Effect of Plant-Fishmeal and Plant By-Product Based Feed on Growth, Body Composition and Organoleptic Flesh Qualities of *Labeo rohita*

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Abstract. Present study was aimed at evaluating the effect of plant-fishmeal feed and/or plant by-product based feed on growth, body composition and flesh quality of *Labeo rohita*. Fish fed on rice polish alone served as control (T_0). Fish feed ingredients were grouped together with two ingredients in each test diet i.e. guar meal and canola meal (T_1), soybean meal and cotton seed meal (T_2), guar meal and cotton seed meal (T_3), soybean meal and canola meal (T_4) and fishmeal and canola meal (T_5). Each treatment including control had two replicates. Earthen ponds (12) with uniform area of 0.03 ha each, were randomly stocked with 100 fish (average weight 200 g) in each. All the ponds were then randomly allotted to individual treatment including control and were fed at 4% of their wet biomass twice a day. Significantly higher growth performance, specific growth rate (SGR) and better food conversion ratio (FCR) in T_3 and T_1 than rest of the treatments suggested that guar meal and cotton seed meal, and guar meal and canola meal are better option to include in future feed formulations for maximum performance and minimum feed wastage. Fish samples showed higher protein values in T_4 , fat in T_2 , moisture contents in control, dry matter in T_1 and ash in T_5 . Nonsignificant differences in flavor, juiciness, and oiliness indicated that the sensory attributes of fish flesh were not affected by feeding fish with blend of various ingredients. Our study revealed that the cotton seed meal which is considered toxic to fish and is restricted in fish feeds perform equally good and can be incorporated in future feed formulations for *Labeo rohita*.

Key Words: Indian major carps, selected feed ingredients, chemical evaluation, Sensory evaluation

INTRODUCTION

Indian major carps are amongst the dominating fish fauna of South Asia and are primary cultureable fish species both in public and private sectors (Khan et al., 2004; Hussain et al., 2011). Labeo rohita the most important member of current fish culture setup is well liked among producers and consumers and therefore has been adopted as major component of fish culture in sub-continent (Chaudhuri et al., 1974). It is highly nutritious and good source of digestible (85-90%) protein with balanced amino acid profile (Rudolf, 1971; Choo and Williams, 2003; Astawan, 2004). Fish is a major source of protein, contributes 26.2% of the total animal meat in Asia and developing countries. Other than nutritional competencies it is an important source of livelihood for local community. Fish culture is on the rise not only in developing

* Corresponding author: <u>khaliduvas@yahoo.com</u> 0030-9923/2014/0001-0253 \$ 8.00/0 Copyright 2014 Zoological Society of Pakistan countries but in developed countries too (Delgado *et al.*, 2002; Louka *et al.*, 2004).

Selection of feed ingredients and their costs have pronounced effect on aquaculture industry (Craig and Helfrich, 2002). Similarly, presence of essential nutrients in appropriate concentrations and elimination/reduction of anti nutrients to the minimum acceptable level is of prime importance for better fish growth and feed conversion ratios (Mokolensang et al., 2003). Feed formulation protocol demands amalgamation of different ingredients from wide variety of sources to achieve feed with desired qualities (Khan et al., 2004). Several researchers (Shabbir et al., 2003; Ali and Salim, 2004; Jabeen et al., 2004; Gull et al., 2005; Inayat and Salim, 2005; Saeed et al., 2005) worked on effects of various ingredients, individually and in combinations on different fish species and found better results when feed ingredients were combined together to formulate fish feeds. Diets become cost effective and several negative growth inhibitory factors in feed ingredients neutralize each other due to their interactive effects (Shioya et al., 2011; Yang et al., 2011). However, the information is scarce for

formulation of artificial feeds for Labeo rohita.

Artificial feed plays a significant role in fish production, sustainability of this industry and production of healthy product for consumers (Shioya et al., 2011; Yang et al., 2011). The sensory attributes viz. color, texture, smell and appearance may also be affected by changes in feed composition irrespective of the type of fish culture practiced (Khan et al., 2011). Population, fish species, spawning period, season, nutrition, postharvest handling, and storage are some of the key factors that influence the quality of fish and its products (Kinsella, 1988; Nielsen et al., 2002). Data is sparse which explains the effect of feed on organoleptic quality of fish flesh. Studies on vellowtail fish (Viyakarn et al., 1992) and rainbow trout (Smith et al., 1988) indicated that soybean protein when used as partial substitute for fish meal did not show any significant differences in flesh quality. Nonetheless studies on some teleosts have shown that nutritional factors, such as dietary protein sources (Kaushik et al., 1995), manuring, commercial diet (Moav et al., 1977), fat sources (Guillou et al., 1995), dietary fat content (Bjerkeng et al., 1997), and vitamin E (Boggio et al., 1985) can influence the physical and organoleptic flesh quality. Considering the breadth and depth of studies conducted so far on different fish species the present study was planned to evaluate the effect of combination of plant-fishmeal and/or plant based feed ingredients on growth, body composition and sensory attributes of juvenile Labeo rohita.

