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Evaluation of dietary protein to carbohydrate ratio on the growth, conversion efficiencies and body composition of Zebrafish

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Abstract
A 45-day feeding experiment was carried out to evaluate the growth, conversion efficiency and body composition of zebrafish by altering the protein-carbohydrate ratio in the diet. One hundred and Eighty Zebrafish (*Danio rerio*) having an average weight of 0.20 ±0.03g were randomly distributed in three experimental groups with 20 fish in each replicate. Fish were fed with three iso-lipidic diets (crude lipid 8%) that contained three different levels of protein and in each protein level, three different levels of carbohydrate (T1:25% protein, 45% carbohydrate; T2- 35% protein and 45% carbohydrate; -T3-45% protein, 25% carbohydrate) were included. The growth performance of the experimental fish was evaluated. The results demonstrated that T1 group exhibited higher growth rate (SGR), feed efficiency ratio (FER) and protein efficiency ratio (PER) but lowest weight gain (% WG), as compared to T2 and T3. Feed conversion ratio was also significantly higher in T1 as compared to T2 and T3 groups. However, there was no significant difference in the protein efficiency ratio between T1 and T2, whereas it was significantly reduced in T3 group. A significant decrease in the whole body moisture, protein content, and increase in whole-body lipid content was observed in group T1. The present study shows that optimum protein levels in the diets may be reduced to 25% protein while carbohydrate levels can be increased up to 45% to formulate the feed with more inclusion of digestible carbohydrate based on the growth performance and protein utilization in zebrafish.

Keywords: zebrafish, growth, protein, carbohydrate

1. Introduction
Aquafeed production is currently one of the fastest expanding agricultural industries in the world, due to the rapid intensification of aquaculture in recent years which has led to the use of formulated feeds. Feeding in aquaculture is considered very important as it accounts for about 50-60% of the variable operating costs in a production cycle where good nutrition is essential to economically produce an affordable, healthy, safe, and a high-quality product.

Aquafeed is used to feed many aquaculture species including omnivorous fishes, carnivorous fishes and crustacean species. The increase in the aquaculture activities of these species has led to an increased demand for fish feed. However, the major consequence of the aquafeed is the rising prices of fishmeal because of a reduction in the supply of important sources of fish which limits its use as the primary protein in fish diets, thus affecting the profitability of many aquaculture enterprises. It is therefore prudent to maximize the non-protein energy sources such as lipid or carbohydrates in fish diets so as to spare dietary protein from being used as energy. The use of carbohydrate-rich ingredients in fish feed is gaining importance because of their ability to spare protein component of the diet [1]. Although the optimal inclusion of dietary carbohydrates is known to increase the retention of protein and lipid in farmed fish [2], fish are known to have a limited capacity for digestion and metabolism of carbohydrates with excessive intake resulting in nutritional problems [3-5]. The maximum dietary carbohydrate inclusion levels recommended for salmonids and marine fish fall within 15-25 percent and 50 percent for herbivorous and omnivorous species [6].

Zebrafish, *Danio rerio*, is a teleost freshwater fish belonging to the family Cyprinidae. It has a fusiform and laterally flattened body, with alternated dark and bright stripes throughout its extension by which it is commonly called zebrafish [7]. It is a usual aquarium ornamental fish but is also extremely important as a research model for nutrition and growth studies in fish [8, 9]. Zebrafish in the wild is omnivorous.

The gut content analysis revealed that its natural diet primarily consists of zooplankton, phytoplankton, filamentous algae and vascular plant material, spores, invertebrate eggs, fish scales, arachnids, detritus, sand and mud [10-12]. Zebrafish can feed on animal and vegetal protein sources, which allows us to infer that it has nutritional pathways similar to those of cultivated herbivores such as carp and tilapia, and to those of carnivores such as trout and salmon.

In this backdrop, the present study was undertaken to evaluate the growth, conversion efficiency and body composition of zebrafish by altering the protein to carbohydrate ratio in the diet.

2. Materials and Methods

2.1 Ethical measures and statement

The animal used in this experiment were cared and treated according to the procedures of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Environment and Forests (Animal Welfare Division), Govt of India on care and use of animals in scientific research. All fishes conduct and treating procedures were approved by the Ethics and Animal Care Committee of the Indian Council of Agriculture research, Central Institute of Fisheries Education, (ICAR-CIFE), Mumbai-61 established norms.

2.2 Experimental design and feeding trial

Animals used for the present study were fingerlings of zebrafish, *D. rerio* with an average weight of 0.20 ±0.03 g. The fishes were acclimatized for a period of 15 days. During acclimation, fishes were fed a diet containing 35% crude protein. One hundred and eighty fishes of *D. rerio* were randomly distributed into three experimental groups in triplicate (T_1-25% protein, T_2-35% protein and T_3-45% protein). The experimental groups consisted of aquarium tanks of 20L capacity (40cmx 20cm x 30cm), containing 20 fishes each following a CRD (completely randomized design). The aeration was maintained in all the experimental groups for 24 h throughout the experiment. The experimental trial was conducted for 45 days. The feed was given to satiation level, twice daily at 9:00 and 17:00 h. Faecal matters and uneaten feed were syphoned out daily with about 75% water exchange. All the physiochemical parameters of water were maintained regularly within the optimum range as required for the *D. rerio*.

