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Evaluation of dietary protein levels for the growing *Aorichthys aor* (Hamilton 1822)

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Abstract
Dietary protein is one of the major determinants of fish growth, especially for the carnivorous economically important species like *Aorichthys aor* (Hamilton), for which lack of proper nutritionally adequate diet is the major constraint. In order to determine protein requirements for optimum growth, four diets with varying protein levels (35-42%) using processed soybean as the main protein source were formulated and fed to the fingerlings of *A. aor* for 90 days. Growth performance, nutrient retention and carcass composition were estimated. Studies revealed significant ($P<0.05$) increase in growth performance, accumulation of protein, fat and energy retention in the fingerlings fed on a diet containing 40% protein in comparison with the fish fed on other diets containing low or high protein levels. Ammonia excretion and o-phosphate production significantly low in fish fed on 40% dietary protein. The optimum dietary protein was found to be in the range of 40% when full fat processed soybean (@ 266g kg^{-1} of diet) is used as the main protein source in practical diets especially when stocking rates are high. Development of diets, reducing the excretion of ammonia and phosphate in the holding water may help in achieving better growth and nutrient retention.

Keywords: ammonia and phosphate excretion, *Aorichthys aor*, nutrition, plant proteins, protein levels

1. Introduction

Aorichthys aor (Hamilton 1822) has great medicinal value, less bone content and high delicious flavors of fish flesh which makes a great demand in intensive culture, but it has problems during intensive and semi-intensive culture and farmers take least interest in culture of this candidate species, because of its feeding and eating habits and nocturnal behaviors. The yield can be enhanced by pond fertilization and also through supplementary feeding. Further, it would require the most economic combination of dietary protein and energy and also dietary protein sources [1]. Lack of sufficient information on these aspects is perhaps one of the major technical constraints to the development of least cost and nutritionally adequate production diets for *A. aor* aquaculture [2]. Dietary protein levels and ration size are known to influence fish growth, feed efficiency and also water quality [3]. The ammonia excreted and orthophosphate production by fed fish appears to be directly related to the source and levels of protein intake [4, 5]. Thus determining the optimum dietary protein levels is important to attain maximum growth and reduce water deterioration problems associated with the use of supplementary feeds with high feeding rates [6]. Several workers have attempted to replace fishmeal with soybean meal, possessing high nutritional value, (SBM) in formulated diets for several fish species [7, 8, 9]. The quality feed is a primary requirement for good growth and production of most fish in aquaculture [10]. However most of them contain endogenous anti-nutritional factors (ANFS) which accentuate digestive losses. These ANFS are labile and are inactivated by heat treatment, which allows dietary level at which such protein sources can be utilized by fish [11]. Therefore, in order to standardize culture technology for this candidate fish species a knowledge of its protein and energy requirements will be very essential. Present experiment therefore, was designed to provide information on growth responses, digestibility, nutrient retention, energy assimilation in *A. aor* when fed on diets containing different levels of protein obtained through the incorporation processed full fat soybean as the protein source.

2. Materials and Methods

2.1. Diet Preparation

Four experimental diets with varying protein (35, 38, 40, and 42%) and energy levels (17.53-18.89 kJ g^{-1}) were formulated using processed full fat soybean (diets 1-4) as the major protein source. Dietary ingredients and proximate composition of formulated diets are given in Table 1 and 2. Soybean was hydro thermic ally treated at 15 1bs pressure at 121ºC for 15 minutes to remove anti nutritional factors (ANFS) such as trypsin inhibitors. After oven drying at 60ºC it was grounded into fine powder and utilized for diet preparation.

2.2. Experimental design

A. aor fingerlings were obtained from “Sultan Fish Seed Farm”, Nilokheri, Karnal (Haryana) India and acclimated for 10 days prior the experimental set up. The water in the aquaria was partially renewed daily with the previously equilibrated water to the desired temperature (25ºC). Each aquarium was stocked with 10 fingerlings weighing 0.40 ± 0.03g. Length-weight measurements were done fortnightly to adjust the feed quantity. The fingerlings were fed twice daily at 0800 h and 1600 h at the rate 3% of their body weight. Any uneaten feed was collected 4 h after feeding and the dry matter content was determined for both supplied and uneaten diet and the data when used for feed consumption calculation. Fecal matter was collected by siphoning from the bottom before the next feeding, freeze-dried and stored until analysis.

