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Responses Of Digestive And Metabolic Enzymes To Dietary Tannin In Labeo Rohita Fingerlings

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ABSTRACT

A 60-days feeding trial was conducted to study the effect of dietary tannin on the response of digestive and metabolic enzyme in *Labeo rohita* fingerlings. One hundred and eighty fingerlings (avg. weight, 3.2g) were randomly distributed among six treatments with each of 3 replicates. Six isonitrogenous (crude protein 33.98 to 35.11%) and isocaloric (394.42 to 394.74 Kcal DE/100g) diets were prepared with graded levels of tannic acid *viz.*, control (0%), T_1 (0.25%), T_2 (0.5%), T_3 (1.0%), T_4 (1.5%) and T_5 (2.0%). Protease activity was inversely related with the dietary tannin concentration. Amylase activity in intestine was significantly ($P<0.05$) inhibited due to dietary tannic acid. Lipase activity also exhibited an inverse relation with dietary tannin. Gluconeogenic enzyme of liver like G6Pase and FDPase activity was significantly ($P<0.05$) lower in tannic acid fed groups compare to the control. FDPase activity also showed an inverse relation with the dietary tannin content. G6PDH activity in liver showed significant ($P<0.05$) increase at lower concentrations, being highest at 0.5% of dietary tannin. Muscle AST and ALT activity was significantly ($P<0.05$) higher at higher concentrations of dietary tannic acid (T_4 and T_5). Muscle ATPase activity showed decrease in its activity with increase in tannic acid in the diet. From this experiment it seems that dietary tannin even at 0.25% level, significantly affects the digestive and metabolic enzyme of *L. rohita* fingerlings. This may be considered by the nutritionists while formulating diet for this species.

KEYWORDS

Tannic acid;
Labeo rohita;
Digestive enzymes;
Metabolic enzymes.

INTRODUCTION

Economical fish production depends on the availability of low cost and nutritionally balanced diet. The high cost of animal protein sources resulted in a constant search for alternative cheaper plant protein sources for computation of nutritionally balanced aquaculture diets. However, presence of various antinutritional factors, primarily tannin, has limited the use of unconventional plant feed ingredients[1,2]. In aquafeed, tannins being most common antinutritional factors present in plant protein sources including oilseeds and leguminous plants; their reduction will render these plants a suitable source of animal/fish feed.

Tannins are a group of polyphenolic substances with various molecular weights and variable complexity, which have received considerable attention with respect to their possible nutritional and physiological effects. The negative effects of tannins are mainly attributed to the binding of tannins with nutrients and enzymes, thereby decreasing the availability of nutrients and thus inhibiting various enzyme activities [3].These are divided into two groups: condensed tannins, that are derivatives of flavonols and hydrolysable tannins, which are esters of a sugar and one or more trihydroxybenzenecarboxylic acids[4]. Hydrolyasable tannins are easily degraded in biological systems and their hydrolysed products enter the blood and cause organ toxicity (particularly liver and kidney) once the level in blood increases beyond the detoxification capability of these organs[3,5,6]. Higher concentration of tannins may induce necrosis of liver[7]. Tannins present in oilseed meals are reported to interfere with protein and dry matter digestibility by inhibiting protease activity[8]. It is also reported to be inhibitors of several enzymes, such as H^+, K^+ - ATPase[9], anti-oxidant enzymes[10]. Tannic acid alone or in combination of metals is found to be toxic to fish due to enzyme inhibition[11]. The presence of tannin in the diet even at low concentration inhibits the activities of digestive enzymes like protease, amylase and lipase in *Labeo rohita* fingerlings[12]. They also reported that protease and lipase activities were reduced more as compared to a-amylase activity. This inhibitory action of tannin on enzyme may affect the efficiency of feed utilization, thereby affecting weight gain.

Rohu (*Labeo rohita*) is a preferable fish among Indian major carps, where plant ingredients dominate in its feed. But presence of tannin may adversely affect its enzyme action and hence nutrient utilization. Though tannin is an integral component of plant ingredients, its action on digestive and metabolic enzymes needs to be studies. Hence this study has been undertaken to study the effect of tannic acid (a hydolysable tannin) on the response of digestive and other metabolic enzymes in *Labeo rohita* fingerlings. This study may be used to quickly study the tannin acid tolerance in terms of digestive and metabolic enzyme responses in *Labeo rohita*.

