

Growth performance study of Koi fish, *Anabas testudineus* (Bloch) by utilization of poultry viscera, as a potential fish feed ingredient, replacing fishmeal

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Abstract

Background In India, fishmeal is habitually considered as the major protein source for fish culture. Rising cost, deteriorating quality and unavailability of fishmeal caused huge difficulty in modern aquaculture, particularly for fish nutrition. Nearly all *Anabas testudineus* cultures faced loss, due to high feed costs, inappropriate feed formulation and management. So it was necessary to find a profitable replacement of fishmeal, which could provide better growth performance to *A. testudineus*.

Results In this study, different types of poultry wastes were tested, and poultry viscera confirmed satisfactory results, having 60.67 % crude protein (% Dry matter basis). Further, a feeding trial was conducted for 60 days in 90-L circular fibre tanks with proper aeration, to evaluate poultry viscera, in the formulated diet for *A. testudineus*. Triplicate groups of fingerlings each were fed four isonitrogenous diets, at 5 % of wet body weight basis. Feed readjusted biweekly. Comparatively, fish accumulated highest and significant ($P < 0.05$) increase in body weight and fat deposition, when dietary fishmeal completely replaced by poultry viscera.

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Discussions Thus, this study revealed that better growth performance in Koi, *A. testudineus* (Bloch) could be achieved through utilization of poultry viscera in the formulated fish feed, compared to fishmeal. Hence, poultry waste recycling could be stimulated also.

Keywords Crude protein · Fishmeal · Koi · Poultry viscera · Proximate composition

Abbreviations

90-L	90 Litre capacity, of the circular fibre fish rearing tank
cm	Centimetre (Length)
CP	Crude protein
DO	Dissolved oxygen in water
E longitude	Eastern longitude
FCR	Feed conversion ratio
FM	Fishmeal
gm	Gram (weight)
NFE	Nitrogen-free extract
N latitude	Northern latitude
PVisc	Poultry viscera
SGR	Specific growth rate
T	Treatment
Temp	Temperature
WB	West Bengal

Introduction

Fish as the prime animal protein source in the nutritional budgeting of West Bengal (Eastern India) deliver high protein, low cholesterol and tasty-soft flesh, etc. About

85–90 % fish protein is digestible and all the dietary essential amino acid is in fish flesh (Cappell et al. 2007).

Climbing Perch (*Anabas testudineus*) is locally known as 'Koi' in West Bengal. It is one of the most popular fishes in West Bengal, due to its traditional role as a medically prescribed diet for sick and convalescents. It contains high amount of available iron and copper essentially needed for haemoglobin synthesis. In addition, it also contains all the essential amino acids (Saha 1971).

Anabas testudineus encompasses a wide range of geographical distribution. This species is also found in India, Pakistan, Burma, Sri Lanka, Thailand, China, Hong Kong, Philippines and Malaysia (Jayaram 1981). It is found in fresh and brackish waters mostly in ponds, swamps and lakes. Climbing perch appears to be visual feeders, feeding primarily during the day (Patra 1993). It is a ferocious predator, omnivorous in feeding habit and tolerate wide range of environmental conditions.

In adverse atmospheric circumstances, this fish proves to be a very good challenger of fish culture. But, often its production fails to fulfil its high market demand, due to lack of proper culture. However, the results of the survey in 2002, more than 50 % climbing perch culture farmers were lost due to high feed costs (Tihn 2003).

There are very little information available based on the nutritional requirement of *A. testudineus*. It is important to know the minimum protein requirement for best growth in formulating a balanced diet as protein is the key nutrient offering growth and other metabolic activities.

Usage of combination of various animal or plant protein ingredients in the place of fish meal in fish diet has been demonstrated successfully (Millamena 2002). Viscera are the large organs inside the body of animals, such as the heart, lungs and stomach, etc. Research findings have revealed that Recycling of wastes from poultry slaughter houses is of economical, biological and environmental importance (Cai et al. 1994; Steffens 1994). However, poultry wastes recycling could be stimulated by the utilization of poultry viscera.

