https://doi.org/10.17221/132/2022-CJAS

Studies on the growth, immunomodulation and gut morphometry of *Labeo rohita* fed pectin

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Citation: Hashmi H.S., Khan N., Iqbal K.J., Fatima M., Anjum K.M., Abbas S., Awais M., Nazir S., Asghar M., De Zoysa M. (2023): Studies on the growth, immunomodulation and gut morphometry of *Labeo rohita* fed pectin. Czech J. Anim. Sci., 68: 178–187.

Abstract: The present study was designed to evaluate the effect of pectin supplemented feeds on the growth, gut morphology and immunology of *Labeo rohita*. Experimental feeds containing various concentrations of pectin including control (0%), T1 (0.5%), T2 (1.0%), T3 (2.0%), and T4 (4.0%) were administered to *L. rohita* twice per day at a rate of 3% body weight. There was a significant ($P < 0.05$) decrease in average final weight, weight gain%, net weight gain and specific growth rate observed in treatment groups in order of control > T1 > T2 > T3 > T4 while feed conversion ratio was significantly ($P < 0.05$) higher in T4 compared to the other groups T3 > T2 > T1 > control. Immunological parameters of peroxidase, protease and antiprotease (trypsin) showed significant ($P < 0.05$) results between control and treated groups. Peroxidase activity was significantly ($P < 0.05$) higher in T4 group fish compared to T3 > T2 > T1 > control group. Total protein, protease and antiprotease activity were significantly ($P < 0.05$) higher in the control compared to treatment groups T1 > T2 > T3 > T4. An increased level of the non-starch polysaccharide pectin in feed caused acute effects on gut morphometry. Villus length, villus width, and an increase in mucosal folds were abnormal but no significant ($P > 0.05$) abnormality was observed in the control group. In the submucosal part of the intestine, hyperplasia was shown in loose connective tissues and in adipose tissue cells in the treated groups. In conclusion, dietary pectin supplementation from 0.5% to 4% had negative effects on the growth, immunological parameters and gut histomorphology of *L. rohita*.

Keywords: rahu; weight gain; serum; plasma protein; peroxidase

Aquaculture has grown at a 5.3 percent annual rate over the last two decades, with an annual output of 114.5 million tonnes in 2018, making it one of the fastest growing food-producing industries (FAO 2020). This vital business accounts for over half of the worldwide fish consumption, produces revenue, and creates employment (Dawood and Koshio 2020). Fish have a high level of balanced

https://doi.org/10.17221/132/2022-CJAS

protein and of the micronutrients (minerals, vitamins, and amino acids) needed for human nutrition, making them more efficient than other domestic animals in converting feed to body protein (Handisyde et al. 2017).

Tropical freshwater carp called Rohu (*Labeo rohita*, Hamilton, 1842) are raised in India and other countries in Asia for commercial purposes. Moreover, 72% of freshwater aquaculture production is made up of carps, with *L. rohita* accounting for around 15% (FAO 2018). Rohu is one of the most significant cultured fish species in carp polyculture systems and among Indian major carps owing to its quick growth, expanding market demand, and capacity to flourish in a range of agroclimatic situations (FAO 2012; Mir et al. 2017). From 1.1 million tonnes (worth USD 1.6 billion) in 2010 to over 2 million tonnes (worth USD 3.4 billion) in 2018, *L. rohita* output rose on a global scale. As a result, it has been considered as a significant aquaculture species, contributing to around 3.7% of the aquaculture production in 2018 (FAO 2020).

In modern aquaculture, fish are fed artificial diets, which calls for the creation of diets that directly affect the quality of the finished product (Hoseinifar et al. 2021). The sustainability of aquaculture is endangered by the loss of production brought on by infectious diseases like *Aeromonas hydrophila*, which may severely harm carps and stunt their growth when they are raised in freshwater aquaculture at high stocking densities. The same circumstances allow for the application of feed additives to enhance fish immunity and growth, resulting in a thriving and high-quality product, while also assuring effective and profitable production. Researchers are searching for and testing substances that may have a favourable influence on fish growth and health as immuno-modulations or stimulants in order to find a solution in light of the aforementioned problems (Hoseinifar et al. 2021; Dadras et al. 2022).

Pectin is a soluble fibre that acts as a structural element found in all terrestrial plants. Pectin may be fermented by the fish microbiome, producing short-chain fatty acids (Hoseinifar et al. 2021). Prebiotics, which are classified as non-digestible carbohydrates, have been shown to improve fish growth and immunity (Guerreiro et al. 2015). Prebiotic characteristics of various pectin types have been investigated in a number of fish species (Hosseini et al. 2020). The target fish, distribution method, degree of inclusion, and pectin source are all likely to have a substantial influence on the treatment effectiveness. Pectin has been shown to have several, advantageous impacts on human health, including a decrease in cholesterol and blood glucose levels, preventing cancer, and enhancing the immune system. It is used as a gelling and stabilizing ingredient in food and cosmetics (Hoseinifar et al. 2021).

