

Replacement of Fishmeal With Blend of Canola Meal and Corn Gluten Meal, and an Attempt to Find Alternate Source of Milk Fat for Rohu (*Labeo rohita*)

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Abstract.- Two laboratory feeding trials were conducted for 60 days using *Labeo rohita*, fingerling (3.7±1.9g and 6.9±1cm) to evaluate the possibility of replacing dietary fishmeal (FM) with a protein blend of canola meal and corn gluten meal (CGM), and to find alternate lipid sources for milk product without compromising the growth of fish. In experiment I, two experimental diets viz.; animal rich protein (ARP) as control diet and plant protein (PP), whereas, in experiment II, two experimental diets were; animal rich fat (ARF) as control diet and plant rich fat (PRF) were fed to *L. rohita* fingerlings, 20 in each group. A balance mixture of plant and animal protein and fat (MPF) was used as common diet for both experiments. The results revealed that there were no significant ($P \geq 0.05$) differences among all feeding groups with respect to specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR) and protein efficiency (PE) in experiment I. Significant ($P \leq 0.01$) difference were detected for percent fat, but protein content was non-significant. PP had high fat content, while MPF showed maximum protein content. In experiment II, significant differences were observed for SGR and WG. MPF showed better growth performance than other groups. Study indicated that partial or complete replacement of FM showed better results in terms of fish growth without compromising the growth rate of fish but complete replacement showed high deposition of fat in the body also. On other hand, a balance mixture of animal and plant lipid showed good outcomes in term of growth than complete replacement of milk lipid with rice polish and canola oil.

Key words: *Labeo rohita*, feeding groups, diet composition, growth parameters.

INTRODUCTION

Dietary Fish Meal (FM) and fish oil are extensively utilized in aquafeeds. It is estimated that in 2006 the aquaculture sector consumed 3724 thousand tonnes of fish meal (68.2% total global fish meal production in 2006) and 835 thousand tonnes of fish oil (88.5% total reported fish oil production in 2006) (Tacon and Metian, 2008). Protein from animal source especially FM is considered to be the better protein source because they have essential amino acids profile that seems to meet the requirements of most of the teleost species, and nutrient bioavailability of FM is also very high (Jobling *et al.*, 2001). Traditionally, fish meal has been the main source of protein in diets for fish fry. However, the increasing cost of FM has restricted its use as a protein source for fry diets (El-Saidy and Gaber, 2003). Various alternative protein sources as partial or complete dietary replacement for FM has

been evaluated in fish diets, *e.g.* poultry by-product (Gaber, 1996), sunflower meal (El-Saidy and Gaber, 2002), soybean meal (Khan *et al.*, 2003) and chuni (Saha and Ray, 1998). Several other protein sources derived from other terrestrial animals are also going to be used in fish feed *e.g.* blood meal, meat and bone meal and worm meal. As these have very high content of protein, these may cause negative effect on the growth of fish at higher than optimum inclusion (Bureau *et al.*, 1999). Partial or complete replacement of FM has been practiced for the last few years. Proteins from plant origin have also different sources. Oilseed meals, prepared from the material remaining after oil has been extracted from soybeans, cotton seed, rape/canola, peanuts, safflower and safflower seeds are the common protein sources (Hardy, 1989; Friedman, 1996). Although ingredients from plant origin are being used in fish feed but can not be replaced totally by plant origin protein as it reduces the growth of fish (Bureau *et al.*, 1998; Burel *et al.*, 1998). Lipids are important components of the diet, supplying both energy and essential fatty acids (Watanabe, 1982) and are efficiently metabolized by most fish species

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(Sargent *et al.*, 1989). Different kinds of oils like silkworm pupa oil and sardine oil are being utilized as good energy source for the carps (*e.g. Cyprinus carpio*) which are cheaper commercially than fish oil (Nandeeshha *et al.*, 1999). A number of studies have demonstrated that increasing dietary lipids above optimum concentration results in increased fish performance and protein utilization by fish. However, an increase in body fat deposition is frequently observed (Lee and Putnam, 1973; Martino *et al.*, 2002). Similar conditions were also in carps such as the Indian carp, *Catla catla*, increasing dietary lipid concentrations can depress growth (Seenappa and Devaraj, 1995). Lipids from different sources including plant and animal origin have been used in fish feeds, and those from both terrestrial and aquatic origin (Hardy, 1989; Jobling, 1994). Looking at protein and fat, various sources of protein and fat have been used in aquaculture (Jobling, 1998). Not only different sources of protein and lipid, protein/lipid ratio in fish diet (Gangadhara *et al.*, 1997) and daily ration size (Khan *et al.*, 2004) are also considered important for better fish performance.

