

Amino acid profile and growth performance of dwarf snakehead, *Channa gachua* (Hamilton 1822) fish as influenced by the alternative protein blends

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There was increased growth performance ($p < 0.05$) of juveniles in response to poultry waste diet (PWD). Lowest FCR was recorded in PWD as compared to other diets ($p < 0.05$) with highest FCR in anchovy fish diet (AFD). The AA composition of four treatment diets and muscle tissue of *C. gachua* fed on these diets was determined. AA concentration in whole-body protein was affected by dietary treatment and fish fed PWD showed comparatively higher concentrations of all essential amino acids (EAAs). In all four diets, the most abundant AA was glutamic acid (7.39 ± 0.7 , 9 ± 0.8 , 7.84 ± 0.7 and 8.1 ± 0.6) and leucine was the most abundant EAA (2.42 ± 0.1 , 2.74 ± 0.2 , 2.64 ± 0.1 and 2.67 ± 0.2). Significant differences ($p < 0.05$) existed between the contents of EAA and non-EAA in all the fish samples. In fish muscle protein, arginine, histidine, glycine, alanine, glutamic acid, cysteine, threonine, valine, leucine, and methionine concentrations were significantly higher in PWD.

[**Keywords:** Amino acid profile, Dwarf snakehead, Poultry waste diet, Glutamic acid, *Channa gachua*]

Introduction

In order to reduce excessive feeding expenses, numerous studies assessed the use of alternative, and cheaper protein sources in fish feeds^{1,2}. Animal byproducts are important contributors to the growth and extension of the world aquaculture feed sector. Several protein sources are available that can be used to replace fishmeal in aquaculture feeds without affecting the growth performance of fish³. Poultry waste has been widely studied as an alternate protein source to fish meal in various fish species' diets^{4,5}. Previous work has established precedence for the partial and total replacement of fishmeal as a protein source with poultry by-product meal, however, the amount of fishmeal replacement is species specific^{6,7}. Menghong et al.⁸ studied the effect of partial replacement of fishmeal with rendered animal protein ingredients: poultry by-product meal and meat and bone meal alone and in combination with lysine and methionine supplements in practical feed for Gibel carp, *Carassius gibelio*. As compared to fish meal,

poultry waste contains a slightly lower concentration of one or more essential amino acids including methionine, lysine and phenylalanine⁹.

Formulation of balanced and cost effective diets depends on the knowledge of basic nutritional requirements of organisms and appropriate feeding practices¹⁰. The requirement of protein concentration is one of the most important aspects of fish nutrition, since essential amino acids provided by the proteins are principally used for growth, metabolism and maintenance especially in young ones¹¹⁻¹³. The amino acid profile forms the quality of a protein and is thus the basis for high protein utilization efficiency and a high growth performance in animals^{14,15}. Dietary administration of amino acid rich protein sources may be alternatives to develop amino acid balanced feeds that can offset environmental impacts on aquaculture animals, improve growth performance, and profitability of the aquaculture industry¹⁶.

Channa gachua (Hamilton 1822) is commonly called dwarf snakehead, frog snakehead or brown snakehead and is important as food fish as well as aquarium fish due to its beautiful colouration¹⁷. Even though it is widely distributed in Asia, it has declined drastically (vulnerable) in India and listed as endangered in Singapore. Therefore, proper culture technique of this air breathing species is required. In this line, the aim of the present study was to determine the effects of four formulated diets differing in the protein source on survival, growth performance and amino acid profile of dwarf snakehead, *C. gachua* juveniles.

Materials and Methods

The percentage of formulated diets basal ingredients purchased from the local market is provided in (Table 1). The protein sources used in the experimental diets included soybean, poultry waste, anchovy and jawala fish. Selected ingredients were powdered with electric pulverizer sieved with 60 µm mesh to obtain fine particles of a uniform size. Following the feed formulation (Table 1), different ingredients were weighed and hand kneaded by adding sufficient quantity of distilled water to make dough². The dough was divided into four parts after steam cooked for 15 minutes in a closed aluminium container at 90–100 °C. To one part soybean was added (control feed) and the remaining three parts were mixed with poultry waste (PWD), anchovy (AFD) and jawala (JFD) respectively. After incorporation of fish oil, the blends were again blended 15 min for thorough mixing for the binding. These blends were passed through a feed extruder to make 3 mm diameter pellets. The pellets were dried in an oven maintained at 60 °C. Dried feed pellets were kept in separate, tightly capped plastic containers.