Hosseini et al. (2020) studied the effects of pectin inclusion on the growth performance and immune response of common carp which was used as an immunostimulant capable of strengthening the immune system and growth promoter. Jiang et al. (2016) also noted that the addition of pectin enhanced the intestinal shape, which increased rat growth performance and nutrition utilization effectiveness. Pectin was also given to sea bream (*Sparus aurata*) larvae by Salem et al. (2016), which enhanced growth and feed consumption. It was also shown by Salem and Abdel-Ghany (2018) that Nile tilapia (*Oreochromis niloticus*) fed apple pectin had improved growth performance. Keeping in view the growing importance of plant products used for combating the disease incidence by enhancing immunity in aquatic organisms, the present study was designed to evaluate the effect of pectin supplemented feeds on the growth, immunomodulation, and gut morphometry of *L. rohita*.

MATERIAL AND METHODS

Experimental design

The experiment was carried out in a fish hatchery, Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Ravi Campus, Pattoki, District Kasur, Punjab, Pakistan. The experimental work was performed for three months (90 days). The trial was conducted in rectangular tanks (1 400 l/tank) having dimensions of 3.048 × 0.762 × 0.609 6 m in a fish hatchery. Each tank was divided into three compartments by fixing plastic sheets for replicates and 15 fishes (average weight of 9.5 g in 31 l of water) were stocked in each compartment (replicate) by following a completely randomized design. *Labeo rohita* fingerlings were used in the experimental trial. For aeration, continuous flow of aerated water was supplied having an average

https://doi.org/10.17221/132/2022-CJAS

temperature of 27 °C. Other water quality parameters [dissolved oxygen (68.04%), pH (8.42 ± 0.02), total dissolved solids (693.27 ± 2.19) and ammonia (0.139 ± 0.07 mg/l) etc.] were monitored on a daily basis during the trial using digital multimeters HANNA (HI 98194; Hanna Instruments Ltd, Leighton Buzzard, UK). Ammonia test was performed using HI 3824 Ammonia Test Kit. During acclimatization, the fish were fed commercial feed (22% crude protein). Water was changed regularly by outlet pipes installed in circular tanks. The fish (*L. rohita*) were acclimatized in five circular tanks for about two weeks. All the fish were weighed individually at the start of the experiment before stocking and the fish were fed at a rate of 3% body weight experimental diets divided into two equal rations per day at 8:00–8:30 AM and 16:00–16:30 PM.

The experiment conducted on *L. rohita* followed general guidelines of the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore-Pakistan.

Feed preparation and analysis

Five experimental diets were prepared, T0 with (0%), T1 (0.5%), T2 (1.0%), T3 (2.0%) and T4 (4.0%) pectin (Pectin from Citrus Peel, Product No. P9135, CAS No. 9000-69-5; Sigma-Aldrich, St. Louis, MO, USA) (Table 1). For the manufacturing of diets, dry pelleted feed having 22% CP was prepared and desired levels of pectin were added. It was ground to a particulate size of 2 mm and mixed using an electrical mixer (KM 280; Kenwood Corporation, Tokyo, Japan). About 600 ml water was added to make dough which was further pelletized using a meat mincer (AG-3060; Anex, Karachi, Pakistan). Pellets were shade dried and then packed in well-sealed plastic zipper bags and used for the study trial.

Feeding trial, growth performance analysis

The initial body weight of fish was taken before stocking. Further sampling was done on a fortnightly basis and feed rationing was adjusted accordingly. On the termination of the trial (90 days) fish growth parameters like final weight, weight gain %, feed conversion ratio (FCR), specific growth rate (SGR), net weight gain and survival rate were determined as per following formula.

The feed conversion ratio was estimated by the following formula.

$$FCR = \text{total feed intake (g)}/\text{net weight gain (g)} \quad (1)$$

$$SGR = \ln(\text{final body weight} - \text{initial body weight})/(n \text{ of days}) \times 100 \quad (2)$$