The main objective of this study was to evaluate the possibility of replacing dietary FM with a protein blend of canola meal and CGM, and to find alternate lipid sources for milk product without compromising the growth of fish.

MATERIALS AND METHODS

Experimental diet

Five almost isocaloric and isonitrogenous experimental diets ($16.76 \pm 0.22 \text{ kJ DE g}^{-1}$) were formulated from Shabir Fish feeds Multan, Pakistan on optimizing energy level with different origin of protein and fat (dry weight). In experiment I, two experimental diets were; ARP as control diet and PP. APR contained 600 g kg^{-1} of FM with very low quantity of plant protein while PP had 417 g kg^{-1} of CGM and 100 g kg^{-1} of canola meal with zero FM. In experiment II, two experimental diets were; ARF as control diet and PRF. AFR contained 60 g kg^{-1} milk fat while in PRF milk fat was replaced by canola oil and rice polish. A balance mixture of plant and animal protein and fat (MPF) was used as common diet for both experiments. MPF contained

230 g kg^{-1} FM, 254 g kg^{-1} CGM and equal proportion of milk and plant lipid. Ingredients and proximate composition of the five experimental diets are presented in Table I. Experimental diets were analyzed using standard AOAC (1995) methods for percent crude protein, crude fat, ash and crude fibre.

Experimental design and feeding trial

Experiments were conducted in Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan. A total of 100 *L. rohita* fingerlings were procured from Al-Madina Fish Hatchery Matital Road Multan and transported in oxygen-filled polythene bags. Fish were acclimated to experimental conditions for two weeks. Then fishes were randomly housed individually in fiberglass RAS (Recirculating aerated system) holding tanks with twenty replicates. Each holding tank had 10 compartments ($61 \times 61 \times 61 \text{ cm}$ each). Average initial weight and length of fishes were taken $3.7 \pm 1.9 \text{ g}$ and $6.9 \pm 1 \text{ cm}$ respectively. Fish were fed twice per day by hand at the rate of 5% body weight (Khan *et al.*, 2004) in two equal portions for whole experimental period of 60 days duration. During the experimental period, the water temperature, dissolved oxygen and pH were $23 \pm 2.7^\circ\text{C}$, $7.56 \pm 0.41 \text{ mg L}^{-1}$, and 7.08 ± 0.43 respectively (Du *et al.*, 2005).

Growth performance

At the end of experiment, fish were weighed and length was taken. SGR, WG, FCR, and PE were calculated by using the formulae in the footnotes of Table II.

Sample collection and analysis

At the end of the experiments, fish were chill-killed by immersing in ice water. Moisture and dry weight (oven dry at 60°C to constant weight), ash content (incinerate at 550°C for 5h in a muffle furnace), fat content (chloroform-methanol method: Cui and Wootton 1988; Salam and Davies 1994) and protein content (Salam and Davies 1994) of all feeding groups based on whole body weight of fish. SPSS and minitab were used for data analysis. Mean values are presented in Tables II and III.

Table I.- Ingredient and proximate composition of experimental diets.

Ingredients (g.kg ⁻¹)	International Feed Number	Diets*				
		Experiment I		Experiment II		
		1 ARP(C)	2 MP	3 ARF(C)	4 PRF	5 MPF
Fish meal	5-09-835	600	230	350	260	230
Canola meal	5-06-145	70	50	100	170	50
Corn gluten (60%)	5-28-242	-	254	71	218	254
Rice bran	4-03-928	100	126	134	100	126
Rice polish	4-03-943	80	210	-	100	210
Soybean meal	5-04-604	70	50	225	50	50
Animal fat ¹ (milk fat)		10	10	60	-	10
Starch	5-01-162a	40	40	40	60	40
Canola oil ²		10	10	-	22	10
Mineral ³ and vitamin ⁴ premixes (Equal proportions)		10	10	10	10	10
Di-calcium phosphate		10	10	10	10	10
Total		1000	1000	1000	1000	1000
Proximate analysis (percent) (Dry weight)						
Dietary energy (kJ g ⁻¹)		16.1	17.5	16.4	16	17.5
Crude protein		39.4	38.3	39.5	38.8	38.3
Crude fat		9.7	9.1	9.6	8.2	9.1
Ash		13.24	9.66	9.32	10.11	9.66
Crude fibre		3.55	4.08	6.38	4.13	4.08
Cost kg ⁻¹ (\$)		0.23	0.17	0.23	0.19	0.17

*Diet is common for both experiments.

