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Protein-Sparing Ability of Carbohydrates from Different Sources in Diets for Fry of Stinging Catfish *Heteropneustes fossilis*

(Keupayaan Jimat Protein Karbohidrat daripada Sumber Berbeza dalam Diet
untuk Anak Keli Stinging *Heteropneustes fossilis*)

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ABSTRACT

The experiments were carried out to evaluate the protein-sparing effect in *Heteropneustes fossilis* fry (0.751 ± 0.01 g) fed for 90 days with six isocaloric diets containing 45, 40 or 35% of casein-protein and 25, 30 or 35% of glucose/dextrin, combined properly as the work purpose. The highest weight gain was recorded in fry fed with 35% dextrin and 35% crude protein level. Interestingly, the value of SGR (2.950±0.017) and PER (1.793±0.03) were significantly ($p<0.05$) increased with reducing protein level from 45 to 35% and with increasing carbohydrate level from 25% to 35%, respectively. However, the poorest growth was recorded in the fry fed with glucose containing diets than dextrin-containing diets. Protein deposition was significantly ($p<0.05$) lower in fry fed with the glucose diets at the protein level of 45% than those fed with 40 or 35%, indicating that *H. fossilis* utilize dextrin easily than glucose. Increase of dextrin content from 35 to 45% did not reduce ($p<0.05$) the weight gain, even reducing the dietary protein from 45 to 35%. This fact suggests that carbohydrate spare protein by using dextrin as energy source.

Keywords: Dextrin; glucose; nutrition; *Heteropneustes fossilis*; sparing effect

ABSTRAK

Kajian telah dijalankan untuk menilai kesan jimat protein dalam pemakanan anak ikan *Heteropneustes fossilis* (0.751 ± 0.01 g) selama 90 hari dengan enam diet isokalori yang mengandungi 45, 40 atau 35% daripada protein kasein dan 25, 30 atau 35% daripada glukosa/dekstrin, digabungkan dengan betul sebagai tujuan kerja. Kenaikan berat badan tertinggi direkodkan dalam pemakanan anak ikan dengan tahap 35% dekstrin dan 35% protein kasar. Menariknya, nilai SGR (2.950±0.017) dan PER (1.793±0.03) masing-masing meningkat secara bererti ($p<0.05$) dengan pengurangan tahap protein daripada 45 kepada 35% dan peningkatan tahap karbohidrat daripada 25 kepada 35%. Walau bagaimanapun, pertumbuhan lemah direkod untuk pemakanan anak ikan dengan diet yang mengandungi glukosa daripada diet yang mengandungi dekstrin. Pemendapan protein adalah ketara lebih rendah ($p<0.05$) dalam anak ikan yang diberi makan dengan diet glukosa pada tahap protein 45% berbanding 40% atau 35% yang menunjukkan bahawa *H. fossilis* menggunakan dekstrin lebih mudah berbanding glukosa. Peningkatan kandungan dekstrin daripada 35 kepada 45% pula tidak mengurangkan ($p<0.05$) pertambahan berat badan, malah mengurangkan diet protein daripada 45 kepada 35%. Fakta ini menunjukkan bahawa karbohidrat jimat protein dengan menggunakan dekstrin sebagai sumber tenaga.

Kata kunci: Dekstrin; glukosa pemakanan; *Heteropneustes fossilis*; kesan jimat

INTRODUCTION

Fish require a well-balanced mixture of essential dietary amino acids for the best growth and maintenance of them. Protein requirement grossly is dependent on species, stage of growth, water temperature, salinity and season among other factors (NRC 1983). Protein-energy ratio, protein digestibility, and the quantity of non-protein energy in the diet also influence the protein requirement of fish (NRC 1983; Wilson & Halver 1986). Since proteins represent the major part of the total feed cost, it is essential that they are incorporated into diets in the proper levels to attain a normal growth and maintenance. An excessive amount of dietary protein is considered biologically detrimental to the species as well as economically wasteful. Cho and Kaushik

(1990) have reported that non-protein energy sources such as dietary carbohydrate and lipid in adequate level can minimize the use of protein as a source of energy.

