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Replacement of Fish Meal With Poultry By–Product Meal (Chicken Intestine) as a Protein Source in Grass Carp Fry Diet

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Abstract.- Present study evaluated the suitability of chicken intestine as an alternate protein source of fish meal for grass carp fry diet. Feed ingredients were collected from the local market and were analyzed for protein, fat, ash, fiber, moisture content and energy. The basal diet (FM_{30}) was formulated with 30% fish meal. Other experimental diets were formulated with chicken intestine meal 7.5% ($FM_{22.5}$), 15.0% ($FM_{15.0}$), 22.5% ($FM_{7.5}$) and 30% (FM_0) replacing 7.5, 15.0, 22.5, and 30% of the fish meal respectively. All the diets were designed to contain equivalent levels of -nitrogen -lipids and energy resulting in diets with 35% protein, 10% lipid and 429-431kcal energy per 100g diet. An eight week feeding trial was conducted under laboratory conditions. $FM_{22.5}$ and $FM_{15.0}$ had almost similar growth as compared to basal diet (FM_{30}) but significantly higher growth was recorded in $FM_{7.5}$ ($P<0.01$) and FM_0 ($P<0.05$) as compared to the basal diet. Lowest feed conversion ratio was observed for treatments with higher growth and vice versa. The results of the present study reveal that although the best growth was achieved in the dietary treatment with 75% chicken intestine meal but 100% chicken intestine meal can also replace fish meal without addition of amino acids and compromising growth and feed conversion ratio in grass carp fry diet.

Keywords: Carp fry diet, poultry by-product meal, grass carp, animal protein source.

INTRODUCTION

Protein is the vital (Williams and Barlow, 1996; Pandian *et al*., 2001) and expensive ingredient of formulated fish feeds. Quality and quantity of proteins in formulated fish feeds are of paramount importance in promoting fish growth (Pandian *et al*., 2001). Fish meal protein is being used globally as dietary protein in formulated fish feeds (Williams and Barlow, 1996; Hardy and Tacon, 2002; New and Wijkstom, 2002; Krishnankutty, 2005; Yigit *et al*.**,** 2006) but major challenge is high cost of fish meal, uncertain availability (Krishnankutty, 2005; Goda *et al*., 2007) and variations in quality (Krishnankutty, 2005). Market and environmental factors suggest that fish meal is financially and environmentally unsustainable as a source of protein for aqua feeds (Muzinic *et al*,. 2006; Subasinghe and Phillips, 2007; Tacon and Nates, 2007).

Animal by-products such as meat, bone meal and poultry by-product meal have considerable potential as feed ingredients in fish production systems (Tacon and Jackson, 1985; Fowler, 1991; Watanabe and Pongmaneerat, 1991; Robaina *et al*., 1997; Bureau *et al*., 2000; Kureshy *et al*., 2000; Millamena, 2002; Wei *et al*., 2004; Fasakin *et al*., 2005; Wei *et al*., 2006) and comparatively less expensive than fish meal (Steffens, 1994; Rodriguez-Sena *et al*., 1996; Bureau *et al*., 1999; Abdel-Warith *et al*., 2001). These animal protein ingredients are good sources of amino acids with high protein content, total digestible dry matter and energy similar to fish meal (Bureau *et al*., 1999; 2000). Therefore, poultry by-product meal is considered a probable replacement for fish meal (Webster *et al*., 1999, 2000; Gaylord and Rawles, 2005; Muzinic *et al*., 2006; Rawles *et al*., 2006 ; Thompson *et al*., 2007). Some studies have shown that poultry by product meal cannot replace more than 50% of fish meal in fish diets (Gallagher and Degani, 1988; Fowler, 1991; Steffens, 1994) but other studies have shown that with the recent improvement of the quality of poultry by product meal, it could replace 75% or even 100% of fish meal without significant decrease in fish growth (Alexis, 1997; Nengas, 1999; Takagi *et al*., 2000).

Chicken intestine is rich in protein but unfortunately not being utilized as protein source in fish feed. Grass carp is one of the popular fish species in fresh water aquaculture due to its tasty flesh comparable with Rohu but faster growth rate. Only a few data is available regarding feeding of grass carp fry.