Considering the above, the present study was conducted with a view to set up the best dietary protein requirement of *A. testudineus* and to assess the result of replacing dietary fishmeal protein by poultry viscera, on the growth performance of *A. testudineus*.

Materials and methods

Experimental sites and study period

During the study period, from April, 2012 to May, 2013 fish feed ingredients were collected and then transported to

the Zoology Laboratory, Visva-Bharati, Birbhum, W.B, India (23°41'30"N Latitude and 87°41'20"E Longitude).

Collection and storage of samples

Samples of feed ingredients such as Poultry viscera, fish meal, rice bran, wheat bran, Mustard oil cakes, wheat flour, etc., were collected, and sun dried properly, then packed in polyethylene bags to prevent initial spoilage and brought to the ASEPAN laboratory Visva-Bharati, then stored in refrigerator.

Proximate composition analysis

Proximate analysis is usually the first step in the chemical evaluation of a feed ingredient, where the material is subjected to a series of relatively simple chemical tests so as to determine the content of moisture, crude protein, lipid, crude fibre, ash, etc.

Estimation of moisture (%)

Moisture is commonly determined by drying a sample at some elevated temperature (100 ± 5 °C) for 30 min and further at 60 °C, until a constant weight was obtained, following [Association of Official Analytical Chemicals (AOAC 1995)].

Determination of ash (%)

Ash is readily determined by ignition from dried sample at about 550 ± 50 °C for 6–8 h in muffle furnace. The residue is weighed and reported as ash, following AOAC (1995).

Determination of crude protein (%)

The crude protein determined by Micro-Kjeldahl method using a Kjeltac system (Tecator, Sweden) through digestion and distillation steps. Kjeltab (containing potassium sulphate and catalysts), H₂SO₄, NaOH, Phenolphthalein, etc., used in this method, following Pearson (1999).

Determination of crude lipid (%)

The lipid content was determined by Soxhlet apparatus. 2gm of sample wrapped in whatman filter paper (No-1) and placed in a thimble connected with Soxhlet apparatus. Initial weight of soxhlet flask recorded and filled with 200 ml petroleum ether, which boiled for 8 h at 60–80 °C through the thimble, by siphoning process. Flask was taken out and allowed to evaporate. The difference in the two



weights of the round joint flask gave the weight of the lipid, following AOAC (1995).

Analysis of fibre (%)

Done by following the method of Pearson (1976).

Nitrogen-free extract (%)

{100 – (% moisture + % crude protein + % crude lipid + % crude fibre + % ash)} following AOAC (1995).

Carbohydrate (%)

[Nitrogen-free extract % + Crude Fibre %] following Hastings (1976).

Caloric value/gross energy (kcal/100 g)

[(Carbohydrate × 4.1/100) + (Protein × 5.65/100) + (Lipid × 9.45/100)]. After (Cho et al. 1982).

Growth parameters

The following parameters were used to evaluate the growth, by using the following formulas according to Castell and Tiews (1980):

Survival rate (%) = $100 \times (\text{final number of fish}/\text{initial number of fish})$.

Specific growth rate (SGR) (%/day) = $100 \times (\text{Ln Wf} - \text{Ln Wi})/\Delta t$.

Feed conversion ratio (FCR) = $\text{feed consumed}/(\text{Wf} - \text{Wi})$. Where: Wf = final weight and Wi = initial weight. Feed consumed = feed given – feed not eaten. Δt = duration (days).

Experimental design

90-L circular fibre tanks were used in this experiment, with proper aeration (Fig. 1). Triplicate groups of fingerlings each were fed four isonitrogenous diets (above 34 % C.P) namely T-1, T-2, T-3 and T-4. Acclimatization period of 15 days was applied to all fishes in laboratory condition, with the feed of 1: 1 mixture of rice bran and mustard oil cake. Stocking rate was 25 fish/tank. Feed ingredients were cleaned under running tap-water, then sun dried for weeks and then stored in air tight plastic container in refrigerator. Diet particle size was used as 0.4 mm to 1.25 mm. Feeds were applied twice a day (08:00 a.m. and 03:00 p.m.) at the rate of 5 % wet body weight basis of the fry. Feed readjusted biweekly. Experimental trial period was of 60 days.

