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UTILIZATION OF CHICKEN INTESTINE AS AN ALTERNATIVE PROTEIN SOURCE IN THE DIET FOR FINGERLINGS OF *CIRRHINUS MIRIGALA*

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ABSTRACT

Suitability of chicken intestine as an alternate protein source was evaluated for the diet of *Cirrhinus mirigala* fingerlings. Percentage protein, fat, ash, fiber, moisture and available energy (Kcal/100g) for the experimental diets were analyzed. Five experimental diets were formulated with chicken intestine meal 0% (FM_{100} Control), 25% (FM_{75}), 50% (FM_{50}), 75% (FM_{25}) and 100% (FM_0) substituting 0, 25, 50, 75, and 100% of the fish meal (FM) respectively. FM_{100} served as control diet. Experimental diets were iso-nitrogenous, iso-lipidic and iso-energic with 40% protein**,** 7.7-8.7% lipid and 425-446kcal energy per 100g diet. Sixtey days feeding trial experiment was performed in static indoor conditions. FM_{75} had almost similar growth as compared to control (FM_{100}) in *Cirrhinus mirigala* fingerlings diets. Significantly higher growth was recorded in FM_{50} as compared to FM_{100} (control) and $FM_{75.}$ Present study showed overall significantly higher growth in FM_{25} ($P<0.01$) and FM_0 ($P<0.05$) as compared to the control. Lowest feed conversion ratio was observed in higher growth dietary treatments and vice versa. The results of the present study concluded that although best growth was achieved in the dietary treatment FM_{25} but without compromising growth 100% chicken intestine could be substituted for fish meal without addition of amino acids in *Cirrhinus mirigala* fingerlings diet.

Key words Carp fingerlings diet; Chicken intestine meal; *Cirrhinus mirigala*; fish meal replacement.

INTRODUCTION

In the aqua feed formulation, protein is the mainbut expensive ingredient and its quality and quantity in fish feeds formulation plays a vital role in promoting fish growth (Pandian *et al*., 2001). However FM as a protein source is used worldwide in the preparation of feeds (Krishnankutty, 2005; Yigit *et al*., 2006) but the major challenge is its availability, quality and cost (Krishnankutty, 2005; Goda *et al*., 2007; Ghazala *et al*., 2011; Tabinda and Butt 2012). FM is unsustainable both environmentally and financially as a protein source for fish feeds (Muzinic *et al*., 2006; Subasinghe and Phillips, 2007; Tacon and Nates 2007).

Bone, meat and PBM meal have been used extensively as an ingredients in fish feeds (Fasakin *et al*., 2005; Wei *et al*., 2004, 2006). PBM is less expensive animal protein source as compared to FM (Abdel-Warith *et al*., 2001; Steffens, 2003). It has high protein value, balanced amino acids profile, total digestible dry matter and total energy comparable to FM (Bureau *et al*., 2000) and therefore can be considered as a probable substitution for FM (Muzinic *et al*., 2006; Rawles *et al*., 2006; Thompson *et al*., 2007).

Low level of ash contents in the diets, no requirements of supplemental amino acids or additional attractants, a good addition and contribution of cholesterol which are inexpensive and finally low cost of PBM as compared to FM, are the main advantages of PBM replacement in fish feed. The cost-effectiveness of substituting PBM for FM varies depending on the local cost of the ingredient, the price difference between FM and the PBM and the rate of substitution (Steffens, 2003; Yu, 2006 and Cruz-Suarez *et al*., 2007)

Chicken intestine is easily available at very low price throughout the year and is protein rich PBM but not been utilized as animal protein source in aqua feed. Present study was carried out to evaluate the impacts of PBM as animal protein source on growth in fingerlings of *Cirrhinus mirigala*. It will help to formulate cheap fish diet that will be available at lower price in the market. It will also save lots of edible fishes (suitable for human consumption) from being converted into FM and contribute significantly towards food security.

MATERIALS AND METHODS

Procurement and analysis of feed ingredients: All feed ingredients used in the preparation of feed formulae were purchased from local market. Chicken intestines were collected in raw form, washed with tap water and converted into meal after sun drying and grinding.

All formulated feeds were analyzed for proximate composition i. e. percentage of protein, fat, moisture, ash, fiber and energy (Kcal/100g) following the methods of AOAC (2005) and Tabinda and Butt (2012). Each determination was done in triplicate and averaged.

All diets were formulated to contain 40% protein, 7.7-8.7% lipid and 425-446 kcal energy per 100g of diet.

