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Inclusion level of deoiled rice bran (DORB) in the diet of *Labeo rohita* (Hamilton, 1882) fingerlings: Effect on growth and gene expression of IGF-I and IGF-II

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ABSTRACT

A 60-day feeding trial was conducted to evaluate the effect of deoiled rice bran (DORB) on the growth performance and liver IGF-I and IGF-II mRNA expression in a freshwater fish, *Labeo rohita*. Six isonitrogenous (30%), isolipidic (6%) and iso-energetic (1.8 MJ/100 g) diets were prepared with different inclusion level of DORB viz., C (control, 0%), T20 (20%), T30 (30%), T40 (40%), T50 (50%) and T60 (60%). Three hundred and fifteen (315) fingerlings with 15 fish per tank having an average weight of 8.0 ± 0.5 g were randomly distributed in seven treatments in triplicates following a completely randomized design. At the end of the experiment, growth and nutrient utilization were evaluated in terms of weight gain% (WG %), SGR, FCR, PER, RNA/DNA ratio along with the gene expression of IGF-I and IGF-II. The weight gain % and specific growth rate were similar in 30, 40, 50 and 60% DORB fed groups, but higher ($p < 0.05$) than the control and commercial diet fed groups. The PER (Protein Efficiency Ratio), LER (Lipid Efficiency Ratio) and FCR (Feed Conversion Ratio) were similar ($p > 0.05$) in 30, 40, 50 and 60% DORB fed groups. The RNA-DNA ratio was significantly lower in T50 group, which was similar to T60 group. Based on second order polynomial regression analysis ($y = -0.008x^2 + 0.5227x + 1.3407$, $R^2 = 0.91$), the expression of IGF-I was found to be maximum at 33% inclusion level. The hepatic IGF-I and IGF-II gene expression were checked using real time PCR normalised against the β-actin gene and found to be maximum at the dietary inclusion level of 33%. Hence, from these results it can be concluded that though growth rate was similar in all the groups fed with DORB at 30 to 60% of inclusion level, but an optimum inclusion level of DORB at 33% is best to support the nutrient utilization and growth performance of *Labeo rohita* fingerlings based on gene expression of IGFI and IGF II in liver.

1. Introduction

Aquaculture is the fastest growing food sector in the world. Global per capita fish availability has reached 20 kg, which is achieved by the significant increase in the aquaculture production both from freshwater and brackish water (FAO, 2016). The Asian subcontinent is dominating in aquaculture production, which include many species like carps, catfishes, shellfish, pangasius, tilapia etc. The IMC (Indian major carps) contributes a lion's share of carp culture in Asian countries. Most of the carp culture in this region is practised as semi-intensive system. This has shifted the carp culture from the non-feed based extensive systems to supplementary or full feed based culture systems. *Labeo rohita* is one of the most popular cultivable carp species among the IMC. Previous studies have shown that feeding is one of the main factor required for faster growth and higher yield of cultured *L. rohita* (Jose et al., 2006; Maity and Patra, 2008).

Due to high rise of ingredient cost, fish farmers are forced to use the cheaper ingredients for feeding the fish. As a practice, DORB is most commonly used by the carp farmers as the major feed ingredient as it is the main agricultural by-product available to the farmers.

Though there are plenty of other potential ingredients are available locally, irregular supply and presence of antinutritional factors makes it difficult to include those ingredients in aquafeed preparations. In carp culture, either mash feed or a combination of mash and pelleted feed is used, where mostly plant protein sources are used (Singh et al., 2004). DORB is predominantly used along with oil cakes in the feed formulation as it is the cheapest agricultural by product, available throughout the year and well accepted by the carps (Veerina et al., 1993).DORB is a fat free rice bran or rice polish and is a good replacer of other aqua-feed ingredients especially the cereals (Limbu et al., 2016). DORB contains 15–17% protein along with 12–15%crude fiber (Sereewatthanawut et al., 2008).

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Among the three major carps rohu *(L. rohita)* has a higher consumer demand, which is mainly fed with DORB as supplementary feed. It is reported that, the cost of aqua-feed can be reduced substantially by using DORB at higher levels (Ramakrishna et al., 2013), but the optimum level of DORB, required for the better growth of *L. rohita*, is still not known. Though, growth study has been done based on the traditional feeding trial with DORB, nothing is clear about the molecular response of growth genes with respect to feeding level of DORB in fish feed.

During the past few years, the researchers are focussing more on molecular tools to verify the data generated from the traditional feeding trial. Molecular tools help us to understand the metabolic response to a particular feed or ingredient of a particular species. It is reported that the growth and muscle development is controlled by insulin like growth factor (IGF) axis (Jones and Clemmons, 1995). The IGF system is composed of IGF-I and IGF-II as well as IGF receptor and its binding protein. Insulin like growth factors, IGF-I and IGF-II are the mitogenic peptides, which are regulated by the nutritional status of fish and have significant role in nutrient metabolism, growth and development (Tatar et al., 2003; Duan, 1998). Growth hormone/insulin like growth factor is known to associate in regulating the growth. Growth in all vertebrate is regulated through increased protein synthesis and decreased lipolysis. IGF-I and IGF-II are produced throughout the body with local and endocrine growth stimulation and endocrine IGFs are produced mainly in liver. Growth hormone-insulin like growth factor system is the main regulator of muscle growth in vertebrate (Glass, 2003; Wood et al., 2005). Looking in to the above prospective, current study was taken to optimize the maximum inclusion levels of DORB in the diet of *L. rohita* by studying the expression of insulin like growth factors in liver of *L. rohita* with response to growth.