2.3. Analytical techniques

The feed ingredients, experimental diets, faecal matter samples, fish carcass (initial and final) were analysed [12]. pH and dissolved oxygen were monitored using automatic analysers (pH meter and DO meter Merck, Germany). At the end of feeding schedule, water samples from each aquarium were collected at two hourly interval over a period of 24 h for estimating the excretory patterns of total ammonia (NH_4-N) and reactive phosphate (o-PO_4) production [13] and calculated [14].

Ammonia/o-phosphate excretion rates (N-NH_4/o-PO_4 mg kg^{-1} fish $2h^{-1}$) were measured at 2 h interval from the aquaria water and calculated as follows:

$$\text{Total ammonia excretion} = \frac{NH_4 - N\ (mg\ l^{-1})\ \text{in aquarium water}}{\text{Fish weight (Kg) per L of water}}$$

$$\text{Reactive phosphate excretion} = \frac{o - PO_4\ (mg\ l^{-1})\text{in aquarium water}}{\text{Fish weight (Kg) per L of water}}$$

$(N\text{-}NH_4/o\text{-}PO_4)_0$ and $(N\text{-}NH_4/o\text{-}PO_4)_{120}$ = concentrations at times 0 and 120 min (2h) post-feeding.

Growth parameters were calculated using standard methods [15]. Gross energy content of the diets and fish were calculated using the average caloric conversion factor of 0.3954, 0.1715 and 0.2364 kJ g^{-1} for lipid, carbohydrate and protein, respectively [16].

2.4. Statistical Analysis

Significant differences among treatment groups were tested by Analysis of variance (ANOVA) followed by Duncan's multiple range tests [17]. Statistical significance were settled at a probability value of $P<0.05$.

3. Results

Studies have revealed that mortality occurred only during the initial days of experiment. The growth performance, digestibility and nutrients retention parameters are shown in Fig. 1 and Table 2. Live weight gain (g), growth percent gain in body weight and specific growth rate (%) d^{-1} increased significantly ($P<0.05$) with each increase in the dietary protein level up to 40% in comparison with fish fed on low (35-38%) or high protein level (42%).

PER, GCE and FCR were significantly ($P<0.05$) improved with each increase in dietary protein levels up to 40% which deteriorated with further increase in protein levels. Crude protein (%), crude fat (%) and total ash (%) were found significantly ($P<0.05$) different in the carcass of fish fed on different experimental diets (Table 3). The dissolved oxygen (DO) fluctuated between 4-5 mg L^{-1} and pH remained alkaline (7.4-7.6). In general, significantly ($P<0.05$) low values in total ammonia and reactive phosphate excretion (mg Kg^{-1} BW d^{-1}) were recorded in fish fed on diet 3 containing 40% protein diet (Table 4). Peak values in N-NH_4^+ excretion occurred at approximately 6 h after the feed was given to the fish and thereafter levels gradually declined. Peak values in o-PO_4 excretion was observed in 8 h after feed was given to the fish and thereafter levels gradually declined (Fig. 2A and 2B).

4. Discussion

Majority of fish species require about 35-40% protein in their diet for optimum growth and metabolism [3]. Generally, the protein requirements for carnivorous fish species are high. Marine fish require more protein in diet [18], whereas freshwater teleosts need comparatively less protein in their diet.

The dietary protein requirements of catfish *Heteropneustus fossilis* has been reported to be 43%[19], when fed on a diet containing hydrothermically processed soybean as major protein source. The optimum requirement of carnivorous fish like *C. batrachus* ranges between 30-40% [20, 1], while the optimum protein requirement for *C. garipenius* has been found to be 40%[21], and 25-44% for *Ictalarus punctatus*[22,23]. Protein requirement seem to be in the order of 40% for *C. garipienus*, *C. batrachus and C. isheriensis*[24] supporting the present results for *A. aor*.

For snakehead fingerlings, the optimum P/E ratio was found to be at 40% protein level based on growth performances, FCR and PER [25]. In case of blue catfish (*Ictalurus furcatus*) it has been shown to be use diets containing high percentages of soybean protein, however, increased amount of soybean protein reduced the growth in juvenile fish [26]. Blue catfish appear to have a lower dietary protein requirement compared to channel catfish [27].