MATERIALS AND METHODS

Experimental site and study trials

This three month study was conducted in earthen ponds of the Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus Pattoki, using juvenile *Labeo rohita* as an experimental animal.

Experimental design

Studies were designed following Completely Randomized Design (CRD). There were 5 treatments and a control with two replicates in each group and whole trial was managed in 12 ponds. 100 fish (initial weight 200g) were randomly stocked in each pond (0.03 ha) and then all these ponds were arbitrarily distributed among 5 treatments and a control. Five experimental diets were prepared, each with two ingredients of known calories which are easily available in the market. The experimental diets were then subjected to proximate analysis. The proportionate ratio of the two was maintained at 1:1 i.e. guar meal and canola meal (T₁), soybean meal and cotton seed meal (T₂), guar meal and cotton seed meal (T₃), soybean meal and canola meal (T₄), fishmeal and canola meal (T₅) and a control diet (T₀) *i.e.* rice polish with two replicates in each. Fish were regularly fed at 4% of wet body weight twice a day.

Growth parameters

The weight and length of individual fish were recorded at the initiation of experiment and then fortnightly to ascertain the growth increments for subsequent feed adjustments. Average increase in weight (AWG), length (AIL) and specific growth rate (SGR) (Hopkins, 1992) was evaluated by following mathematical formulae;

AWG = Final average weight (g) - initial average weight (g) AIL = Final average length (cm) - initial average length (cm)

SGR (%) =
$$\frac{\ln (\text{Final wet body weight}) - \ln (\text{Initial wet body weight})}{\text{Number of days}} \ge 100$$

Water quality parameters

Dissolved oxygen (DO), pH, electrical conductivity, water temperature, salinity and total dissolved solids (TDS) were monitored daily at 09:00 A.M. and 02:00 P.M. Water temperature and DO were measured by DO meter (YSI 55 Incorporated, Yellow Springs, Ohio, 4387, USA), pH by pH meter (LT-Lutron pH-207 Taiwan) and electrical conductivity, salinity and TDS by salinity meter (Condi 330i WTW 82362 Weilheim Germany).

Proximate analysis

The technique Near Infrared Reflectance Spectroscopy (NIRS 5000 model, Foss Tecator, Sweden) was used for proximate analysis of feed ingredients and fish. The principle of NIRS is that bonds between organic molecules absorb a specific wavelength range of light in the near infrared region, and the near – infrared color of the sample provides information about its composition. Before submitting to analytical set-up, feed and fish were dried, finely ground in pestle and mortar and then placed in sampler cups. The cups were placed in NIRS machine for two minutes which displayed values for fat, moisture, protein and ash (Martinez *et al.*, 2003). Data sheet was collected and preserved for further processing and future usage.

Fish preparation for organoleptic evaluation

At the end of feeding trial, 10 fish were randomly picked up from the total catch of each treatment, degutted and well cleaned with fresh tap water for further processing. After preliminary dressing the flesh was uniformly filleted with fillet size of 3.8 x 6.6 cm having an average weight of 28.50g. One tablespoon iodized salt was sprinkled on each fillet and manually rubbed uniformly on its entire surface and then steamed in Orient microwave oven (Model OW-720ADL) at medium high temperature for 12 minutes. The cooked samples were placed them at safe place and allowed to equilibrate with room temperature before serving to panel of judges for sensory evaluation of these samples (Khan *et al.*, 2011).

Sensory evaluation

Fillets were presented to 12 member semitrained panel, randomly selected from students and faculty members of University of Veterinary and Animal Sciences, Lahore, Pakistan for organoleptic test. The descriptors for various sensory attributes were color and its intensity (whitish/creamish) (typical of steamed fish flesh), flavor, intensity of perceived taste of typical steamed fish flesh, juiciness(intensity of juiciness of steamed fish flesh while chewing), tenderness (intensity of softness perceived at the time of chewing), oiliness (intensity of oiliness that perceived taste of a typical steamed fish flesh) and finally overall acceptability (accumulative impression of steamed fish taken from above tested attributes). Prepared samples were presented in glass plates coded with three digit random numbers. Mineral water was provided for convenient rinsing of mouths after tasting and testing each sample to avoid unintentional mixing of the taste and confounding effects of different treatment groups. The panelists were asked to rank their acceptance for above sensory attributes according to hedonic scale: 1 =dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 =like very much; 9 = like extremely. Sensory tests for fish from each treatment were performed on the same day under white incandescent lights (Meilgaard *et al.*, 2007).