Feeding experiments were conducted at the Centre for Aquaculture Research and Extension (CARE), St. Xavier's College, Tirunelveli, Tamil Nadu, India. Dwarf snakehead *C. gachua* juveniles (n = 240) collected from CARE were stocked and acclimatized for two weeks prior experimentation. Three tanks were randomly assigned for each diet (triplicate) using 12 tanks (bottom surface 0.3 m², total water volume 150 l) and there was no significant difference in the fish weight among replicates, at the start of the experiment (3.23 ± 0.08 g, weight; n = 20 per tank). Before 48 h of the experiment, the juveniles were starved to empty their gut contents. To examine the effect of feed on growth and survival of juveniles, four types of diets viz.,

control diet; poultry waste diet (PWD); jawala fish diet (JFD); anchovy fish diet (AFD) were selected for feeding the fish for 60 days. Each tank was provided with four PVC pipes of 20 mm diameter for hiding purposes.

For the feeding trial experiment, pellet diet were offered at 5% body weight per day and dispensed 2 times a day at 09:00 and 16:00 h in equal proportion. Fishes were sampled and weighed initially and thereafter fortnightly for growth estimation. Five individuals from each tank were randomly collected, measured directly on a measuring board and weighed using an electronic balance (sensitivity 0.001 g). Unconsumed feed and fecal matter were siphoned out with daily water exchange up to 20% during the experimental period. The photoperiod was a 12L: 12D cycle. Water quality parameters viz., water temperature (28.5– 30.7 °C), dissolved oxygen (4.3–6.5 mg l⁻¹), pH (7.0–8.3) and ammonia (0.16 mg l⁻¹) in the rearing water were monitored daily. Aeration was supplied 24 h.

The experimental diets were analysed for proximate composition of dry matter, crude protein, crude lipid, fibre and ash using standard AOAC methods¹⁸. Briefly, dry matter was determined by drying feed sample at 100 °C to constant weight. Nitrogen content was determined by the Kjeldahl method¹⁸ using KEL PLUS – Elite ExVA – diigestion and distillation apparatus and the protein content was calculated by multiplying the nitrogen content with a factor of 6.25 (Nitrogen x 6.25). Crude fibre was estimated by loss on ignition of dried residue after successive digestion with 5% H₂SO₄. The crude fat was determined by Soxhlet method¹⁸ using petroleum ether (60–80 °C) as solvent in a SOCS PLUS-SCS 08R system. Total carbohydrate was estimated by using the method of Roe, using TCA extracted sample¹⁹. Ash content was determined by combusting samples in a muffle furnace at 500–550 °C for 16 h¹⁸. By subtracting the sum of crude protein, crude fat, ash and crude fibre from the total dry matter content, Nitrogen free extract (NFE) was calculated.

About 0.5 g sample (diet and muscle tissue) was weighed and put into flat bottomed flask (100 ml) containing 1 ml Norleucine standard solution and 5 ml performic acid kept in ice bath. After oxidation procedure (16 h) carried out in a refrigerator, sodium meta bisulphite (0.84 g), 6N HCl (30 ml) and anti-bumping granules were added to this mixture. This mixture was hydrolysed for 24 h in PEG bath at 130 °C. On completion of the reaction, samples were allowed to cool and 4 M lithium hydroxide (30 ml) was

added into the mixture. pH of the sample was adjusted to 2.1 and the mixture was made up to 100 ml final volume. About 5 ml of the sample was filtered through 2 µm filter and this was run through a Biochrom 20 Amino Acid Analyser. The data was collected in the form of chromatograms. The analyser was ion exchange with several buffers at varying pH running through the system. Each sample took 4 h to run through the system. The analysis was carried out by a commercial analysis laboratory (Research Laboratory, Coimbatore, India).

