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# Utilization of sustainable agri-waste watermelon rind for fishmeal in *Labeo rohita* diets: Effects on nutritional indices, hemato-biochemical properties, histoarchitechtural traits, amino acid and fatty acid profiles

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ABSTRACT

Fish meal (FM) remains an essential protein source with certain limitations like cost and sustainability in the aquaculture industry. Underutilized agricultural wastes with nutraceutical potential could be the feasible solution. This study paved the way for new avenues in the form of economically sustainable low-cost feed for betterment of aquaculture, by evaluating watermelon rind (WMR) as fish meal replacement in diets for *Labeo rohita* (rohu). Dried water melon rind was incorporated into five isonitrogenous diets (control, 25% fish meal replacement (FMR), 50% FMR, 75% FMR, and 100% FMR then fed to triplicates groups of *L. rohita* fingerlings. The experiment was conducted for 60 days. There were significant ($p<0.05$) improvements in nutritional indices of fish fed with 50% FMR. The digestive enzyme analysis showed a promising result in fish fed with 50% FMR diet compared to the control and other experimental diets indicating that fishmeal replaced with 50% FMR diet will be suitable for the digestion in the experimental fish rohu. Finally, histological alterations of organs showed a reduction in the damage of tissues in fish fed with WMR inclusion diets. Enrichment of amino acids (AA) and fatty acids (FA) was observed in the fish muscle. Overall, our results confirm that the 50% fishmeal replacement diet with watermelon rind is a promising alternative for fish meal displaying no adverse effects. Thus, the study concludes that the partial replacement of fishmeal by WMR provides new insights into nutrient utilization, and growth performances, digestibility, and biochemical compositions of freshwater fish *L. rohita*.

## 1. Introduction

The increasing world population leads to greater demand for fish and its byproducts as a major food protein source (Hassaan et al., 2019). Aquaculture is one of the promising sectors for generating income, securing food and supporting livelihood (Hu et al., 2022). Sustainable industrialization of this sector provides significant economic contribution to societies (Jahan et al., 2021). The global aquaculture sector has grown rapidly, resulting in increased demand for fish feed. Fishmeal (FM) is the most common protein source in fish feed; however, the production of fishmeal has not increased substantially in the past 20 years. Due to the rising demand, the price of FM is steadily increasing (Rahmah et al., 2022). The aquaculture sectors are extremely concerned on account of FM demand and price, and it is the need of the hour to find viable substitutes for fishmeal in fish feed (Zhu et al., 2022a). In an intensive farming systems, aquaculture feed cost accounts for 75–95% of overall production cost (El-Sayed et al., 2015). Around 70% of the farmers depend on commercial fish feed in worldwide aquaculture production (Li et al., 2021). Therefore, replacing FM in fish feed continues to be a crucial area of research. FM replacement has been made possible by a number of interesting alternative feed components (Das et al., 2022). The goal of research is to find substitutes for fishmeal, such

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as plant, animal, organic, and valorized agricultural wastes with nutraceutical characteristics that are not detrimental to fish health (Gupta et al., 2020). These sources have recommended as viable alternatives to fishmeal because of their wide availability and lack of adverse effects on growth and production of fish (Kari et al., 2023).

Plant-based agriculture waste such as peels, seeds, pods, and leaves have been used to replace fishmeal to achieve sustainability in terms of economics and productivity. Various plant sources like barley, cassava leaves, moringa leaves, and sesame seed meal are used as fishmeal replacement in diets for various aquaculture species (Zaretabar et al., 2021; Olude et al., 2021; Churniya et al., 2021; Hussain et al., 2018; Jimoh, 2021). Plant-based diets are not readily accepted by some species as fish meal is highly palatable (could compromise the fish in the aspects of feed intake and growth performance due to limited levels of certain essential amino acids like lysine and methionine) (Ranjan et al., 2023). The concentrations of the amino acids in the plant-based diets influence the growth of the rohu, likely due to the fact that the essential amino acid concentrations were lower compared to the fishmeal (Goswami et al., 2020). Conversely, the oxidative stress brought by the environmental factors might damage polyunsaturated fatty acids (PUFA) like α-linolenic and linoleic acids, which are necessary for the integrity of the fish's cell membrane. Thus, variations in PUFA affect the taste of the fish (Fatima et al., 2019). The animal-based waste products have also been used as a substitution for the fishmeal in the fish feed. The increasing use of animals for food creates a huge amount of waste such as poultry by-product, and feather meal (Psofakis et al., 2020). Blending animal and plant-based alternative sources together have been used for the complete replacement of fishmeal in the preparation of low-cost fish feed (Chen et al., 2020). In order to utilize resources sustainably, agricultural wastes having significant nutritional profile could be the sustainable alternative sources for replacing fishmeal in the diets of various aquaculture species (Kari et al., 2023). Either various agricultural wastes like vegetables and fruits have been used to reduce the quantity of fishmeal partially or completely to produce fish feed (Savonitto et al., 2021). The agriculture, food and beverage sectors have produced novel products from these biowastes (Kassim et al., 2022; Turhal et al., 2019). The discovery and sustainable development of plant ingredients as replacement to FM have attracted the global aquaculture industry's interest, largely due to lower cost and global availability (Kari et al., 2021).

