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Specific growth rates of *Clarias batrachus* (Linn.) and *Anabas testudineus* (Bloch), by poultry waste diet, apart from fishmeal: A comparative study

Pratyush Bhaskar, Saroj Kumar Pyne and Arun Kumar Ray

Abstract
This comparative study on growth of Indian air-breathing fishes, *Clarias batrachus* (Linn.) and *Anabas testudineus* (Bloch) was performed in 42-L aquariums with water recirculation system for 60 days. Triplicate groups of fingerlings each were fed four isonitrogenous compound diets, at 5% of wet body weight basis. Fishmeal in control diet was 100% replaced by poultry wastes (skin, intestine and viscera) in three experimental diets respectively, though other ingredients remained same. Effects of poultry wastes were judged on the basis of comparative growth performances (S.G.R%, F.C.R, P.E.R) of both fish types. Proximate composition confirms, nutrients in poultry viscera are superior to fishmeal. P.E.R, S.G.R%, weight gain and survival rate of both fish types were recorded highest with poultry viscera based compound diet, which are significantly different from control diet. So, this treatment is preferred and is recommended, for both fish types, because the use of poultry viscera as a nutritious feed supplement helps to reduce feed cost ensuring optimal fish growth, and stimulate waste management also.

Keywords: Indian air-breathing fishes, crude protein, nutrition, poultry viscera

1. Introduction
Fish is the conventional food source, because it is palatable, tender, and of high nutritive importance. In West Bengal of India, air-breathing fishes like *Clarias batrachus* (Linn.) and *Anabas testudineus* (Bloch) are marketed fresh and live, due to their traditional role as a medically prescribed diet for sick and convalescents. They contain high amount of iron and copper essentially needed for haemoglobin synthesis. In addition, they also contain all the essential amino acids [12, 31]. By nature, both are predators, omnivorous in feeding habit and tolerate a wide range of environmental conditions, especially they can survive in low oxygen content ponds [1, 30]. The qualities of both fishes as food, includes firm textures and taste beside their high levels of nutrients, which play an important role for their selection in this experiment.
There is little dietary information available for *C. batrachus*, but no such definite information available based on the nutritional requirement of *A. testudineus*. So, it is important to find out the optimum dietary protein requirement for best growth for these fishes, as protein is the key nutrient offering growth and other metabolic activities. Fishes have used protein efficiently as energy source; hence they convert protein to energy better and faster than livestock. This gives fish a higher productive energy value, attributable to the efficient manner in which it excretes nitrogen [20]. In the context of energy budget, overall, 29% of consumed energy in fishes goes for growth [9].
Fish require essential nutrients to grow efficiently, either naturally or artificially in aquaculture, which enable the fish to grow adequately for the enhancement of health in humans [5]. Fish feed is the single most expensive item in fish production [1]. The high cost of fishmeal makes commercial production of catfish capital intensive as it accounts for between 30% and 60% of variable operating cost [17]. Some studies concentrated on the trial of non-conventional sources of animal protein to substitute fishmeal in fish diets, such as hydrolyzed feather meal, meat bone meal, blood meal and fish and chicken viscera [10, 22]. So, poultry viscera as the non-conventional sources of animal protein, being enriched with essential nutrients, can replace fishmeal as an alternative aqua-feed stimulating the waste recycling.

Along with this background, this study was conducted to: (a) Determine the optimum inclusion level and effect of Poultry waste (skin, intestine and viscera) meal in compound diets and (b) Examine the comparative growth performance of *C. batrachus* and *A. testudineus* fed on varying inclusion levels of Poultry waste meal.

2. Materials and methods

2.1 Collection and storage of samples: Samples of feed ingredients such as poultry wastes (skin, intestine and viscera), fish meal, rice bran, wheat bran, mustard oil cakes, etc were collected, processed, then packed in polyethylene bags to prevent initial spoilage and stored in refrigerator. Three-week old hatchery bred fingerlings were collected from local market and used for this study.