Table 1– Ingredients used and proximate composition of the experimental diets (g kg⁻¹ DM)

Ingredients	Control	PWD	AFD	JFD
Soy Flour	25	10	10	10
Tapioca Flour	20	10	10	10
Wheat Flour	20	10	10	10
Rice Flour	20	10	10	10
Rice Bran	10.7	7.5	7.5	7.5
Fish Oil	2.3	2.3	2.3	2.3
Vitamin / Mineral Mix*	2	2	2	2
Anchovy (%)	0	0	48	0
Jawala (%)	0	0	0	48
Poultry waste (%)	0	48	0	0

*Vitamin-mineral premix (mg Kg⁻¹ diet): 80:riboflavin, 6.0:biotin, (IU) vitamin A, 6000: vitamin ANCHOVY DIET, 2000: vitamin E, 6000 IU, 0.1: nicotinic acid, pantothenic acid, 180 menadione, 0.6: thiamin hydrochloride, 15: pyridoxine, 40: inositol, 400: astaxanthin, 60: choline chloride, Zinc (as sulphate) 72, iron (as sulphate) 36, manganese (as sulphate) 12, selenium (as selenate) 0.2, chromium (trivalent, as chloride) 0.8, iodine (as iodate) 1.2, copper (as sulphate) 24, cobalt (as chloride) 0.6 and molybdenum (as molybdate) 0.2.

Weight gain (WG), specific growth rate (SGR), mean growth rate (MGR), food conversion ratio (FCR) and survival rate were calculated employing the following equations:

$$\text{Weight gain (g)} = (W_2 - W_1)$$

$$\text{SGR (\% WG/d)} = 100 (\ln W_2 - \ln W_1) / t$$

$$\text{MGR (mg g}^{-1} \text{ d}^{-1}) = 1000 (W_2 - W_1) / 0.5(W_1 + W_2) t$$

$$\text{FCR} = \text{dry food consumed (g)} / \text{wet weight gain (g)}$$

$$\text{Survival rate (SR)} = (\text{No. of live fishes} / \text{No. of fishes introduced}) \times 100$$

where, Ln= Natural log, W₁ = Initial weight, W₂ = Final weight, t = Culture period in days.

All statistical analyses were carried out using Statistica 6.0 and SPSS 10.0. Data was expressed as the mean±SE of triplicates. The means within each treatment and among treatments were compared using Turkey's test of multiple comparison with a 0.05 % significance level.

Results

All four formulated diets significantly affected growth performance and feed utilisation of *C. gachua* juveniles (Table 2). Feed utilization of *C. gachua* juveniles was significantly affected by protein level. AFD, JFD and control fed fishes attained significantly low ($P < 0.05$) level of survival and growth when compared to PWD fed group. SGR (% WG/d) was significantly higher in fish fed on PWD (2.63 ± 0.09) followed by control group (2.39 ± 0.13) whereas SGR was lowest in fish fed on JFD (2.27 ± 0.17). Similar trend was observed for MGR. Maximum MGR ($\text{mg g}^{-1} \text{ d}^{-1}$) was recorded in PWD (21.94 ± 0.08) which was significantly different ($p < 0.05$) from control diet (20.54 ± 0.16). Minimum MGR was found in JFD fed juveniles (19.77 ± 0.21). The best FCR (1.09 ± 0.11) obtained from PWD (40.32% crude protein) was significantly different from other diets, while the lowest FCR (1.25 ± 0.12 , $P < 0.05$) was obtained from AFD (37.03% crude protein). Survival rate (76%) was significantly different ($p < 0.05$) in PWD than other treatments whereas the lowest value (70%) was recorded in AFD (Table.2)

The chemical composition of formulated feeds is provided in Table 3. Among the four formulated diets, the highest protein content was significantly ($P < 0.05$) elevated in PWD (40.32%) followed by AFD (37.03%) and JFD (35.78%), whereas protein contents are significantly lower in control diet (25.89%). However, the carbohydrate and lipid contents were significantly higher ($P < 0.05$) in PWD and AFD incorporated feeds, respectively.

