed throughout Indian sub-continent, inhabiting swampy and marshy water bodies. The fish has a high marketability due to its superior nutritive value, on is cultured extensively. Lack of information on its nutritional requirements is a major constraint in the development of intensive culture of this fish. Information on the nutrition of this species seems limited only to its protein requirements and lipid utilisation (Akand *et. al.* 1989, Anwar and Jafri 1992, Firdaus 1993). Information on carbohydrate and lipid utilisation and appropriate carbohydrate to lipid ratio in *H. fossilis* fed fish meal based practical diets is restricted. It is thus imperative to determine the optimum dietary carbohydrate to lipid ratio in this species that produces the best growth. The objective of this study was to investigate dietary carbohydrate to lipid interactions and their influence on growth, feed and protein utilisation and body composition leading to optimisation of CHO:L ratio for *H. fossilis*.

Materials and methods

Experimental diets

Five experimental isonitrogenous (35% CP) and isoenergatic (17.06 kJg^{-1} GE) diets were formulated with a P/E ratio 20.50 mg protein kJ^{-1} GE based on results from previous studies of optimised P/E ratio of *H. fossilis* . The non-protein energy was adjusted by varying the ratios of lipid and carbohydrate in the diets so that the carbohydrate to lipid ratios (CHO:L, g/g) ranged from 0.60 to 3.44. Diets are referred to by two numbers separated by a "/", the first number being the percentage energy from dietary lipid and the second number the percentage energy from dietary carbohydrate. Composition of the experimental diets and their proximate composition are shown in Table 1.

Table 1. Formulation and proximate composition of the experimental diets (% dry weight) for cat fish, *Heteropneustes fossilis*

	Diets (Lipid/ Carbohydrate, % Energy)				
Diet number (Lipid / CHO), (%)	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Ingredients					
Fish meal[1]	40.00	40.00	42.00	48.50	54.50
Mustard oil cake[2]	17.00	17.00	17.00	9.00	2.00
Rich bran (auto)[3]	26.00	26.00	17.50	9.75	1.70
Starch[4]	00	5.17	12.62	21.70	30.70
Alpha cellulose[5]	12.00	9.06	8.38	8.55	8.60
Soybean oil[6]	2.50	0.27	00	00	00
Binder (Carboxymethyl cellulose)[7,8]	2.30	2.30	2.30	2.30	2.30
Vitamin and minerals premix[9]	0.20	0.20	0.20	0.20	0.20
Proximate composition					
Crude Protein	35.02	35.02	35.00	35.02	35.04
Crude Fat	17.05	15.58	13.40	11.16	8.92
Ash	12.42	12.42	11.58	10.88	10.13
Fiber	15.98	13.04	11.68	10.46	9.25
NFE	10.18	15.35	20.47	25.56	30.72
GE (kJ g^{-1})	17.05	17.06	17.05	17.07	17.06
P: GE ratio	20.50	20.50	20.50	20.52	20.52
CHO: L ratio (g/g)	0.60	0.98	1.53	2.29	3.44

NFE= Nitrogen free extractives, calculated as 100 – (% protein + % Lipid + % Ash + % Fibre);
GE= Gross energy content; P: GE ratio= Protein to energy ratio in mg protein kJ^{-1} of GE.
CHO: L g/g ratio= % wt. in CHO/ % wt. in lipid.
[1] Crude protein: 60.05; crude fat: 4.54; fibre: 0.65; ash: 26.87.
[2] Crude protein: 34.78; crude fat: 9.03; fibre: 8.25; ash: 9.16.
[3] Crude protein: 16.39; crude fat: 28.42; fibre: 11.10; ash: 14.05.
[4] Marck Ltd., India. [5] Sigma, UK. [6] Teer, City group, Bangladesh.
[7] Carboxymethyl cellulose – Sodium salt, high viscosity.
[8] BDH, Poole, UK. [9] Novartis (BD) Ltd.

The required amount of ingredients along with vitamin and mineral premix were weighed (Table 1) and mixed homogenously. During mixing, oil was gradually poured into the mixture to assure homogeneity. Adequate amount of water was added to moisten the mixture to get a definite dough texture and then the mixture was extruded through 1 mm diameter die of a pellet machine (Hobart mixture machine, Model A200). The resultant diets were broken into smaller pieces and then sun dried. The experimental diets were separately packed in air-tight polyethylene bag and stored in a deep freeze for further use.