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Present study was carried out to evaluate the chicken intestine as a protein source and replace fish meal with chicken intestine as an alternate source of animal protein in grass carp fry diet in order to save fish from being converted to fish meal.

MATERIALS AND METHODS

Feed ingredients

Chicken intestine meal, fish meal, soya bean meal, gluten, rice polish, corn flour, wheat flour, ascorbic acid, carboxyl-methyl cellulose, α-cellulose, cod liver oil were used for formulation of fish diets.

Prior to formulation of diets all ingrediets were analyzed in triplicate for proximate composition (Table 1) following the methods of AOAC (1990). Crude protein content was determined using protein analyzer (inkjel M Behr Labor-Technik), lipids by soxlet solvent extraction unit (KB 8 Gerhardt Bonn), ash by muffle furnace (Carbolite CWF 1200), fiber contents by crude fiber apparatus (OSK 1352OA, Ogawa Seiki Co), moisture by oven (memmert GmbH + Co. KG D-91126 Schwabach FRG) after drying at 105 Ċ till constant weight and energy was determined using bomb calorimeter (K-C2000 basic IKAR WERKE).

Feed preparation

Five test diets were formulated to contain 35% protein (Akand *et al.*, 1991), 10% lipid (National Research Council, 1983) and 429-431kcal energy per 100g diet (Table II). To evaluate the chicken intestine as a dietary protein source for grass carp fry the fish meal in the basal diet was incrementally replaced with poultry by product meal (0, 25, 50, 75, 100%). Cod liver oil was supplemented to the test diets as needed to equalize lipid levels. To balance the lipids and energy, cooking oil (9 Kcal per ml) was also added according to the requirement. α- methyl cellulose was used as filler (Mohanta, *et al.*, 2008) and carboxy-methyl cellulose was added as feed stabilizer at a rate of 2%. Dry ingredients in different ratios were mixed and homogenized with oil. After mixing, all the ingredients were ground in grinding mill (KMF 10 Basic IKA^R WERKE) and again analyzed for biochemical components following above procedures. The powdered feeds were stored at -20°C in air tight plastic bags.

Procurement of fish

Grass carp (*Ctenopharyngodon idella*) fry was procured from Himalaya Fish Hatchery, Muriedeky and transported to the SDSC laboratory. Fish fry was transported in oxygen-filled polythene bags. After giving a prophylactic dip in $KMnO_4$ solution fish fry was stocked in indoor glass aquaria. Fish fry was acclimatized in lab conditions for a period of two months and grown to the desired weight and size. During this time period, fish fry were fed with basal diet @ 2% body weight four times a day.

Experimental procedure

A static indoor rearing system was used to conduct the experiment at Sustainable Development Study Center (SDSC) Laboratory, GC University Lahore. Experiment was conducted in 15 rectangular glass aquaria (3'×1.5'×1') of 90 L capacity containing 77L of water. Artificial aeration was provided to each tank to maintain adequate levels of dissolved oxygen. A constant photoperiod of 12 hours light and 12 hours dark (12L: 12D) was maintained with the help of artificial lightening system. After acclimatization, fish fry (average weight 0.76±0.06) were assigned into groups of 10 fish in each experimental aquarium. Each fish group was placed in an individual experimental aquarium.

Fish were weighed collectively at the beginning and fortnightly for each aquarium to determine gain in weight (each treatment comprised of 3 aquaria and 30 fish). Fortnightly bulk weights were used to adjust the daily feed ration for the following 2 weeks and so on. All fish were fed 4 times a day at equal intervals (8.00 AM, 11.00 AM, 2.00 PM, 5.00 PM) manually at a fixed feeding rate of 2.0% wet body weight per feeding per day (Du *et al.*, 2006**)**. To determine the feed consumption, any left over unconsumed feed was siphoned out 1 h after feeding and weighed after oven drying. Each treatment group had three replicates and was fed according to the experimental protocol. Feces were removed everyday in the morning and evening by siphoning from the bottom of each aquarium 3 hours after feeding to remove uneaten food and feces.

