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Mitigation of immunosuppressive and oxidative stress effect of dietary gelatinized starch in *Labeo rohita* fingerlings by elevation of rearing temperature within optimum range

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ABSTRACT

The present study was conducted to investigate the strategy to mitigate the immunosuppressive and oxidative stress effect of gelatinized starch in fingerling of *Labeo rohita*. Fingerlings were either maintained at ambient water temperature (26 °C) or exposed to 32 °C for one week and then subjected to 26 °C for four weeks. Both groups were fed with isoproteinous (30% crude protein) diets containing gelatinized (G) or non-gelatinized (NG) starch. After 5 weeks of feeding trial, fingerlings were challenged by *Aeromonas hydrophila* and survival rate was recorded for the next 7 days. Serum cortisol and glucose content was significantly ($p < 0.05$) higher in G starch fed group and decreased with the increase in temperature from 26 to 32 °C, which was consistent for next four week after decrease in temperature from 32 to 26 °C. Lower respiratory burst activity and serum total protein and globulin content in G starch fed group at 26 °C significantly ($p < 0.05$) increased after elevation of temperature from 26 to 32 °C and levelled off to NG starch fed group. Liver superoxide dismutase (SOD) and catalase (CAT) activity of G starch fed group was significantly higher in group reared at 32 °C compared to 26 °C. After challenge, fish fed G starch showed lower survival rate than that of fish fed NG starch. Subsequently, exposure of elevated temperature (32 °C) for one week significantly increased the survival rate of G starch fed group and levelled off to NG starch fed group. The results of this study indicated that dietary G starch may cause metabolic stress of fingerling *L. rohita*, as might consequently lead to the decrease antioxidant abilities, depressed immunity and reduced resistance to *A. hydrophila* infection. Subsequently, exposure of elevated temperature (32 °C) for one week mitigate immunosuppressive and oxidative stress effect of dietary G starch.

1. Introduction

Efficiency of carbohydrate utilization by fish is associated with dietary level and the technological treatment applied [1]. The application of technological treatments such as gelatinization improves its digestibility and potential as an energy source [2–4]. Digestibility of native starch is rather low (30–50%), whereas that of gelatinized starch is higher (50–90%). This has been shown in carp by Mohapatra et al. [5], Yengkokpam et al. [6,7] and Kumar et al. [8].

Indian major carp, *Labeo rohita*, an omnivorous fish can utilize carbohydrate up to 43% in the diet [9] without any detrimental effect on health status [10]. Further, it has been reported that fish fed higher levels (45–50%) of gelatinized starch have higher carbohydrate digestibility (83%), indicating better utilization of carbohydrate by herbivores/omnivores such as carps [7]. However, Kumar et al. [10] found an interesting relationship between the types of starch: gelatinized (G) or non-gelatinized (NG) in the diet and immunity status of *L. rohita* that NG starch fed groups registered maximum immunoprotection than their G starch counter parts. Gelatinization results in hydratization and shortening of starch chain, which makes available of high digestible starch instance at the gut absorption site. This considerable amount of

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digestible starch such as glucose enters the body before sufficient elevation of the activities of carbohydrate metabolic enzymes, which in turn restrict the use of these highly digestible starch and results in accumulation of high glucose due to negative physiological effect caused by digestible starch saturation [11]. Fish with excess glucose are assumed to be under constant metabolic stress [11,12], which leads to suppress immune function [13–15].

In the natural aquatic environment of fish, carbohydrate sources are relatively scarce therefore their metabolic system related to carbohydrate utilization become in quiescent state [16]. Hence, carbohydrate utilization in fish may be augmented by increase in their metabolic activity. Metabolic rate of fish increased exponentially as temperature increased [17]. The optimum range of 31 °C–33 °C is the optimum for better growth in *L. rohita* fry [18]. Kumar et al. [10] revealed that metabolic activities in *L. rohita* fingerlings are triggered by exposure to elevated temperature within optimum range from 26 °C to 32 °C for one week and increased metabolic rate prolonged for three weeks. The increased metabolic activity was in response of enhanced hepatic glucokinase and pyruvate kinase activity, the enzymes responsible for the uptake of glucose by the hepatocyte. Therefore, it was hypothesized that triggered metabolism by short term exposure of elevated temperature within optimum range may mitigate the stress and immunosuppressive effect of dietary gelatinized starch in *L. rohita* fingerlings.

