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Feeding higher level of de-oiled rice bran causes stress to *Labeo rohita* fingerlings

Sarvendra Kumar, N.P. Sahu*, N. Shamna, Amit Ranjan

ICAR-Central Institute of Fisheries Education, Versova, Mumbai 400 061, India

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ABSTRACT

A 60-day feeding trial was conducted with different inclusion levels of de-oiled rice bran (DORB) to study the digestive enzyme activity, physio-metabolic changes and oxidative stress in *Labeo rohita* fingerlings. Six iso-nitrogenous (30% crude protein), iso-lipidic (6% crude lipid) and iso-energetic (380.14 Kcal/100 g) semi-purified diets were prepared with the graded levels of DORB viz. 0 (control), T20 (20%), T30 (30%), T40 (40%), T50 (50%) and T60 (60%). Three hundred and fifteen (315) fingerlings with an average weight of 8 ± 0.5 g were distributed randomly in seven treatments in triplicates, following a completely randomised design (CRD). The digestive enzyme, protease and amylase activities were higher in 20, 30 and 40% DORB fed groups but the Lipase activity did not vary significantly whereas, SOD and GST activity was significantly lower ($p < 0.05$) in the same groups. The T50 and T60 groups showed significantly higher ($p < 0.05$) hepatic LDH (Lactate dehydrogenase), GST (Glutathione-s-transferase) and SOD activity, while RBC (Red blood cell) and WBC (White blood cell) content were found significantly lower in these groups. The catalase activity did not vary significantly ($p > 0.05$) among treatment group. Serum glucose content was higher in the T40, T50 and T60 groups, while serum total protein, albumin, NBT (Nitroblue tetrazolium) and haemoglobin levels were not significantly different among the treatments. From the study, it can be concluded that DORB causes stress to fish when fed at higher inclusion level (50 & 60%).

1. Introduction

Labeo rohita, is a commercially important and widely cultured freshwater fish in Asia in general and the Indian subcontinent in particular (Khan et al., 2003; Iqbal et al., 2014). Fish feed has always been a top priority for success of intensive aquaculture and higher fish production. The feed cost depends on the ingredient availability and the aqua feed industry relies heavily on the major conventional ingredients because of their protein quality, balanced amino acid and fatty acid profile. Rising cost and uncertain availability of conventional ingredient compels the aquaculture nutritionists and feed manufacturers to use less expensive and more readily available plant protein sources as substitutes. In aquaculture, agro-industrial by-products, such as rice bran, copra meal, palm kernel and locally available ingredients including sweet potato and cassava can be effectively used as substitutes to the imported grains, thereby lowering the feed cost. Protein is the most expensive ingredient in the diet, and its quality is a very important aspect in aquaculture (Garcia-Ulloa Gomez et al., 2008). Species-specific feed formulations and can support optimum and cost effective fish production (Craig and Helfrich, 2002). Formulation and preparation of balanced and cost effective fish feed, which is acceptable to fish farmers, has always been a challenging task for both the aquaculturists and nutritionists. In herbivorous fish culture systems, the protein of plant origin is preferred to animal origin (Singh et al., 2004).

De-oiled rice bran (DORB) constitutes 6–10% by weight of rice and is more nutritious than any other agri-products. De-oiled rice bran is a fat free rice bran or rice polish and is a good replacement for other aqua feed ingredients, such as maize or other cereals (Limbu et al., 2016). DORB contains CP (crude protein) - 15.30%; CL (Crude lipid) - 0.33%; CF (Crude fiber) - 14.45%; Ash - 6.01% and NFE (Nitrogen free extract) - 63.88% (Kumar et al., 2017). It is comparable with other cereal by-products for their amino acid composition. DORB is the major ingredient used in carp feeds and is used either singly or in combination with other ingredients, even upto a 90% inclusion level (Veerina et al., 1993).

The growth performance of fish dependent on the digestive and metabolic capacity required to support the tissue protein synthesis (Blier et al., 2002). The type and source of each ingredient and nutrient can alter the enzyme secretion and its enzyme activity from the digestive tract. The digestive enzymes play a key role in nutrient

* Corresponding author at: Central Institute of Fisheries Education, Versova, Mumbai 400 061, India.
E-mail address: npsahu@cife.edu.in (N.P. Sahu).

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utilization and growth performance of the fish (Suarez et al., 1995). The activity of these digestive enzymes leads to break down of large nutrient to small nutrient which in turn, is associated with better digestion and absorption of nutrients (Moraes and Bidinotto, 2000; Debnath et al., 2007).

The ingredients can be best evaluated by the physiological responses of the fish. Hence, the metabolic and stress linked enzymes can indicate the metabolic and physiological changes associated with the introduction of an ingredient in the diet of fish.