Table 1. Feed formulation and ingredient composition of experimental diets

Ingredients (g/kg)	Control	T1	T2	T3	T4
Wheat bran	101.50	96.50	91.50	81.50	61.50
Soybean meal	120	120	120	120	120
Canola meal	90	90	90	90	90
Rapeseed meal	90	90	90	90	90
Guar meal	70	70	70	70	70
Rice polish	240	240	240	240	240
Maize grain	205	205	205	205	205
Corn gluten (30%)	40	40	40	40	40
Rice gluten	20	20	20	20	20
Molasses	10	10	10	10	10
Palm oil	5	5	5	5	5
Yeast	0.50	0.50	0.50	0.50	0.50
Choline	1.0	1.0	1.0	1.0	1.0
Vitamin mix[1]	1.0	1.0	1.0	1.0	1.0
Mineral mix[2]	1.0	1.0	1.0	1.0	1.0
DCP	5.0	5.0	5.0	5.0	5.0
Pectin%	0	5	10	20	40
Total	100	100	100	100	100
Proximate composition					
Moisture	102	103	104	104	103
Crude protein	223	224	222	222	223
Fat	65	65.3	65.2	65.1	65
Ash content	80	81	81.5	81.9	81.8
Fiber	60	60.8	60.9	61	61.1

Control = 0% pectin; T1 = 0.5% pectin; T2 = 1% pectin; T3 = 2% pectin; T4 = 4% pectin

[1]Mineral mixture contained the following per kilogram; 23 750 mg manganese, 75 000 mg zinc, 5 000 mg copper, 2 000 mg cobalt, 2 750 mg iodine, 100 mg selenium, 200 000 mg magnesium

[2]Vitamin premix contained the following per kilogram; 4 000 000 IU vitamin A, 480 000 IU vitamin D_3, 2 400 mg vitamin E, 2 400 mg vitamin K_3, 4 000 mg vitamin B_1, 4 000 mg niacin, 10 000 mg calcium D-pantothenate, 4 000 mg vitamin B_6, 10 mg vitamin B_{12}, 100 mg D-biotin, 1 200 mg folic acid, 40 000 mg vitamin C, 60 000 mg inositol

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$$\text{Weight gain \%} = (\text{final weight} - \text{initial weight})/(\text{initial weight}) \times 100 \quad (3)$$

$$\text{Net weight gain} = \text{final weight (g)} - \text{initial weight (g)} \quad (4)$$

$$\text{Survival rate \%} = 100 \times (\text{final fish number}/\text{initial fish number}) \quad (5)$$

Collection of serum and blood plasma

Fish were starved for 24 h at the end of the experimental period. Fish from each group were randomly selected and anaesthetized using buffered tricaine methanesulphonate (MS-222) (100 µg/ml). To determine immune response, a blood sample was collected from the caudal vein and transferred into a sterile centrifuge tube, clotted, and centrifuged at 4 000 rpm for 15 min at 4 °C for serum separation. The separated serum was stored at –20 °C until required for the estimation of antioxidant activity. Whole blood was drawn into EDTA-treated tubes, which are commercially available. Cells from plasma were centrifuged at 1 000–2 000 *g* for 10 min using a chilled centrifuge. Plasma was drained off platelets by centrifugation at 2 000 *g* for 15 minutes. Immediately, the plasma was transferred into a new polypropylene tube with a Pasteur pipette. For the measurement of total protein, the sample was kept at a temperature of –20 °C or below.

Analysis of plasma protein and immune responses

Peroxidase test. The enzymatic activity assay was performed by evaluating the capability of an enzyme to reduce the hydrogen peroxide (H_2O_2) concentration at A_{470} nm according to method of Civello et al. (1995). Blank solution (3 ml) was added to the cuvette and observed in a spectrophotometer. It was then adjusted to zero at a wavelength of 470 nm. In the first minute, the cuvette with the buffered substrate solution was observed in a spectrophotometer and then a newly prepared extract of the enzyme (0.06 ml) was added to initiate the reaction. The absorbance was observed after 3 minutes.

$$\text{Activity (ml)} = (\Delta A/3)\ (26.6 \times 60)\ 3\ 000 \quad (6)$$

Antiprotease activity. A quantity of 10 µl of serum sample from liver and 10 µl (5 mg/ml) of trypsin solution were mixed with each other. Both the solutions were mixed in the same vial at the same time, and then incubated at 22 °C for about 20 minutes. After 20 min of incubation, the sample was collected from the incubator, then 100 µl of the solution with 0.1 M of phosphate buffer with (pH 7.0). The pH level was maintained with the help of addition of a few drops of diluted HCl, and 125 µl of 2% (w/v) azocasein was added. The solution was then incubated at 22 °C for about 1 hour. An aliquot of 500 µl of about 10% (v/v) trichloroacetic acid (TCA) was added to stop the reaction. The reaction was stopped after the addition of TCA, and then the solution was centrifuged for 15 min at 6 000 rpm. The supernatant was transferred to tubes containing 400 µl of 1N sodium hydroxide solution. The optical density (OD) at 450 nm was obtained using a spectrophotometer (M-10145517; Thermo Scientific™, Waltham, MA, USA). The antiprotease activity of inhibition was expressed in percentage (%age) of trypsin inhibitory activity (Zuo and Woo 1997) [(optical density of control – optical density of sample)/control optical density × 100].

