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Full Length Research Paper

Effect of dietary carbohydrate to lipid ratios on growth, feed conversion, protein utilisation and body composition in climbing perch, *Anabas testudineus*

M. Z. Ali*, M. Zaher, M. J. Alam and M. G. Hussain

Bangladesh Fisheries Research Institute, Freshwater Station, Mymensingh- 2201, Bangladesh, India.

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An 8-week feeding trial was conducted in a static indoor rearing system with 15 cylindrical fibre glass tanks (80 cm diameter, 75 cm deep, 70-L each) to investigate the optimum carbohydrate to lipid ratio (CHO:L ratio) in climbing perch, *ANABAS TESTUDINEUS* (2.15 ± 0.07 g). Five isonitrogenous (40% crude protein) and isoenergetic (17.71 kJ g^{-1} gross energy (GE)) fishmeal based diets with varying carbohydrate to lipid (CHO: L g/g) ratios of 0.58, 0.99, 1.53, 2.29 and 3.00 diets 1 to 5, respectively were tested. These diets had a fixed protein to energy ratio (P : E ratio) of 22.60-mg protein kJ^{-1} GE. They were fed to triplicate groups of 50 fish at 5% body weight per day and the feed quantity was adjusted fortnightly. Diet 1, containing 11% carbohydrate and 17% lipid with a CHO: L ratio of 0.58 produced the poorest (P<0.05) growth rate, and feed and protein efficiency. Increasing dietary carbohydrate to 24%, concomitant with a reduction in lipid content to 10.50% that resulted in a CHO: L of 2.29 significantly improved (P<0.05) growth rate, and feed and protein efficiency. But, these did not differ from diets 2, 3 and 5, containing CHO:L ratios of 0.99, 1.53 and 3.00. Apparent net protein utilization (ANPU) in fish fed diet 5 was significantly higher (P<0.05) than those receiving diets 1 and 2, but did not differ from that of diets 3 and 4. Higher lipid deposition (P<0.05) in whole body was observed with decreasing dietary CHO: L ratios and increasing lipid levels. Whole body protein of fish fed varying CHO: L diets did not show any discernible variations among the dietary treatments. This study revealed that climbing perch, *ANABAS TESTUDINEUS* can perform equally well on diets containing carbohydrate ranging from 14.43 to 28.81%, with 9.60 to 14.64% lipid or CHO: L g/g ratios of 0.99 to 3.00.

Key words: Climbing perch, *Anabas testudineus*, carbohydrate to lipid ratio, protein utilization, dietary lipid, carbohydrate level.

INTRODUCTION

In aquaculture, prime consideration is generally given to the dietary protein component to produce optimal fish growth. Equally important is the inclusion of appropriate levels of non-protein energy sources that determines the efficiency of protein utilisation (Wilson and Halver, 1986). Carbohydrate and lipid are the major non-protein energy sources in fish diets. Any imbalance with respect to non-protein energy sources and /or levels of inclusion may have a direct effect on growth, conversion efficiencies, nutrient retention, and body composition. Interestingly, no requirement for dietary carbohydrate has been demonstrated in fish, although certain species exhibit reduced growth rates when fed carbohydrate-free diets (Wilson, 1994). Therefore, carbohydrate and lipid in fish diet should be included at appropriate levels to maximise the use of dietary protein for growth. In testing isonitrogenous and isoenergetic diets (that is, the same P/E ratio) with various carbohydrates to lipid ratios, the varying net protein utilization and protein efficiency ratio reflects the ability of fish to use these nutrients to spare protein. In general, net protein utilization and protein efficiency ratios peak at some point between extremes of dietary lipid and carbohydrate, sometimes nearer the

*Corresponding author: E-mail: zulfikar_bfri@yahoo.com. Tel: +88-01711 780422. Fax: +880-91 66559/ 62628.