2. Materials and methods

2.1. Experimental set up

Present study was carried out at ICAR-Central Institute of Fisheries Education, Mumbai, India. Three hundred and fifteen (315) fingerlings (avg. wt. 8.0 g ± 0.5) were equally distributed in seven treatments in triplicates following a completely randomized design (CRD). The experiment was conducted for a period of 60 days in 21 rectangular plastic tubs (57 × 36 × 47 cm, 75 L capacity) and was fed to apparent satiation level twice daily. One third of the water was exchanged at every alternate day. All experimental diets (Table 1) were isonitrogenous (30%), isolipidic (6%) and isoenergetic (1.8 MJ/100 g) with different inclusion level of DORB viz., C, T20, T30, T40, T50 and T60. In addition, one commercial diet with 29.88 ± 0.30% CP and energy (1.82 MJ/100 g) was used to compare the growth and nutrient utilization of the fish with the experimental diets.

2.2. Proximate composition

Proximate composition of feed and fish were analysed according to the standard methods of AOAC (1995). The experimental diets and whole fish samples were dried to constant weight at 100 ± 2 °C in hot air oven to determine the moisture content. Crude protein content was determined using micro-Kjeldahl method (Kelplus, PELICAN, India), whereas crude lipid was determined by soxhlet extraction method (SOCS plus, SAS-AS 08, PELICAN, India). The ash content was measured using muffle furnace and fiber estimation was done in Fiber tech (Tulin equipments, India) apparatus and further ashing was done using a muffle furnace at 550 °C for 5 h.

2.3. Growth parameters

The growth of fish was assessed by weighing the fish at every fifteen days interval and the various growth parameters were assessed at the end of the experiment by using the following formula:

Table 1
Formulation of the experimental diets (%).

Ingredients	C	T20	T30	T40	T50	T60
Casein[a]	27.50	24.00	23.00	21.60	20.30	19.00
Gelatin[a]	7.00	6.00	5.10	4.90	4.30	4.20
Dextrin[a]	16.50	12.50	10.00	8.00	6.20	4.00
Starch[a]	29.98	20.48	16.28	11.48	5.98	1.78
DORB[b]	0.00	20	30	40	50	60
Sunflower oil[d]	3.00	3.00	3.00	3.00	3.00	3.00
Cod liver oil[d]	3.00	3.00	3.00	3.00	3.00	3.00
Cellulose[a]	10.00	8.00	6.60	5.00	4.00	2.00
CMC[a]	1.00	1.00	1.00	1.00	1.00	1.00
Vit. min. mix[c]	1.90	1.90	1.90	1.90	1.90	1.90
Vitamin C[a]	0.10	0.10	0.10	0.10	0.10	0.10
BHT[a]	0.02	0.02	0.02	0.02	0.02	0.02

Abbreviations: T:-Treatments; C:-Control CMC: - Carboxymethyl cellulose; BHT: - Butylated hydroxyl toluene; DORB-Deoiled rice bran (CP-15.30%; CL-0.33%; CF-14.45%; ASH-6.01% and NFE-63.88%).

[a] Purified ingredients procured from Himedia Pvt., India.

[b] Purchased from Vaighai agro products, India.

[c] Composition (quantity/kg): Vitamin A, 550,000 IU; Vitamin D_3, 110,000 IU; Vitamin B_2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B_6, 1000 mg; Vitamin B_{12}, 6 mcg; Calcium Pantothenate, 2500 mg; Nicotinamide, 10 g; Choline Chloride, 150 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 L-lysine, 10 g; DL-Methionine, 10 g; Selenium 50 ppm.

[d] Procured from local retail shop.

$$WG\% = (\text{final wt.} - \text{initial wt.}) \times 100/\text{initial wt.}$$

$$SGR = (\text{Log}_e \text{ final weight} - \text{Log}_e \text{ initial weight}) \times 100/\text{Number of days}$$

$$PER = \text{Weight gain (g)/protein intake (g)}$$

$$FCR = \text{Feed given dry weight/weight gain}$$

$$LER = \text{Weight gain (g)/lipid intake (g)}$$

$$GSI = (\text{weight of gastrointestinal tract/whole body weight of fish}) \times 100$$

$$HSI = (\text{weight of liver/whole body weight of fish}) \times 100$$

2.4. Total RNA isolation and cDNA synthesis

Liver tissues were collected for the gene expression study and stored immediately in RNAlater™ solution (Qiagen, Netherlands).Total RNA was isolated from the stored liver tissue ~50 mg using 1 ml TRIzol (Invitrogen, USA) reagent following the manufacture's protocol. RNA concentration and purity (260/280)was checked by Nanodrop spectrophotometer (Thermoscientific USA).The RNA samples were treated with DNase (Fermentas, USA) according to the protocol given by the manufacturer to remove the residual DNA during preparation. Complementary DNA (cDNA) was synthesized from the total RNA using oligo dT primers following the protocol of first strand cDNA synthesis kit (Fermentas, USA) and was used immediately for PCR or stored at − 20 °C for further use.

2.5. Cloning of IGF-I and IGF-II from liver tissue

Polymerase chain reaction was carried out in thermal cycler (AB applied Biosystem 2720 model) using 2 × PCR master mix (Thermo scientific).The oligomer primers (Table 4) for amplification of IGF-I and IGF-II were designed from the sequence of IGF-I of *Danio rario* (Acc. No. - NM_131825) and IGF-II of *Cyprinus carpio* (Acc. No.-KP663718) using Gene Runner 3.05 software. A reaction volume of 25 µl containing 12.5 µl 2 × PCR master mix, 1 µl of (10 pmol) each primer (IGF-I and IGF-II), 1 µl cDNA and remaining nuclease free water were prepared for PCR amplification. The condition of PCR for IGF-I had initial denaturation at 94 °C for 5 min, 30 cycle of 94 °C for 45 s, 57 °C for 45 s and