Carbohydrate is easily available in the feed and is a cheap source of energy in the diet. Fish growth can be affected by different levels, sources and complexity of dietary carbohydrates (Mollah & Alam 1990; Orire & Sadiku 2014; Tung & Shiau 1991; Zhou et al. 2015). However, the utilization of carbohydrate as a protein-sparing energy source if compared with lipid has received less attention in the feeding of omnivorous fish (Page & Andrews 1973; Takeuchi et al. 1979). A clear knowledge upon the optimal level of protein as dietary carbohydrate is used to explore the protein sparing effect may be useful

to minimize the cost of fish feeds (Shiau & Peng 1993; Watanabe et al. 1987).

The stinging catfish, *Heteropneustes fossilis*, is an omnivorous freshwater catfish farmed in several Asian countries and commercially relevant (Akand et al. 1989). It is very delicious and demandable fish due to its high nutritive and medicinal value (Bhatt 1968). The market price of *H. fossilis* still beyond the poor people means though it is cultured commercially in Bangladesh. Earlier very few studies were conducted on the biology including length-weight relationship, fecundity, stocking density by using formulated feed (Das et al. 1989; Mia 1984; Narejo et al. 2005). However, the protein-sparing effect of carbohydrate from different sources including glucose and dextrin in *H. fossilis* remains to be clarified. Since feeding, which constitutes the largest operational cost (50-70% of the total operational costs), is the most important determinant of production and ultimately economic condition of aquaculture farms. However, proteins, the most expensive dietary item in fish feeding, should be partially replaced by providing adequate energy through dietary carbohydrates and lipids (Mohanta et al. 2007), which should reduce the production cost. Carbohydrate utilization probably 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 (NRC 1993; Wilson 1994).

Therefore, the present investigation was conducted to evaluate the effect of different levels of dietary

carbohydrates at variable protein levels on growth, conversion efficiencies, body composition and nutrient retention in fry of *H. fossilis*.

MATERIALS AND METHODS

DIETS

Six experimental diets were prepared with two sources of carbohydrates (glucose and dextrin). The diets were prepared with three levels of protein (45, 40 and 35%) and three levels of carbohydrate (25, 30 and 35%). Glucose or dextrin was used as carbohydrate source performing six isocaloric diets with the following combination - 45% protein, 25% glucose for carbohydrate (P45C25G); 45% protein, 25% dextrin for carbohydrate (P45C25D); 40% protein, 30% glucose for carbohydrate (P40C30G); 40% protein, 30% dextrin for carbohydrate (P40C30D); 35% protein, 35% glucose for carbohydrate (P35C35G); and 35% protein, 35% dextrin for carbohydrate (P35C35D). Major portion of protein and carbohydrate sources were synthetic casein, gelatin and glucose, dextrin, respectively, except fishmeal which was used as a common ingredient. The composition and proximate analysis of experimental diets are shown in Table 1. The vitamin and mineral premixes were used according to Halver (1976).

The diets were prepared by thoroughly mixing the air-dry ingredients in a mixer moisturized with warm water (75-85°C) and blended to obtain semisolid slurry.

TABLE 1. Experimental diets composition and ingredient analyses used for *H. fossilis* fry

Ingredients (%)	Treatments					
	P45C25G* ³	P45C25D* ⁴	P40C30G* ⁵	P40C30D* ⁶	P35C35G* ⁷	P35C35D* ⁸
Casein (75% CP)	40.50	40.50	36.00	36.00	29.33	29.33
Gelatin (84% CP)	8.03	8.03	7.14	7.14	7.14	7.14
Glucose	25.00	-	30.00	-	35.00	-
Dextrin	-	25.00	-	30.00	-	35.00
Cellulose	2.61	2.61	3.00	3.00	4.67	4.67
Common ingredients* ¹	23.86	23.86	23.86	23.86	23.86	23.86
Total (g)	100.00	100.00	100.00	100.00	100.00	100.00
<i>Proximate analysis (%)</i>						
Moisture	12.82	12.95	13.78	12.98	13.82	13.41
Crude protein	45.15	44.85	39.96	40.23	35.06	35.15
Lipid	9.15	9.35	8.95	9.48	9.05	8.86
Ash	7.43	7.34	7.18	7.06	7.14	7.51
NFE* ²	38.27	38.46	43.91	43.23	48.75	48.48