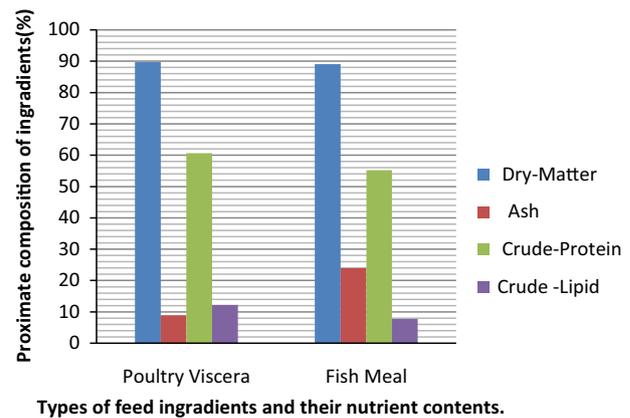


Fig. 1 Nutritional comparison between PVisc and FM (% dry matter basis): PVisc poultry viscera, FM fishmeal

Sampling and data analysis

25 fishes were sampled (Randomly) every 14th day to determine fish growth rate from each treatment, by the help of glass nylon hapa. 1 h after feeding, faeces, waste particles of food and dead bodies of fish were siphoned out for measurement. The survival rate of fish was calculated at 60th day. Feed ingredients, formulated diets, fish body carcass, etc., were analysed for proximate composition following the AOAC (1995) procedures. Water quality parameters were determined by APHA (1992) procedures. All the structured designs and data were analysed using MS-Excel one-way ANOVA. This was followed by Duncan's new multiple range test (Duncan 1955) to identify the level of significance of variance ($P < 0.05$) among the treatment means. Standard deviations (\pm SD) of treatment means were also calculated.

Results

Table 1 shows highest protein content in poultry viscera (60.67 %) that is much more than in Fishmeal (55.19 %). Highest lipid contents observed in poultry skin (12.89 %). Highest ash contents observed in poultry litter (15.98 %). In this study, the estimated protein, lipid and ash contents of poultry viscera are 60.67, 12.25 and 08.93 %, respectively those are slightly higher than the findings of Cai et al. (1994). The estimated Lipid contents of Poultry Intestine are 10.41 % which is more or less similar with the findings (07.64 %) of Tabinda and Butt (2012).

Feed formulation and proximate chemical composition of the control and experimental diet (%)

Replacement of fish meal with poultry viscera in compound diet was performed on the culture of *A. testudineus*

Table 1 Proximate composition of different poultry wastes and FM (% dry matter basis)

Ingredients	Dry matter	Ash	Crude protein	Crude lipid
Poultry viscera	89.70 ^b ± 0.15	08.93 ^a ± 0.40	60.67 ^a ± 0.41	12.25 ^a ± 0.22
Poultry intestine	88.4 ^b ± 0.73	06.25 ^a ± 0.07	53.77 ^a ± 0.53	10.41 ^a ± 0.65
Poultry skin	89.59 ^{ab} ± 0.52	07.97 ^a ± 0.19	31.09 ^a ± 0.35	12.89 ^{ab} ± 0.28
Poultry litre	87.03 ^{ab} ± 0.64	15.98 ^a ± 0.56	19.01 ^a ± 0.74	02.13 ^b ± 0.33
Fish meal	89.10 ^a ± 0.23	23.95 ^a ± 0.38	55.19 ^b ± 0.53	07.77 ^b ± 0.42

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

FM fish meal

for 60 days. In the feeding treatments fish meal was replaced by 33.3, 66.6, and 100 % of poultry viscera, respectively, as the experimental diets. The diet containing 100 % fish meal (0 % poultry viscera) was set as the control diet to compare performances with those fed with other experimental diets (Tables 2, 3, 4).