Protease immune activity. Casein solution (0.5 ml) and enzyme extract (0.5 ml) were present in the reaction mixture incubated at 37 °C for 20 minutes. An aliquot of 1 ml of TCA was added and kept for an hour at 2 °C to stop the reaction. The OD of the supernatant was calculated at 280 nm after samples were centrifuged and filtered. By using the same procedure a reagent blank was prepared without adding any substrate. The standard curve of tyrosine showed the proteolytic enzyme activity and it was expressed as mole of tyrosine released/min/mg protein at 37 °C (Kunitz 1947).

Total protein. Total protein was estimated by the method of Lowry (Lowry et al. 1951). The total plasma protein content was determined by the commercial protein kit (P 5656; Sigma-Aldrich, St. Louis, MO, USA) based on the principle of the Lowry reaction. Bovine serum albumin was used as a standard and the data were expressed in g/dl.

Analysis of gut morphometry

Six fishes were captured randomly from each treatment for studying the gut morphology. After taking blood for immunological parameters they were dis-

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sected in an aseptic environment to remove the gut (the intestine was removed in a biosafety cabinet which was disinfected with ethanol). The fish from each replicate were euthanized with an overdose (3 000 mg/l) of clove oil for 40–60 s and then scarified for the collection of tissue samples. A tissue sample of 5 cm was taken for intestinal morphology. The sample of the tissue section was washed properly with normal saline, thereafter it was preserved in 10% formalin solution. Intestine samples were fixed in Bouin's fluid as described. The samples were washed in 70% ethanol for 24 h and then they were dipped in ethanol in different specific absorptions for 20 minutes. The samples were dipped and soaked in xylene for 30–35 minutes. They were placed in xylene at 65 °C in an oven for 3 h and then soft paraffin was substituted for xylene in the oven for 3 h at 65 °C. Then it was replaced with hard paraffin in the oven for 24 h at the same temperature. The microtome was fixed at 2–3 µm in order to slice the samples and then they were fixed on the glass slide. Mayer's albumen was added and stained with haematoxylin stains (in powder form) 1.0 g, sodium iodate (2.0 g), potassium alum (50 g), citric acid (1.0 g), chloral hydrate (50.0), distilled water (1.0 l). The slides of the distal part of the intestine were examined under the microscope at 10× magnification. The gut morphological parameters were studied, examined and measured using the Labomed Pixel Pro Microsoft computer-assisted programme (Labomed Inc., Los Angeles, CA, USA). The measurement of distal parts was examined at 10× magnification (Khanal et al. 2021).

Statistical analysis

Obtained data from different morphometric and physicochemical parameters were subjected to one-way analysis of variance ANOVA. One-way ANOVA was used to compare the changes between treated and control groups by using SPSS v20.0 (IBM Corp, Armonk, NY, USA). Duncan's multiple range test was used to compare means at the significance level of 0.05.

RESULTS

Growth performance

Initial weights of *L. rohita* fingerlings were kept nearly constant ($P > 0.05$) in all treatments. A significant ($P < 0.05$) decrease in final weight, weight gain %, specific growth rate and net weight gain was observed in all pectin supplemented groups in order of T1 > T2 > T3 > T4, as compared to the control, while feed conversion ratio increased ($P < 0.05$) in order of control < T1 < T2 < T3 < T4. As the pectin supplementation increased, growth performance decreased ($P < 0.05$), while survival rate was 100% in the control and all pectin groups (Table 2).

Immunological parameters

Peroxidase activity increased significantly ($P < 0.05$) as the dietary pectin level increased in order of T1 < T2 = T3 < T4 as compared to the control, while there was a non-significant difference ($P > 0.05$) in peroxidase activity between T2 and T3. The total protein level significantly ($P < 0.05$) decreased as the supplementation level of pectin increased in treatment groups (T2 ≤ T1 < T3 = T4) as compared to the control, but there was a non-significant difference ($P > 0.05$) between T1, T2 and

Table 2. Growth parameters of experimental fish (*Labeo rohita*) that received the feed with different levels of pectin

Parameter	Control	T1	T2	T3	T4	*P*-values
Initial weight (g)	9.45 ± 0.10	9.63 ± 0.04	9.63 ± 0.03	9.55 ± 0.11	9.56 ± 0.00	0.467
Final weight (g)	26.44 ± 0.11^{e}	24.24 ± 0.29^{d}	22.46 ± 0.21^{c}	20.03 ± 0.01^{b}	18.90 ± 0.05^{a}	0.00
Net weight gain (g)	16.98 ± 0.18^{e}	14.61 ± 0.30^{d}	12.83 ± 0.18^{c}	10.47 ± 0.12^{b}	9.33 ± 0.05^{a}	0.00
Weight gain (%)	179.68 ± 3.79^{e}	151.79 ± 3.41^{d}	133.16 ± 1.47^{c}	109.64 ± 2.59^{b}	97.62 ± 0.60^{a}	0.00
SGR (%)	1.142 ± 0.01^{e}	1.025 ± 0.01^{d}	0.940 ± 0.00^{c}	0.82 ± 0.01^{b}	0.75 ± 0.00^{a}	0.00
FCR	1.50 ± 0.03^{a}	1.78 ± 0.04^{b}	2.02 ± 0.02^{c}	2.46 ± 0.05^{d}	2.76 ± 0.01^{e}	0.00
Survival rate (%)	100	100	100	100	100	–