*¹ Common ingredients: fish meal-17.50 g; cod liver oil-1.61 g; carboxymethylcellulose-2.25 g; Vitamin premix contains (mg/kg of dry diet): ascorbic acid 1000mg, inositol 600mg, choline chloride 2700mg, nicotinic acid 200mg, pyridoxine 40mg, thiamine 40mg, riboflavin 50mg, calcium pantothenate 117mg, biotin 1mg, folic acid 10mg, cyanocobalamin 0.5mg, alpha tocopherol 20mg; Mineral premix contains (g/Kg of dry diet): calcium carbonate 4 g, potassium phosphate 2.8 g, calcium orthophosphate 1.60 g, ferrous sulphate 1.5 g, magnesium sulphate 1.6 g, sodium phosphate 1 g, zinc sulphate 0.24 g. Both premixes were prepared with Cellulose to include at 2.5% of the experimental diets.

*² NFE=Nitrogen free extract, calculated as 100-(% protein+% lipid+% ash).

*³ 45% protein, 25% Glucose as Carbohydrate source

*⁴ 45% protein, 25% Dextrin as Carbohydrate source

*⁵ 40% protein, 30% Glucose as Carbohydrate source

*⁶ 40% protein, 30% Dextrin as Carbohydrate source

*⁷ 35% protein, 35% Glucose as Carbohydrate source

*⁸ 35% protein, 35% Dextrin as Carbohydrate source

Afterward, they were spread on a plastic sheet with the help of a brush and allowed to dry overnight. After drying, the flakes were removed and stored in airtight plastic container until required.

EXPERIMENTAL DESIGN

A total of 600 fry were collected from Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. They were acclimatized indoor for 1 week in a 300 L plastic tank and fed with casein-gelatin based purified diet. The experiment was designed following six treatments (P45C25G, P45C25D, P40C30G, P40C30D, P35C35G and P35C35D) with three replicates each where a group of 30 fry was stocked. Fry were fed with the diets at the rate of 4% of the body weight twice a day. Sampling of fishes was done every fortnightly interval and feeding regime was adjusted accordingly. The initial and final weights of the fry were recorded using electronic weighing balance.

WATER QUALITY MEASUREMENT

One-fourth of the total volume of water was removed daily to flush out excreta and unfed diet and replenished by the same volume of water. Water parameters (temperature, dissolved oxygen and pH) were recorded using 'Lutron PDO-519' and 'EZDO PH-5011', respectively, on alternative days. The temperature ranged from 27.3-27.8°C, pH between 6.8 and 7.1 and dissolved oxygen between 5.9 and 6.7 mg/L.

CHEMICAL ANALYSIS

A total of 24 numbers of fry were used for the determination of initial body composition before the commencement of the experiment. At the end of the experiment, 5 fishes from each treatment were analyzed for final body composition. The experimental diets were analyzed for crude protein (N Kjeldhal \times 6.25), crude lipid (solvent extraction with petroleum ether B. P. 40-60°C for 10-12 h) and ash content

(oven incineration at 650°C for 4-6 h) determination according to AOAC (1984).

STATISTICAL ANALYSIS

Growth performance data of *H. fossilis* at each treatment were analysed by one-way analysis of variance (ANOVA) after confirmation of homogeneity of variance. Tukey (HSD) mean separation test were used to determine the differences among the means. Significant differences were stated at ($p < 0.05$) level unless otherwise noted (Zar 1999). All statistical analyses were performed using statistical software SPSS 16.5.0 for Windows (SPSS Inc. Chicago, IL USA).

RESULTS

The food conversion ratio (FCR) and growth efficiency data for *H. fossilis* fry are shown in Table 2. The fish appeared healthy throughout the experimental period and nothing but some mortality less than 15% was observed. The FCR (1.613 ± 0.08), daily increment in weight (0.306 ± 0.003), specific growth rate (SGR) ($2.950 \pm 0.017\%$) and protein efficiency ratio (PER) (1.793 ± 0.03) were significantly ($p < 0.05$) higher in the fish fed with dextrin-containing diet (P35C35D) as compared to the fish fed the glucose-containing diets. However, the poorest values for FCR (2.810 ± 0.02), daily weight gain (0.192 ± 0.003 g), SGR ($2.360 \pm 0.098\%$) and PER (1.397 ± 0.03) were observed in the fish fed with the glucose-containing diet (P45C25G). The initial and final body compositions of fry from all the six treatments are shown in Table 3. Whole-body crude protein and lipid content of the fry increased significantly ($p < 0.05$) but the ash content remained low showing no significant changes in the case of the fish fed with dextrin-containing diet (P35C35D). With the increasing level of glucose and dextrin in the diet, values of protein and fat were significantly increased.