Water quality monitoring

Temperature, dissolved oxygen and pH were monitored three times a day. Dissolved oxygen was measured by dissolved oxygen meter (HANNA-HI 9145), pH with a pH meter (WTW D82362 Wellheim, Germany) and temperature using a mercury thermometer. Total ammonia (NH3 –N), NO_3 –N, NO_2 –N, chloride, total alkalinity and total hardness were measured following standard methods (APHA, 1998).

Growth indices

The various growth indices such as weight gain, FCR (food conversion rate and percent survival) were calculated according to Ali (2001), Goda *et al.* (2007) and Hernandez *et al*. (2008).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using computer software SPSS, Version13. Standard deviation (±SD) was calculated to identify the range of means and differences between the means of treatments were examined using Duncan's multiple range test.

RESULTS

The average initial body weight of *Cetenopharyngodon idella* fry (7.67-7.73g per 10 fish) in all the treatment groups was same ($P>0.05$). Overall weight gain ranged between 9.97 and 14.55g and % weight gain was between 129.31% and 188.22% for different treatments after 60 days of experiment. Comparative growth response (% weight gain) of fish fed the diets FM_{30} (148.23%) and $FM_{22.5}$ (150.710%) was almost similar ($P>0.05$). Significantly higher growth was observed in $FM_{7.5}$ (188.22) ($P<0.01$) and FM_0 (162.45%) ($P<0.05$) but non-significantly lower growth ($P>0.05$) was observed in FM_{15} (129.31%) as compared to the basal diet (Table III).

Overall feed conversion ratio (FCR) ranged between 2.10 and 2.70, lowest for $FM_{7.5}$ and highest for FM_{15}. Fish fed the diets FM_{30} (2.54) and $FM_{22.5}$ (2.47) had almost similar FCR ($P>0.05$) while FM_{15} (2.70) had significantly higher FCR ($P<0.05$) than FM_{30} (basal diet). Significantly lower FCR was observed in $FM_{7.5}$ (2.10) ($P<0.01$) and FM_0 (2.23) ($P<0.05$) than FM_{30} (basal diet) (Table III). There was no mortality of fish and survival was 100% (Table III).

The overall temperature ranged from 28.47 to 28.81 C°, dissolved oxygen 6.86 to 6.92 mg/L, pH 8.44 to 8.58, ammonia 0.061 to 0.083 mg/L, nitrite 0.029 to 0.055 mg/L, nitrate 4.74 to 5.29 mg/L, phosphate 0.086 to 0.100 mg/L (Table IV), total alkalinity 339 to 394 mg/L, total hardness 221 to 337 mg/L and chloride 80 to 89 mg/L for different treatments. Values of these parameters did not significantly differ ($P>0.05$) among different treatments.

DISCUSSION

Fish meal has been completely replaced by terrestrial protein sources in production diets of various freshwater fishes (Webster and Lim, 2002). In present study best growth was recorded for diets containing 22.5% chicken intestine ($FM_{7.5}$). The complete replacement of fish meal (FM_0) also resulted in better growth than basal diet (FM_{30}). The inferior growth performance of $FM_{30,}$ could be due to high ash content of diet that might have decreased the digestibility as well as nutrient utilization by the fish. Similar growth depression was observed in different fish species when their diets contained 100% by-catch fishmeal as the source of animal protein (Bureau *et al*., 1999; Kureshy *et al.*, 2000; Chaimongkol and Boonyaratpalin, 2001; Yang *et al*., 2004; Giri *et al*., 2010).

Du *et al.* (2009) reported 35.45 to 41.05% growth of juvenile grass carp (average initial weight 3.30 to 3.62g) at 35% protein, 9 to 12% lipids with varying energy to protein (P/E) ratio (9.16 to 9.93) during 70 days period. Present studies reported significantly higher ($P<0.001$) growth performance (129.31 to 188.22%) of grass carp fry (average initial weight 0.76 ± 0.06) at same protein but higher lipid (10.620 to 10.894%) level and P/E ratio (12.26 to 12.31) during 60 days.

Emre *et al.* (2003) reported significantly decreased growth in Mirror carp (*Cyprinus carpio*) after increased incorporation of poultry by product meal in the formulated diet After 10 weeks of experimental trial, average weight gain of carp fingerlings fed the control diet was significantly

Table I.- Proximate composition (Mean±SD) of feed ingredients.