Table 1. Formulation and proximate composition of the experimental diets (% dry weight) for climbing perch, *A. testudineus* (Thai koi).

Diet number (Lipid / CHO), (%)	Diet				
	1 (80/20)	2 (70/30)	3(60/40)	4 (50/50)	5(40/60)
Ingredient					
Fishmeal[1]	47.25	48.50	55.60	61.00	64.00
Mustard oil cake[2]	20.00	20.00	10.55	4.00	00
Rich bran (auto)[3]	22.00	16.80	10.00	2.90	00
Starch[4]	00	6.20	15.00	23.35	29.85
Alpha cellulose[5]	7.35	6.00	6.35	6.25	3.65
Soybean oil[6]	0.90	00	00	00	00
Binder (Carboxymethyl cellulose)[7,8]	2.30	2.30	2.30	2.30	2.30
Vitamin and minerals premix(Megavit- Aqua)[9]	0.20	0.20	0.20	0.20	0.20
Proximate composition					
Crude protein	40.06	40.03	40.15	40.08	40.17
Crude fat	16.71	14.64	12.52	10.50	9.60
Ash	13.44	12.93	12.33	11.65	11.39
Fiber	11.26	9.45	8.45	7.21	4.03
NFE	9.66	14.43	19.16	24.04	28.81
GE (kJ g^{-1})	17.71	17.71	17.69	17.72	18.19
P:GE ratio	22.60	22.60	22.63	22.59	22.01
CHO: L ratio (g/g)	0.58	0.99	1.53	2.29	3.00

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: 62.50; crude fat: 15.00; fibre: 0.60; ash: 17.80. [2] Crude protein: 36.00; crude fat: 15.00; fibre: 8.25; ash: 9.70. [3] Crude protein: 15.00; crude fat: 26.00; fibre: 9.10; 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.

lipid or carbohydrate extreme.

Climbing perch, *Anabas testudineus* (Thai koi) is a promising species for aquaculture by virtue of its omnivorous feeding habit, air-breathing characteristics and rapid growth rate. The fish commands high demand and market price due to its superior nutritive value and delicious taste. It is hardy, can survive in adverse aquatic environments and can be cultured at high stocking densities. *A. testudineus* is a newly introduced species in aquaculture of Bangladesh; lack of information on its nutritional requirements is a major constraint in the development of intensive culture of this fish. A recent survey showed that more than 50% Thai koi culture farmers have lost due to high feed costs (Tihn, 2003). Information on the nutrition of this species seems limited only to its protein requirement and lipid utilisation. Knowledge on carbohydrate and lipid utilization and appropriate carbohydrate to lipid ratio (CHO: L) in climbing perch, fed fish meal based practical diets is restricted. It is, thus, imperative to determine the optimum dietary carbohydrate to lipid ratio that produces the best growth in this species. The objective of this study was to investigate dietary carbohydrate to lipid interactions and their influence on growth, feed and protein utilization and body composition, in order to determine the optimum CHO:L ratio for climbing perch.

MATERIALS AND METHODS

Experimental diets

Five isonitrogenous (40% CP) and isoenergatic (17.71 kJg^{-1} GE) diets were formulated with a P/E ratio 22.60 mg protein kJ^{-1} GE based on the results from previous studies of optimised P/E ratio for *A. testudineus.* 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 0.58 to 3.00. 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. The required amount of ingredients along with vitamin and mineral premix were weighed 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 pellets were made into bite size to be suitable for the fish. 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