Notes: different levels of replacement of fish meal with poultry viscera.

63.44 %, more or less similar to the findings of Fisheries Research Institute, FRI (1989). During the present study the crude lipid content was recorded in the range between 09.22 and 16.49 % in poultry viscera, more or less similar to the findings of Hasan and Amin (1997).

Triplicate groups of fingerlings each were fed four iso-nitrogenous (34–35 % C.P) diets namely T-1, T-2, T-3 and T-4 which is, however, similar with the treatments of

<u>Feed Treatments</u>	<u>Levels of Replacement of F.M with P.Visc</u>	<u>Diet-Type</u>
T-1-----	100 % Fish meal, 0 % Poultry viscera-----	Control Diet.
T-2-----	66.6 % Fish meal, 33.3 % Poultry viscera ----	} Experimental Diet.
T-3-----	33.3 % Fish meal, 66.6 % Poultry viscera ----	
T-4-----	---0 % Fish meal, 100 % Poultry viscera-----	

Discussion

In this study, the common fish feed ingredients like fish meal, mustard oil cake, rice bran, wheat bran and wheat flour were used for proximate analysis, which are usually available throughout the year and all over India. Low cost and good quality supplementary feeds proved essential for superior practices, and it was the key demand of farmers to reduce production cost. For formulation of fish feed, information about feed ingredient's price, nutritive value, application strategies, and its seasonal variation on quality and availability were crucial. The present study attempted to collect this information.

Due to rising cost, uncertainty and unavailability of fishmeal, it was an immense problem in modern aquaculture, especially in relation to fish nutrition, to find a desirable replacement for fish meal. Researches on poultry viscera have revealed interesting results and more works were being done by scientists from all over the world.

The analysed crude protein content of the poultry viscera was estimated in the range between 57.90 and

Adhikary et al. (2009) and this level is higher than that of 30 % reported for *A. testudineus* by Chareontesprasit and Jiwyam (1996).

The results showed that the growth of *A. testudineus* fry varied significantly ($P < 0.05$) with different diets. At the end of feeding trial, highest S.G.R (1.39) and survival rate (85 %) were found where fish were fed on T-4 (treatment where fishmeal 100 % replaced by poultry viscera). The poorest growth rate was shown by T-1 (control treatment, where fishmeal was not replaced by poultry viscera). These results are more or less similar with the findings of Doolgindachabaporn (1994).

FCR was highest (3.82) in T-1 and gradually decreased (2.10) in T-4 with the increasing dietary protein levels. Similar trend observed in Tilapia (Jauncey 1982) and *Puntius gonionotus* (Wee and Ngamsnae 1987). Doolgindachabaporn (1994) found that the FCR value of *A. testudineus* ranges from 1.8 to 3.0. Similarly, FCR values in the present study were comparable with FCR values (01.29–01.62) in rainbow trout, by the nutritional diet of co-dried fish silage (Hardy et al. 1984). Potongkam (1972)

Table 2 Formulation and proximate chemical composition of the control and experimental diets (%)

	Ingredients	Experimental (replacement of FM by PVISC)			
		Control T-1	33.3 % T-2	66.6 % T-3	100 % T-4
Feed formulation of the control and experimental diets (%)					
	Fish meal (FM)	30.00	20.00	10.00	00
	Poultry viscera (PVisc)	00	10.00	20.00	30.00
	Mustard oil cake	15.50	15.50	15.50	15.50
	Rice bran	29.90	29.10	29.60	29.50
	Wheat bran	19.00	19.80	19.30	19.40
	Vitamin premix	00.50	00.50	00.50	00.50
	Mineral premix	00.10	00.10	00.10	00.10
	Sunflower oil	05.00	05.00	05.00	05.00
	Total	100	100	100	100
Proximate chemical composition of the control and experimental diets (% dry matter basis)					
	Dry matter	90.97	91.29	90.92	91.13
	Crude protein	34.33	34.37	34.90	35.88
	Crude fat	07.62	08.8	09.20	09.68
	Crude ash	06.58	06.92	07.66	06.36
	Crude fibre	02.51	02.30	02.30	02.13
	Nitrogen-free extract	39.93	38.90	36.86	37.08
	Gross energy (kcal/100 g)	439.97	446.27	444.68	454.95