S.No	Ingredients	Protein (%)	Fat (%)	Fiber (%)	Ash (%)	Moisture (%)	Energy (Kcal/100g)
1	Chicken intestine	70.000±0.001	7.640±0.002	0.210±0.001	4.330±0.001	6.660±0.001	529.8±0.01
2	Fish meal	57.000±0.001	11.780±0.001	2.400±0.002	21.30±0.002	9.780±0.001	453.80±0.02
3	Soya bean	46.000±0.001	14.440±0.002	11.240±0.001	7.430±0.001	9.800±0.002	429.20±0.01
4	Rice polish	16.000±0.001	11.370±0.002	1.920±0.001	9.420±0.002	8.760±0.002	444.60±0.02
5	Corn flour	10.750±0.001	8.400±0.002	1.550±0.002	1.260±0.002	7.340±0.001	426.50±0.03
6	Wheat flour	6.000±0.002	2.760±0.001	1.100±0.002	1.080±0.002	7.980±0.001	419.00±0.02
7	Gluten	27.000±0.002	1.550±0.001	7.170±0.001	8.340±0.002	10.900±0.002	413.80±0.02

Values are expressed as mean of triplicate samples

Table II.- The formulation and chemical composition of the tested diets.

Ingredients	Basal diet	Tested diets			
Fish meal/chicken intestine meal (%)	100/0	75/25	50/50	25/75	0/100
Treatment No.	(FM_{30})	($FM_{22.5}$)	(FM_{15})	($FM_{7.5}$)	(FM_{0})
PBM[1]	0.00	7.50	15.00	22.500	30.00
Fish meal[2]	30.00	22.50	15.00	7.5000	0.00
Soyabean meal[3]	21.05	28.50	20.00	25.500	19.20
Rice polish[4]	11.15	-	18.00	6.000	13.30
Glutin[5]	21.50	6.00	12.00	14.000	3.00
Wheat flour[6]	10.50	18.67	10.50	13.000	-
Corn flour[7]	-	10.00	-	-	21.00
Corn oil[8]	0.3	-	-	-	-
Carboxyl-methyl cellulose[9]	2.00	2.00	2.00	2.000	2.00
α-methyl cellulose[10]	0.50	1.83	4.50	6.500	8.50
Ascorbic acid[11]	0.050	0.05	0.05	0.050	0.05
Cod liver oil[12]	2.00	2.00	2.00	2.000	2.00
Vitamin premix[13]	1.00	1.00	1.00	1.000	1.00
Chemical composition (on as fed basis)					
Crude protein	35.002	35.000	35.000	35.000	35.027
Crude fat	10.894	10.786	10.682	10.620	10.652
Crude fiber	5.629	5.460	4.599	4.384	3.630
Ash	11.700	6.315	5.705	5.223	4.675
Moisture	9.122	8.328	8.116	7.282	6.878
OM[14]	88.300	93.685	94.295	94.777	95.325
T-CHO[15]	42.404	47.899	48.613	49.157	49.646
Gross energy (Kcal/100g)	429.190	430.317	430.275	429.695	431.370
P:E ratio (mg/kcal)	81.553	81.335	81.343	81.453	81.199

[1]Fresh chicken intestine was collected from Tolinton Market, Lahore, washed and converted into meal after sun drying and grinding; [2]Fish meal (Pakfish pure, Karachi); [3]Soya bean meal (Ishan Herbotech International, India); [4]Rice polish (Barry Rice Mill, Mureidke); [5]Gluten (Rafhan Company, Faisalabad); [6]wheat flour (Sufi Flour Mill, Lahore); [7]Corn flour (Faisalabad Trading Company, Okara); [8]Corn Oil (Super Habib, Company); [9]Carboxyl Methyl Cellulose (China); [10]α-methyl Cellulose (Taiwan); [11]Ascorbic acid (Merck); [12]Cod Liver Oil (Alia Pharmaceuticals, Pvt., Ltd.)