*Labeo rohita* (Rohu) is a commercially significant Indian freshwater carp that is frequently farmed in the aquaculture sector, notably in South Asia, because of its swift development rates, nutritional value, and cultural prominence (Ashaf-Ud-Doulah et al., 2021). Rohu is also considered as one of the top ten freshwater aquaculture species cultivated worldwide (Mehar et al., 2022). Of the total worldwide production of aquaculture, *L. rohita* constitutes 3.7% of the global supply (FAO, 2020) and are widely cultivated in polyculture systems (Mansour et al., 2021). Watermelon is one of the largest cultivated members of the Cucurbitaceae family, with significant commercial importance and widespread consumption. About 3 billion pounds of watermelon were produced worldwide, primarily in China, Turkey, and India (Manivannan et al., 2020). Watermelon rind accounts for about 40% of the overall watermelon bulk; it is frequently discarded directly into the environment as waste, which is a source of pollution (Du and Ramirez 2022). The watermelon rind is rich in minerals, vitamins and phytochemicals which possess anti-oxidant, antimicrobial and anti-inflammatory properties due to the presence of alkaloids, flavonoids, phenolic flavonoids, and terpenoids (Osinubi et al., 2020; Zamuz et al., 2021). Watermelon is rich in protein, minerals, crude fibre, and fat (Ho et al., 2018; Egbuonu, 2015). Minerals present in watermelon rind include iron, manganese, phosphorus, calcium, sodium, potassium, zinc, copper, magnesium and vitamins A, $B_1$, $B_2$, $B_3$, $B_6$, and C are found in relatively high concentrations (Gladvin et al., 2017). However, the rind of a watermelon has few applications. Therefore, the goal of this research was to efficiently utilize the nutrients in watermelon rind through replacement of fishmeal in the diets of freshwater fish rohu (*Labeo rohita*). Either in the form of partial or complete in the diets and analyze its effects on growth, hematological, biochemical, antioxidant, digestive enzyme activities, and histological alterations of the fish

## 2. Materials and methods

### 2.1. Preparation of watermelon rind extract and its primary phytochemical screening

The watermelon rind (WMR) was collected, washed, and sliced. The sliced WMR was air dried for 3–4 days and pulverized using a mixer/mechanical grinder. The watermelon rind powder was stored in an airtight container for further use (Farag et al., 2020). Watermelon rind powder was extracted with various solvents based on their polarity such as methanol, ethanol, acetone, ethyl acetate, petroleum ether and hexane in order to quantify the phytochemicals and secondary metabolites. The extract was prepared in the ratio of 1:10 (w/v) (Arumugam et al., 2022a). The mixtures were maintained in a shaker to extract the bioactive metabolites for 24 h. The extracts underwent filtering and kept at −20° C for further analysis.

#### 2.1.1. Primary phytochemical screening of watermelon rind extracts

The primary phytochemicals such as alkaloids, (Auwal et al., 2014), flavonoids, saponins, steroids, tannins, terpenoids (Roghini and Vijayalakshmi. 2018), phenols (Tepal, 2016), phenolic flavonoids (Ahmed et al., 2018), and phytosterols (Harborne. 1998) of various extracts of watermelon rinds were investigated and analyzed by the respective methods.

### 2.2. Proximate analysis of watermelon rind with feed ingredients and formulated diet

AOAC (2005) methods were followed to analyze the proximate composition of watermelon rind powder, other feed ingredients and prepared experimental diets. The moisture content was analyzed after drying in oven at 135°C for 2 h; crude protein was determined using Kjeldhal wet chemical method; crude fibre was analyzed using the ceramic filter fibre method; crude fat (ether extract) was analyzed using modified Randall method; the total ash was determined by using the method of IS 14827–2000; gross energy was calculated.