Table 2– Survival and growth of *Channa gachua* juveniles fed on different feeds

Parameters	Control	PWD	AFD	JFD
Initial Weight (g)	3.23 ± 0.24	3.07 ± 0.15	3.26 ± 0.20	3.37 ± 0.27
Final Weight (g)	13.6 ± 0.30	14.9 ± 0.18	13.5 ± 0.24	13.2 ± 0.44
Weight gain (g)	10.37±0.23	11.83±0.19	10.24±0.12	9.83±0.31
**FCR	1.23±0.12	1.09± 0.11*	1.25± 0.12*	1.20± 0.15
**SGR (%)	2.39±0.13	2.63± 0.09*	2.36± 0.12	2.27±0.17*
**MGR (mg g ⁻¹ d ⁻¹)	20.54±0.16	21.94±0.08	20.36±0.15	19.77±0.21
Survival (%)	72	76*	70	75

Each value is mean ± SD of 10 individuals. *Significant at (P < 0.05).

**FCR = Food Conversion Ratio; **SGR = Specific Growth Rate; **MGR = Mean Growth Rate

Table 3– Proximate chemical analysis (% diameter basis) of four diets fed to *Channa gachua*

COMPONENT (%)	DIETARY TREATMENTS			
	Control	PWD	AFD	JFD
Crude protein	25.89	40.32	37.03	35.78
Crude carbohydrate	12.05	12.25	13.93	13.23
Crude fibre	2.22	2.00	2.38	2.10
Crude lipid	5.54	6.12	5.54	7.97
Total ash	15.04	14.45	19.02	17.05

*Values are means of two replications per diet

Fig. 1 A shows the amino acid compositions for each diet. Overall, the amino acid content in the control diet, PWD, AFD and JFD showed the presence of EAA such as arginine (1.92±0.1, 2.85 ± 0.3, 2.3±0.3 and 2.02±0.2), histidine (0.72±0.08, 0.92±0.06, 0.82±0.04 and 0.81±0.05), isoleucine (1.28±0.07, 1.75±0.08, 1.42±0.04 and 1.43±0.05), leucine (2.42 ±0.1, 2.74±0.2, 2.64±0.1 and 2.67±0.2), lysine (1.46±0.1, 1.91±0.2, 1.82±0.2 and 1.92±0.1), methionine (0.39±0.04, 0.87±0.05, 0.50±0.02 and 0.71±0.02), phenylalanine (1.52±0.1, 1.93±0.2, 1.7±0.08 and 1.57±0.07), threonine (1.24 ±0.2, 1.81±0.1, 1.33±0.2 and 1.6±0.1), tyrosine (1.01±0.1, 1.51±0.05, 1.08±0.08 and 1.12±0.3), valine (1.39±0.06, 1.96±0.04, 1.08±0.07 and 1.12±0.08) and non-EAA viz., alanine (1.4±0.5, 2.76±0.4, 1.56±0.5 and 1.43±0.3), aspartic acid (3.21± 0.5, 4.06±0.4, 3.86±0.4 and 3.72±0.5), cysteine (0.49±0.02, 0.81±0.01, 0.6±0.01 and 0.63±0.02), glutamic acid (7.39±0.7, 9±0.8,

7.84±0.7 and 8.1 ± 0.6), glycine (1.4±0.05, 1.88±0.04, 1.6±0.05 and 1.68±0.03), proline (2.16±0.2, 2.69±0.1, 2.23±0.1 and 2.34±0.2) and serine (1.56±0.04, 2.52±0.1, 1.8±0.05 and 1.8±0.07). Glutamic acid and aspartic acid were significantly higher among all AA concentrations and are both non-EAAs. Leucine and arginine constituted the highest EAA concentration. The overall amino acid level was significantly lower in the control diet when compared to PWD, AFD, and JFD.