EXPERIMENTAL

Experimental animal

Prior to start of the experiment *Labeo rohita* fingerlings (avg. wt 3.2 gm) were procured from Palghar fish farm, Maharastra, India, and given prophylactic dip treatment in $KMnO_4$ solution (50mgL^{-1}). The fish were transported in a circular container (500 L) with sufficient aeration to the experimental site of Fish Nutrition and Biochemistry Lab, Central Institute of Fisheries Education, Mumbai. They were carefully transferred to another circular tank (1000L) and left undisturbed for overnight. The stock was acclimatized under aerated conditions for 25 days at 26-28^{0}C water temperature. During these periods the fishes were fed with a basal diet containing 35% protein.

Diet Preparation

Six iso-nitrogenous (CP: 33.98-35.11%) and isocaloric (394.42-397.74 K cal DE 100g^{-1}) diets supplemented with graded levels of tannin (Qualigens chemicals, Glaxo India Pvt. Limited, Mumbai, India) viz., T_1 (0.25%), T_2 (0.5%), T_3 (1.0%), T_4 (1.5%) and T_5 (2.0%) and control group (no tannic acid) were prepared.

The composition of the experimental diets is given in TABLE-1. Diets were prepared by mixing thoroughly the finely ground ingredients except the vitamin-mineral mixture and vitamin C, with water

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TABLE 1: Composition of the Experimental Diets (%)

Ingredient	% Inclusion (g/100g feed)					
	Control	T_1 (0.25%TA)	T_2 (0.5%TA)	T_3 (1.0%TA)	T_4 (1.5%TA)	T_5 (2.0%TA)
Fish meal	10.0	10.0	10.0	10.0	10.0	10.0
Soymeal	44.0	44.0	44.0	44.0	44.0	44.0
Coconut oil cake	9.5	9.5	9.5	9.5	9.5	9.5
Sunflower oil cake meal	18.4	18.4	18.4	18.4	18.4	18.4
Wheat flour	4.5	4.5	4.5	4.5	4.5	4.5
Corn flour	4.5	4.5	4.5	4.5	4.5	4.5
Carboxymethyl Cellulose	2.0	2.0	2.0	2.0	2.0	2.0
Sunflower oil : Cod liver Oil(1:1)	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin + Mineral Mix (EMIX PLUS)[1]	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin C (S d-fine chem.)	0.1	0.1	0.1	0.1	0.1	0.1
Cellulose	2.0	1.88	1.63	1.13	0.63	0.13
Tannic acid Qualigens chem.	Nil	0.12	0.37	0.87	1.37	1.87

1-Composition of vitamin mineral mix (EMIX PLUS) (quantity/2.5kg), Vitamin A 55,00,000 IU; Vitamin D_3 11,00,000 IU; Vitamin B_2 2,000 mg; Vitamin E 750 mg; Vitamin K 1,000 mg; Vitamin B_6 1,000 mg; Vitamin B_{12} 6 mcg; Calcium Pantothenate 2,500 mg; Nicotinamide 10 g; Choline Chloride 150 g; Mn 27,000 mg; I 1,000 mg; Fe 7,500 mg; Zn 5,000 mg; Cu 2,000 mg; Co 450 mg; Ca 500 g; P 300g; L- lysine 10 g; DL- Methionine 10 g; Selenium 50 ppm; Selenium 50 ppm

to make a dough. The dough was then transferred to an aluminum container and steam cooked in pressure cooker at 15 PSI for 15 min. The vitamin mineral mixture and vitamin C were mixed after cooling. Pellets were prepared by a hand pelletizer with 2 mm dia. Finally the pellets were air dried for sometime and kept in oven at 60^0C till complete drying. After drying, the pellets were packed in airtight polythene bags and labeled properly.

Experimental Design

One hundred eighty (180) fingerlings of *Labeo rohita* were randomly distributed in 6 experimental groups, in triplicate, following a completely randomized design (CRD). All the groups were fed their respective diets to satiation. The feeding trial was conducted for 60 days. Daily ration were fed in two split doses (10:00 AM and 6:00 PM).