The optimum dietary protein level which resulted in maximum growth in *A. aor* in terms of specific growth rate and live weight gain was found to be 40%, which is in accordance with the results [28] reporting that optimum protein requirements of *H. fossilis* were 39% based on the diets fed to the fish. In the present studies high values in growth parameters (SGR, live weight gain, growth per day) carcass protein, fat and energy were observed in *A. aor* fingerlings fed on 40% dietary protein, which were no or less significantly different from those of fish fed on 42% dietary protein. On the other hand significantly ($P<0.05$) low FCR values seen in fish fingerlings fed on 40% dietary protein. Despite there were no significant differences in the initial weights of fish, growth per cent gain in body weight, live weight gain (g), and specific growth rate (SGR) and proximate carcass composition, all increased proportionally with increasing dietary protein level up to 40% and thereafter decreased with increase in protein level. This type of apparent growth depressing effect of high protein diets has been also reported for mrigala fry [29] as well as for other tropical fish species such as grass carp [30], tilapia [31], catfish *Pangasis sutchi* fry [32], and *Puntius goniotous* [33], Indian cat fish *H. fossilis* [28].

Results of present investigation have also revealed that the FCR values decreased, while those of PER increased with each increase in protein content of diets up to 40%, with further increase in dietary protein level, an increase in FCR values and decrease in PER was observed. PER showed a negative and a linear relationship with the dietary protein level in *Oreochromis niloticus* [34]. Many other authors [31, 35] have also reported an increase in FCR and decrease in PER with increase in dietary protein contents beyond the optimum level. PER decreased with increasing dietary protein content affecting the efficiency of feed utilization [36].

Present studies have further revealed that the carcass composition of fish was also significantly affected by the dietary protein level. Fish fed on a diet containing 40% protein had significantly higher carcass protein in comparison to fish fed on low protein diets 35 and 38% crude protein). The majority of fish species require 40-50% protein in the diet [10]. Fish have the ability to handle protein in excess that is required for growth and maintenance by deamining amino acids bronchially and excreting ammonia. Present studies on *A. aor* have also established that when protein levels in diets exceed the limits of digestibility are deaminized and are excreted as ammonia. Higher levels of total ammonia and reactive phosphate were observed in the holding water when fingerlings were fed on diet containing 42% protein. An increase in the dietary protein (beyond 40%) did not enhance the growth of carcass protein contents, which may be attributed to the non-availability of sufficient quantity of proteolytic enzyme in gut [37, 38]. Protein levels above the optimum requirements may result in decreased growth rate because of a reduction in dietary energy available for growth, due to the energy required to deaminate and excrete excess of amino acids. Since excretory rates of metabolites depends not only on the fish species, but also on the size, temperature, salinity (fresh/marine water) of the medium and also on the experimental conditions [39], therefore, absolute values cannot be compared between different species, however, trends in excretion/production of metabolites and relative magnitude can be compared.

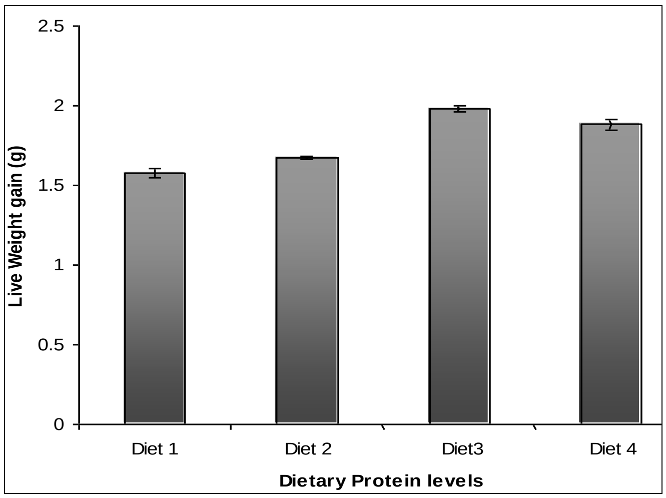
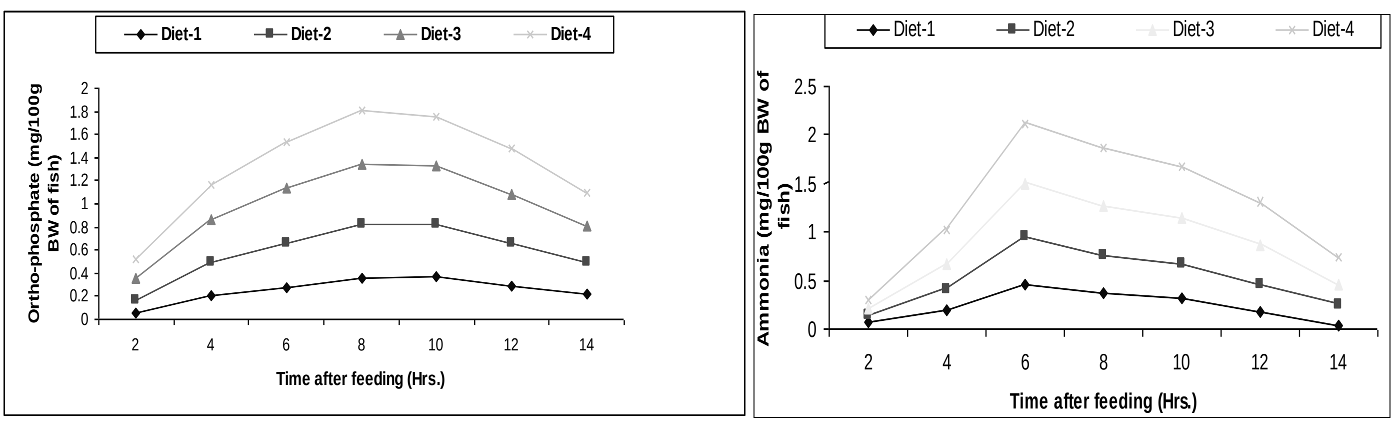