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism. The antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) are the primary enzymes of the antioxidant defence against oxidative damages generated by these ROS. During stress, the energy requirement in animal body increases and biomolecules like glucose, lipids and protein, also start mobilizing to meet the energy demand depending on the intensity of stress (Javed and Usmani, 2015). This can cause variation in the activity of enzymes like LDH. Therefore, inclusion of DORB in diet can have an effect on metabolic and oxidative stress in *L. rohita* which should be studied in detail. Similarly, the possible adverse effects of diets on the immune response of fish have also been studied (Waagbo, 1994; Kiron et al., 2004). The analysis of blood indices such as Red blood cell (RBC), White blood cell (WBC), Haemoglobin (Hb), glucose etc. is a valuable parameters in assessing the condition of fish, as it provides a reliable index of their physiological condition (Alyakrinskyaya and Dolgova, 1984).

While the predominant concern about the effects of various alternative plant proteins in fish is on growth performance, relatively few studies have monitored the dietary influence on the biochemical index of fish, such as changes in protein metabolism, enzyme activities (Krogdahl et al., 2003; Kumar et al., 2012), hepatic metabolism (Vilhelmsson et al., 2004) and oxidative status. These are essential to provide an indication of disturbance by specific ingredient to the metabolic function and nutrient utilization in fish (Kumar et al., 2011; Lin and Luo, 2011). Thus, the assessment of metabolic parameters in fish serves as an indicator of nutritional status and it is also contribute to the nutrition and farming of these animals.

To our knowledge, no studies have been conducted on the digestive enzymes and metabolic profile in the *Labeo rohita*, as a means to optimize the DORB utilization. Studies on the digestive, metabolic, oxidative stress enzyme and serum parameter in fish might elucidate some aspects of their nutritional physiology and, thus, could support developing feed formulation and feeding strategies for fish feeding and diet formulation. Thus, the aim of the current study was to find out the physio-metabolic responses, digestive enzyme activity and oxidative stress in *Labeo rohita* fingerlings fed different inclusion levels of DORB.

2. Materials and methods

2.1. Experimental setup

The present study was carried out at ICAR-Central Institute of Fisheries Education, Mumbai. Three hundred and fifteen (315) fingerlings (avg. wt. 8 ± 0.5 g) were equally distributed in seven treatments in triplicates containing fifteen fish in each tank. The experiment was conducted for a period of 60 days in 21 rectangular plastic tubs (57 × 36 × 47 cm, 75 l capacity) and were fed to an apparent satiation level twice daily. All experimental diets (Table 1) were iso-nitrogenous (30%), iso-lipidic (6%) and iso-energetic (380 Kcal/100 g) with different inclusion level of DORB viz., C, T20, T30, T40, T50 and T60.

2.2. Proximate analysis of diet

Proximate composition of all diet was analysed in triplicates (AOAC, 1995) and is presented in Table 2. The moisture content was determined by drying at 105 °C to a constant weight. Crude protein content was determined using the Micro-Kjeldahl method (Kelplus, PELICAN, India), whereas crude lipid was determined by soxhlet's extraction method (SOCS plus, SAS-AS 08, PELICAN, India), ash content was measured using muffle furnace at 550 °C for 6 h and fiber estimation was done in Fiber-tech (Tulin equipments, India) apparatus and further ashing was done using a muffle furnace.

Table 1
Compositions 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
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
Vitamin mineral 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
DORB[b]	0.00	20.00	30.00	40.00	50.00	60.00
Total	100	100	100	100	100	100

Abbreviations: T: - Treatments; C: - Control; CMC: - Carboxymethyl cellulose; BHT: - Butylated hydroxyl toluene; DORB - De-oiled rice bran.

[a] Purified ingredients procured from HImedia Ltd., India.

[b] Purchased from Vaighai agro products, India.

[c] Composition (quantity/kg): Vitamin A, 55,00,000 IU; Vitamin D_3, 11,00,000 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.

2.3. Collection of serum

At the end of experiment, blood was drawn from the caudal vein using a syringe without any anti-coagulant and transferred immediately into 1.5 ml eppendorf tube and allowed to clot for 2 h at room temperature. After clotting, the blood was centrifuged for 15 min at 6000 rpm and the supernatant was collected to another eppendorf tube for analysis. Serum total protein, albumin and glucose were estimated by using total protein kit, albumin kit and glucose kit (Erba® Diagnostic Mannheim; Transasia Bio-medicals Ltd., Solan, HP, India). Blood samples were collected without using EDTA anticoagulant for the NBT test.

2.4. Nitroblue tetrazolium (NBT) assay

The respiratory burst activity was studied in terms of optical density of formazon blue precipitate formed in Nitroblue tetrazoleum (NBT) assay following the method of Stasiak and Baumann (1996). The optical density was recorded in an ELISA reader at 620 nm.

2.5. Haemoglobin

The level of haemoglobin in blood was analysed by estimating cyanmethaemoglobin using Drabkins Fluid (Qualigens Diagnostics, division of Glaxo SmithKline Pharmaceutical Limited). The optical density (OD) was measured using a spectrophotometer at a wavelength of 540 nm, and the final concentration was calculated by comparing it with the standard cyanmethaemoglobin (Qualigens diagnostics). The haemoglobin concentration was then calculated by using the following formula:

$$\text{Haemoglobin(g/dl)} = [\text{OD(T)/OD(S)}] \times (251/1000) \times 60$$

where, OD (T), optical density of test; OD(S), optical density of standard.