### 2.3. Experimental diets

The isonitrogenous (34%) fishmeal replacement (FMR) diets and the control diet were formulated using Pearson's square method with various inclusion levels of watermelon rind (WMR). The control diet was prepared with fishmeal, groundnut oil cake, soybean meal, wheat flour, tapioca flour, and corn flour. Four fishmeal replacement diets were prepared in various concentrations of replacing fishmeal in the formulated diet; 0 g/100 g (control diet without watermelon rind), 6.25 g/100 g (25% FMR), 12.5 g/100 g (50% FMR), 18.75 g/100 g (75% FMR), and 25 g/100 g (100% FMR). The selected ingredients and their proximate compositions are shown in Table 1. Five experimental diets contained vitamin and mineral mixes to ensure that all the diets had the optimum dietary requirement of *Labeo rohita* fingerlings (Fawole et al., 2016). Tapioca flour and egg albumin were added as a binder. The formulated fishmeal replacement (FMR) diets were given in Table 2. The collected ingredients were combined and ground into a homogeneous mixture. Sterilized water and palm oil were then added to produce dough, which was then passed through a hand pelletizer with a 2 mm diameter to make the pellets. The pellets were air dried and kept at −20°C for experimental trial.

**Table 1**
Proximate composition of feed ingredients and watermelon rind.

| S. No | Ingredients | Moisture (%) | Crude Protein (%) | Crude Fibre (%) | Crude Fat (%) | Total ash (%) |
|---|---|---|---|---|---|---|
| 1 | Fish meal | 4.63 | 49.8 | 0.25 | 3.69 | 38.65 |
| 2 | Soybean meal | 6.3 | 47.11 | 3.36 | 1.27 | 6.64 |
| 3 | Groundnut oil cake | 6.23 | 30.99 | 4.46 | 8.97 | 6.8 |
| 4 | Wheat bran | 8.21 | 13.87 | 13.15 | 4.21 | 6.42 |
| 5 | Tapioca flour | 8.55 | 10.49 | 1 | 1.94 | 0.42 |
| 6 | Corn flour | 10.18 | 8.54 | 1.73 | 3.76 | 1.85 |
| 7 | Watermelon rind powder | 9.35 | 12.66 | 60.27 | 1.74 | 10.7 |

**Table 2**
Preparation of fishmeal replaced experimental diets inclusion of watermelon rind (g/100 g).

| S. No | Ingredients (g/ 100 g) | Control | 25% FMR Diet | 50% FMR Diet | 75% FMR Diet | 100% FMR Diet |
|---|---|---|---|---|---|---|
| 1 | Fish meal | 25 | 18.75 | 12.5 | 6.25 | 0 |
| 2 | Water melon rind | 0 | 6.25 | 12.5 | 18.75 | 25 |
| 3 | Soybean meal | 30 | 35 | 37 | 47 | 55 |
| 4 | Groundnut oil cake | 15 | 17 | 25 | 18.5 | 12 |
| 5 | Wheat bran | 10 | 7 | 4 | 2.5 | 2 |
| 6 | Tapioca flour | 8 | 7 | 3 | 2.5 | 2 |
| 7 | Corn flour | 10 | 7 | 4 | 2.5 | 2 |
| 8 | Palm oil | 1 | 1 | 1 | 1 | 1 |
| 9 | Vitamin and mineral Mix* | 1 | 1 | 1 | 1 | 1 |
| | Total | 100 | 100 | 100 | 100 | 100 |

*Vitamin and mineral mix (Each serving of soft gel capsule): Energy-5.75 Kcal; Carbohydrate-0.09 g; Protein-0.0095 g; Fat-0.594 g; Saturated fatty acids-0.12 g; Eicosatetraenoic acid-90 mg; Docosahexaenoic acid- 60 mg; Alpha Lipoic acid- 30 mg; Vitamin C- 25 mg; Vitamin E acetate- 8 mg; Betacarotene- 2.4 mg; Vitamin $B_2$-1.5 mg; Vitamin $B_1$-0.8 mg; Folic acid- 50 mcg; Vitamin $B_{12}$-0.8 mcg; Zinc-9 mg; Selenium-30 mcg; Chromium- 30 mcg.

### 2.4. Experimental fish and feeding trial

The 60-day feeding trial experiment was conducted in the aquarium facility, laboratory of aquabiotics/nanoscience, Department of Animal Science, Bharathidasan University, India. *Labeo rohita* (rohu) fingerlings were procured from Nathan fish farm, Thanjavur, and acclimatized for 2 weeks by feeding with control diet. A total of 375 rohu fingerlings (2.00 ±0.50 g) were distributed into five groups in 15 FRP (Fibre-reinforced plastic) tanks with the capacity of 120 L in biological triplicates. During the experimental period fish were fed twice daily (07:00 AM and 07:00 PM) with formulated diets (5% of their body weight) manually. Throughout the experimental trial, water quality parameters (physio-chemical) were routinely monitored within the cultivable range (dissolved oxygen 8.5±0.5 mg $L^{-1}$; temperature 27±0.5°C; pH 7.0) (Hussain et al., 2011; Guy et al., 2018).