Per kg vit premix: 4,000,000 IU vitamin A, 480,000 IU vitamin D3, 40,000 mg vitamin E, 2,400 mg vitamin K3, 4,000 mg vitamin B1, 6,000 mg vitamin B2, 40,000 mg niacin, 10,000 mg Ca-pantothenate, 4,000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1,200 mg folic acid, 40,000 mg vitamin C and 60,000 mg inositol

Per kg min premix: 23,750 mg Mn, 75,000 mg Zn, 5,000 mg Zn, 2,000 mg Co, 2,750 mg I, 100 mg Se, 200,000 mg Mg

Table 3 Growth parameters and survival of *A. testudineus*

Parameters	Treatments			
	T-1	T-2	T-3	T-4
Initial weight (g)	0.91 ^a ± 0.04	0.91 ^a ± 0.04	0.92 ^a ± 0.07	0.92 ^a ± 0.07
Final weight (g)	4.61 ^b ± 0.07	5.79 ^{a,b} ± 0.05	5.87 ^a ± 0.14	6.31 ^{a,b} ± 0.07
FCR	3.82 ^a ± 0.03	2.70 ^{a,b} ± 0.02	2.63 ^{b,c} ± 0.08	2.10 ^c ± 0.04
Survival rate (%) (S.G.R)	83 ^a ± 0.08	84 ^a ± 0.10	83 ^a ± 0.05	85 ^a ± 0.07
	1.17 ^b ± 0.04	1.33 ^{a,b} ± 0.06	1.34 ^{a,b} ± 0.03	1.39 ^b ± 0.06

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

Table 4 Water quality parameters for different treatments

Parameters	Treatments			
	T-1	T-2	T-3	T-4
Average Temp. (°C)	29.8 ^a ± 0.03	29.7 ^a ± 0.22	29.8 ^a ± 0.43	29.8 ^a ± 0.12
Average pH	7.9 ^b ± 0.04	7.6 ^{a,b} ± 0.03	7.7 ^a ± 0.05	7.8 ^{a,b} ± 0.23
Average D.O (mg/l)	6.1 ^a ± 0.06	6.3 ^{a,b} ± 0.04	6.0 ^{b,c} ± 0.04	6.1 ^c ± 0.04

Water quality parameters were determined by APHA (1995) procedures. Values are ±SD of three replications. Figures in the same row having different superscript are significantly different ($P < 0.05$)

reported that FCR of climbing perch fed on trash fish and pellet was 2.07 and 1.89, respectively.

In the present study, the water quality parameters were found to be within the acceptable level for fish culture. Recommendation for water quality usually specifies DO greater than 3 mg/l for growth of channel catfish (Weeks and Ogburn 1973). The pH values obtained in this study

(7.6–7.9) fell within the suitable range according to Swingle (1967) and Boyd (2000).

My current research focused on the evaluation of poultry viscera as a prospective replacement for fish meal in the culture of Koi, *A. testudineus*. It is hoped that the results will reveal more clues that will justify poultry viscera as a potential replacement for fish meal. The experimental



results suggested that T-4 (treatment where fishmeal 100 % replaced by poultry viscera) can be recommended for the intensive culture of *A. testudineus*.

Conclusion

Poultry viscera can 100 % replace fish meal potentially, in the compound diet for Koi, *A. testudineus*, only by proper formulation and sun-dry processing. Applying these feed fish farming becomes more profitable to the poor fish farmers by lowering the feed costs to a certain degree.

Author contribution All authors, have made reasonable effort on all parts of the work necessary for the development of this manuscript in accordance with their expertise. All authors read and approved the final manuscript.

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Conflict of interest The authors declare that there is no conflict of interest.

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