Preparation of Experimental Diets: In order to formulate five iso-energic, iso-nitrogenous and iso-lipidic diets, different feed ingredients were mixed (Table 2) and FM was replaced with PBM as a dietary protein source for *Cirrhinus mirigala* fingerlings. Experiment comprised of 5 dietary treatments i.e. PBM 0% (FM_{100} Control), 25% (FM_{75}), 50% (FM_{50}), 75% (FM_{25}) and 100% (FM_0) in order to compare growth performance in the fingerlings of *C. mirigala* influenced by two different protein sources. Fish oil was added in equal proportions in the experimental diets for sufficient supply of fatty acids. Two ml of cod liver oil was also added in each diet in order to provide essential fatty acids. Alpha-methyl cellulose was added according to Mohanta *et al*. (2008) and carboxy-methyl cellulose was added to stabilize the feeds. One percent vitamin premix was also added as nutrient supplement. Dry ingredients in different ratios were mixed and homogenized, grinded in grinding mill for experimental feed preparation (KMF 10 BasicIKA^R WERKE) and analyzed for its proximate composition (AOAC, 2005). The powdered feeds were stored at -20 °C in ziplock bags (Tabinda and Butt, 2012; Ghazala *et al*., 2011).

Procurement of fish: *Chirrinus mirigala* fingerlings were procured from Central Fish Seed Hatchery, Lahore and transported to the SDSC laboratory in oxygen-filled polythene bags. *C. mirigala* fingerlings were stocked in indoor glass aquaria, after giving a prophylactic dip in $KMnO_4$ solution with concentration of 2mg/l for 30 seconds only (Marecaux, 2006) and acclimatized for a period of 15 days. During acclimatization fingerlings were fed with control diet @ 3% body weight twice a day.

Experimental Design: Experiment was conducted in a static indoor rearing system in 15 rectangular glass aquaria (3'×1.5'×1') of 90 L capacity containing 77L of water. To maintain dissolved oxygen above 5mg/l artificial aeration was provided to each aquarium. A constant photoperiod (12L: 12D) was maintained. Fish fingerlings were weighed (average weight 6.58 $\pm$ 0.036g) after acclimatization, and were assigned into groups of 10 fish in each experimental aquaria and each treatment was comprised of 3 replicates including oneexperimental group of control (FM_{100}) (Adewolu *et al*., 2010).

Fish were weighed after every fortnight to determine weight gain. During the experiment fortnight bulk weights of the fish were used to adjust the daily feed ration for the following fortnight and so on. All fish were fed manually 4 times a day at equal intervals 8.00 AM, 11.00 AM, 2.00 PM, 5.00 PM @ 2.0% wet body weight per feeding per day (Du *et al*., 2006). Throughout the experiment, any left over feed was siphoned out 1 h after feeding and weighed after oven drying in order to determine feed consumption. Feces were removed in the morning and evening after 3 hours of feeding by siphoning from the bottom of each aquarium.

Physicochemical Parameters: On daily basis water temperature, dissolved oxygen and pH were monitored. Temperature was recorded using digital thermometer (IR Thermometer Smart Sensor), DO (dissolved oxygen) was recorded using DO meter (HANNA-HI 9145) and pH was determined using pH meter (WTW D82362 Wellheim, Germany). Phosphate, nitrate, nitrite, total ammonia, chloride, total alkalinity and total hardness were measured following standard methods (APHA, 2005).

Growth indices: Total WG, percent WG, SGR, FCR and survival rate were calculated according to Kureshy *et al*. 2000, SGR according to Emre *et al*. 2003 and FCR according to Hernandez *et al*., 2008.

Data analysis: All the data were analysed for variance (ANOVA) using computer software SPSS, Version 13. Duncan's multiple range test was used and the range of means and differences between the means of treatments were examined.

RESULTS

Ten *Cirrhinus mirigala* fingerlings with average initial body weight (6.58 $\pm$ 0.036 per fish) were reared in all the treatment groups. Overall WG ranged between 9.290g and 12.113g and %WG was between 140.700% and 184.650% for different treatments after 60 days of experimental study. Comparative response of growth (% WG and SGR) of fish fed with diets FM_{100} (140.740% and 9.290g) and FM_{75} (146.73% and 9.574g) was almost similar. However, FM_{50} (161.740% and 10.426g) ($P<0.01$) had significantly higher growth as compared to control (FM_{100}) (140.70% and 9.290) but non significant to FM_{75} (146.73% and 9.574). Overall highest growth was observed in FM_{25} (184.650% and 12.113g) ($P<0.01$), FM_0 (175.300% and 11.556g) ($P<0.05$) as compared to control (FM_{100}) (140.70% and 9.290) as well as to FM_{75} (146.73% and 9.574g) and FM_{50} (161.740% and 10.426g). However, non-significantly higher growth ($P>0.05$) was observed in FM_{25} (184.650% and 12.113g) ($P<0.01$) as compared to FM_0 (175.300% and 11.556g) ($P<0.01$) (Table 3).