### 2.5. Growth parameters

The growth parameters like survival rate (SR), specific growth rate (SGR), weight gain (WG), length gain (LG), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (K), hepatosomatic index (HSI) and viscero-somatic index (VSI) were determined using the following formulae.

$$\text{Specific growth rate (SGR, \%)} = \frac{\{(\log 10W_f - \log 10W_i)\} \times 100}{\text{experimental days}}$$

$$\text{Length gain (LG, cm)} = \{(L_f - L_i)/L_i\}$$

$$\text{Weigth gain (WG, g)} = \{(W_f - W_i)/W_i\}$$

$$\text{Feed conversion ratio (FCR)} = W_d/(W_f - W_i)$$

$$\text{Protein efficiency ratio (PER)} = (W_f - W_i)/\text{protein intake}$$

$$\text{Condition factor (K)} = \text{body weight}/\text{lenght}^3 \times 100$$

$$\text{Hepatosomatic index (HSI, \%)} = (W_l/W_b) \times 100$$

$$\text{Viscerosomatic index (VSI, \%)} = (W_v/W_b) \times 100$$

where $W_f$ is the final body weight, $W_i$ is the initial body weight; $L_f$ is the final length, $L_i$ is the initial length; $W_d$ dry feed intake; $W_l$ is the weight of the liver, $W_b$ is the weight of the body, and $W_v$ is the weight of the viscera.

### 2.6. Sample collection

The experimental fish samples were collected on 30th and 60th day of the feeding trial. Experimental fish were starved for 24 h before sampling. From each FRP tank, 7 fish were randomly collected for sampling from experimental setup in triplicates. Weight of the fish was measured using an electronic weighing balance. Collected fishes were anesthetized using clove oil. Blood samples were collected in EDTA tubes for hematological analysis and serum samples were prepared from blood samples through centrifugation (5000 rpm for 5 mins) for biochemical analysis. On the 60th day, the experimental fish fed with experimental diets were dissected, and liver, intestine, and muscle samples were collected for analysis of the enzymatic activity (digestive enzymes and antioxidant enzymes ). The collected samples were immediately transferred to −20°C to prevent postmortem changes. The organ samples were kept in 10% formalin and further processed for histology.

### 2.7. Hematological parameters

Hematological indices were evaluated using the standard protocols and procedures such as white blood cell (WBC) by Miale (1962), red blood cell (RBC) counted via hemocytometer, hemoglobin (HB) level were investigated by following the protocol of Wasnik et al. (2014), mean corpuscular hemoglobin (MCH) using Ahmed and Sheikh, (2019), Hematocrit (HCT) using Satheeshkumar et al. (2012), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were estimated by the protocol of Satheeshkumar et al. (2011). MCH, MCHC, and MCV were expressed using the formula (1), (2), and (3) respectively.

$$\text{MCH} = \text{Hemoglobin} \times 10/\ \text{RBC count} \quad (1)$$

$$\text{MCHC} = \text{Hemoglobin} \times 100\ /\ \text{HCT} \quad (2)$$

$$\text{MCV} = \text{Hematocrit} \times 100\ /\ \text{RBC count} \quad (3)$$

### 2.8. Serum biochemical

The bilirubin, total cholesterol (TC, A111–2–1), glucose, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total protein, triglycerides (TAG, A110–2–1) and alkaline phosphatase were analyzed using ERBA, biochemical analyzer XL 640, USA.

### 2.9. Preparation of samples for enzymatic activities

The collected liver and intestine samples were homogenized using a tissue homogenizer, then PBS buffer was added to the homogenate in 1:10 ratio. The homogenized samples were vortexed and subjected to centrifugation (Eppendorf, Centrifuge 5804 R) at 12,000 rpm for 20 mins at 4 °C. Then, the supernatant was collected and stored at −20°C for further enzymatic assays.

#### 2.9.1. Digestive enzyme activities

*2.9.1.1. Amylase activity.* Amylase activity was measured using 1% starch as a substrate. The reaction mixture contained 1 mL of 1% starch and 1 mL of 0.1 M phosphate buffer having pH 7 and 0.5 mL of sample supernatant. The reaction mixture was incubated for 10 min at 40°C. 2 mL of distilled water and 2 mL of dinitrosalicylic acid (DNSA) were added to the reaction mixture to terminate the reaction. Then the test tubes were subjected to heating in a boiling water bath for exactly 5 mins. After cooling, the absorbance was read at 600 nm and maltose was used as the standard (Bhilave et al., 2014).