Statistical analysis

The data were subjected to ANOVA using SAS (statistical package; version 16.0) at $p \le 0.05$. Duncan's Multiple Range Test (DMRT) was applied to compare means when the level of variation between dietary treatments was significant.

RESULTS AND DISCUSSION

Fish growth and its associated qualities depend upon quality and quantity of feed. Quality and cost effective feed is not only hard to formulate and develop but impossible if we do not have proper set of known and well tested feed ingredients. The main objective of these series of experiments was to select different ingredients from the bulk available in the market for the convenience of the farmer and manufacturer which can be used blindly for well being of fish and increase fish production at low cost. During present study significantly higher growth performance, SGR and better FCR were observed in T_3 and T_1 than rest of the treatments and control which suggested that fish fed on guar meal and cotton seed meal, and guar meal and canola meal performs better than the rest of the combinations (Table III). Our findings are in line with similar observations of others when fed Labeo rohita with other fish varieties on different feed ingredients (Ashraf et al., 2008; Ahmad et al., 2012; Abid and Ahmed, 2009a,b).

Proximate analysis of experimental diets revealed highest protein in T_5 than T_2 , T_3 , T_4 , T_1 and T_0 in decreasing order (Table II) while better growth was observed when fish were fed with T_3 diet (Table III). Our findings contradict the work of Abid and Ahmed (2009a) who observed significantly higher (P<0.05) weight gain (26.17g) with increasing crude protein levels in fish feed. Our studies indicate that protein is not the only factor in fish feed which promotes fish growth and ameliorates nutritional value of fish flesh. Li et al. (2000) observed significant differences among various protein rich artificial feeds when offered to channel catfish (Ictalurus punctatus). On the other hand, Hasan et al. (2005) could not observe any difference in growth and body composition of fish when reared on agro based diet and compared it with fishmeal based diet in common carp.

 Table I. Experimental diets were prepared using the formula.

Treatment	Feed ingredients	Percentage (%)	
T_1	Guar Meal and Canola meal	50:50	
	(GM+CM)		
T_2	Soybean Meal and Cotton	50:50	
	Seed Meal (SBM+CSM)		
T ₃	Guar meal and cotton seed	50:50	
	meal (GM+CSM)		
T_4	Soybean meal and canola meal	50:50	
	(SBM+CM)		
T ₅	Fishmeal and canola meal	50:50	
	(FM+CM)		
T_0	Rice Polish (RP)	100	

Table II.- Proximate analysis of feed combinations.

Analysis	T ₁	T ₂	T ₃	T ₄	T ₅	T ₀
Protein %	36.76	38.44	37.63	37.57	40.35	6.07
Moisture	7.07	9.68	7.13	9.62	7.33	4.92
%						
Fat %	1.77	1.42	1.60	1.35	4.87	3.15
Ash%	8.22	12.48	12.35	8.35	15.59	6.3
kcal/g	4.09	4.07	4.08	4.07	4.15	4.13

Proximate analysis of ground *Labeo rohita* from treated and control ponds revealed highest protein in T_4 , originally fed on low protein diet, fat in T_2 , moisture content in control, dry matter in T_1 and ash in T_5 ponds (Table IV) which confirms findings of Khan *et al.* (2012) who observed non-significant differences in major carp body composition fed on artificial feed with different

crude protein levels. It further revealed that protein deposition in fish does not reflect the protein composition of diet rather it may be interaction among nutrients, their anti-nutritional level, acceptability/palatability of each ingredient and its behavior in digestive system (Moraes and Bidinotto, 2000; Lim and Dominy, 1991).

Significantly higher SGR P<0.05 was observed in fish fed T_3 diet followed by T_1 , T_0 , T_2 , T_3 and T_4 (Table III) in decreasing order. Our results are contradictory to the findings of Abid and Ahmed (2009a) who observed non-significant differences in SGR between treatments in which fish were fed with increasing crude protein levels of feed. FCR is the best parameter to assess the acceptability of feed and its ultimate performance in fish (Inayat and Salim, 2005). In present study better FCR was observed in treatment T_3 (2.602±0.35) when compared to T₀ (4.700±0.23) (Table III). Our results confirm the previous findings of Abid and Salim (200) and Ashraf et al. (2008) in mrigal (Cirrhinus mrigala) and rohu (Labeo rohita) (Abid and Ahmed, 2009a,b) who found similar outcomes by using feed ingredients in combination. Li et al. (2000) also reported significantly higher FCR when they compared plant based feed with animal based feed. On the other hand, Hasan et al. (2005) reported nonsignificant differences on plant origin feed when compared with animal origin feed in common carp. The results of present study are in line with the work of Latif et al. (2008) who combined feed ingredients to prepare experimental diets and obtained similar results.