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

Diet number (Lipid / CHO), (%)	Diet				
	1 (80/20)	2 (70/30)	3 (60/40)	4 (50/50)	5 (40/60)
Initial body wt. (g)	2.18^{a}± 0.04	2.15^{a} ± 0.07	2.15^{a} ± 0.07	2.15^{a}± 0.07	2.15^{a} ± 0.07
Final body wt. (g)	9.45^{b} ± 0.07	10.82^{ab}± 0.05	11.40^{ab} ± 0.14	12.65^{a} ± 0.07	10.82^{ab} ± 0.09
Weight gain (g)	7.28^{b} ± 0.11	8.67^{ab} ± 0.12	9.25^{ab} ± 0.07	10.50^{a} ± 0.14	8.67^{ab} ± 0.02
Weight gain (%)	334.57^{b} ± 10.32	403.34^{ab} ± 18.86	430.42^{ab} ± 10.87	488.75^{a} ± 22.65	403.23^{ab} ± 12.27
Specific growth rate (SGR) (% day)	2.45^{b} ± 0.04	2.70^{ab} ± 0.06	2.78^{ab} ± 0.03	2.96^{a} ± 0.06	2.69^{ab} ± 0.04
Food conversion ratio (FCR)	1.88^{a} ± 0.03	1.72^{ab} ± 0.02	1.45^{bc} ± 0.08	1.35^{c} ± 0.04	1.75^{ab} ± 0.05
Protein efficiency ratio (PER)	2.70^{a} ± 0.08	2.70^{a} ± 0.10	2.80^{a} ± 0.05	2.85^{a} ± 0.07	2.66^{a} ± 0.08
Apparent net protein utilization (ANPU, %)	33.45^{c} ± 0.44	35.70^{bc} ± 1.55	42.52^{ab} ± 2.10	44.50^{ab} ± 3.05	45.80^{a} ± 3.20

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

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 the test tanks. About sixty percent of the water in the system was replaced biweekly to avoid accumulation of waste. Water quality parameters such as temperature (25.00 to 34.60°C), pH (6.70 to 7.80), dissolved oxygen (5.40 to 7.50 mg/L) and ammonia (0.12 to 0.29 mg/L) remained within acceptable ranges for climbing perch (Viveen et al., 1985; Hoffman et al., 1991). Eight hundred 12-week old (average weight 2.25 ± 0.07 g) *A. testudineus* fingerlings collected from the Freshwater Station, of Bangladesh Fisheries Research Institute, Mymensingh were given a prophylactic treatment in 3% NaCl solution for 10 min, maintaining sufficient oxygen supply through artificial aeration. Before starting the experiment the fish were acclimatized to the experimental conditions for one week.

Experimental procedure

Fish were randomly assigned into groups of 50 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 end of the experiment and bulk-weighed by tank fortnightly in between, using a digital electronic balance (KERN, Model No EMB-2000-0). 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 9.30, 13.00 and 17.00 h, at the rate of 5% body weight, sub-divided equally. Prior to weighing, fish were caught with a fine mesh scoop net and excess water from fish body was removed by gently blotting on a soft tissue paper. The experimental tanks were cleaned during the 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) was analysed in triplicate following AOAC (1990) methods. Nitrogen-free extract (NFE) was calculated by the difference method of Hastings (1976). 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 × 100]

% Weight gain = (Final body weight - Initial body weight / Initial body weight × 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 × 100

The growth performance, feed utilization, and whole body composition data were analysed 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 afterwards.

RESULTS

Neither mortality nor external clinical symptoms occurred in any treatment during the study. Growth responses and feed and protein utilization of fish fed the experimental diets are shown in Table 2. The average increase in fortnightly fish weight is shown in Figure 1. There was a trended of increasing growth with increased inclusion of dietary carbohydrate and concomitant reduction in dietary lipid level (increasing CHO:L ratio). This trend was not maintained above CHO: L ratio of 2.29; diet 4 (carbohydrate 24.04% and lipid 10.50%), produced the best fish growth. Growth performances in terms of % weight gain and SGR of fish fed diet 4 was significantly higher (P<0.05) than those fed diets 1, but did not differ from diets 2, 3 and 5 (Table 2). Food conversion ratio (FCR) values were better as the dietary carbohydrate level increased with concomitant reduction in dietary lipid level. Fish fed diet 4, containing CHO: L ratio of 2.29 g/g showed significantly (P < 0.05) superior FCR, but did not differ from diet 3 (Table 2).