*2.9.1.2. Protease activity.* The protease activity was determined using the standard casein hydrolysis method (Kunitz, 1947). The reaction mixture contained 0.25 mL of casein in water as substrate. To the substrate, about 0.1 mL of the sample was added. Then, 0.25 mL of the tris-HCl buffer was added and incubated at room temperature for 60 min. After the incubation, about 0.6 mL of 8% TCA was added. Then, the reaction mixture was incubated for 1 h at 2°C. After that, the reaction mixture was centrifuged (Eppendorf, Centrifuge 5804 R) at 1800 x g for 10 min. The supernatant was collected and the absorbance was read at 280 nm. Tyrosine was used as the standard.

*2.9.1.3. Trypsin activity.* The substrate was N-a-benzoyl DL-arginine p-nitro anilide hydrochloride (BAPNA), in a buffer of tris HCl (0.04 M; pH 8.2) with 0.02 M $CaCl_2$. The reaction mixture contained 0.1 mL of crude sample extract and 2.9 mL of substrate buffer solution, which were incubated for 10 min at 25°C. Trypsin activities were assessed using p-nitro aniline as the standard reference and an increase in absorbance was read at 410 nm (Chong et al., 2002).

### 2.10. Antioxidant activity

#### 2.10.1. Lipid peroxidation [thiobarbituric acid reactive substance (TBARS)]

TBARS was determined by Kamyshnikov (2004) method. Initially, 2 mL of distilled water was added to the 0.1 mL liver, muscle and intestine sample tissue homogenate separately. Then, 1 mL of 0.8% 2-thiobarbituric acid (TBA) reagent and 1 mL of 20% TCA were added. The reaction mixture was then heated at 95°C and incubated for 10 mins. The reaction was halted by submerging the reaction mixture in freezing water. The reaction mixture was then centrifuged for 10 mins at 3000 ×g using an Eppendorf Centrifuge 5804 R. The supernatant was collected and absorbance was read at 540 nm. The extinction coefficient of $1.56\times10^5$ $mM^{-1}$ $cm^{-1}$ was used to calculate the μmol of TBARS.

#### 2.10.2. Superoxide dismutase activity (SOD)

The superoxide dismutase activity was determined by adapting the method of Marklund and Marklund (1974). This assay consisting of 100 μL of the liver, muscle and intestine sample separately mixed with 0.15 mL of chloroform. Then, the reaction mixture was centrifuged for 15 mins at 13000 rpm. After that, 2 mL of ethylenediaminetetraacetic acid (EDTA) buffer was added to the supernatant. 0.5 mL of pyrogallol and 1 mL of distilled water were added to the mixture. At 470 nm, the reaction mixture's absorbance was measured. One unit of SOD activity is the quantity of the enzyme necessary to inhibit 0.1 mM pyrogallol solution oxidation by 50% at a temperature of 25°C. The units of the enzyme activity were $mg^{-1}$ $protein^{-1}$.

#### 2.10.3. Catalase activity

The catalase activity (CAT) was determined by the method of Koroliuk et al. (1988) with slight modification in accordance with the sample. The reaction mixture consisted liver, muscle and intestine homogenate diluted in the incubation medium consisting of a 1:10 v/v solution of hydrogen peroxide and ammonium molybdate, and the enzyme activity was evaluated by the reduction in hydrogen peroxide absorbance at 410 nm. Catalase activity was measured in nmol $H_2O_2$ per $min^{-1}$ $mg^{-1}$ of protein.

#### 2.10.4. Reduced glutathione (GSH) activity

The GSH content was measured by the method of Moron et al. (1979). The supernatant was obtained from the liver, muscle and intestine sample after centrifugation in which 0.5 mL of 10% TCA was added. Then, 4 mL of disodium hydrogen phosphate (0.3 M, pH 8.0) and 0.5 mL of 0.04% DTNB (5–5' – dithiobis (2-nitrobenzoic acid) were added. The yellow color formation was observed at the end of the reaction, and its absorbance was read at 412 nm. The results were represented as μg of GSH used per mg of protein per min.