Fig. 1: Live fish weight (g) in *Aorichthys aor* (Ham.) fingerlings fed on four experimental diets (1-4) with varying protein levels (35.0 to 42.0%).



2A 2B

Fig 2: Excretory patterns of orthophosphate (2A) and total ammonia (2B) (mg 100g^{-1} Body Weight of fish) in holding waters for fish *Aorichthys aor* (Ham.) fingerlings fed on four formulated diets with varying protein levels (Diet 1-35%; Diet 2-38%; Diet 3-40% and Diet 4-42%).

Table 1: Ingredient contents (g Kg^{-1}) and proximate composition (% dry wt. basis) of diet.

Ingredients	DIET 1 (35% protein)	DIET 1 (38% protein)	DIET 1 (40% protein)	DIET 1 (42% protein)
Ingredient Composition				
Ground nut cake	650	650	650	573
Rice bran	109	58	32	75
Wheat flour	109	58	32	75
Processed full fat soybean*	112	214	266	317
Mineral mixture**	10	10	10	10
Proximate Composition				

Crude protein (%)	35.20 ± 0.35^{D}	38.23 ± 0.20^{C}	40.06 ± 0.27^{B}	42.43 ± 0.35^{A}
Crude fat (%)	7.45 ± 0.08^{C}	8.67 ± 0.08^{B}	9.11 ± 0.10^{B}	10.17± 0.19^{A}
Crude fiber (%)	6.51 ± 0.26^{A}	6.24 ± 0.18^{A}	6.20 ± 0.05^{A}	5.35 ± 0.17^{B}
Total ash (%)	5.73 ± 0.03^{B}	5.57 ± 0.24^{A}	5.45 ± 0.03^{A}	5.80 ± 0.08^{D}
Moisture (%)	7.68 ± 0.63^{C}	7.17 ± 0.13^{A}	7.18± 0.02^{A}	7.35 ± 0.03^{B}
Nitrogen Free extract (%)	36.55 ± 0.47^{A}	33.33 ± 0.38^{B}	30.62 ± 0.22^{C}	28.12± 0.18^{D}
Gross energy (kJ g^{-1})	17.53 ± 0.08^{C}	18.18± 0.04^{B}	18.31± 0.02^{B}	18.89± 0.89^{A}

*Soybean was hydrothermically processed in an autoclave at 121^{0} C (15 lbs for 15 minutes) to eliminate anti nutrient factor (Garg *et al*., 2002).
**Each Kg has nutritional value: copper 312 mg, cobalt 35 mg, magnesium 2.114 g, iron 979 mg, zinc 2mg, iodine 15 mg, L-Methionine 1.920 g, L-lysine mono hydrochloride 4.4 g, calcium 30%, phosphorous 8.25%.
All values are means ± S.E of mean.
Mean with same letter in the row are not significantly (P<0.05) different.
Data were analysed by Duncan's Multiple Range test

Table 2: Growth performances of fish fed on four experimental diets.