Experimental system and animals

The experiment was conducted in a static indoor rearing system with 15 cylindrical fibre glass tanks (80 cm diameter, 75 cm deep, 70-L each). Artificial aeration was used to maintain an adequate level of dissolved oxygen in each test tank. About sixty percent of the water in the system was replaced biweekly to avoid accumulation of waste products. Water quality parameters such as temperature (26.00-35.50^{0}C), pH (6.80-7.90), dissolved oxygen (5.70-7.80 mg/L) and ammonia (0.12-0.30 mg/L) remained within acceptable ranges for stinging catfish, *Heteropneustes fossilis* (Viveen *et al.* 1985; Hoffman *et al.*1991). Seven hundred 10-week old (average weight 1.72±0.02 g) shing fingerlings were collected from local fish vendors of Mymensingh. The collected fish were given a prophylactic treatment with salt (3% NaCl) solution for 10 minutes. During treatment sufficient oxygen supply was maintained through artificial aeration. Before starting the experiment the fish were acclimatized to the experimental condition for one week.

Experimental procedure

Fish were randomly assigned into groups of 40 per 70-L cylindrical fibre glass tank. Each dietary treatment had three replications and the experiment was conducted for 8 weeks. The fish were individually weighed at the start and at the end of the experiment and bulk-weighed by tank fortnightly in between. Fortnightly bulk weights were used to adjust the daily feed ration for the following 2 weeks. The fish were offered the test diets three times daily at the rate of 5% of their body weight and sub-divided into three equal feeds at 9.30, 13.00 and 17.00 h. Prior to weighing, fish were caught with a fine mesh scoop net and excess water was then removed from fish body by gently blotting on a soft tissue paper. Weight of fish in each sampling was measured by bulk weighing them using a digital electronic balanc. The sampled fish were handled very carefully. The experimental tanks were washed and cleaned during sampling time. At the onset of the experiment, 15 fish were sacrificed for analysis of initial carcass composition. At the termination of the experiment, 4 fish were taken from each replication for determination of whole body composition.

Analytical methods and analysis of data

Proximate composition of diet ingredients, diets and whole body fish (carcass) were analysed in triplicate following AOAC (1990) methods. Nitrogen-free extract (NFE) was calculated by difference. Gross energy was calculated according to Jauncey (1998).

Specific growth rate (SGR), %weights gain, food conversion efficiency (FCR), protein efficiency ratio (PER) and apparent net protein utilisation (ANPU) were calculated as follows:

SGR (%/day) = [(Ln. Final body weight – Ln. Initial body weight)/days x 100]
% Weight gain = (Final body weight – Initial body weight/Initial body weight) x 100
FCR= Food fed (g dry weight)/ Live weight gain (g)
PER = Live weight gain (g)/Crude protein fed (g dry weight)
ANPU(%) = (Final carcass protein – Initial carcass protein)/Total dry protein consumed x 100

The growth performance, feed utilization, and whole body composition data were analyzed using one way ANOVA. Paired mean comparisons among the treatments were made using Duncan's Multiple Range Tests (Duncan 1955). A significance level of ($p<0.05$) was used. Standard deviation (±SD) was calculated to identify the range of means. Percentage data were arc-sine transformed (Zar 1984) prior to ANOVA and reversed afterward.

Results

No mortality nor external clinical symptoms occurred in any treatment during the study. Growth responses, feed and protein utilisation of fish fed the experimental diets are shown in Table 2. The increased in average fortnightly fish weight is shown in Fig. 1. There was a trend of increasing growth with increasing inclusion of dietary carbohydrate and concomitant reduction dietary lipid level (increasing CHO:L ratio). Growth performances in terms of weight gain, %weight gain and SGR of fish fed diet 5 was significantly higher ($p<0.05$) than fish fed diets 1, 2 and 3, but did not differ ($p>0.05$) from diet 4 (Table 2). Food conversion ratio (FCR) values were better as the dietary carbohydrate level increased with concomitant reduction dietary in lipid level. Fish fed diet 5, containing CHO:L ratio of 3.44 g/g showed significantly ($p<0.05$) superior FCR but did not differ ($p>0.05$) from diet 4 (Table 2).

Protein utilisation efficiencies on the basis of PER and ANPU increased significantly ($p<0.05$) with increasing CHO:L ratio. Fish fed diet 5, significantly ($p<0.05$) highest PER value but did not differ ($p>0.05$) from diet 4. ANPU fish fed diet 5 was significantly higher ($p<0.05$) than diets 1 and 2 but did not differ ($p>0.05$) from diets 3 and 4 (Table 2). Except for body lipid content, whole body composition (body protein and ash) was not affected ($p>0.05$) by the dietary treatments. There was an overall trended of decreasing carcass lipid with decreasing inclusion level of dietary lipid (increasing CHO:L ratio, g/g). Body lipid content of fish fed diet 2 was significantly ($p<0.05$) higher than that of fish fed diets 4 and 5 (containing the lower dietary lipid) but did not differ from diets 1 and 3 (Table 3).