Physico-chemical parameters of water

Water quality parameters like temperature, pH,

TABLE 2: Effect of dietary tannin on digestive enzymes in intestine of *Labeo rohita* fingerlings.

Treatment	Protease	Amylase	Lipase
Control	$0.12^{a} \pm 0.011$	$0.03^{a} \pm 0.004$	$2.45^{a} \pm 0.39$
T_1	$0.10^{ab} \pm 0.004$	$0.02^{b} \pm 0.003$	$2.19^{a} \pm 0.14$
T_2	$0.10^{ab} \pm 0.003$	$0.02^{b} \pm 0.001$	$1.99^{a} \pm 0.13$
T_3	$0.09^{bc} \pm 0.003$	$0.02^{b} \pm 0.003$	$1.03^{b} \pm 0.23$
T_4	$0.08^{bc} \pm 0.009$	$0.02^{b} \pm 0.001$	$0.85^{b} \pm 0.22$
T_5	$0.08^{c} \pm 0.001$	$0.02^{b} \pm 0.002$	$0.78^{b} \pm 0.13$

Mean value containing different superscript in same column vary significantly ($p < 0.05$)

Enzyme (amylase and lipase) activity expressed as Mean ± SE (n=3) units/mg protein

Protease activity expressed as Mean ± SE (n=3) units/mg protein/min.

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TABLE 3: Effect of dietary tannin on glycolytic enzymes in the liver of *Labeo rohita* fingerlings.

Treatment	G-6-Pase (liver)	F-1,6-DPase(liver)	LDH (liver)	G6PDH (Liver)
Control	$294.35^{a} \pm 21.03$	$532.59^{a} \pm 7.86$	$2.10^{c} \pm 0.04$	$3.08^{c} \pm 0.12$
T_1	$152.16^{b} \pm 5.08$	$536.08^{a} \pm 25.53$	$2.75^{b} \pm 0.15$	$4.16^{b} \pm 0.28$
T_2	$156.50^{b} \pm 12.66$	$432.48^{b} \pm 16.92$	$3.80^{a} \pm 0.18$	$6.02^{a} \pm 0.33$
T_3	$154.54^{b} \pm 15.25$	$206.19^{c} \pm 9.30$	$3.60^{a} \pm 0.20$	$2.74^{c} \pm 0.12$
T_4	$177.08^{b} \pm 14.15$	$206.19^{d} \pm 12.32$	$3.42^{a} \pm 0.23$	$3.00^{c} \pm 0.14$
T_5	$143.40^{b} \pm 13.10$	$121.28^{e} \pm 9.31$	$3.31^{a} \pm 0.15$	$2.97^{c} \pm 0.08$

Data expressed as Mean ± SE (n=3), Mean value containing different superscript in same column vary significantly ($p < 0.05$)
Enzyme (G-6-Pase) activity expressed as nano moles phosphorous released /mg protein/min at 37 ^{0}C
Enzyme (LDH)activity expressed as micromoles/mg protein/min at 27 ^{0}C
Enzyme (G6PDH)activity expressed as micro moles phosphorous released /mg protein/min at 37 ^{0}C

TABLE 4: Effect of dietary tannic acid on enzymes of protein and energy metabolism in muscle of *Labeo rohita* fingerlings.

Treatment	AST(Muscle)	ALT(Muscle)	ATPase(Muscle)
Control	$0.09^{b} \pm 0.008$	$0.035^{b} \pm 0.002$	$738.76^{a} \pm 45.24$
T_1	$0.06^{b} \pm 0.004$	$0.03^{b} \pm 0.003$	$642.50^{ab} \pm 38.24$
T_2	$0.08^{b} \pm 0.010$	$0.03^{b} \pm 0.002$	$593.55^{b} \pm 43.47$
T_3	$0.08^{b} \pm 0.015$	$0.04^{a} \pm 0.003$	$405.18^{c} \pm 44.53$
T_4	$0.14^{a} \pm 0.024$	$0.05^{a} \pm 0.003$	$392.66^{c} \pm 21.04$
T_5	$0.15^{a} \pm 0.004$	$0.04^{a} \pm 0.004$	$388.52^{c} \pm 21.12$