2.2. Proximate composition analysis

- Estimation of Moisture (%), following (AOAC, 1995) [3].
- Determination of Ash (%), following AOAC (1995) [3].
- Determination of Crude Protein (%), following Pearson (1999) [28].
- Determination of Crude Lipid (%), following AOAC (1995) [3].
- Analysis of Fibre (%), following Pearson (1976) [26].
- Nitrogen Free Extract (%): {100 - (% Moisture + % Crude Protein + % Crude Lipid + % Crude Fibre + % Ash)}, following AOAC (1995) [3].
- Carbohydrate (%): [Nitrogen free extract % + Crude Fibre %], Hastings (1976) [26].
- Caloric Value / Gross Energy (kcal / 100gm): [(Carbohydrate X 4.1 /100) + (Protein X 5.65 /100) + (Lipid X 9.45 /100)] After (Cho, Slinger, & Bayley, 1982) [16].

2.3 Growth Parameters were used to evaluate the growth of fishes, according to Castell & Tiews (1980) [13].

2.4 Experimental design

Same type of experimental design was applied for both types of fishes. 42-L glass aquariums were used in this experiment, with proper recirculation system and aeration within an indoor hatched rearing system. Triplicate groups of fingerlings each were fed four isonitrogenous diets (33.9 ± 2% C.P), namely T-1, T-2, T-3 and T-4. Diet T-1 was set as the control diet with fish meal as the major protein source. While, experimental diets T-2, T-3 and T-4 were formulated with poultry skin, poultry intestine and poultry viscera as the major protein sources respectively. Acclimatization period of 15 days was applied to all fishes in laboratory condition, with the feed of 1: 1 mixture of rice bran and fishmeal (Patra & Ray, 1988) [27]. Stocking rate was 30 fishes / tank. To process the poultry wastes, those were cleaned in running tap water, then passed through hot air oven at 70°C for 30 min and then sun dried for weeks. Other selected feed ingredients were also processed in the same way. After that, all the dried and crushed feed ingredients were mixed in a proper ratio and grinded by mixer. The whole lot of resultant mixture of dry feed components was homogeneously blended, moistened with luke warm water and kneaded well until the texture reached a stiff dough consistency. The dough was compressed though a pelletizer equipped with 1 mm dies. The resulting strands were air dried initially and then dried overnight at 45°C in a hot air oven. The dried pellets were crumbled into suitable pellet length, screened to remove fines and stored in plastic jars in a refrigerator until used. These pellets were used to spread out on polythene paper and allowed to sun-dry for 5 hrs/day during the entire study period. 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 fish. Feed readjusted biweekly. From the 7th day of rearing, antibiotic treatment (2-3 gm of Terramycin) was used to control the occurrence of bacterial diseases.

The fingerlings were checked once a week and inspected for signs of undernourishment or disease. Dead fishes were immediately removed from the aquariums. Experimental trial period was of 60 days.

2.5 Sampling and data analysis

On every 14th day, 25 fishes were sampled randomly to determine fish growth rate from each treatment, by the help of glass nylon hapa. 2 hrs after feeding, faeces, waste particles of food and dead bodies of fish were siphoned out for measurement and later faeces were stored in air tight plastic container in refrigerator for further studies. Total length and weight were recorded using a graph paper attached Petri dish and a Metler AJ 100 digital balance respectively. After careful measurement the fingerlings were released in the respective place. The survival rate of fish was calculated at 60th day. Feed ingredients and formulated diets were analysed for proximate composition following the AOAC (1995) [3], procedures. Water quality parameters were determined by APHA (1992) [4], procedures. All the data were analysed using MS-Excel one-way ANOVA. This was followed by Duncan's new multiple range test (Duncan, 1955) [19], to identify the level of significance of variance ($P<0.05$) among the treatment means. Standard deviations (±SD) of the treatment means (of three determinations) were also calculated.

3. Results and discussions

Rising cost and unavailability of fish meal, forced to find a profitable replacement of it for the sake of aquaculture. The present study was attempted to collect the information of few easy available and low cost fish feed ingredients, with their nutritional value, application strategies and its quality along with their effects on fish growth performance.