#### 2.10.5. Glutathione peroxidase (GPx) activity

With a little modification, the Rotruck et al. (1973) technique was used to quantify the glutathione peroxidase activity. 0.5 mL of sodium phosphate buffer (0.4 M, pH 7) was mixed with 0.1 mL of 10 mM sodium azide, 0.2 mL of 4 mM reduced glutathione, 0.1 mL of 2.5 mM hydrogen peroxide, and 0.1 mL of liver, muscle and intestine tissue homogenate to create the reaction mixture. Following that, the mixture was diluted with distilled water to a volume of 2 mL and incubated for 3 mins at 37ºC. 0.5 mL of 10% TCA was added and the reaction mixture was centrifuged (Eppendorf, Centrifuge 5804 R) for 10 mins at 7500 ×g. Disodium hydrogen phosphate (0.3 mM) was added to 4 mL of the supernatant after it had been collected. Dithio-bisnitrobenzoic acid, a final ingredient of 1 mL was added into it. A spectrophotometer (Synergy HT, BioTeck) was used to measure the absorbance at 412 nm to compare the colour formed against the blank. GSH concentrations in tissues were reported as g/mg protein.

### 2.11. Muscle amino acid composition

The lyophilized muscle samples of *L. rohita* fed with formulated WMR diets were subjected to acid hydrolysis for quantifying amino acid concentration using liquid chromatography (Cortés-Herrera et al., 2018). Chromatographic conditions were: injection volume was 5 μL; eluent A was 200 mL (20: 80 acetonitrile: 25 mM potassium phosphate, pH 3.3) eluent B was 80: 20 CAN: 25 mM potassium phosphate, pH 3.3; flow rate, 1 mL/min; gradient rate 0 – 75% over 15 mins, fluorescence detector, excitation at 250 nm, emission at 395 nm; column (DENALI C18; 5 μm, 4.6 mm×150 mm (cat no. DEN-5C18–15046); Lachrome Hitachi, Japan (Ishida et al., 1981; Mohanty et al., 2014).

### 2.12. Muscle fatty acid composition

The muscle fatty acids of *L. rohita* fed with experimental FMR diets were measured using a comprehensive protocol (Siddiqua and Khan. 2022). 75 mg of freeze-dried muscle samples were placed in a 20 mL tube with a lid. Followed by 1 mL of diethyl ether was added and blended with 1 mL of 0.5% methanolic potassium hydroxide (1 N), rapidly agitated for 15 min, and heated in a water bath for 20 mins at 75°C. 1 mL of 1 N HCl was added, allowed to cool, and then heated for further 20 mins in a 75°C water bath. After that, 2 mL of petroleum ether was added, agitated continuously for about a minute, and allowed to separate into two layers. Fatty acid methyl esters were discovered in the top layer, which was separated, decanted, and dried in a water bath for

around 20 mins. The tube was then filled with 0.5 mL of n-heptane. Finally, the amount of all the samples were measured using a Shimadzu QP-2010 Plus coupled with a Thermal Desorption System TD 20 GC-MS. Column material: fused silica; size: 30 m x 0.25 mm; stationary phase: macrogel 20,000 R; carrier gas: helium R; split ratio: 1:200. The injection volume was 1 μL. The initial column temperature was 170–225°C for 55 mins, then 225°C for 75 mins, whereas the temperature at the injection port was 250°C and 280°C in the detector, respectively (Ragonese et al., 2009).

### 2.13. Histoarchitectural analysis

The liver, muscle, and intestine were dissected from the experimental fish on the 30th and 60th days of the experiment for histological analysis. These organs were immediately transferred to a vessel containing 10% formalin solution. The buffered parts were embedded in paraffin and stained with hematoxylin and eosin for histological studies (Humason. 1962; Rajkumar et al., 2016).

### 2.14. Statistical analysis

The results were provided as mean±standard error of the mean (SEM) and analysed with SPSS 16.0 software (SPSS, Chicago, IL, USA). The triplicate findings were compared using one-way analysis of variance (ANOVA) with Duncan's multiple range test (DMRT). The degree of significance accepted was $p<0.05$. Graphs were created with Origin Pro 9.0 (Northampton, US) and visualized with GraphPad prism (GraphPad Software, San Diego, CA, USA).

## 3. Results

### 3.1. Primary phytochemical screening of watermelon rind (WMR)

The primary phytochemical analysis of watermelon rind extracts exhibited the presence of alkaloids, phenols, flavonoids, phenolic flavonoids, phytosterols, proteins, saponins, tannins, and terpenoids. The WMR extracts from polar solvents like methanol, and ethanol, and mid-polar like acetone exhibited an abundant presence of flavonoids, alkaloids, saponins, phenols, and terpenoids. Phytosterols and flavonoids were present in extracts of non-polar solvents like petroleum ether and hexane. Terpenoids were found in all the solvent extracts of WMR except hexane (Table 3).