Table 2. Mean growth performance, feed and protein utilization of *Heteropneustes fossilis* fed diets containing various lipid to carbohydrate ratios for 8 weeks

	Diets				
Diet number (Lipid / CHO), (%)	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Initial body wt. (g)	1.72^{a} ±0.02	1.72^{a} ±0.03	1.73^{a} ±0.01	1.72^{a} ±0.02	1.73^{a} ±0.01
Final body wt. (g)	5.78^{c} ±0.11	7.08^{b} ±0.04	7.55^{b} ±0.08	8.35^{a} ±0.07	8.75^{a} ±0.10
Weight gain (g)	4.06^{c} ±0.08	5.36^{b} ±0.06	5.82^{b} ±0.08	6.64^{a} ±0.06	7.03^{a} ±0.11
Weight gain (%)	236.72^{c} ±2.02	311.41^{b} ±8.81	336.42^{b} ±1.52	386.90^{a} ±2.90	405.82^{a} ±1.99
Specific growth rate (SGR) (% day)	2.43^{c} ±0.01	2.83^{b} ±0.04	2.95^{b} ±0.02	3.17^{a} ±0.01	3.24^{a} ±0.02
Food conversion ratio (FCR)	1.80^{a} ±0.02	1.66^{b} ±0.01	1.59^{b} ±0.10	1.35^{c} ±0.03	1.29^{c} ±0.05
Protein efficiency ratio (PER)	2.75^{c} ±0.09	2.79^{c} ±0.10	2.90^{ab} ±0.13	2.98^{ab} ±0.09	2.83^{a} ±0.10
Apparent net protein utilization (ANPU, %)	33.45^{c} ±0.44	35.70^{c} ±1.55	42.52^{ab} ±2.10	44.50^{ab} ±3.05	45.80^{a} ±3.20

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p<0.05$).

Table 3. Whole body composition (% wet wt. basis) of *Heteropneustes fossilis* at the start and end of the experiment

		Diets				
Diet number (Lipid/CHO), (%)	Initial (all fish)	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Moisture	78.16	73.85^{a} ±0.16	74.15^{a} ±0.55	73.30^{a} ±0.15	74.80^{a} ±0.65	74.98^{a} ±0.09
Crude Protein	14.24	16.60^{a} ±0.51	15.85^{a} ±0.43	16.75^{a} ±0.37	16.48^{a} ±0.74	16.58^{a} ±0.63
Crude Lipid	2.25	4.65^{ab} ±0.45	4.98^{b} ±0.85	4.79^{ab} ±0.47	3.64^{ab} ±0.40	3.48^{a} ±0.52
Ash	2.96	3.32^{a} ±0.70	3.24^{a} ±0.07	3.30^{a} ±0.23	3.15^{a} ±0.56	3.18^{a} ±0.28

Note: Values are ± SD of three replications. Figures in the same row having different superscript are significantly different ($p<0.05$).

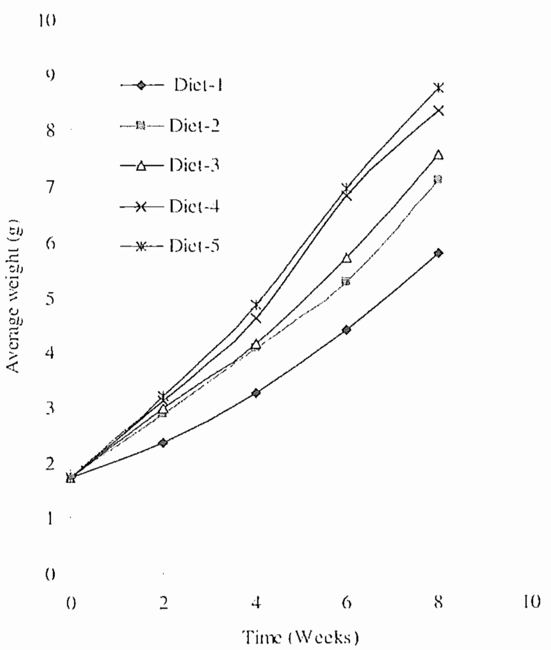

Fig. 1. The mean fortnightly growth response of *Heteropneustes fossilis* fed diets containing various lipid to carbohydrate ratios for 8 weeks
Diets 1, 2, 3, 4 and 5 contained CHO:L ratio (g/g) 0.60, 0.98, 1.53, 2.29 and 3.44