Table 1, represents the feed formulation and proximate composition of the control and experimental diets. Poultry viscera based experimental diet (T-4) having dietary protein level (35.88%), followed by fishmeal based control diet (34.33%), while 32.07% dietary protein level recorded in poultry skin based experimental diet (T-2) as reported for *A. testudineus* by Chareontesprasit & Jiwyam (1996) [15]. But these isonitrogenous compound diets (T-1 to T-4) contain lower protein level than as applied for *C. batrachus* cage culture (>40% C.P) by Borthakur & Goswami (2007) [7].

Table 2, describes that, on the 1st day the body length and weight of *C. batrachus* were observed as 9.88 ± 0.06 cm and 8.33 ± 0.14 gm respectively, while on 60th day the body length and weight were increased to 12.58 ± 1.98 cm and 17.87 ± 2.19 gm respectively, by the effect of fishmeal based control diet (T-1). Starting with similar body length and weight (9.87 ± 0.12 cm and 8.32 ± 0.31 gm) respectively, on 60th day, by the effects of poultry viscera based experimental diet (T-4), the body length and weight of *C. batrachus* were increased to 12.79 ± 1.37 cm and 19.26 ± 1.31 gm respectively. For *A. testudineus* by the effects of poultry viscera based experimental diet (T-4) the body length and weight were observed as 1.43 ± 0.09 cm and 0.92 ± 0.02 gm respectively, on the 1st day, while on 60th day the body length and weight were increased to 06.82 ± 1.01 cm and 06.31 ± 0.63 gm respectively. Weight gain of 1.69 gm more recorded in T-4 than in T-1 for *A. testudineus*. In this study, biweekly feed adjustment (5% body weight basis), and the pattern of length and weight increases in both fishes follow similar trends observed in *Tilapia* (Juancey, 1982) [25], and in *Puntius gonionotus* (Wee & Ngamsnae, 1987) [34]. During 35th to 45th day of study period, both fish showed a rapid growth in weight and length than the rest of the study period, similar to the study of Chakraborty & Haque (2014) [14].

Table 3, shows the growth performance of *C. batrachus* and *A. testudineus* fingerlings were varied significantly (P<0.05) with different experimental diets. At the end of feeding trial, for *C. batrachus* and *A. testudineus*, highest S.G.R % / day were recorded as 1.40 and 1.39 (Fig.1) and Survival rate (83% and 85%) of fishes were recorded in poultry viscera based experimental diet (T-4) respectively. F.C.R was recorded highest (2.64 and 1.46) in T-1 (fishmeal based control diet) which decreased (2.41 and 1.27) in T-4 (poultry viscera based experimental diet), respectively for *C. batrachus* and *A. testudineus*. F.C.R values in the present study for *A. testudineus* (01.27 - 01.46) was comparable with F.C.R values as in rainbow trout (01.29–01.62), by the nutritional diet of co-dried fish silage reported by Hardy, Shearer, & Spinelli (1984) [23]. Doolgindachabaporn (1994) [18] and Potongkam (1972) [29], also, found that the F.C.R value of *A. testudineus* ranges from 1.8 to 3.0. P.E.R was recorded highest as 1.15 and 2.20 in T-4, respectively for *C. batrachus* and *A. testudineus*, similar trends, as observed in Tilapia (Jauncey, 1982) [25], and in *Puntius gonionotus* (Wee & Ngamsnae, 1987) [34]. For *C. batrachus* S.G.R%, F.C.R, P.E.R and survival rate values are slightly lower than that reported by Borthakur & Goswami (2007) [7]. Highest body weight was gained as 10.94 gm and 5.39 gm by the dietary effects of T-4 during 60 days of study period, respectively for *C. batrachus* and *A. testudineus*.

In Table 4, temperature (29.7±0.1 °C) and alkalinity (184.4±0.2 mg/L) of water were recorded within acceptable range, however higher than as reported by Borthakur & Goswami (2007) [7]. Values of D.O recorded within an acceptable range of 06.00 to 06.10 mg/L (as recommendation of greater than 3 mg/L for growth of channel catfish) according to Weeks & Ogburn (1973) [35]. In this study, the pH values (7.6 –7.7) were fell within the suitable range according to Swingle (1967) [32], and Boyd (2000) [8].