Control = 0% pectin; T1 = 0.5% pectin; T2 = 1% pectin; T3 = 2% pectin; T4 = 4% pectin

$^{a–e}$Means with different superscripts in a row show statistically significant ($P < 0.05$) differences

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T3, T4. Similarly, protease and antiprotease activity significantly ($P < 0.05$) decreased with the increasing level of pectin inclusion in the diets as compared to the control. For protease activity, T3 and T4 showed a non-significant difference ($P > 0.05$), while for antiprotease activity, T1, T2 and T3, T4 showed a non-significant difference ($P > 0.05$) between them (Table 3).

Gut histology

Gut morphological parameters like villus height (VH), villus width (VW), villus surface area (VSA) and *muscularis externa* (ME) decreased as the pectin level increased in treatment groups. But VH and VSA in T2, T3 and T4 showed non-significant ($P > 0.05$) differences, while significant as compared to the control. While VW and ME showed non-significant differences ($P > 0.05$) between all treatment groups, control, T1, T2, T3 and T4 (Table 4, Figure 1).

T1, T2, T3 and T4 showed the impaired distal part of the intestine as compared to the control group. Abnormal mucosal fold development was observed in all treatments. In the submucosal part of the intestine, hyperplasia was shown in loose connective tissues and in adipose tissue cells. Increased levels of non-starch polysaccharide pectin caused acute effects on gut morphometry. But in the control group, neither abnormality nor damage was observed in the mucosa externa and in microvilli (Figure 2).

DISCUSSION

The present study indicated that with the increase of dietary pectin from 0.5% to 4% the growth performance significantly ($P < 0.05$) decreased and the gut health and immunomodulation in *L. rohita* fingerlings were also affected. Similar effects were reported after the use of high levels of some other non-starch polysaccharides (NSPs) in the species like Nile tilapia (*O. niloticus*) (> 8% guar gum) (Amirkolaie et al. 2005) and rainbow trout (*Salmo gairdneri*) (> 10% guar galactomannan and alginates) (Storebakken 1985). When the fish were fed the high levels of feed supplemented with other NSPs, it further caused the depression in their growth performance and decreased the feed utilization. The same study has been done previously when xylan (4%) non-starch polysaccharide was supplemented to diet and it resulted in the lowering of feed intake in turbot fed 4% dietary pectin (Liu et al. 2015). Although in the present study the devastative effect occurred at 0.5% to 4% inclusion of the dietary pectin in fish feed, it was in consistence with the preceding studies on pigs (7.5% xylan) (Morel et al. 2001). Another study also

Table 3. Immunomodulation of experimental fish administered the feed with different levels of pectin

Parameters	Control	T1	T2	T3	T4	*P*-values
Peroxidase (IU/ml)	0.89 ± 0.00^{a}	1.62 ± 0.02^{b}	1.73 ± 0.00^{c}	1.72 ± 0.01^{c}	1.91 ± 0.00^{d}	0.00
Total protein (g/dl)	3.77 ± 0.0^{c}	3.55 ± 0.01^{b}	3.67 ± 0.00^{bc}	3.32 ± 0.09^{a}	3.17 ± 0.06^{a}	0.00
Protease (µ/ml)	12.88 ± 0.04^{d}	12.50 ± 0.00^{c}	12.06 ± 0.06^{b}	11.05 ± 0.02^{a}	10.90 ± 0.06^{a}	0.00
Antiprotease (µ/ml)	0.84 ± 0.02^{c}	0.68 ± 0.05^{b}	0.64 ± 0.01^{b}	0.47 ± 0.01^{a}	0.39 ± 0.01^{a}	0.00

Control = 0% pectin; T1 = 0.5% pectin; T2 = 1% pectin; T3 = 2% pectin; T4 = 4% pectin

[a–d]Means with different superscripts in a row show statistically significant ($P < 0.05$) differences

Table 4. Gut morphology of *Labeo rohita* administered the feed with different levels of pectin