The overall range of SGR (SGR) was between 1.46 and 1.75. Highest SGR was determined in FM_{25} and lowest in $FM_{100.}$ There was no significant difference ($P>0.05$) among SGRs of FM_0 (1.69) and FM_{25} (1.75). FM_{100} had significantly lower SGR (1.46) than FM_0 (1.69) ($P<0.05$) and FM_{25} (1.75) ($P<0.01$) and FM_{50}

(1.58) ($P<0.05$) but non significantly lower SGR ($P>0.05$) as compared to FM_{75} (1.51) (Table 3).

Table 1. Different formulae of experimental diets used for *Cirrhinus mirigala*

Ingredients	**Experimental Diets**				
FM/PBM (%)	100/0	75/25	50/50	25/75	0/100
Treatment No.	**(FM_{100})**	**(FM_{75})**	**(FM_{50})**	**(FM_{25})**	**(FM_{0})**
FM	40.00	30.00	20.00	10.00	0.000
PBM	0.00	10.00	20.00	30.00	40.00
Corn Flour	0.00	0.00	10.00	10.000	0.000
Glutin	31.00	21.00	14.00	14.000	1.000
Rice Polish	4.50	0.00	0.00	0.000	24.00
Soyabean Meal	13.00	20.50	20.00	20.00	20.00
Wheat Flour	6.50	13.5	10.00	10.000	0.000
Carboxyl Methyl Cellulose	2.00	2.00	2.00	2.000	2.00
α-methylCellulose	0	0	0.95	0.95	9.95
Corn Oil	0.3	-	-	-	-
Cod Liver Oil	2.00	2.00	2.00	2.000	2.00
Vitamin Premix	1.00	1.00	1.00	1.000	1.00
Ascorbic Acid	0.05	0.05	0.05	0.05	0.05

Table 2. Chemical composition (on as fed basis) of feed formulae of *Cirrhinus mirigala*

	Chemical Composition				
Crude protein	39.970	40.010	40.055	41.355	41.310
Crude fat	7.991	7.952	8.097	7.679	8.672
Ash	11.810	6.428	5.790	5.410	4.755
Moisture	9.011	8.220	8.020	6.991	6.811
Crude fiber	5.690	5.582	4.692	4.418	3.720
[1]T-CHO	31.568	37.440	38.038	38.556	38.448
[2]OM	79.529	85.402	86.190	87.59	88.43
P:E ratio (mg/kcal)	93.918	94.082	92.198	92.570	96.654
Gross energy (Kcal/100g)	425.584	425.269	434.442	446.742	427.402

[1]Total carbohydrate (TCHO) =OM-CP+CL [2]Organic matter (OM) = Total Ash-DM

Overall range of FCR was between 1.99 and 2.87, lowest for FM_{25} and highest for FM_{100}. Fish fed with diets FM_{100} (2.87) and FM_{75} (2.48) had non significantly different ($P>0.05$) FCRs. Similarly FM_{50} (2.14) had non significantly lower FCR ($P<0.05$) than FM_{75} and FM_{100} (control). Significantly lower FCR was observed in FM_{25} (1.76) and FM_{0} (1.99) ($P<0.05$) than FM_{100} (control) (Table 3). Fish survival ranged between 98-100% in different treatments (Table 3).

Table 3. Growth performance of *Cirrhinus mirigala* fingerlings on different feed formulae

[1]FM/CI (%)	**Experimental Diets**				
	100/0.0	75/25	50/50	25/75	0.0/100
Treatment No.	**(FM_{100})**	**(FM_{75})**	**(FM_{50})**	**(FM_{25})**	**(FM_{0})**
Average initial weight (g)	6.610±0.09*	6.525±0.02*	6.605±0.02*	6.560±0.06*	6.592±0.14*
Average final weight (g)	15.910±1.84*	16.099±1.07*	17.031±0.64*****	18.673±2.66****	18.148±1.78***
Weight gain (g)	9.290±1.77*	9.574±1.07*	10.426±0.65******	12.113±2.70****	11.556±1.88**
% weight gain	140.70±21.56*	146.73±13.69*	157.85±8.70*****	184.65±35.93****	175.30±26.53**
SGR	1.46±0.09*	1.51±0.69*	1.58±0.17*****	1.75±0.11****	1.69±0.33**
FCR	2.87±0.31*	2.48±0.10*	2.14±0.15*****	1.76±0.30****	1.99±0.31**
Survival (%)	99	99	98	100	100

[1]FM/CI=FM/Chicken intestine

Values are expressed as mean of triplicate groups of ten fishes.