Fig.1 shows highest crude protein content in poultry viscera (60.67%) that is much more than in Fishmeal (55.19%), similar to the findings of Fisheries Research Institute, FRI (1989) [21], and slightly higher than the findings of Cai, Pancorbo, Barnhart, Sander, & Merka (1994) [11]. Highest lipid content was observed in poultry skin (12.89%) and the crude lipid range was recorded as 09.22% to 16.49% in poultry viscera, more or less similar to the findings of Hasan & Amin (1997) [24] and Tabinda & Butt (2012) [33]. Gross-energy content in poultry viscera (490.72 kcal/100 g) also shows much more values than in Fishmeal (402.41 kcal/100 g).

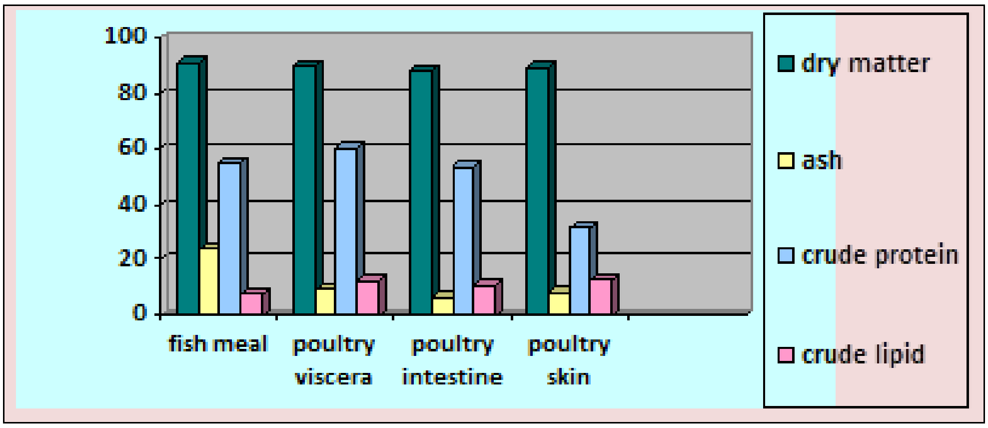

Fig 1: Proximate composition of different protein sources (%D.M basis).
*Y axis represents % of Dry. Matter, Ash, Crude Protein, & Crude Lipid, X axis represents the protein sources.

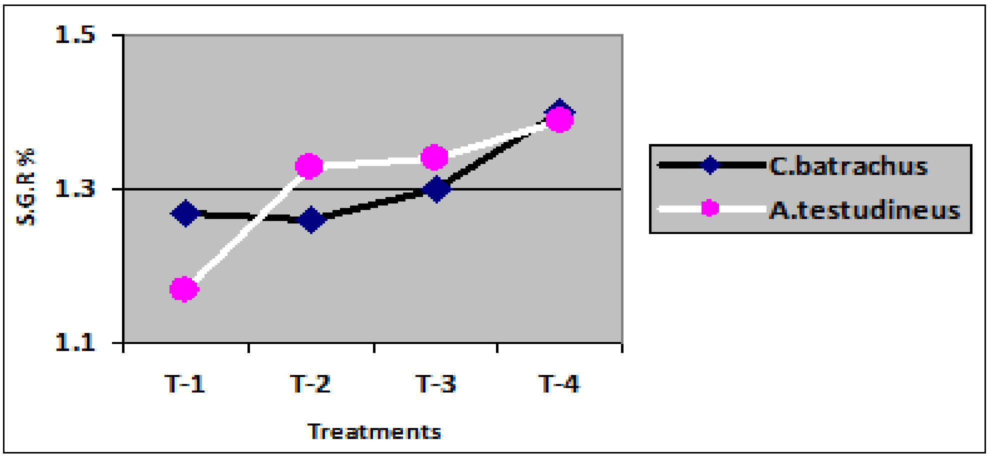

Fig 2: Comparison of growth performance [by S.G.R (%/day)] of *C. batrachus and. A. testudineus* for 60 days, by the effects of same experimental treatments.
*S.G.R = Specific growth rate (% / day), T = Dietary treatments.

Table 1: Formulation and Proximate composition of. Control and Experimental diets. (%.Dry matter basis).