### 3.2. Proximate analysis of feed ingredients with watermelon rind and prepared diet

The proximate composition and energy values of the five isonitrogenous formulated fishmeal replacement (FMR) diets using watermelon rind were provided in Table 4. The higher moisture content was observed in 100% FMR diet (12.22%). The crude protein concentration was approximately 34% in all the formulated diets. In the case of crude fibre, 100% FMR diet exhibited higher concentration (8.79%). Crude fat concentration was higher in the control (7.77%) and 25% (7.77%) FMR diet and lowest value (5.37%) was observed in the 100% FMR diet. The total ash concentration was higher (6.28%) in the 100% FMR diet. The total energy was higher in the control diet (4324 Kcal/kg).

### 3.3. Growth parameters

The replacement of fishmeal with watermelon rind created significant changes in terms of the growth parameters of the experimental fish (Table 5). The control, 25%, and 50%, FMR diets showed a 100% survival rate. The specific growth rate (SGR) varied from the control diet to the 100% FMR diet. The 50% FMR diet showed higher SGR followed by the 25% FMR diet and control diet. The highest weight gain (WG) and length gain (LG) were recorded in 50% FMR diet fish. The lowest feed conversion ratio (FCR) was recorded in the 50% FMR diet and the highest was recorded in the 100% FMR diet. The protein efficiency ratio (PER) was comparatively higher in 50% FMR diet and lower in the 100% FMR diet. During the experimental study, the condition factor (K) was maintained at 1 in all the experimental diet fed fish. In the current study, the replacement of fishmeal with WMR significantly ($P<0.05$) varied concerning the control diet on the growth parameters of rohu.

### 3.4. Hematological parameters

In the experimental fish, the hematological parameters varied from the control diet to the experimental diet. The hematological profile was significantly improved from the 30th day to the 60th day (Fig. 1). Comparatively, fish fed with 50% FMR diet possess higher red blood cells (RBC) on 60th day compared to the 30th day. Fish fed with the FMR diets including control diets didn't exhibit any variations in the range of RBCs in their blood. The WBC count was significantly ($P<0.05$) altered to that of control and other groups on the 30th and 60th day. At both the 30th and 60th day sampling of fish, 75% FMR diet fed fish exhibited higher WBC level than other diets. In the case of hematocrit, there were no significant differences on 30th day. At the 60th day sampling, hematocrit was prominent in control followed by the 50%, 25%, 75%, and 100% FMR diets. The hemoglobin level was improved during the experimental study; the 50% FMR diet showed a lower level on 30th day, but it was higher on 60th day, while fish fed with other diets displayed a similar range.

### 3.5. Serum biochemical parameters

Overall, the biochemical profile of experimental fish was increased from the 30th day to 60th day (Fig. 2). During the study period, fish fed with 50% FMR diet exhibited reduced bilirubin, glucose, and triglycerides, but total protein concentrations were higher in control and

**Table 3**
Primary phytochemical screening of watermelon rind using various solvents.

| Phytochemicals | Methanol | Ethanol | Acetone | Ethyl acetate | Petroleum ether | Hexane |
|---|---|---|---|---|---|---|
| Alkaloids | +++ | +++ | ++ | + | - | - |
| Carbohydrates | +++ | +++ | ++ | - | - | - |
| Flavonoids | +++ | +++ | +++ | - | ++ | + |
| Phenols | +++ | ++ | + | - | - | - |
| Phenolic flavonoids | + | - | - | - | - | - |
| Phytosterols | + | - | - | - | ++ | +++ |
| Proteins | + | + | + | - | - | - |
| Saponins | +++ | ++ | + | - | - | - |
| Steroids | + | - | - | - | - | - |
| Tannins | - | - | - | - | + | + |
| Terpenoids | +++ | ++ | ++ | ++ | + | - |

[+ Presence (+++-High, ++-Moderate, +-Mild), – absence]

**Table 4**
Proximate composition of formulated fishmeal replacement (FMR) diets by watermelon rind.

| S. No | FMR Diets | Moisture (%) | Crude Protein (%) | Crude Fiber (%) | Ether Extract (%) | Total Ash (%) | Gross Energy (Kcal/kg) |
|---|---|---|---|---|---|---|---|
| 1 | Control | 11.33 | 34.37 | 3.03 | 7.77 | 5.22 | 4324 |
| 2 | 25% | 11.88 | 34.18 | 4.51 | 7.77 | 5.36 | 4275 |
| 3 | 50% | 11.66 | 34.19 | 6.34 | 6.95 | 5.01 | 4246 |
| 4 | 75% | 11.79 | 34.18 | 6.88 | 7.42 | 5.86 | 4145 |
| 5 | 100% | 12.22 | 33.45 | 8.79 | 5.37 | 6.28 | 3912 |