Parameters	Control	T1	T2	T3	T4	*P*-values
VH (µm)	191.16 ± 10.84^{c}	145.34 ± 15.71^{b}	119.69 ± 14.04^{ab}	99.7 ± 15.93^{a}	83.24 ± 14.08^{a}	0.00
VW (µm)	31.26 ± 4.41	27.77 ± 4.19	22.86 ± 4.78	17.03 ± 1.92	19.11 ± 2.00	0.068
ME (µm)	21.06 ± 2.38	18.10 ± 2.55	12.83 ± 1.71	15.01 ± 2.21	13.25 ± 1.55	0.06
VSA (µm)	$9\,393.24 \pm 1\,501.1^{c}$	$6\,065.81 \pm 676.25^{b}$	$4\,089.91 \pm 657.82^{ab}$	$2\,755.54 \pm 607.39^{a}$	$2\,400.50 \pm 300.67^{a}$	0.00

Control = 0% pectin; ME = *Muscularis externa* (µm); T1 = 0.5% pectin; T2 = 1% pectin; T3 = 2% pectin; T4 = 4% pectin; VH = villus height (µm); VSA = villus surface area (µm); VW = villus width (µm)

[a–c]Means with different superscripts in a row show statistically significant ($P < 0.05$) differences

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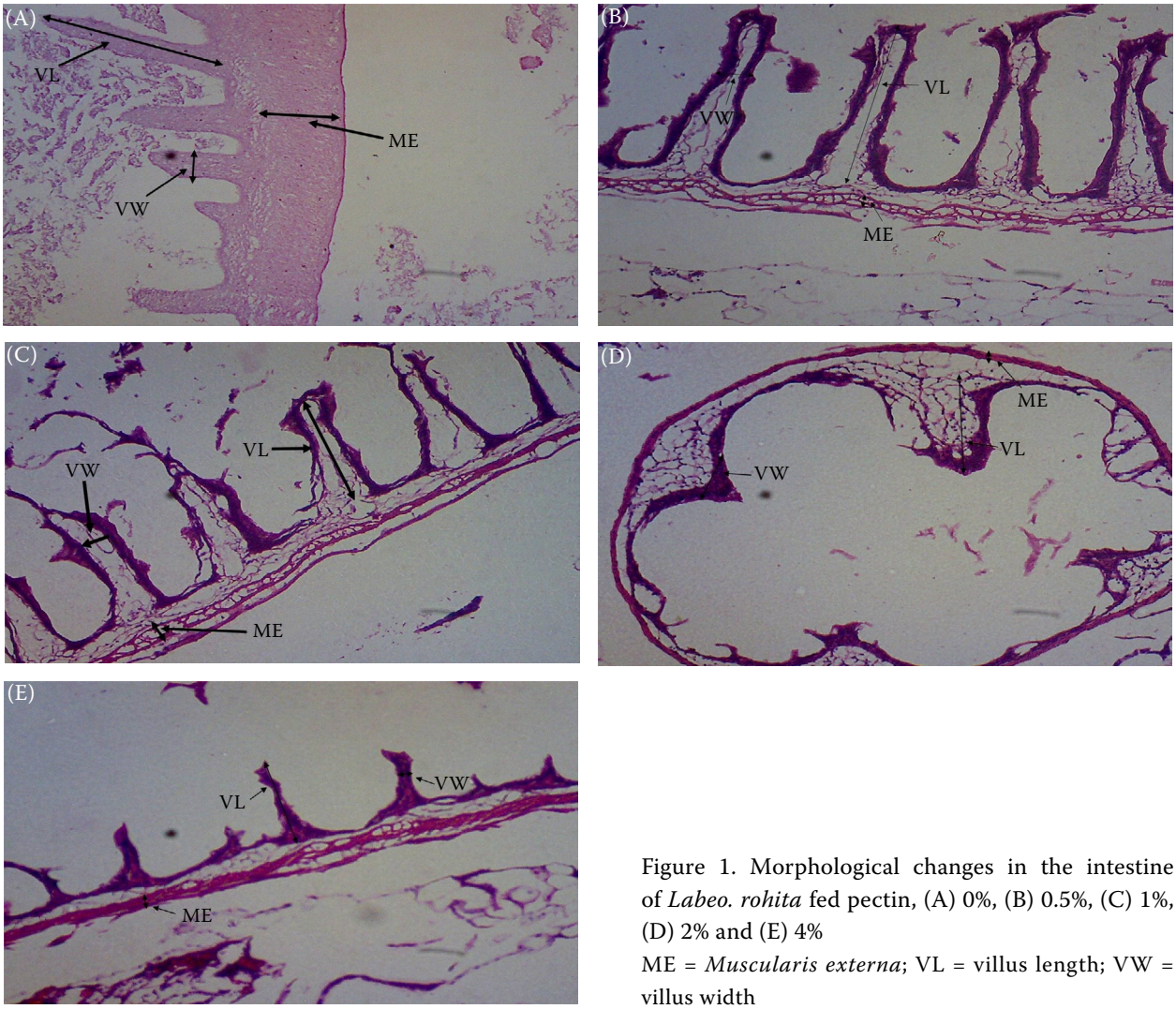