[13]Vitamin premix each Kg contains: Vitamin A= 4,000,000 IU, Vitamin D_3=100,000 IU, Vitamin E=2,000mg, Vitamin K_3=750mg, Vitamin B_1=600mg, Vitamin B_2=2,000mg, Vitamin B_6=600mg, Vitamin B_{12}=10,000mcg, Vitamin C=2,000mg, L- Lysine=10,000mg, DL Methionine=25,000mg, copper carbonate=2,500mg, cobalt carbonate=550mg, ferrous carbonate=4,000mg, manganese carbonate=50,000mg, zinc carbonate=5,000mg, potassium iodide=150mg, coline chloride=110,000mg, nicotinic acid=9,000mg, folic acid=225mg, calcium Pantothenate=3,500mg and butylated hydroxytoluene (BHT) =125mg.

[14]Organic matter (OM) was calculated by subtracting total ash from DM.

[15]Total carbohydrate (TCHO) was calculated by subtracting CP and CL from OM.

Table III.- Effect (Mean±S.E) of replacing fish meal with chicken intestine meal on growth performance of grass carp fry.

Ingredients	Basal diet	Tested diets			
Fish meal/chicken intestine meal (%)	100/0	75/25	50/50	25/75	0/100
Treatment No.	(FM_{30})	($FM_{22.5}$)	(FM_{15})	($FM_{7.5}$)	(FM_{0})
Average initial weight (g)	7.67 ± 0.09^{a}	7.71 ± 0.02^{a}	7.71 ± 0.02^{a}	7.73 ± 0.06^{a}	7.67 ± 0.14^{a}
Average final weight (g)	19.04 ± 1.84^{a}	19.33 ± 1.07^{a}	17.68 ± 0.64^{c}	22.28 ± 2.66^{b}	20.13 ± 1.78^{ac}
Weight gain (g)	11.37 ± 1.77^{a}	11.62 ± 1.07^{a}	9.97 ± 0.65^{c}	14.55 ± 2.70^{b}	12.46 ± 1.88^{ab}
% weight gain	148.23 ± 21.56^{a}	150.71 ± 13.69^{a}	129.31 ± 8.70^{c}	188.22 ± 35.93^{b}	162.45 ± 26.53^{ab}
FCR	2.54 ± 0.31^{a}	2.47 ± 0.10^{a}	2.70 ± 0.15^{c}	2.10 ± 0.30^{b}	2.23 ± 0.31^{ab}
Survival (%)	100	100	100	100	100

Values are expressed as mean of triplicate groups of ten fishes.
Means with different superscript letters within a row are significantly different (P<0.05)

Table IV.- Water quality parameters (Mean±S.E) for different treatments.

Fish meal/chicken intestine meal (%)	Temperature (Ċ)	pH	Dissolved oxygen (mg l^{-1})	Ammonia (mg l^{-1})	Nitrite (mg l^{-1})	Nitrate (mg l^{-1})	Phosphate (mg l^{-1})
FM_{30}	28.60 ± 0.51^{a}	8.56 ± 0.17^{a}	6.88 ± 0.26^{a}	0.072 ± 0.00^{ab}	0.055 ± 0.00^{a}	5.17 ± 0.02^{a}	0.100 ± 0.00^{a}
$FM_{22.5}$	28.76 ± 0.53^{a}	8.47 ± 0.15^{a}	6.87 ± 0.30^{a}	0.070 ± 0.00^{ab}	0.047 ± 0.00^{a}	4.89 ± 0.03^{a}	0.086 ± 0.00^{a}
FM_{15}	28.56 ± 0.52^{a}	8.58 ± 0.16^{a}	6.86 ± 0.29^{a}	0.083 ± 0.00^{b}	0.060 ± 0.00^{b}	5.29 ± 0.04^{a}	0.092 ± 0.00^{a}
$FM_{7.5}$	28.47 ± 0.51^{a}	8.44 ± 0.14^{a}	6.92 ± 0.30^{a}	0.061 ± 0.00^{a}	0.029 ± 0.00^{c}	4.74 ± 0.06^{a}	0.086 ± 0.00^{a}
FM_{0}	28.81 ± 0.52^{a}	8.55 ± 0.15^{a}	6.88 ± 0.36^{a}	0.066 ± 0.00^{a}	0.037 ± 0.00^{b}	4.93 ± 0.06^{a}	0.093 ± 0.00^{a}

Means with different superscript letters within a column are significantly different (P<0.05)
FM30, control diet containing 30% fish meal and 0% chicken intestine meal; FM 22.5, 22.5% fish meal and 7.5% chicken intestine meal; FM 15,15% fish meal and 15.0% chicken intestine meal; FM 7.5, 7.5% fish meal and 22.5% chicken intestine meal; FM 0, 0% fish meal and 30% chicken intestine meal.