Feed formulation for the Control & Experimental diets (%).				
Ingredients/Parameters	**Control Diet**	**Experimental Diets**		
	T- 1	**T- 2**	**T- 3**	**T- 4**
Fish Meal (F.M)	30.00	-	-	-
Poultry Viscera (P.Visc)	-	-	-	30.00
Poultry Intestine	-	-	30.00	-
Poultry Skin	-	30.00	-	-
Mustard oil cake	15.50	15.50	15.50	15.50
Rice bran	28.90	28.10	28.60	28.50
Wheat bran	19.00	19.80	19.30	19.40
Vitamin & Mineral premix*	00.60	00.60	00.60	00.60
Chromic oxide (Cr_2O_3)	01.00	01.00	01.00	01.00
Sunflower oil	05.00	05.00	05.00	05.00
Total	100	100	100	100
Proximate composition of the Control & Experimental diets (% Dry matter basis).				
Dry matter	90.97	90.39	90.79	91.13
Crude protein	34.33	32.07	33.99	35.88
Crude fat	07.62	10.37	08.90	09.68
Crude ash	06.58	06.96	07.63	06.36
Nitrogen free extract	39.93	38.70	37.96	37.08
Gross energy(kcal/100 g)	439.97	447.23	441.24	454.95

* Vitaminetes Forte, Roche Products Ltd., 24/28 Pt. M. M. Road, Mumbai 400 034, India.

Table 2: Biweekly changes in mean body length and weight. of. *C. batrachus*, and. *A. testudineus* by different dietary treatments.

Dietary Treatments	**Fish Types**	**Experimental Days**									
		1 st		**15 th**		**30 th**		**45 th**		**60 th**	
		Length (cm)	**Weight (g)**	**Length (cm)**	**Weight (g)**	**Length (cm)**	**Weight (g)**	**Length (cm)**	**Weight (g)**	**Length (cm)**	**Weight (g)**
T-1	*C. batrachus*	9.88 ± 0.06	8.33 ± 0.14	10.47 ± 0.65	9.13 ± 0.36	11.15 ± 1.80	12.79 ± 1.11	11.84 ± 1.01	14.93 ± 2.37	12.58 ± 1.98	17.87 ± 2.19
	A. testudineus	1.42 ± 0.11	0.91 ± 0.01	1.87 ± 0.03	1.41 ± 0.13	3.79 ± 0.08	2.28 ± 0.25	4.70 ± 0.93	3.68 ± 0.95	5.39 ± 1.01	4.61 ± 0.94
T-2	*C. batrachus*	9.83 ± 0.05	8.31 ± 1.11	10.40 ± 0.58	9.13 ± 1.09	11.16 ± 1.03	12.81 ± 0.92	11.85 ± 0.87	14.82 ± 1.77	12.49 ± 1.28	17.83 ± 1.22
	A. testudineus	1.41 ± 0.10	0.91 ± 0.02	2.01 ± 0.09	1.68 ± 0.05	4.07 ± 0.13	2.92 ± 0.83	4.98 ± 1.03	4.35 ± 0.63	5.94 ± 0.97	5.29 ± 1.04
T-3	*C. batrachus*	9.79 ± 0.06	8.31 ± 0.41	10.50 ± 0.55	9.11 ± 1.07	11.19 ± 0.87	12.88 ± 1.77	11.83 ± 1.05	15.52 ± 1.19	12.60 ± 0.45	18.10 ± 1.70
	A. testudineus	1.43 ± 0.17	0.92 ± 0.03	2.20 ± 0.04	1.85 ± 0.14	4.30 ± 0.23	3.51 ± 0.93	5.30 ± 0.98	4.90 ± 0.99	6.45 ± 1.03	5.87 ± 0.92
T-4	*C. batrachus*	9.87 ± 0.12	8.32 ± 0.31	10.52 ± 0.67	9.15 ± 1.03	11.21 ± 1.09	13.12 ± 1.04	11.89 ± 0.79	15.96 ± 1.22	12.79 ± 1.37	19.26 ± 1.31
	A. testudineus	1.43 ± 0.09	0.92 ± 0.02	2.29 ± 0.10	1.99 ± 0.04	4.72 ± 0.21	3.68 ± 0.71	5.75 ± 0.63	5.33 ± 0.83	6.82 ± 1.01	6.31 ± 0.63

Data are mean (± SE) of 3 determinations. T = Treatment.