The analyses of the muscle tissues of *C. gachua* revealed the presence of twelve amino acids of which lysine, arginine, histidine, threonine, valine, isoleucine, leucine and methionine are EAA and cysteine was the only non-EAA. More particular amino acid analysis on the tissue of the fish perfectly reflected their utilization of certain amino acids, high levels of arginine (2.52±0.01, 3.04±0.01, 2.92±0.02 and 2.92±0.01), cysteine (2.16±0.01, 2.19±0.02, 2.12±0.02 and 2.08±0.01), lysine (1.16±0.1, 1.44±0.01, 1.52±0.02 and 1.44±0.3), threonine (1.12±0.02, 1.16±0.01, 1.12±0.01 and 1± 0.01) and histidine (1.28±0.06, 1.76±0.01, 1.24±0.02 and 1± 0.03) are expressed in *C. gachua* juveniles fed on control diet, PWD, AFD, and JFD (Fig. 1 B).

The concentration of EAA viz, lysine, arginine, threonine, valine, leucine and methionine in fish muscle protein was lower in control diet (p < 0.05), whereas the concentration of isoleucine was significantly higher. The amino acid contents viz., histidine, arginine, leucine, glycine and alanine were found to be higher in *C. gachua* fed with PWD when compared to other diets.

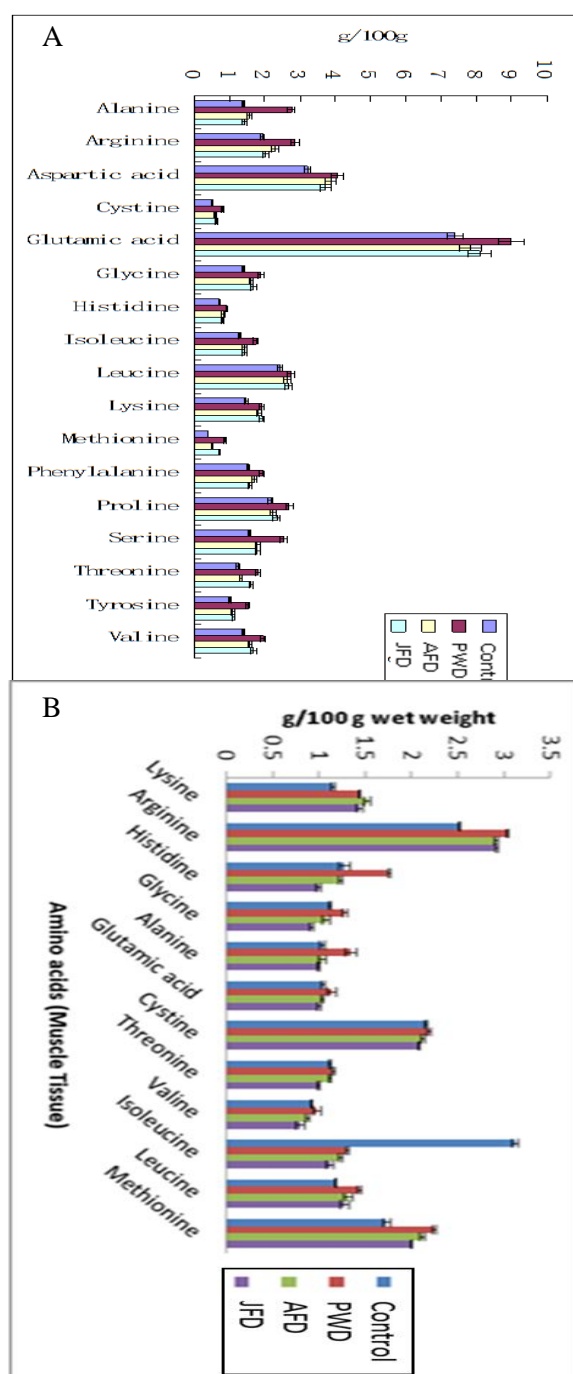


Fig.1–Amino acid profile of the experimental diets (A) and tissue (g/100 g wet wt.) of *C. gachua* fed on experimental diets (B) Results were plotted as mean \pm standard error, n= 3.

As per essential amino acid index (EAAI) of the muscle tissue, higher value (246.89) has been recorded in PWD containing 40% of crude protein followed by 212.46 for *C. gachua* fed 35% protein diet (JFD), 208.86 for fish fed 37% protein diet (AFD) and the least value of 185.65 for Control diet.