Data expressed as Mean ± SE (n=3). Mean value containing different superscript in same column vary
significantly ($p < 0.05$)
Enzyme (ALT) activity expressed as nanomoles of sodium pyruvate released /mg protein/min at 37 ^{0}C
Enzyme (AST) activity expressed as nanomoles of oxaloacetate released / mg protein/min at 37 ^{0}C
Enzyme (ATPase) activity expressed as nanomoles phosphorous released /mg protein/min at 3

dissolved oxygen, free carbon dioxide, total hardness, ammonia, nitrite and nitrate were recorded at every 15 day during the entire experimental period. Temperature and dissolved oxygen were measured using a digital dissolved oxygen meter (Merck, Germany). The pH was measured by a digital pH meter (Lab India). Nitrate, nitrite, free carbon dioxide and ammonia were estimated by using standard methods of APHA[13]. Total carbonate hardness was estimated by carbonate hardness test kit (Merck, Germany). The temperature, pH, dissolved oxygen, total hardness, ammonia, nitrite and nitrate were found within the range of 23.3^{0}C - 28.4^{0}C, 7.6 - 8.5, 5.6 - 7.6 mg L^{-1}, negligible, 228 - 245 mg L^{-1}, 0.14-0.27 mg L^{-1}, 0.001 - 0.005 mg L^{-1} and 0.02 - 0.07 mg L^{-1}, respectively which was within the normal range for rearing of *L. rohita*.

Tissue Homogenate Preparation

The muscle, liver and intestine of the fishes were removed carefully and were weighed. It was homogenized with chilled sucrose solution (0.25 M) in a glass tube using Teflon coated mechanical tissue homogenizer. The tube was continuously kept in ice to avoid heating. The homogenate was centrifuged at 5000 rpm for 10 min at 4^{0} C in a cooling centrifuge. The supernatant was stored at 4^{0}C until use. A 5% homogenate was prepared for muscle, liver and intestine. The intestinal content was removed before homogenization.

Enzyme Assays

Protease

Protease activity was determined by casein digestion method as described by Drapeau[14]. The enzyme reaction mixture consisted of 1% casein in 0.05M Tris PO_4 buffer (pH – 7.8) and incubated for 5 min at 37^{0}C. Ten min latter reaction was stopped by adding 10% TCA. Then the sample was filtered after 10 min of the reaction. Adding tissue homogenate just before stop the reaction and with no incubation was made the reagent blank. One unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to $0.001A_{280}$ per min at 37^{0}C and pH 7.8.

Amylase

The reducing sugars produced due to the action of glucoamylase and a- amylase on carbohydrates was estimated using Dinitro-salicylic-acid (DNS) method[15]. The reaction mixture consisted of 1% (w/v) starch solution, phosphate buffer (pH-6.9) and the tissue homogenate. The reaction mixtures were

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incubated at 37°C for 30 min. DNS was added after incubation and kept in boiling water bath for 5 min. After cooling, the reaction mixture was diluted with distilled water and absorbance was measured at 540 nm. Maltose was used as the standard. Amylase activity was expressed as mmole of maltose released from starch per min at that temperature.

Lipase

The lipase activity was assayed by the method of Cherry and Crandell [16]. Two test tubes labeled as Test (T) and Control (C) were taken and into each of the two tubes 3ml of distilled water and 1ml of homogenate were added. One of the tubes (C) was placed in boiling water for 5 min at 100^{0}C and then cooled. This was served to inactivate the lipase in control. Then 0.5ml of phosphate buffer solution (pH-7) and 2ml of olive oil emulsion was added to both the tubes; shaken well and incubated at 37^{0}C for 24hrs. Then 3ml of 95% alcohol and 2 drops of phenolphthalein solution were mixed. Each of the tubes was titrated with 0.05N NaOH up to the appearance of permanent pink colour. The volume (ml) of N/20 NaOH solution required for 100mg intestinal tissue in the experimental tube minus the volume (ml) of N/20 NaOH solution required for the same amount of intestinal tissue in the control tube represented the units of intestinal lipase activity per g tissue. One unit will hydrolyze 1.0 micro-equivalent of fatty acid from a triglyceride in 24 hrs at pH 7.7 at 37°C.