In present study physico-chemical water parameters such as temperature, dissolved oxygen, pH, salinity, TDS and electrical conductivity remained within the desired range (Table V) and well corroborated with previous findings (Ali *et al.*, 2000; Abid and Ahmed, 2009a,b).

Sensory evaluation showed that color, flavor, juiciness, tenderness, oiliness and overall acceptability of fried fish flesh showed nondifferences irrespective significant of diet composition among various dietary treatments (Table VI). Some earlier researchers have reported similar sensory attributes when fish was fed on varying protein levels (Rora et al., 2005; Koshio et al., 1994). Findings of Khan et al. (2011), further

PLANT BASED FISH FEED

Treatment	Weight gain %	Weight gain (g)	Length increase (cm)	FCR	SGR %
T ₁	58.29	246.16 ± 6.17^{a}	21.96 ± 0.95^{a}	3.03 ± 0.04^{bc}	0.42 ± 0.05^{ab}
T ₂	43.11	162.66 ± 20.00^{b}	13.54 ± 0.72^{b}	4.69±0.91 ^{ab}	0.27 ± 0.05^{b}
T_3	65.99	294.00±30.00 ^a	24.27 ± 0.83^{a}	2.60±0.35°	0.52 ± 0.08^{a}
T_4	42.08	159.09 ± 4.90^{b}	14.34 ± 0.65^{b}	4.91±0.55 ^a	0.26 ± 0.03^{b}
T ₅	43.24	161.00±6.33 ^b	13.10 ± 0.91^{b}	4.65±0.19 ^{ab}	0.27 ± 0.02^{b}
To	43.71	164.66 ± 4.66^{b}	14.57 ± 1.29^{b}	4.70±0.23 ^{ab}	0.27 ± 0.02^{b}

Table III.- Comparison of growth increments and attached growth indicators in Labeo rohita.

Table IV.- Proximate composition of Labeo rohita (on dry weight basis) fed on different dietary combinations.

Sr. No.	СР %	Fat %	Dry matter %	Ash %
T_1	70.00 ± 1.75^{ab}	$4.46\pm0.15^{\circ}$	29.76 ± 6.33^{a}	16.33 ± 3.21^{a}
T_2	$63.00\pm0.87^{\circ}$	6.73 ± 0.25^{a}	25.92 ± 1.22^{ab}	$15.00{\pm}1.00^{a}$
T_{3}	65.62 ± 3.50^{bc}	6.23 ± 0.20^{b}	24.18 ± 1.53^{ab}	15.00 ± 3.00^{a}
T_4	74.81 ± 2.18^{a}	5.80 ± 0.30^{b}	24.11 ± 2.49^{ab}	16.00 ± 2.00^{a}
T ₅	69.12±6.12 ^{abc}	$4.93 \pm 0.20^{\circ}$	23.46 ± 1.01^{b}	22.33 ± 5.50^{a}
T ₀	65.18 ± 3.06^{bc}	$4.53 \pm 0.35^{\circ}$	22.43 ± 1.36^{b}	18.33 ± 6.50^{a}

Table V.- Ranges of various physicochemical parameters observed during the course of trial.

Treatment	DO	pH	Temperature	EC	TDS	Salinity
Morning						
T_1	5.86 ± 0.06	8.33±0.04	34.55±0.42	2.64 ± 0.03	1001.56±11.76	0.87 ± 0.02
T_2	5.89 ± 0.08	8.34±0.05	34.67±0.47	2.60 ± 0.04	974.69±33.04	0.91±0.03
$\overline{T_3}$	5.85±0.09	8.30±0.04	34.64±0.37	2.61±0.03	972.83±9.11	0.96 ± 0.01
T_4	5.82 ± 0.07	8.30±0.03	34.91±1.54	2.65 ± 0.06	980.72±8.32	0.84 ± 0.05
T_5	5.95±0.7	8.37±0.18	33.73±0.92	2.59 ± 1.08	947.17±31.09	0.81 ± 0.01
T_0	5.79 ± 0.06	8.37±0.03	34.67±0.34	2.58±0.03	992.09±12.31	0.94 ± 0.02
Evening						
T_1	5.36±0.06	8.13±0.04	35.58±0.42	2.74±0.03	1006.90±11.76	0.89 ± 0.02
T_2	5.39±0.08	8.14±0.05	35.70±0.47	2.70±0.04	980.03±9.54	0.92 ± 0.05
$\overline{T_3}$	5.35±0.09	8.10±0.04	35.67±0.39	2.71±0.03	978.17±9.11	0.96 ± 0.01
T_4	5.32±0.07	8.10±0.03	35.94±0.44	2.75±0.03	986.05±8.32	0.98 ± 0.01
T_5	5.45 ± 0.07	8.17±0.05	35.26±0.56	2.70 ± 0.05	980.52±9.32	0.82 ± 0.04
T_0	5.29±0.06	8.17±0.03	35.70±0.34	2.68±0.03	997.43±12.31	0.94 ± 0.02