Discussion

Traditionally, fish meal is the key and preferred protein source used in aquaculture feeds because of its excellent nutritional composition. However, the increasing demand and limited supply make fish meal an expensive protein source²⁰. The growing demand, increasing price and inconsistency of supply are limiting the use of fish meal, putting a great pressure on the feed industries to find economical alternative source of protein. Alternative protein sources such as Poultry waste have been tested using different fish models²¹⁻²³.

The nutritional value of the feed plays an important role in fish culture as it reflects on the consumer organisms²⁴. Estimation of proximate composition of fish diet is important in understanding their physiological function, metabolic rate, nutritive value and energy transfer²⁵. Ash, fibre, lipid and protein contents showed significant ($p < 0.05$) differences among the experimental diets (Table 2).

In the present study, PWD showed better results in terms of survival rate, weight gain FCR, SGR and MGR. In aquaculture a feed with FCR of 1:1 and 1:2 are considered as very efficient²⁶. Values of FCR in the present study (1.09-1.25) were similar to previous reports of Rouse and Kahn²⁷, Jones and Ruscoe²⁸, Thompson et al.²⁹ and Webster et al.³⁰. The diet PWD containing 40.32% crude protein was found to meet the protein requirement of *C. gachua*, which seems to be very close to the requirement observed for *H. longifilis*³¹ with 45.36% and hybrid *H. longifilis*³² with 45%.

It was observed that animal by product like poultry could replace 30% to 50% of fish meal in Cuneate drum diet without affecting the SGR, final BW and FCR⁵. Replacement of fish meal with poultry by product at 80% and 100% levels in the pacific white shrimp and sunshine bass diet respectively does not have negative effects on the FCR and BW⁷. Gallagher and LaDouceur³³ observed that juvenile Palmetto bass fed with a diet containing 12% fish meal and 36% low-ash poultry by product had weight gains similar to fish fed with a diet containing 47% fish meal. Poultry by products seems to be a good dietary protein alternative to fish meals in fish diet.

The success of fish culture largely depends on the availability of the essential amino acids as fish cannot synthesize these

amino acids and these are to be chiefly obtained from the diet³⁴. During the present analysis, totally 17 amino acids were observed in experimental diets with glutamic acid, aspartic acid, arginine, leucine, proline, lysine, threonine, alanine, serine and glycine being prominent among them (Fig. 1 A). Only very limited information is available on the amino acid content of the formulated diets used in the present experiment, but the role of amino acids in the growth and maintenance of fish growth is well understood. The diet, PWD, has a high amount of individual amino acids compared to that of other feeds. The amino acid profile of muscle tissue of *C. gachua* fed on these diets also perfectly reflected the utilization of amino acids available in the diet. Methionine plays a vital role in the promotion of fish growth³⁵. Hence, the comparatively high percentage of methionine in PWD (Fig. 1 A) would have contributed to the increase in the specific growth rate of juveniles fed. Chen et al.³⁶ reported that amino acids viz., phenylalanine, glycine, histidine and alanine reduce the oxidative stress in the organisms and promote the growth of the organism. The presence of high percentage of arginine in PWD as compared to other diets would have stimulated the release of hormones such as somatotropin and insulin in *C. gachua* juveniles and enhance the size of the fish³⁷. Histidine is involved in many metabolic functions including production of histamines, which take part in allergic and inflammatory reactions. It plays an important role in maintaining the osmoregulation and energy production³⁸. This suggests that the specific functions governed by these amino acids were performed well in the juveniles fed with PWD. Replacement of fish meal with poultry waste appears to provide sufficient levels of essential amino acids to meet the necessary requirements of *C. gachua*.

Conclusion

Present investigation assessed the nutritive value of four formulated feeds as a diet in *C. gachua* culture. It was experimentally proved that, poultry waste can be an alternative to fish meal in murrel diets and PWD showed acceptable results in terms of acceptance, nutritional value and other factors. In the view of selecting a profitable supplement in feed management practices, use of poultry waste may be recommended as an alternative ingredient in feed formulation of freshwater murrel culture

since it is a bio-waste, polluting the environment.

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