Growth parameters	DIET 1 (35% protein)	DIET 2 (38% protein)	DIET 3 (40% protein)	DIET 4 (42% protein)
Initial weight (g)	0.39±0.018^{B}	0.42±0.01^{A}	0.40±0.00$^{A B}$	0.42±0.00^{A}
Final weight (g)	1.97±0.12^{C}	2.09±0.00^{B}	2.32±0.02^{A}	2.30±0.01^{A}
Live weight gain (g)	1.58±0.03^{D}	1.67± 0.01^{C}	1.92±0.02^{A}	1.88± 0.03^{B}
Survival rate (%)	73.61±0.20^{A}	73.77±0.00^{A}	73.61±0.20^{A}	72.01±0.00^{A}
Growth (%) gain in BW	405.12±12.6^{B}	422.50±10.4^{B}	475.33±5.08^{A}	447.61±5.72^{A}
Growth per day (%) in BW	2.23±0.01^{B}	2.26±0.02^{B}	2.35±0.01^{A}	2.30±0.01^{A}
Specific growth rate (SGR)	1.75±0.02^{C}	1.84±0.01^{B}	2.12±0.015^{A}	2.10±0.12^{A}
Feed conversion ratio (FCR)	2.95±0.21^{A}	2.86±0.32^{B}	2.18±0.33^{c}	2.75±0.14^{B}
Gross conversion efficiency (GCE)	0.03±0.08^{B}	0.04±0.00^{B}	0.05±0.20^{A}	0.05±0.08^{A}
Protein efficiency ratio (PER)	0.04±0.00^{C}	0.05±0.00^{B}	0.06±0.20^{A}	0.04±0.08^{C}

All values are mean ± S.E of mean.
Means with same letter in the row are not significantly (P<0.05) different.
Data were analysed by Duncan's Multiple Range test.

Table 3: Proximate carcass composition of fish fed on four experimental diets

Proximate composition	Initial value	DIET 1 (35% protein)	DIET 2 (38% protein)	DIET 3 (40% protein)	DIET 4 (42% protein)
Moisture (%)	85.23±0.7	79.46±0.7^{A}	77.67±0.5^{A}	76.58±0.1^{A}	76.04±0.2^{A}
Crude protein (%)	11.24±0.31	12.87±0.54^{C}	13.38±0.5^{B}	15.18±0.26^{A}	14.48±0.1^{AB}
Crude fat (%)	2.12±0.02	3.49±0.13^{C}	3.44±0.14^{C}	4.14±0.65^{B}	4.93±0.05^{A}
Total ash (%)	2.01±0.64	2.20±0.70^{B}	3.23±0.61^{A}	3.15±0.01^{A}	3.12±0.02^{A}
Nitrogen free extract (%)	1.23±0.02	1.62±0.00^{A}	1.54±0.02^{AB}	1.42±0.65^{B}	1.22±0.06^{C}
Gross energy (kJ g^{-1})	2.25±0.19	4.68±0.17^{B}	4.78±0.10^{B}	5.45±0.73^{A}	5.56±0.04^{A}

All values are mean ± S.E of mean.
Means with same letter in the row are not significantly (P<0.05) different.
Data were analysed by Duncan's Multiple Range test.

Table 4: Effects of fish fed on four experimental diets on water quality characteristics

Physico-chemical parameters	DIET 1 (35% protein)	DIET 2 (38% protein)	DIET 3 (40% protein)	DIET 4 (42% protein)
Dissolved oxygen (DO) (mg L^{-1})	4.4±0.002	4.5±0.003	4.5±0.005	4.6±0.001
pH	7.2±0.003	7.3±0.003	7.5±0.005	7.40.002
Total ammonia (mg Kg^{-1} BW d^{-1})	1323.16±9.04^{C}	1364.65±10.02^{C}	1442.05±9.05^{B}	1636.24±10.45^{A}
Reactive phosphate (mg Kg^{-1} BW d^{-1})	865.32±11.02^{C}	902.22±10.23^{C}	1016.92±10.50^{B}	1236.25±10.02^{A}

All values are mean ± S.E of mean.
Means with same letter in the row are not significantly (P<0.05) different.
Data were analysed by Duncan's Multiple Range test.

5. Conclusion

The results of this study clearly demonstrated that processed soybean can be recommended as an alternative protein source for *A. aor*. The optimum dietary protein was found to be in the range of 40%. However, further studies using different sources of plant origin proteins are needed to find out the most suitable, economically viable and environmental friendly diet which can further reduce the excretion of ammonia and phosphate in the holding water, helping in achieving better growth and nutrient retention.

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