Mean±SE

support our findings who did not find any difference in meat quality of fish either reared on natural or artificial feed in fish raised in poly culture system. In another study, Khan *et al.* (2012a) had similar findings when fingerlings of Indian major carps were grown up on plant based artificial feeds under monoculture system. But contrary to our study they also reported that in monoculture system, the sensory attributes like juiciness and tenderness scores of *Labeo rohita* differed significantly in treatments versus control. Javed *et al.* (1995) also found significant differences among the taste scores of three fish species *C. catla, C. mrigala* and *L. rohita.* Findings of Jayaram *et al.* (1980) and Tahir (2008), further support our observations who reported non-significant differences in taste and overall quality of all the three Indian major carps when cultured in manured ponds and/or fed on

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Feed ingredients	Color	Flavor	Tenderness	Juiciness	Oiliness	Overall acceptability
8						▲ ∨
T_1	6.50 ± 0.23^{a}	6.50±0.35 ^a	6.94±0.31 ^a	6.58 ± 0.31^{a}	6.58 ± 0.46^{a}	6.66±0.28 ^a
T_2	6.25 ± 0.25^{a}	6.41 ± 0.43^{a}	6.58±0.35 ^a	6.25 ± 0.44^{a}	5.91 ± 0.46^{a}	6.33 ± 0.22^{a}
T ₃	6.89 ± 0.22^{a}	6.91±0.33 ^a	6.75±0.32 ^a	6.66±0.39 ^a	6.66±0.35 ^a	6.97 ± 0.24^{a}
T_4	6.50 ± 0.26^{a}	6.75 ± 0.35^{a}	6.41±0.39 ^a	6.41 ± 0.35^{a}	6.25 ± 0.44^{a}	6.33±0.35 ^a
T ₅	6.25 ± 0.37^{a}	6.83 ± 0.29^{a}	6.58±0.39 ^a	6.91±0.33 ^a	5.91 ± 0.54^{a}	6.83 ± 0.34^{a}
T_0	6.75 ± 0.37^{a}	6.66±0.35 ^a	6.50±0.35 ^a	6.08 ± 0.37^{a}	6.00±0.32 ^a	6.58±0.33 ^a

Table VI.- Organoleptic/sensory score of fish flesh against various parameters tested.

Note: - The results show organoleptic evaluation test marks out of ten,

Figures with same superscript letters in columns are not significantly different from each other at p>0.05

artificial feed. Similarly, findings of Khan et al. (2011) are quite in line with our results who observed no difference in taste, texture and aroma when fish diet was supplemented with either poultry fat or fish oil. Hassan (1996) while working on Indian major carps in similar context under natural food and artificial feeding environment could not identify any difference in sensory and organoleptic qualities of fish flesh from two different and independent sources. From previous as well as current studies it can be deduced that type of fish, culture environment and type of feed offered have significant bearing on flesh quality. These differences narrow down with changes and modifications in habitats, feeding manipulations, species proximity, sensitivity of the experimental studies and training of the panelists who can validate subtle differences. Nevertheless majority of such type of studies previous as well as current incline towards non-significance. Therefore, like previous authors we also end up with the inference that different feed sources do not have much impact on sensory qualities of fish flesh. Nonetheless until and unless discrete differences among various ingredient including macro as well as micronutrients are not critically visualized it will remain unresolved puzzle.

It can be concluded from the present study that protein is not the only nutrient which enhances growth of herbivorous fish but feed quality, its acceptability to the fish and water quality of rearing unit also play a pivotal role in this direction. All these factors mentioned above in this contact will be focused in our future research. Guar meal and cotton seed meal, and guar meal and canola meal resulted better as compared to the other feed ingredients and can be used for future *Labeo rohita* feed formulations.

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