2.3 Diet preparation

Three iso-lipidic diets (crude lipid 8%) were prepared to contain three different levels of protein and carbohydrate (T1-25% protein, 45% carbohydrate; T2-35% protein and 45% carbohydrate; T3-45% protein, 25% carbohydrate) (Table 1). Casein and gelatin were used as protein sources while starch and dextrin as carbohydrate sources. All the ingredients were blended thoroughly with water to make dough except the vitamin-mineral mix (PREMIX PLUS, India). The dough was then conditioned properly for 1 h followed by steaming for 5 min in a pressure cooker. The vitamin-mineral premix was added just after cooling and mixed thoroughly. The final dough was then pressed through a pelletizer (Ace Exports, Mumbai, India) to produce uniform pellets which were dried and stored at 4°C for later use. Proximate analysis of the diets and carcass tissue was done by standard methods of AOAC Interational at Fish Nutrition Laboratory, ICAR-CIFE Mumbai.

2.4 Sample Collection

The ethical guidelines of ICAR-Central Institute of Fisheries Education, Mumbai India for the animal care were strictly followed. The sampling was done at the end of the 45-day feeding experiment. Fish were starved overnight before weighing on an electronic balance.

2.5 Growth Performance

The growth performance and feed utilization efficiency were calculated using the following formulae:

Weight gain (%) = (FW − IW) × 100/ IW

FCR = Feed given (DW)/body weight gain (WW)

FER = Body weight gain (WW)/ Feed given (DW)

SGR (%) = [ln (FW) − ln (IW)] / [N] × 100

PER = Body weight gain (WW) / protein fed

Where; FW =final weight, IW = initial weight, DW = dry weight,

WW = wet weight, ln= natural log and N =number of culture days.

2.6 Statistical Analysis

The statistical analysis of the data was carried out by using Microsoft Excel and statistical software Package (SPPS V16.0, USA) in which data were subjected to one way ANOVA and Duncan's multiple range tests were used for post hoc comparison ($P<0.05$) to determine the significant differences between the means.

3. Results

3.1 Body Composition

The body moisture, protein, lipid and ash did not show any significant difference among the different experimental groups (Table 3).The moisture content of the experimental fishes varied from 74.51±0.33 to 75.57±0.31.The observed crude protein of different experimental groups varied from 15.45±0.18 to 16.14±0.19. The ether extract of different groups that were recorded varied from 4.22±0.10 to5.46±0.08. The total ash content varied from 4.15±0.15 to 4.63±0.19

3.2 Growth performance and conversion efficiency

Increasing levels of dietary protein from 25% to 45% led to increased weight gain (WG %) but decreased specific growth rate (SGR), although no significant differences were detected in protein levels above 35% ($P<0.05$) (Table 4). The feed conversion ratio (FCR) was significantly lower ($P<0.05$) in the treatment group fed with low protein (25%) and high carbohydrate (45%) levels and highest at an optimal level (Table 4). The feed efficiency ratio decreased along with the protein increment in the diets, but for protein levels 25% and35%, no significant differences were found (Table 4). The protein efficiency ratio (PER) was higher for the lowest dietary protein levels of 25% and 35% and decreased significantly ($P<0.05$) as the dietary protein level increased to 45% (Table 4).

Table 1: Composition of different experimental diets (g%)

Ingredients	T_1	T_2	T_3
Casein	22.7	32	41
Gelatin	5.5	7.5	9.8
Starch	20	15	10
Dextrin	25	20	15
Cellulose	14.3	13	11.7
Cod liver oil	4	4	4
Sunflower oil	4	4	4
VM-MM premix	2	2	2
CMC	2	2	2
Choline Chloride	0.4	0.4	0.4
BHT	0.1	0.1	0.1
Total	100	100	100

VM: Vitamin mix MM: Mineral mix
Composition of vitamin mineral mix (PREMIX PLUS) (quantity/kg)
Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6 mg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn,27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L-lysine,10g; DL-Methionine,10g; Selenium, 125 mg.

Table 2: Proximate composition (Dry matter basis) of different experimental diets

Treatments	CP (%)	EE (%)	Ash (%)	CF (%)	NFE (%)	DE (Kcal/100g)
T_1	24.77±0.51	7.77±0.16	6.15±0.49	16.49±0.21	44.82±0.16	379.78±1.8
T_2	34.55±0.53	7.89±0.13	6.90±0.53	15.75±0.26	34.91±0.12	375.29±1.8
T_3	44.89±0.57	7.64±0.16	6.97±0.51	15.63±0.15	24.87±0.24	373.37±1.8

Data expressed as Mean ± SE, n=3.