Discussion

The results of the present study demonstrated that growth performance and feed conversion efficiency are influenced by the dietary carbohydrate to lipid ratios in *H. fossilis*. From the growth performance data it observed that the the optimal dietary CHO:L ratio in *H. fossilis* which produced good growth rate and feed utilisation ranged from 2.29 to 3.44, which is smaller than the optimum ranges reported for African cat fish, hybrid *Clarias* catfish and walking catfish. In African catfish diets containing isonitrogenous (40% CP) and isocaloric (20 kJ g^{-1}GE), with CHO:L ratios ranging from 1.7 to 3.40 reported the production of fish with significantly improved growth performance and feed utilisation (Ali and Jauncey 2004). Jantrarotai *et al.* (1994) who fed hybrid *Clarias* catfish isonitrogenous (33% CP), isocaloric (12 kJ g^{-1} DG) diets containing CHO:L ratios ranging from 3.83 to 11.24, reported the production of fish with no significant difference in weight gain, feed conversion, or protein and energy deposition. Similarly, in channel catfish diets containing 24% CP and 12 kJ g^{-1} ME, with CHO:L ratios ranging from 0.45 to 4.5 there were no significant effects on fish performance (Garling and Wilson 1977). An investigation in walking catfish (*Clarias batrachus*) diets containing 40% CP and 14 kJ g^{-1} ME, with CHO:L ratios ranging from

0.02 to 3.38 reported the production of fish with significantly improved growth performance and feed utilisation (Erfanullah and Jafri 1998).

In the present study, the differences observed in optimal ranges of dietary CHO:L ratios from other species mentioned above due to different isonitrogenous, isocaloric diets containing varying levels of non-protein energy (varying CHO:L ratios). The differences also observed in growth and feed or protein efficiency indicate on ability of the stinging cat fish, *H. fossilis* to adapt to increasing levels of dietary carbohydrate (26-31%) which appears to be lower than the African cat fish (38%) (Ali and Jauncey 2004) but almost similar that reported for channel catfish (28%) (Garling and Wilson 1977) and walking catfish (27%) (Erfanullah and Jafri 1998).

Reduced growth rate and feeding or protein efficiency was found in stinging cat fish, *H. fossilis* fed the highest-lipid lowest-carbohydrate of diet 1, containing 10% carbohydrate and 17% lipid (CHO:L ratio 0.60). This could be the result of reduced feed consumption by fish due to the high dietary lipid level because of excessive food energy. This may prevent intake of the necessary amounts of protein and other nutrients required for maximum growth. Similar observations have been reported for African cat fish (Ali and Jauncey 2004), walking catfish (Erfanullah and Jafri 1998) and hybrid Clarias catfish (Jantrarotai *et al.* 1994). Crude fibre content (14%), as cellulose, in diet 1 is unlikely to be the cause of poor performance of *H. fossilis*. Since the diets were isonitrogenous and isoenergetic, the increase in PER and ANPU with increasing dietary carbohydrate (corresponding to increasing CHO:L ratios) could be attributed to the relative amounts of non-protein energy sources. This may indicate that *H. fossilis*, despite being omnivorous, can utilise dietary carbohydrate more efficiently than lipid although it is difficult to be certain. Because the diets were isoenergetic, higher lipid levels could be attained only by increasing the fiber content of the diet. There are several reports indicating that high dietary fiber levels reduce the utilisation of other nutrients (Hilton and Atkinson 1982; Anderson *et al.* 1984), but this view has been challenged (Jantrarotai *et al.* 1994). Thus, it remains unclear if reduced growth in fish fed the high lipid diets was due to inefficient lipid utilisation by fish, as compared with carbohydrate utilisation, or to the deleterious effects of the high dietary fiber level.

The different CHO:L ratios of the experimental diets had no effect on protein and ash content of the whole body of *H. fossilis*. However, increasing dietary lipid to 13% or higher (diets 1–4) resulted in increased total body lipid. Similar results have been reported in walking catfish, *C. batrachus* (Erfanullah and Jafri 1998), African cat fish (Ali and Jauncey 2004), hybrid Clarias catfish (Jantrarotai *et al.* 1994) and channel catfish (Garling and Wilson 1977). The inverse relationship between dietary CHO and whole body lipid content was interesting, since increased CHO did not produce undesirable fat accumulation in the body of the fish. This is in agreement with results reported for walking catfish, *C. batrachus* (Erfanullah and Jafri 1998), African cat fish (Ali and Jauncey 2004), hybrid Clarias catfish (Jantrarotai *et al.* 1994), channel catfish (Garling and Wilson 1977), tilapia (El-Sayed and Garling 1988) and Indian major carps (Erfanullah and Jafri 1998b).

On the basis of growth performance, feed and protein utilisation and whole body composition, it may be stated that the diet 5 of 40% lipid energy (8.92% lipid), 60% carbohydrate energy (30.72% carbohydrate) and carbohydrate to lipid ratio (CHO:L ratio g/g) of 3.44 performed best. In conclusion, this study reveals that stinging cat fish, *Heteropneustes fossilis* can perform equally well on isonitrogenous and isocaloric diets (35% protein, 17.05 kJ g^{-1} GE) containing carbohydrate ranging from 26 to 31% with 9 to 11% lipid or at CHO:L ratios of 2.29 to 3.44.

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