(P<0.05) higher (42.63±0.66) compared to the fish fed 33% (30.14±0.06), 67% (25.91±0.48) and 100% PBM (19.77±0.07) respectively when initial weight of fish was 15.40±0.03g. Poor growth performance may be due to limiting amino acid content, difficult digestion of poultry by product meal containing feather, connective tissue and skin contents, subjection of the product to high temperature (150-200ºC) for a long time (10 hours) during the processing, or combination of all.

Results of Emre *et al.* (2003) are contradictory as compared to the results obtained during present studies.

Giri *et al*. (2010) in their 84 days study period obtained 24.85, 33.66, 47.24, 47.65 and 46.82 % growth in *Clarias batrachus* (Linn.) fingerlings (initial weight 13.0-13.60g/fish) as compared to growth in control (22.67%) replacing 20, 40, 60, 80 and 100% fish meal with chicken vicera meal respectively. Present studies resulted in significantly higher (P<0.001) growth (148.23 to 188.22 % in 60 days) as compared to the results obtained by them.

Alexis *et al.* (1985) reported that fish meal could be partially replaced with poultry by-products in diet of rainbow trout with no effect on growth. Sevgili and Ertürk (2004) reported 20% replacement of fish meal with poultry by product meal into formulated diet of rainbow trout. Abdel-Warith *et al.* (2001) reported 40% replacement of fish meal with poultry by product meal without effectively altering growth in the diet of African catfish. Yildrim *et al.* (2009) reported significantly high growth than control for *Tilapia Zilli* at 50% replacement of fish meal with poultry by product meal. Yang *et al.* (2006) reported 66.5% replacement of fish meal with poultry by product meal in the diet of Gibel carp. Growth results obtained by Alexis *et al.* (1985), Sevgili and Ertürk (2004), Abdel-Warith *et al.* (2001), Yildrim *et al.* (2009) and Yang *et al.* (2006) are significantly low as compared to the results obtained during present studies. But findings of Gouveia (1992), Davis and Arnold, (2003) are comparable to our findings. Gouveia (1992) reported 80% replacement with

poultry by product meal in rainbow trout diet; Davis and Arnold (2000) reported that 80% fish meal alteration in formulated feeds of *L. vannamei* but Hao and Yu (2003) evaluated 80% replacement of fish meal with poultry by product meal and meat and bone meal in the diet of juvenile catfish without any harmful effect.

Results reported by Gropp *et al.* (1979) are not consistent with results obtained during present studies. Gropp *et al.* (1979) for rainbow trout reported that poultry by product meal formulated diets produce equivalent growth comparable to the control with addition of amino acids. But the results obtained by Steffens (1988), Hasan *et al.* (1993) Hasan and Das (1993), Appelbaum *et al.* (1996), El-Sayed (1998), Kureshy *et al.* (2000) and Webster *et al.* (2000) are consistent with the results obtained during present studies. Steffens (1988) in carp, Hasan *et al.* (1993) in catla, Hasan and Das (1993) in rohu and El-Sayed (1998) in tilapia reported that complete replacement of fish meal by poultry by product could be possible when high-quality poultry by product meal are used.

Present study indicates that 100% replacement of fish meal with chicken intestine could be done in grass carp fry diet. All the water quality parameters like temperature, dissolved oxygen and pH did not significantly differ ($P<0.05$) among all the treatments and were within desired range as reported by Boyd (1982) and Shah *et al.* (1998). Ammonia and nitrite concentrations were within acceptable limits for fish growth and health (Boyd, 1981). Nitrate and phosphate, total alkalinity and total hardness also remained within the suitable range for fish culture (Boyd, 1982; Renukaradhya and Varghese, 1986).

CONCLUSION

Chicken intestine can 100% replace fish meal in the diet of grass carp (*Ctenopharyngodon idella*) fry without any processing and addition of amino acids.

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