Protein utilization efficiencies on the basis of PER and

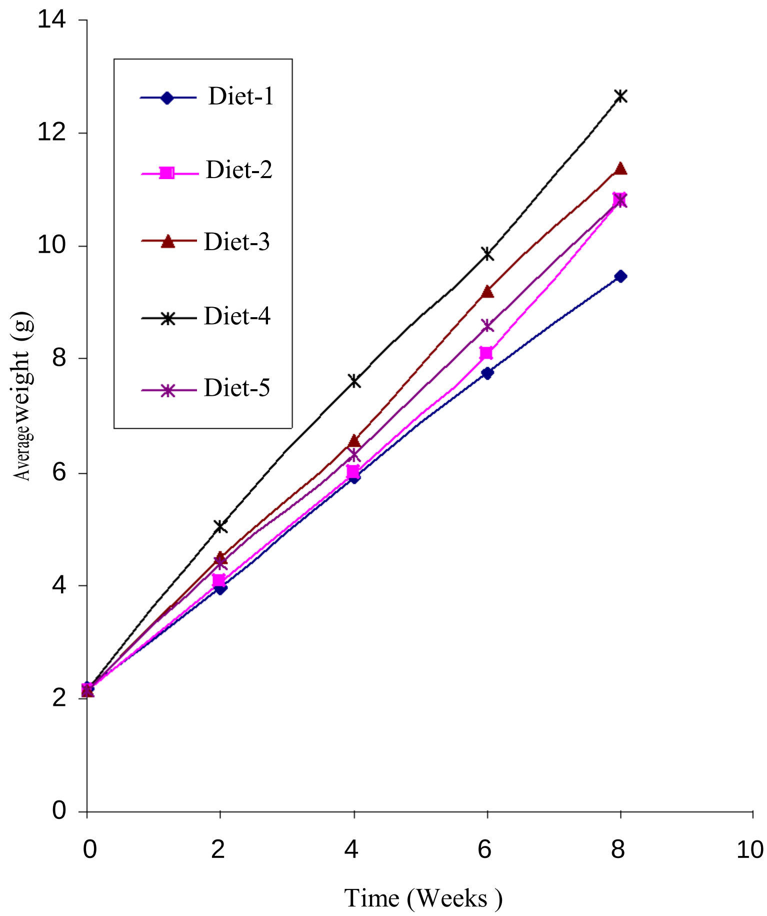

Figure 1. The mean fortnightly growth response of *A. testudineus* fed diets containing various carbohydrate to lipid ratios for 8 weeks. Diets 1, 2, 3, 4 and 5 contained CHO: L ratio (g/g) 0.58, 0.99, 1.53, 2.29 and 3.00.

Table 3. Whole body composition (% wet wt. basis) of *A. testudineus* 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	77.68	69.48^{b} ± 0.15	70.15^{b} ± 0.73	71.76^{ab} ± 0.52	72.79^{ab} ± 0.64	73.60^{a} ± 0.23
Crude protein	14.55	16.20^{a} ± 0.41	15.95^{a} ± 0.53	16.37^{a} ± 0.35	16.58^{a} ± 0.74	15.88^{a}± 0.53
Crude lipid	5.35	9.15^{a} ± 0.22	8.95^{a} ± 0.65	8.56^{ab} ± 0.28	8.15^{b} ± 0.33	7.82^{b} ± 0.42
Ash	3.45	3.54^{a} ± 0.40	3.36^{a} ± 0.07	3.30^{a} ± 0.19	3.15^{a} ± 0.56	3.28^{a} ± 0.38

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

ANPU increased with increasing CHO: L ratio. Fish fed diet 4, recorded comparatively higher PER value than other diets. ANPU of fish fed diet 5 was significantly higher (P<0.05) than that of diet 1, but did not differ 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 dietary lipid inclusion (increasing CHO:L ratio, g/g). Body lipid content of fish fed diet 1 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 2 and 3 (Table 3).