Glucose-6-Phosphatase(G6Pase)

The G-6-Pase activity in the liver tissue was assayed by the method of Marjoric[17]. The assay mixture consisted of 0.3ml of malate buffer (pH 6.5), 0.1 ml of 0.1M glucose 6-phosphate solutions and 0.1 ml of tissue homogenate and was incubated for 15 min at 37^{0}C. The reaction was terminated by addition of 1 ml of 10% TCA solution. One milli litre of the aliquot of the supernatant was used for phosphate (Pi) estimation by method of Fiske and Subbarow[18].

Fructose1,6-DiPhosphaase(FDPase)

The FDPase activity in the liver tissue was assayed by the method outlined by Freeland and Harper[19]. The reaction mixture was comprised of 0.1 ml of 0.05M FDP (pH 7-7.3), 0.1 ml of 0.5M $MgSO_4$, 0.2ml of tissue homogenate and 0.6ml of borate buffer (pH 9.5). The mixture was incubated at 37^{0}C for 30 min and the reaction was terminated by addition of 1 ml of 10% TCA solution. One milli liter of the aliquot of the supernatant was used for phosphate (Pi) estimation by method of Fiske and Subbarow [18].

Lactate Dehydrohenase(LDH)

The LDH activity in the liver tissue was assayed by the method of Wroblewski and Ladue[20]. Total 3 ml of the reaction mixture comprised of 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH dissolved in 1ml of phosphate buffer solution), 0.1ml of tissue homogenate and 0.1 ml sodium pyruvate. The reaction was started after addition of substrate sodium pyruvate. The OD was recorded at 340nm at 30 seconds interval for 3 min. The enzyme activity was expressed as units/mg protein/min at 25^{0}C where 1 unit was equal to D0.01 OD/min.

Glucose-6-Phosphate dehydrogenase(G6PDH)

The G6PDH activity in different tissues was assayed by the method of De Moss[21]. Total 3ml of the reaction mixture comprised of 1.5 ml of 0.1M Tris buffer (pH 7.8), 0.2ml of 2.7mM NADP, 0.1ml of tissue homogenate, 1.05ml of distilled water and 0.1ml of 0.02M glucose-6-phosphate (G6P). The reaction was started by adding glucose-6-phosphate as substrate. The OD was recorded at 340nm 15 seconds interval against distilled water. The G6PDH activity was expressed as units/ mg protein/ minute. One unit was equal to D0.01OD/ min/ ml at 25^0 C.

Aspartate amino transferase(AST) and Alanine amino transfearse(ALT)

The AST activity was assayed in different tissue homogenates as described by Wooten[22]The substrate comprised of 0.2M D, L- aspartic acid and 2mM a-ketoglutarate in 0.05M phosphate buffer (pH 7.4). In the experimental and control tubes, 0.5ml of substrate was added. The reaction was started by adding 0.1ml of tissue homogenate. The assay mixture was incubated at 37^{0}C for 60 min. The reaction was

terminated by adding 0.5ml of 1mM 2,4 dinitrophenyl hydrazine (DNPH). In the control tubes the enzyme source was added after DNPH solution. The tubes were held at room temperature for 20 min with occasional shaking. Then 5ml of 0.4ml NaOH solution was added, the contents were thoroughly mixed. After 10 min, the OD was recorded at 540nm against the blank. The procedure adopted for ALT activity was same as that for AST activity except the substrate comprised of 0.2 M D,L- alanine instead of aspartic acid.

Adenosine triphospatase(ATPase)

The total adenosine triphosphatase was assayed according to the modified method of Post and Sen[23]. The reaction mixture comprised of 1.0 ml of 0.1 M Tris-HCl buffer (pH 7.8), 0.1 ml of 100 mM NaCl, 0.1 ml of 20 mM KCl, 0.1 ml of 3mM $MgCl_2$, 0.5 ml of 5 mM ATP and 0.1 ml homogenate. The mixture was incubated for 15 minutes and the reaction was terminated by the addition of 1 ml 10% TCA. After centrifugation, 2.0 ml of supernatant was processed for estimation of inorganic phosphate by the method of Fiske and Subbarow[18]. Enzyme activity was expressed as nanomoles phosphorous released /mg protein/min at 37 ^{0}C

Protein Estimation

Quantification of protein in the different tissues was carried out using Lowry's method[24].