ARP(C), Animal rich protein (Control for experiment. I); PP, Plant protein; ARF(C): Animal rich fat (Control for experiment. II); PRF, Plant rich fat; MPF, Mixture of protein and fat from animal and plant source.

¹Blue band (Unilever Pakistan Limited) contains skimmed milk, milk fat, salt stabilizer, preservatives, Vit. A, B, D and calcium.

²Season canola oil (Wali Oil Mills Lahore, Pakistan) contains Fat profile 6%: saturated fat 62%, Polysaturated fat (linolic acid) 11%.

³SB mineral mix (SB Pharma, Rawalpindi, Pakistan) contains (kg⁻¹); Copper 5x10³mg; Iron 5x10⁴mg; Manganese 6.2x10⁴mg; Zinc 3x10⁴mg; Iodine 5x10²mg and Selenium 1x10²mg.

⁴SB vita-L (SB Pharma) contains (kg⁻¹); A 5x10⁶IU; D₃ 5x10⁶IU; E 7.5x10³mg; K₃ 5x10²mg; B₁ 1x10³mg; B₂ 2.5x10³mg; B₆ 1.5x10³mg; B₁₂ 10mg; Niacin 1.5x10⁴mg; Biotin 2.5x10mg; Pantothenic acid 4x10³mg; Folic acid 5x10²mg; Antioxidant 5 x10³mg and Carrier(upto) 1 x10³g.

RESULTS AND DISCUSSION

In experiment I, non significant ($P \geq 0.05$) differences among all feeding groups with respect to SGR, WG, FCR and PE were observed. Significant ($P \leq 0.01$) difference was detected for percent fat, but protein content was non-significant. MPF showed better results in terms of growth performance, percent fat and percent protein content. Similarly, PP also showed good growth performance but had high fat content reducing the protein content of the body. In experiment II, significant ($P \leq 0.01$) differences were observed for SGR and WG, while

FCR and PE were non-significant ($P \geq 0.05$). MPF showed better growth performance showing highest value for SGR and WG which was not significantly different from control (ARF) group. Significant results were observed in percent fat and percent protein content of fish. ARF showed better growth but had more fat content and less protein content than PRF. All the results are reported in Tables II and III.

Present study revealed that the FM could be replaced partially or totally with blend of CGM and canola meal without compromising the growth of *L. rohita* in terms of SGR, WG, FCR and PE in

Table II.- Mean values and standard deviation (parenthesis) of SGR, WG and PE of *Labeo rohita* for all feeding groups.

Growth parameters	Feeding Groups					
	Experiment I			Experiment II		
	1 ARP(C)	2 PP	5 MPF	3 ARF(C)	4 PRF	5 MPF
SGR ¹ (%day ⁻¹)	1.350 (0.156)	1.602 (0.366)	1.745 (0.382)	1.641 ^b (0.272)	1.238 ^a (0.374)	1.745 ^b (0.382)
WG ² (%)	125.66 (20.89)	167.08 (62.17)	191.5 (61.92)	171.07 ^{ab} (45.34)	115.42 ^a (51.94)	191.5 ^b (61.92)
FCR ³	2.085 (0.589)	2.202 (0.82)	1.858 (0.75)	2.376 (0.76)	2.444 (0.69)	1.858 (0.75)
PE ⁴ (g)	0.135 (0.040)	0.133 (0.047)	0.159 (0.054)	0.122 (0.041)	0.116 (0.032)	0.159 (0.054)

Note: Feeding groups 5 is common for both experiments.

All the vales are mean. Values sharing common superscripts do not different significantly.

¹Specific Growth rate (%day⁻¹) = (ln final weight – ln initial weight) × 100/ days

²Weight Gain (%) = (final weight – initial weight) × 100/ (initial weight)

³FCR = [total food intake (g)/total weight gain (g)]

⁴Protein Efficiency = Final weight – Initial weight / protein intake

For other abbreviations see Table I.