Figure 1. Morphological changes in the intestine of *Labeo. rohita* fed pectin, (A) 0%, (B) 0.5%, (C) 1%, (D) 2% and (E) 4%

ME = *Muscularis externa*; VL = villus length; VW = villus width

supported our study in which dietary pectin of high levels (3%) lowers the growth performance and it may decrease feed utilization in chickens (Choct et al. 1996). It has been revealed that dietary supplementation of 5% pectin caused lower growth performance and decreased feed utilization in the marine flatfish like juvenile turbot (Camire et al. 1990). Similar observation was also reported in sea bream (*Chrysophrys major*) (3–12% carboxymethylcellulose) (Morita et al. 1982). In the present study, it is revealed that basal feed with 22% CP significantly increased the growth as compared to pectin supplemented feed.

The protease, antiprotease activity and total protein level in the control group have shown better results than in treated groups. Pectin-free feed or a low level of pectin may be considered better than a higher level of pectin (4%), which caused negative impacts on immunological activities. In the previous study, it was mentioned that generally decreased immunity and increased viscosity stress in the digestive organs were caused by higher levels of NSPs (Camire et al. 1990), which may harm the digesta of fish by suppressing the secretion of digestive enzymes and immunological activity (Sinha et al. 2011). In another study it has been revealed that the higher level of dietary pectin (4%) caused the depression in the activity of immunological parameters (Leenhouwers et al. 2006). Ikegami et al. (1990) reported that the highest level of NSPs may cause the binding of a large quantity of the digestive enzymes which may depress the interaction of these enzymes with other nutrients. It has been revealed that the action of immunological activity was depressed by adding dietary pectin and this contributes to the decreased growth performance and feed utilization in fish. Additionally, immunological parameters like total protein, peroxidase,

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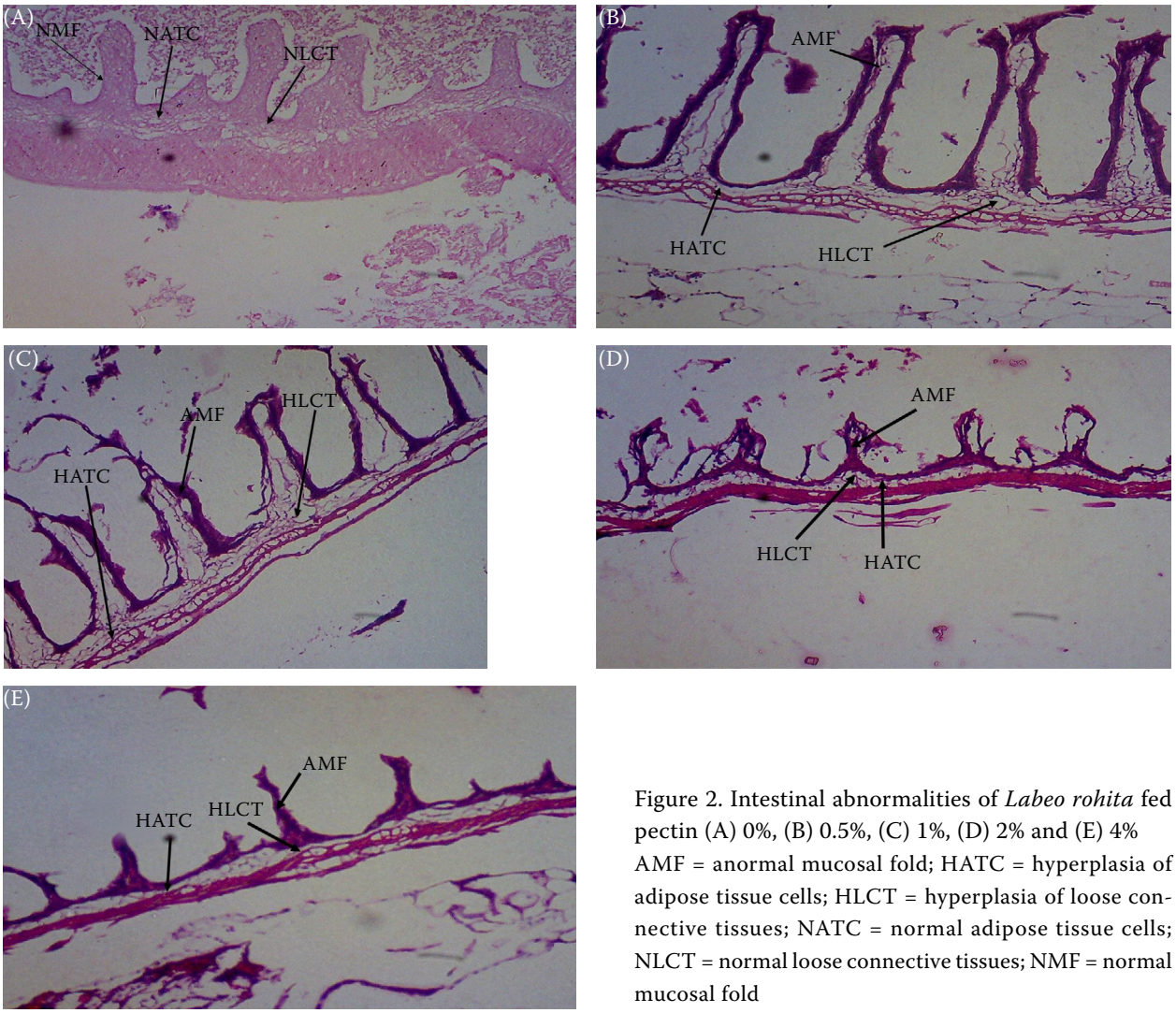