TABLE 2. Growth parameters of *H. fossilis* fed with distinct protein-carbohydrate ratios

Growth Parameters	Treatment					
	P45C25G	P45C25D	P40C30G	P40C30D	P35C35G	P35C35D
AIW* ¹	0.751 \pm 0.005	0.749 \pm 0.001	0.745 \pm 0.006	0.748 \pm 0.010	0.750 \pm 0.002	0.747 \pm 0.004
AFW* ²	11.252 \pm 0.89 ^a	12.983 \pm 0.21 ^b	11.688 \pm 0.26 ^a	15.685 \pm 0.15 ^c	15.516 \pm 0.25 ^c	19.080 \pm 0.15 ^d
DWG* ³	0.192 \pm 0.003 ^a	0.229 \pm 0.003 ^c	0.207 \pm 0.004 ^b	0.274 \pm 0.003 ^c	0.246 \pm 0.004 ^d	0.306 \pm 0.003 ^f
FCR* ⁴	2.810 \pm 0.02 ^c	2.187 \pm 0.04 ^c	2.330 \pm 0.09 ^d	1.813 \pm 0.06 ^b	2.230 \pm 0.12 ^{cd}	1.613 \pm 0.08 ^a
PER* ⁵	1.397 \pm 0.03 ^a	1.460 \pm 0.02 ^b	1.563 \pm 0.02 ^c	1.733 \pm 0.04 ^c	1.630 \pm 0.02 ^d	1.793 \pm 0.03 ^f
SGR* ⁶	2.360 \pm 0.098 ^a	2.527 \pm 0.015 ^{bc}	2.403 \pm 0.012 ^{ab}	2.633 \pm 0.186 ^{cd}	2.720 \pm 0.020 ^d	2.950 \pm 0.017 ^e
Survival	92.00 \pm 5	95.00 \pm 3	89.00 \pm 7	90.00 \pm 3	87.00 \pm 4	91.00 \pm 6

[Means of three replicate groups \pm S.D.M.]

Values bearing different superscript in a column are significantly different at $p < 0.05$

*¹ Average Initial Weight (g)

*² Average Final Weight (g)

*³ Daily Weight gain (g)

*⁴ Food conversion ratio (FCR) = mass of feed consumed (dry)/increase in animal mass (wet)

*⁵ Protein efficiency ratio (PER) = Increase in animal mass (wet)/mass of protein fed (dry)

⁶ Specific growth rate (SGR) = 100(ln final weight - ln initial weight)/culture period in days

TABLE 3. Body composition and nutrient retention efficiencies in *H. fossilis*

Body composition (% w/w basis)	Treatments						
	Initial fish	P45C25G	P45C25D	P40C30G	P40C30D	P35C35G	P35C35D
Moisture	77.46±0.14	78.16±1.34 ^{cd}	77.02±0.94 ^{bcd}	78.77±0.52 ^d	76.44±0.74 ^{bc}	75.41 ±1.00 ^b	73.39±1.15 ^a
CP* ¹	12.53±0.11	13.54±0.48 ^a	15.18±0.17 ^c	14.26±0.08 ^b	16.33±0.07 ^c	15.77±0.09 ^d	17.73±0.35 ^f
Fat	2.36±0.09	2.21±0.14 ^a	3.30±0.22 ^b	3.23±0.25 ^b	4.69±0.81 ^c	3.61±0.41 ^b	5.52±0.24 ^d
Ash	3.85±0.17	3.97±0.12 ^d	3.58±0.38 ^{cd}	3.25±0.28 ^{bc}	2.43±0.23 ^a	2.93±0.41 ^{ab}	2.99±0.22 ^b
PRE * ²	-	15.94±0.46 ^a	21.83±0.49 ^c	19.17±0.53 ^b	27.77±0.49 ^d	19.72±0.35 ^b	32.68±0.42 ^c