Means with different asterisk within a row are significantly different ($P<0.05$)

Throughout the experiment temperature ranged from 21.90 to 22.70 ºC, DO (dissolved oxygen) 6.87 to 7.02, pH 7.63 to 7.76, phosphate 1.69 to 2.19, nitrate 7.52 to 8.98, nitrite 0.15 to 0.24, total ammonia 0.02 to 0.05, chloride 80.920 to 90.531, total alkalinity 343 to 391 and total hardness 217 to 340 mg/L for different treatments (Table 4). No significant difference was observed (P>0.05) among different treatments regarding water quality parameters.

Table 4. Physicochemical parameters (Mean ± S.E) of water during experimental Trial in *Cirrhinus mirigala*

Treatments	Temp (Ċ)	DO ($mg\ l^{-1}$)	pH	Phosphate ($mg\ l^{-1}$)	Nitrite ($mg\ l^{-1}$)	Nitrate ($mg\ l^{-1}$)	Ammonia ($mg\ l^{-1}$)
FM_{100}	22.70±2.00*	6.88±0.26*	7.76±0.19*	2.17±0.00*	0.21±0.00*	8.90±0.02*	0.02±0.00*
FM_{75}	22.27±1.57*	6.87±0.30*	7.74±0.15*	2.18±0.00*	0.22±0.00 *	8.94±0.03*	0.03±0.00*
FM_{50}	22.56±2.40*	6.86±0.29*	7.76±0.09*	2.18±0.00*	0.24±0.00**	8.98±0.04*	0.05±0.00**
FM_{25}	22.21±1.67*	6.92±0.30*	7.69±0.11*	2.00±0.00*	0.15 ±0.00***	7.52±0.06*	0.03 ±0.00*
FM_0	21.90±1.81*	7.02±0.36*	7.63±0.26*	2.11±0.00*	0.20 ±0.00**	8.16 ±0.06*	0.04±0.00*

Means with different asterisk within a column are significantly different (P<0.05)

DISCUSSION

According to the present study results, highest growth was observed in terms of percentage WG, SGR and FCR in FM_{25} diet for *Cirrhinus mirigala* as compared to the control FM_{100} without any supplementation of amino acids and processing. Similar results were achieved for *Scophthalmus maeoticus* (Turker *et al*., 2005), *Nibea miichthioides* (Yang *et al*., 2006), *Morone chrysops* and *M. saxtilis* (Rawles *et al*., 2006) and *humpback grouper* (Shapawi *et al*., 2007; Goda *et al*., 2007; Giri *et al*., 2009). Percentage WG in fish fed with diets FM_{100}, FM_{75}, was significantly lower ($P<0.05$) from the FM_{50}, FM_{25} and FM_0. Takagi *et al.* (2000) reported similar findings. According to them complete substitution of FM with PBM resulted in better growth as compared to the control in yearling red sea bream diet.

In present study, SGR was significantly higher (p<0.05) in FM_0, FM_{25} and FM_{50} as compared to FM_{75} and FM_{100}. Similar results were reported by Samocha *et al*. (2004). They reported significant difference in SGR at high inclusion of PBM in the diet *L. vannamei*. Similarly Shapawi *et al.* (2007) also found high SGR with the inclusion of PBM upto 75-100%.

FCR in fish fed diets FM_{100} and FM_{75} was significantly higher (P<0.05) and slightly decreased by increasing PBM level in fish diets. Study of Giri *et al*., (2009) in catfish *Clarias batrachus* (Linn.), Goda *et al*., (2007) in African catfish (*Clarias gariepinus)* and Shapawi *et al*., (2007) in humpback grouper also support our results. They reported relatively low FCR by increasing percentage replacement of FM with chicken intestine.

Better response of the *Cirrhinus mirigala* to FM_{25} and FM_0 may be due to the good quality of the diet ingredients used in terms of nutrient profile, their digestibility and palatability. Therefore, with similar protein/energy dietary ratios, 100% replacement of FM could be possible without compromising survival and growth performance of the *Cirrhinus mirigala*. Results of Samocha *et al*. (2004) and Tan *et al*. (2003) are in agreement with the results obtained from present study.

Studies of Takagi *et al*. (2000), Davis and Arnold (2000) also support our results. According to them, by using good quality of high protein value PBM, it could be possible to replace 75% or even 100% of FM without compromising on the fish growth. Sealey and Hardey (2011) reported that complete replacement of FM with PBMcould be possible in the feed formulation by using good quality PBM without any addition of amino acids. Similarly, Kureshy *et al.,* (2000) in juvenile red drum and Webster *et al.,* (2000) in sun shine bass reported upto 100% replacement of FM with PBM which is in agreement with present study.

All the water quality parameters did not significantly differ (P<0.05) among different treatments and were within desired range as reported by Boyd (2000).

Conclusion: PBM can replace FM by 75-100 % in formulating diet for fingerlings of *Cirrhinus mirigala* without using any additional amino acids as well as processing.

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