Figure 2. Intestinal abnormalities of *Labeo rohita* fed pectin (A) 0%, (B) 0.5%, (C) 1%, (D) 2% and (E) 4%
AMF = anormal mucosal fold; HATC = hyperplasia of adipose tissue cells; HLCT = hyperplasia of loose connective tissues; NATC = normal adipose tissue cells; NLCT = normal loose connective tissues; NMF = normal mucosal fold

protease and antiprotease activities in fish were significantly different in all treatments.

However, there has been no evidence of the effects of high levels of dietary pectin on the distal intestine of the fish *L. rohita*. In the present study neither damage nor lesions were reported on the intestines of fish fed the control diet (0% pectin) of experimental feed. Furthermore, the enlarged surface area of the intestine was observed in fish fed lower levels of pectin (0.5%) and in pectin-free dietary group. In different studied groups of current research, there was a simple kind of mucosal folds named simple folds, which were regularly altered respectively. The epithelial cells of the surface layer were covered with mucosa and have microvilli on their borders. The *lamina propria* along with submucosa gradually present under the mucosa represented different length and width with decreased cellularity. In the present study all the treated groups (T1, T2, T3 and T4) showed the impaired distal part of the intestine except the control where abnormal mucosal fold development was noticed. In the submucosal part of the intestine, hyperplasia was seen in loose connective tissues and in adipose tissue cells. There was a clearcut difference observed in all treatments. Increased levels of non-starch polysaccharide pectin caused acute effects on gut morphometry. Abnormal villus length and abnormal increase of mucosal folds were observed in histological studies. But in the control group no abnormality was found. No damage was observed in the mucosa externa and in microvilli in the control group. In the previous study, the microbial disintegration of NSPs occurred in the distal intestine (Choct et al. 2010), which has great importance in nutrient digestion and assimilation in animals. In the previous study, the impaired integrity of the fish distal intestine was observed when the diet

https://doi.org/10.17221/132/2022-CJAS

was supplemented with a higher level of non-starch polysaccharide (xylan). Furthermore, the morphometric investigation revealed the reduction in the surface area of the intestine when higher than 4% dietary pectin was supplemented in the diet. Viscosity stress was also noticed to be induced by the higher NSP level in digesta which may lead to somatic erosion in the intestine and atrophy of the intestinal folds because of the rise in cell loss from the villous apex by apoptosis (Sinha et al. 2011). Similar results were also reported in chickens (≥ 2.5% gum xanthan) (Iji et al. 2001) and pigs (≥ 4% carboxymethylcellulose). Similarly, a previous study showed that the supplementation of a suitable level of dietary NSPs may raise the viscosity stress in the digesta which increases the secretion of digestive juices and activity of enzymes in fish (Ikegami et al. 1990). In the present study neither damage nor lesions were reported on the intestine of fish fed the control diet. Previous studies showed that comparable enhancements with a moderate level of NSPs were reported in rats (2.5% pectin) (Andoh et al. 1996) and chickens (2.5% guar gum and 2.5% gum xanthan) (Iji et al. 2001). The increased thickness of the intestinal muscularis was perceived in fish fed the control diet, it may boost the intestinal activity and finally increase the feed intake of fish.

CONCLUSION

The present study revealed that the effect of pectin at different levels (0.5–4%) supplemented in the commercial feed of 22% CP had negative effects on *L. rohita* growth, gut morphology, and immunology. A detailed study is needed to investigate the mechanisms of such effects of pectin at further levels.

Conflict of interest

The authors declare no conflict of interest.

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Received: August 15, 2022
Accepted: February 1, 2023
Published online: April 5, 2023