[Means of three replicate groups±S.D.M.]; values bearing different superscripts in a column are significantly different at $p < 0.05$

*¹Crude protein

*² Protein retention efficiency = $100 \times [(\text{mean final body weight (g)} \times \% \text{ nutrient of final body weight}) - (\text{mean initial body weight (g)} \times \% \text{ nutrient of initial body weight})] / [(\text{nutrient intake (g-1)} \times \text{kcal g-1})]$

DISCUSSION

Protein-sparing effect of carbohydrate over the growth of fishes has been reported since the mid 70's in channel catfish (Garling & Wilson 1976) and afterwards in rainbow trout (Pieper & Pfeffer 1980) and European eel (Degani 1987; Hidalgo et al. 1993). Decrease in dietary protein level from 45 to 35% and concomitant increase in dextrin from 35 to 45% did not affect the weight gain in *H. fossilis*, indicating that dextrin can spare some protein when the dietary protein is low. Carbohydrates utilization in fishes is affected by their source, complexity and presence of carbohydrate-metabolizing enzymes (NRC 1983; Stone et al. 2003; Wilson 1994). In the present investigation, significant growth was observed in *H. fossilis* fry fed with the diet containing dextrin. This may be due to the fact that herbivorous and omnivorous fishes are able to use oligo and polysaccharides easier than using di or monosaccharides (Shiau & Peng 1993; Tung & Shiau 1991). On the contrary, the reduced growth recorded in fry fed with the diet containing glucose is in agreement with the findings in other fish species (Erfanullah & Jafri 1995; Orire & Sadiku 2014; Shiau & Peng 1993; Zhou et al. 2015). Pieper and Pfeffer (1980) have suggested that a great percentage of absorbed glucose may not be utilized as an effective source of energy in fish before adequate insulin is available to facilitate its utilization which were supported by Furuichi and Yone (1982) as well as Hilton and Atkinson (1982).

Some authors have recommended 34 to 37% dietary protein level in the diet for optimum growth of *H. fossilis* fry. However, in the present experiment when fish were fed with 45% protein, sparing effect of protein by carbohydrate was not as distinct as in low protein levels (40%, 35% CP) in the diet, indicating that the protein-sparing effect generally occurs when the dietary protein is sub-optimum and it could have been possibly even more evident if an intermediate dietary protein level (i.e. 42% or 37%) would have been assayed. Similar trend was observed in *Cirrhinus cirrhosus*, fed lower protein (35% & 30%) compared with the optimum protein (40%) level in the diet (Singh et al. 2006) as well as in tilapia, fed with sub-optimum protein (28% & 24%) compared with the optimum protein (32%) level in the diet (Shiau & Peng 1993).

The PER increased with an increase of carbohydrate level among diets but repeated trend was observed towards increased PER as dietary carbohydrate (dextrin) increased. On the contrary, although there were no negative results obtained for the glucose-containing diets but significantly low PER (1.397) indicate poor utilization of glucose for energy purpose in *H. fossilis* fry.

The variations in the body composition of *H. fossilis* fry fed with the test diets were observed to be significant (Table 3). The whole-body protein of *H. fossilis* fry increased with dietary protein and correlated negatively with body moisture. Similar findings have been reported for other fish species (Hidalgo et al. 1993; Khan & Abidi 2011). Moisture content showed an inverse relationship with carcass lipid when dietary protein level was increased. A parallel observation has been reported for other fishes (Degani & Viola 1987; Hidalgo et al. 1993). In the present investigation, it was also observed that higher levels of dietary carbohydrate in the diet increases the whole body lipid in fish which may be deposited into the body through *de novo* lipogenesis.

In conclusion, from the present study it is possible to suggest 35% dietary dextrin as a good source of carbohydrate whether combined with 35% of protein in the diet of *H. fossilis* fry. The introduction of dietary dextrin in the feeding of *H. fossilis* make possible to reduce the levels of an expensive dietary item, the proteins and all the secondary and inconvenient effects from the high levels of such macronutrient in the fish feeding.

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