Statistical Analysis

The data were statistically analyzed by statistical package SPSS version 14. Main effects of tannin at graded levels were analyzed by one-way analysis of variance (ANOVA). Comparison between two treatments was done by Duncan's Multiple Range Test (DMRT) at the 5% probability levels.

RESULTS

Digestive enzyme activities were significantly ($P<0.05$) affected following the addition of tannic acid in the diet (TABLE-2). A significant reduction in protease activity of intestine was observed at 1% or above tannin supplementation, compared to the control group. Protease activity showed an inverse relation with the dietary tannin concentration. However, amylase activity was significantly ($P<0.05$) inhibited in the tannin treatment groups than the control, though there was no difference ($P>0.05$) within the treatment groups. Intestinal lipase activity was also significantly ($P<0.05$) inhibited by dietary tannin.

Liver glucose-6-phosphatase activity reduced significantly ($P<0.05$) in treatment groups as compared to the control group. However, there was no variation ($P>0.05$) within the treatment groups. Like glucose-6-phosphatase, fructose-1,6-diphosphatase also showed significant ($P<0.05$) reduction in its activity with increasing tannin concentration in the diet (TABLE-3).

Lactate dehydrogenase (LDH) activity in liver of experimental groups varied significantly ($P<0.05$) due to dietary supplementation of tannic acid (TABLE-3). The lowest activity (2.10 ± 0.04) was recorded in the control group. Though supplementation of tannin enhanced the LDH activity but there was no variation ($P>0.05$) among the T_2, T_3, T_4 and T_5 groups. Glucose-6-phosphatedehydrogenase (G6PDH) activity in the liver of the experimental fishes showed significant ($P<0.05$) variation due to tannic acid in the diet (TABLE-3). It showed an increasing trend upto supplementation of 0.5% tannic acid in the diet after which its activity decreased and remained at par with the activity in the control group.

Asparate amino transferase (AST) and alanine amino transferase (ALT) activity in muscle showed significant ($P<0.05$) variation due to the dietary tannic acid (TABLE-4). Higher AST activity was recorded in T_4 and T_5 groups compared to the rest of the groups including the control. ALT activity in muscle followed similar trend as that of AST activity.

Total Adenosine tri phosphatase (ATPase) activity in muscle of *Labeo rohita* fingerlings showed significant ($P<0.05$) changes following dietary tannic acid supplementation, highest being in control group, which varied significantly from treatment groups (TABLE-4). Lowest ATPase activity (343.00 ± 22.20) was found in T_3 group, which was similar to T_4 and T_5 group. A gradual decrease of ATPase activity was noticed due to increase concentration of

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dietary tannin.

DISCUSSION

Tannins are reported to form less digestible complex with dietary proteins [8]. They may bind and inhibit the endogenous proteins such as digestive enzymes[24] and can adversely affect the enzyme activities[26,27,28,29]. In the present study, protease, amylase and lipase activities were studied in the intestine of *Labeo rohita* fingerlings. Protease, amylase and lipase activities were found to be significantly affected by dietary tannin. There was inhibition in amylase and protease activity in the present study due to dietary tannin. Similar results were reported by Maitra and Ray[12], who obeserved that á-amylase activity was reduced by 9.3% to 34.2% and protease by 24.07 % to 90.21% when fed with 6.25-200µg of tannin (*Acacia* leaf meal extract) in *Labeo rohita* fingerlings. In the present study lipase activity exhibited an inverse relation with dietary tannin. Maitra and Ray[12] reported that tannin even at low concentration causes inhibition of activities of protease, amylase and lipase. Tannins forming complex with digestive enzymes interfere with normal digestion. There is evidence to support that endogenous proteins as well as enzymatic proteins form a considerable portion of excreted nitrogen when animals are fed with tannin rich diets[30].