2. Material and methods

2.1. Gelatinization of corn

The corn was ground to fine powder and made into dough by adding required amount of water followed by cooking in as autoclave at 15 psi for 1 h so as to get maximum gelatinization. The cooked corn was then spread over a tray and dried in an oven at 60 °C. The dried mass was then pulverized in a hammer mill with a 0.5 mm screen and stored in airtight containers until use. The degree of gelatinization of corn was determined as Guraya and Toledo [19]. A known amount (0.2 g) of corn powder was mixed with 15 ml of 0.2N potassium hydroxide followed by intermittent stirring for 30 min. The pH of the mixture was adjusted to 5.5 using 2N phosphoric acid and the volume was made upto 100 ml with distilled water. Next, 100 μl of aliquot was transferred to a test tube and diluted to 5 ml with distilled water. Then 50 μl of standard iodine solution (4% KI, 1% I_2) was added and the absorbance of the solution was taken at 600 nm (A_1) against the reagent blank. Another aliquot was made by the same procedure by mixing 0.2 g of dried corn powder in 15 ml of 0.6N potassium hydroxide and the absorbance was taken at 600 nm (A_2) as above. The degree of gelatinization was calculated as follows: Gelatinization % = $A_1/A_2 \times 100$. The measured gelatinization ratio in gelatinized corn was 92%.

2.2. Diets

Two isoprotein (30% crude protein) and isocaloric (400 kcal/100 gm) semipurified diets with 50% corn meal either gelatinized (G) or non gelatinized (NG) were prepared. All the ingredients except the gelatin, vitamin mineral mixture, and vitamin C were mixed in a big plastic bowl. Gelatin crystals were mixed in luke warm water so as to form a jelly like substance. The mixed ingredients were then combined with the dissolved gelatin to form dough with the addition of necessary quantity of water. After that oil was added and mixed well. The dough was then allowed to set for 1 h for proper conditioning followed by steaming for 5 min in a pressure cooker. The vitamin mineral mixture and vitamin C were added after cooling. Pellets were prepared using a hand pelletizer having 2 mm diameter size. Finally the pellets were air dried for some time and kept in oven for 4 h at 50 °C till complete drying. After drying, the pellets were packed in airtight polythene bags and labelled properly. Ingredient compositions of the experimental diets are presented in Table 1.

2.3. Experimental animals

L. rohita (Cypriniformes; Cyprinidae) fingerlings were procured from Prem Fisheries Consultancy, Gujarat, India. Fish were transported in a circular container (500 L) with sufficient aeration to the wet laboratory of the Fish Nutrition and Biochemistry Lab, Central Institute of Fisheries Education, Mumbai, India. Fish were carefully transferred to a circular tank (1000 L) and were left undisturbed the whole night. In order to ameliorate the handling stress the fingerlings were given a mild salt treatment (3% NaCl) on the next day. About 50% of water was exchanged every day. The stock was acclimatized under aerated conditions at ambient temperature (26 °C) for a period of 15 days and was fed with a practical diet containing 30% crude protein.

2.4. Experimental design

Four hundred *L. rohita* fingerlings (average weight 7.93 g) were randomly distributed into four treatments groups with four replicates each, following a completely randomized design in 16 tanks (150 L). Half of the experimental groups were maintained at ambient temperature (26 °C), whereas the other half were exposed to 32 °C by using thermostatic water heater (range up to 50 °C, DTC-PID – 50L × 51B × 52H, General Trading Corporation, Mumbai, India) for one week, thereafter they were maintained at ambient temperature (26 °C). The water temperature of the treatments exposed to 32 °C was decreased to 26 °C within 24 h. Continuous aeration was provided to all the tanks from a compressed air pump

Table 1
Formulation and nutrient composition of the different experimental diets.

Ingredients (%)	Experimental diets	
	NG	G
Fish meal[a]	20	20
Corn flour[a]	50	50
Cellulose[b]	5	5
Casein + Gelatin (4:1)[c]	15	15
Soybean oil	4	4
Cod liver oil	2	2
CMC[d]	2	2
Vit-Min Mix[e]	1.99	1.99
Vitamin C[f]	0.01	0.01
Proximate composition of diet (% DM basis)		
Dry matter	92.67	93.24
Crude protein (CP)	30.05	30.00
Ether extract (EE)	8.55	8.35
Total carbohydrate (TC)	51.61	52.11
Total ash	9.79	9.54
Gross energy (Kcal/100 gm)	403.59	403.59

[a] Procured from Central poultry farm, Mumbai, India.
[b] Sd Fine Chemicals (Mumbai, India).
[c] Casein fat free: 75% CP (HImedia ltd, India). Gelatin: 96% CP (HImedia ltd, India).
[d] Carboxymethylcellulose (Sd Fine Chemicals Ltd., India).
[e] Vitamin-mineral mix (Emix™ plus) (quantity 2.5 kg^{-1}): 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 mg; Calcium panthothenate-2500 mg; Niacinamide-10 gm; Choline chloride-150 gm; Mn-27,000 mg; Iodine-1000 mg; Fe-7500 mg; Cu-2000; Zn-5000 mg; Co-450 mg; Ca-500 g; P-300 g; Se-50 ppm; L-Lysine-10 g; DL-methionine-10 g.
[f] Stay C (Hoffman La Roche, Nutley, N.J., USA) 15% ascorbic acid activity.