Table 3: Whole body composition of *Danio rerio* in different experimental groups (% wet weight basis).

Treatment	Moisture (%)	Protein (%)	EE (%)	Ash (%)
T_1	74.51^b±0.33	15.45^b±0.18	5.46^a±0.08	4.63^a±0.19
T_2	75.13^a±0.30	16.09^a±0.16	4.42^b±0.15	4.41^a±0.37
T_3	75.57^a±0.31	16.14^a±0.19	4.22^b±0.10	4.15^a±0.15

Mean values in the same column with different superscripts (a,b) differ significantly ($P<0.05$). Data expressed as Mean ± SE, n=3

Table 4: Growth parameters of experimental fishes in different treatment groups

Treatments	Wt. Gain %	SGR	FCR	FER	PER
T1	95.32^b ±7.79	1.35^a ± 0.08	2.25^b ± 0.40	0.15^a ± 0.01	2.36^a ± 0.09
T2	106.14^a ± 6.62	0.27^b ± 0.03	3.43^a ± 0.44	0.15^a ± 0.00	2.29^a ± 0.14
T3	108.58^a ± 11.90	0.25^b ± 0.01	3.66^a ± 0.69	0.12^b ± 0.01	1.75^b ± 0.06

Mean values in the same column with different superscripts (a,b) differ significantly ($P<0.05$).
Data expressed as Mean ± SE, n=3;

4. Discussion

The present study was carried out to evaluate the growth, conversion efficiencies and body composition of zebrafish by altering the protein-energy ratio in the diet. The results of this study demonstrated that the weight gain percentage improved as the dietary protein level increased from 25% to 45% respectively, suggesting that the fish efficiently utilized the high protein diets by converting them into body tissue protein. Similar trends and utilization of protein were reported by Kpundeh *et al.*, [13] in GIFT tilapia when the dietary protein levels were increased from 25% to 45% with maximum weight gain occurred at 41.6% protein. However, Viola and Zohar [14] reported that when protein levels increased further from 35% to 45% respectively, weight gain percentage of GIFT tilapia did not increase significantly. These results are thus in accordance with the findings of the present study.

Moreover, lower the feed conversion ratio (FCR) of the feed, the higher the efficiency (FER) and vice-versa. In the present study, the lowest feed conversion ratio (FCR) and hence the highest feed efficiency was recorded in the fish feed that contained 25% protein and 45% carbohydrate. However, in juvenile arctic char, Amoah [15] reported the lowest FCR from the fish feed that contained the highest protein content (42%) compared to the feed that has the lowest protein content (26%). Shiau and Peng [16] have reported that decreasing dietary protein level from28% to 24% and increasing starch or dextrin in the diet from 37% to 41% did not reduce weight gain or feed efficiency ratio in tilapia, suggesting that starch or dextrin can spare some protein when the dietary protein is low.

In the present study, the specific growth rate (SGR) and protein efficiency ratio (PER) decreased significantly ($p<0.05$) as the dietary protein level increased from 25% to 45% with the highest value observed in fish fed 25% protein. The PER was higher for the lowest dietary protein levels of 15 and 20% and decreased as dietary protein level increased [17]. High PER in fish fed diets containing low protein and high carbohydrates have been reported in carp [18-20] tilapia [21], and European eel [22, 23]. Usually, when fish are fed protein levels below optimum requirements, the fish needs to consume more feed to make up for a protein required for growth and metabolism. At high and optimum protein levels, less feed is required to maintain a balance between the energy for growth and metabolism. This is an indication that protein efficiency ratio (weight gain/protein fed) is reduced with increased dietary protein levels.

The body composition of zebrafish at the end of the growth trial showed marked differences in different treatments (Table 3). Moisture increased with increases in dietary protein, showing an inverse relationship with carcass lipid as has been reported for other fishes [18, 19, 22]. Body protein increased with dietary protein and correlated inversely with body moisture. Similar observations have been reported for other fish species [22-24]. Increase in the body lipid at higher levels of dietary carbohydrate is also in agreement with earlier studies [18, 20, 21, 25, 26]

From this study, the percentage weight gain in zebrafish was found to be directly proportional to the increased dietary protein levels while the rate of growth (SGR) and the protein efficiency ratio (PER) were inversely proportional to the dietary protein levels. Therefore, it can be suggested that optimum protein levels may be reduced to 25% and that of the carbohydrate may be increased up to 45% in the diets based on their effect on growth performance and protein utilization.

5. Conclusion

Considering the main aim of the present study, our conclusions are dietary carbohydrate requirements for zebrafish were estimated at about 45% for maximum growth rate (SGR), feed efficiency ratio (FER) and protein efficiency ratio (PER). In addition to this optimum protein levels in the diets may be reduced to 25% while carbohydrate levels can be increased up to 45% to formulate the feed with more inclusion of digestible carbohydrate based on the growth performance and protein utilization in zebrafish.

6. Declaration of interest

All authors report no conflict of interest related to this manuscript and above research work.

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