Table 3: Comparative growth performance and survival of *C. batrachus* and. *A. testudineus* fed on similar experimental diets for 60 days.

Parameters	***Fish Types***	**Dietary Treatments**			
		T-1	**T-2**	**T-3**	**T-4**
Mean Initial Weight (g)	*C. batrachus*	08.33^{a}	08.34^{a}	08.32^{a}	08.32^{a}
	A. testudineus	0.91^{a}	0.91^{a}	0.92^{a}	0.92^{a}
Mean Final Weight (g)	*C. batrachus*	17.87 ± 0.80^{c}	17.83 ± 0.72^{c}	18.19 ± 1.01^{b}	19.26 ± 0.78^{a}
	A. testudineus	04.61± 0.06^{d}	05.79± 0.09^{c}	05.87± 1.06^{b}	06.31± 0.75^{a}
Weight gain (g)	*C. batrachus*	09.54 ± 0.28^{c}	09.49 ± 0.33^{c}	09.87 ± 0.26^{b}	10.94 ± 0.38^{a}
	A. testudineus	03.70± 0.96^{d}	04.88± 0.99^{c}	04.95± 0.41^{b}	05.39± 0.96^{a}
F.C.R	*C. batrachus*	02.64 ± 0.05^{a}	02.63 ± 0.08^{ab}	02.59 ± 0.06^{b}	02.41 ± 0.04^{c}
	A. testudineus	01.46± 0.08^{a}	01.35± 0.06^{b}	01.33± 0.05^{b}	01.27± 0.03^{c}
P.E.R	*C. batrachus*	01.10 ± 0.04^{b}	01.09 ± 0.03^{ab}	01.11 ± 0.03^{a}	01.15 ± 0.04^{a}
	A. testudineus	02.00± 0.05^{c}	02.16± 0.07^{b}	02.15± 0.05^{b}	02.20± 0.04^{a}
S.G.R (% / day)	*C. batrachus*	01.27 ± 0.06^{b}	01.26 ± 0.06^{b}	01.30 ± 0.03^{b}	01.40 ± 0.02^{a}
	A. testudineus	01.17± 0.11^{c}	01.33± 0.16^{b}	01.34± 0.10^{b}	01.39± 0.09^{a}
Survival-Rate (%)	*C. batrachus*	81 ± 2^{b}	80 ± 3^{c}	83 ± 2^{a}	83 ± 2^{a}
	A. testudineus	82 ± 3^{d}	84 ± 3^{b}	83 ± 2^{c}	85 ± 2^{a}

*Survival Rate (%) = 100 x (Final Number of Fish / Initial number of Fish), Specific Growth Rate (S.G.R) (% / Day) = 100 x (Ln Wf - Ln Wi) /Δt, Feed Conversion Ratio (F.C.R) = Feed Consumed / (Wf - Wi), [Where: Wf = Final Weight & Wi = Initial Weight, Feed Consumed = Feed Given - Feed Not Eaten, Δt = Duration (Days)]. Data are mean (± SE) of 3 determinations. Data in the same row with different superscripts are significantly (P<0.05) different.

Table 4: Water Quality Parameters For Different Treatments (Mean ± S.D).

Parameters	Dietary Treatments			
	T-1	T-2	T-3	T-4
Average-Temp. (°C)	29.8 ± 0.03	29.7 ± 0.04	29.8 ± 0.03	29.8 ± 0.10
Average-pH	07.6 ± 0.03	07.6 ± 0.04	07.7 ± 0.05	07.6 ± 0.04
Average-D.O (mg/L)	06.1 ± 0.11	06.1 ± 0.15	06.0 ± 0.08	06.0 ± 0.18
Alkalinity mg/L	184.4 ± 1.07	184.6 ± 1.08	184.2 ± 1.10	184.1 ± 1.12

*Water quality parameters were determined by APHA (1995) procedure.

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