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 *A. testudineus*. From the growth performance data, it is observed that the optimal dietary CHO:L ratio in climbing perch which produced good growth rate, and feed and protein utilisation ranged from 0.99 to 3.00, which is lower than the optimum ranges reported for the African catfish (*Clarias gariepinus*), hybrid *Clarias* catfish (*Clarias macrocephalus* × *C. gariepinus*), channel catfish and walking catfish. In African catfish, isonitrogenous (40% CP) and isocaloric (20 kJ g^{-1}GE), diets with CHO:L ratios ranging from 1.70 to 3.40 resulted in significantly improved growth performance and feed utilisation (Ali and Jauncey, 2004). An investigation in walking catfish (*Clarias batrachus*) employing diets containing 40% CP and 14 kJ g^{-1} ME, with CHO:L ratios ranging from 0.02 to 3.38 produced fish with significantly improved growth performance and feed utilization (Erfanullah and Jafri, 1998).

The differences observed in optimal ranges of dietary CHO:L ratios of this study from other species aforementioned are due to the varying levels of non-protein energy contents. The growth and feed and protein efficiency recorded in the present study indicate the ability of the climbing perch to adapt to increasing levels of dietary carbohydrate (28.81%) which appears to be almost similar to that of the walking fish, *C. batrachus* (27%), (Erfanullah and Jafri, 1998) and channel catfish (28%) (Garling and Wilson, 1977), but lower than that reported for African catfish (38%) (Ali and Jauncey, 2004).

Reduced growth rate and feed and protein efficiency was found in climbing perch fed diet 1, containing the lowest-carbohydrate and highest-lipid (9.66% carbohydrate and 16.71% lipid, CHO:L ratio 0.58). This could be the result of reduced feed consumption by fish due to the high dietary lipid level because of excessive food energy, preventing the intake of necessary amounts of protein and other nutrients required for maximum growth. Similar observations have been reported for African catfish (Ali and Jauncey, 2004), walking fish (Erfanullah and Jafri, 1998) and hybrid Clarias catfish (Jantrarotai et al., 1994). Crude fiber content (11%), as cellulose, in diet 1 is unlikely to be the cause of poor performance of fish. Even though there are reports indicating that high dietary fiber levels reduce the utilization of other nutrients (Hilton and Atkinson, 1982; Anderson et al., 1984), this view has been challenged (Jantrarotai et al., 1994). 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 possibly indicates that *A. testudineus*, being omnivorous, can utilize dietary carbohydrate more efficiently than lipid. However, it remains unclear if reduced growth in fish fed the high lipid diets was due to inefficient lipid utilization as compared with carbohydrate utilization, 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 fish. But, increasing dietary lipid to 12.52% or higher (diets 1 to 3) resulted in increased total body lipid. Similar results have been reported in 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 observation is in agreement with results reported for African catfish (Ali and Jauncey, 2004), hybrid *Clarias* catfish (Jantrarotai et al., 1994), channel catfish (Garling and Wilson, 1977), tilapia (El-Sayed and Garling, 1988) and walking catfish (Erfanullah and Jafri, 1998).

On the basis of growth performance, feed and protein utilisation and whole body composition, it may be stated that diet 4 with 50% lipid energy (10.50% lipid), 50% carbohydrate energy (24.04% carbohydrate) and a carbohydrate to lipid ratio (CHO:L ratio g/g) of 2.29 performed the best. In conclusion, this study reveals that climbing perch, *A. testudineus* can perform equally well on isonitrogenous and isocaloric diets (40% protein, 22.60 kJ g^{-1} GE) containing carbohydrate ranging from 14.64 to 28.81% with 9.60 to 14.64% lipid content or at CHO:L ratios of 0.99 to 3.00.

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