Table III.- Mean values and standard deviation (parenthesis) of various body constituents of *Labeo rohita* for four different feeding groups.

Body constituents (%)	Feeding Groups					
	Experiment. I			Experiment. II		
	1 ARP(C)	2 PP	5 MPF	3 ARF(C)	4 PRF	5 MPF
Water	73.3 (1.2)	73.4 (0.9)	73.4 (1.5)	78.1 (3.8)	75.8 (6.1)	73.4 (1.5)
Dry weight	26.74 (1.23)	26.64 (0.94)	26.64 (1.51)	21.93 (3.76)	24.16 (6.09)	26.64 (1.51)
Ash (wet weight)	2.80 (0.43)	3.36 (0.49)	2.55 (0.36)	2.57 (0.48)	2.73 (1.01)	2.55 (0.36)
Fat (wet weight)	12.99 ^{ab} (3.87)	14.73 ^b (2.86)	10.35 ^a (2.67)	10.16 ^{ab} (2.67)	7.38 ^a (2.22)	10.35 ^b (2.67)
Protein (wet weight)	13.68 (3.32)	11.82 (3.57)	16.24 (2.99)	11.70 ^a (3.13)	16.71 ^b (4.21)	16.24 ^b (2.99)

Note: Feeding groups 5 is common for both experiments.

All the vales are mean. Values sharing common superscripts do not different significantly.

Mean sharing the same letter do not different significantly at P_≥0.05

For other abbreviations see Table I.

experiment I. But complete replaced with plant protein showed high deposition of fat in fish body. These results were also agreed with previous findings that partial replacement of fish meal with plant protein gave better results as compared to complete replacement. Giri *et al.* (2000) reported similar results working on catfish *Clarias batrachus* fingerlings showed good growth with FM diet followed by partial or complete replacement with fish viscera, chicken viscera and also only plant protein. The suitability of partial or complete replacement of FM with other protein sources in terms of growth performance is highly variable among fish species and experimental conditions (El-Sayed, 1999). The increase in SGR and WG in *L.*

rohita fed the mixture of FM and plant protein used in the present study appeared to be due to improved palatability of the diet as suggested by Mohsen and Lovell (1990) working on channel catfish. Although there were no significant differences among MPF, PP and ARP for growth parameters, but PP showed good growth with unexpectedly high content of body fat having corn gluten as a major plant protein source. This was due to high (95- 96%) digestibility of corn gluten by carps and trout and high lipid content of CGM as reported by Pongmaneerat and Watanabe (1991) and Robaina *et al.* (1997). Similarly, A-S Goda *et al.* (2007) working on Nile tilapia *Oreochromis niloticus* noticed higher fish body fat using high quantity of corn gluten as

protein source. In second experiment, MPF and ARF showed better growth performances which were significantly higher than PRF. The main reason for this difference was seemed to be due the presence of high amount of canola oil as lipid source; which is not considered as a good energy source for fish growth. These results also agreed with previous researches that glucosinolates are the main factors in canola (Duncan, 1991) and are known to affect mineral utilization and cause thyroid abnormalities in rainbow trout, salmon, carp and tilapia resulting poor growth (Hossain and Jauncey, 1989; Davies *et al.*, 1990). Vegetable oil has been used to replace the animal oil as a fat source in fisheries, reporting limited effect on growth but marked consequences on lipid composition and metabolism (Bell *et al.*, 2001). Our results also matched with those given in El-Kerdawy and Salama (1997), who fed fish diets in which 50% of the fish oil and replaced it with soybean oil and there were no reported differences, although fish fed a diet of 50% linseed oil and rapeseed oil level weighed less. Similarly high quantity of plant oil in fish feed worsened fish performance as reported by Alexis (1997). Protein content was good in PRF which was not significantly different from MPF suggesting that partial of complete replacement of milk fat with plant lipid had no effect on protein content of the fish. This result was also in agreement with Raso and Anderson (2003) worked on barramundi (*Lates calcarifer*) to check the effect of different oils sources on the growth and body composition showing no differences in protein content of fish.

In conclusion, this study clearly indicated that for *L. rohita*, a mixture CGM and canola meal could be used for the partial replacement of FM reducing the cost of feed formulation. Similarly, milk fat could be partially replaced with canola oil without compromising on the growth of fish.

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