In the present study, two key gluconeogenic enzymes namely glucose-6-phosphatase (G6Pase) and fructose-1,6-diphosphatase (FDPase) were studied in the liver tissue after feeding graded levels of dietary tannic acid. G6Pase is an enzyme, which catalyzes the conversion of glucose-6phosphate to glucose. The brain tissues essentially require glucose. Gluconeogenesis is the process by which glucose is produced from non-carbohydrate sources like proteins, fatty acids and glycerols etc. G6Pase activity in liver of *Labeo rohita* fingerlings in experimental groups were significantly reduced as compared to the control group. Another key enzyme of gluconeogenic pathway is fructose-1,6-diphosphatase, which is involved in dysphosphorylation of fructose-1.6-diphosphate to fructose-1-phosphate. In the present study FDPase activity in liver tissue decreased in treatment groups compared to the control group. Reduction of enzyme activity may be due to inhibition of denovo enzyme protein synthesis by the metabolites of tannin or by irreversible binding of such compounds in enzyme substrate complex. Reduction in gluconeogenic activity is presumed to be the nutritional regulation to check utilization of amino acid for glucose production as tannin causes reduced availability of protein due to complex formation.

Lactate dehydrogenase (LDH) is an important enzyme in glycolytic pathway. LDH converts pyruvate to lactate in the presence of coenzyme NADH that is converted to NAD^+. In presence of enough oxygen pyruvate enters the Kreb's cycle but under anaerobic condition, LDH helps in ATP production by converting pyruvate to lactate. Thus LDH assay serves as a useful stress indicator. LDH activity increases with stress viz. temperature stress[31], starvation stress[32] and confinement stress[33]. LDH activity increases with increase energy demand. In the present study LDH activity in liver increased significantly in treatment groups as compared to control group.

G6PDase is a key regulatory enzyme of the pentose phosphate shunt. It converts glucose6phosphate to 6-phosphogluconolactone using $NADP^+$ as a coenzyme and releasing NADPH in the process. G6PDase activity may increase when there is higher need of NADPH. It is an important constituent for the production of long chain fatty acids and cholesterol steroids. G6PDase activity was studied in liver. In the present study G6PDase activity showed an initial increase and reached a maximum at 0.5% dietary tannin level followed by decrease to the level similar to control. Tannins at high concentration usually inhibit the activity of enzymes, but at low concentration they often stimulate enzyme activity[34].

In current study, activities of two amino transferases i.e. aspartate amino transferase (AST) and alanine amino transferase (ALT) in muscle were estimated. AST and ALT activities showed significant increase only in higher tannin fed groups (T_4 and T_5) indicating more synthesis of non-essential amino acid. This might be utilized for energy required for higher tannin fed groups due to stress. In a similar experiment by Varanka et al.[11] observed increased

AST and ALT activities in the plasma of common carp (*Cyprinus carpio*) following exposure to tannic acid. Similar results of increased AST and ALT activities were reported when Indian major carps subjected to confinement stress[33].

ATPase is responsible for the transport of ions through the membrane and regulates Na^+/K^+ gradient along the cell membrane[34]. ATPase hydrolyses the high-energy phosphate (ATP) and utilizes the energy for maintaining ionic gradient across the plasma membrane[35]. It helps in active transport of Na^+ and K^+ respectively outward and inward across the membrane. Total ATPase activity was studied in muscle tissue. Significant decrease in total ATPase activity was found in muscle of *Labeo rohita* fingerlings in treatment groups. The decrease in the ATPase activity might have occurred due to unavailability of substrate ATP. Similar results reported by Murakami et al.[9] who observed that tannins are inhibitors of H+ and K^+ ATPases.

It seems that dietary tannin forms complex with proteins and hence it affects the enzyme activities. As expected all the digestive enzyme were inhibited due to dietary tannin. Enzymes of gluconeogenic pathway like G6Pase and FDPase activity in liver inhibited by dietary tannin. Dietary tannin also enhanced liver LDH activity in treatment groups. G6PDH activity showed initial increased activity in liver at lower concentrations followed by decrease at higher concentrations of tannin to level similar to control. Enzymes of protein metabolism like AST and ALT in muscle were significantly increased at higher dietary tannin concentrations, whereas ATPase activity decreased with increase in dietary tannin. Inhibition of gluconeogenic enzyme due to dietary tannin seems to be interesting for fish but needs further studies. From evidences of study it can be concluded that dietary tannin content as low as 0.25% significantly affects both the digestive and metabolic enzymes of *L. rohita* fingerlings. Hence care must be taken for the tannin content of the ingredients while formulating feed. More detailed studies are required for testing the tolerance level of dietary tannin with different species as literatures in these aspects are scanty.

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