**Table 5**
Growth performance of *L. rohita* fed diet containing various inclusion levels of the watermelon rind.

| Nutritional Indices | Control | 25% FMR | 50% FMR | 75% FMR | 100% FMR |
|---|---|---|---|---|---|
| SR (%) | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 96 ±2.30$^{a}$ | 85.66 ±3.48$^{b}$ |
| SGR (%) | 0.31 ±0.05$^{bc}$ | 0.35 ±0.01$^{b}$ | 0.52 ±0.01$^{a}$ | 0.41 ±0.02$^{d}$ | 0.42 ±0.01 $^{cd}$ |
| LG (cm) | 1.90 ±0.02$^{b}$ | 1.86 ±0.02$^{b}$ | 2.24 ±0.01$^{a}$ | 1.41 ±0.05$^{c}$ | 1.27 ±0.05$^{d}$ |
| WG (gm) | 3.82 ±0.11$^{d}$ | 5.10 ±0.17$^{b}$ | 8.31 ±0.17$^{a}$ | 4.95 ±0.17$^{b}$ | 4.46 ±0.08$^{bc}$ |
| FCR | 2.61 ±0.12$^{a}$ | 2.00 ±0.34$^{c}$ | 1.37 ±0.06$^{e}$ | 1.95 ±0.73$^{d}$ | 2.33 ±1.80$^{b}$ |
| PER | 1.09 ±0.11$^{b}$ | 1.42 ±0.02$^{c}$ | 2.10 ±0.03$^{a}$ | 1.46 ±0.03$^{d}$ | 1.25 ±0.01$^{e}$ |
| K | 1.01 ±0.01$^{a}$ | 0.96 ±0.03$^{a}$ | 0.97 ±0.02$^{a}$ | 1.01 ±0.03$^{a}$ | 1.05 ±0.03$^{a}$ |
| HSI (%) | 2.11 ±0.01$^{b}$ | 2.03 ±0.30$^{b}$ | 2.51 ±0.05$^{a}$ | 1.48 ±0.06$^{c}$ | 1.23 ±0.02 $^{cd}$ |
| VSI (%) | 5.65 ±0.73$^{e}$ | 6.85 ±0.60$^{d}$ | 8.09 ±0.10$^{c}$ | 8.93 ±0.15$^{b}$ | 10.09 ±0.10$^{a}$ |

SR, survival rate; SGR, specific growth rate: LG, length gain; WG, weight gain; FCR, feed conversion ratio; PER, protein efficiency ratio; K, condition factor (CF); HSI, hepatosomatic index; VSI, viscerosomatic index. Each value represents the mean±standard error of the mean (n=3) and different superscript letters indicate a significant difference between the groups ($p<0.05$) using one-way Analysis of variance (ANOVA) followed by Duncan's multiple range (DMRT) as post hoc test (SPSS).

all other experimental diets. The bilirubin level was found in the similar range in all the diet fed fish on 30th day. However on 60th day, control and 50% FMR diet fed fish exhibited lower bilirubin content than other FMR diet fed fish. Cholesterol levels were maintained in similar range in the all the diet fed fish on both 30th and 60th days. Glucose levels were gradually increased on 60th day compared to the 30th day of the experimental trial. The SGOT was increased from control to the 100% FMR diet on the 30th day. On the 60th day, the SGOT level was decreased in fish fed with the 25%, 50%, 75% and 100% FMR diet compared to the control diet. The serum glutamic pyruvic transaminase (SGPT) was lowest in fish fed with 50% FMR diet than other diet fed fish on the 30th day; however on 60th day, the SGPT levels were increased in all the diet fed fish compared to the control. In the case of total protein, fish fed with 50% FMR diet showed a slight increase in the protein value followed by all other experimental diets on both 30th and 60th day of the experimental trial. The alkaline phosphatase (ALP) was higher in fish fed with 75% and 100% FMR diet followed by the 25% FMR and control diet. 50% FMR diet exhibited lowest range of ALP on 30th day of the experimental trial. On the 60th day, 25% FMR diet fed fish exhibited higher value compared to all other diet fed fish. On 30th day, the triglyceride level was slightly increasing from control to 25% and 50% FMR diet fish then it tends to decrease in the 75% and 100% FMR diet fed fish. However, on 60th day, 100% FMR diet fish exhibited higher triglycerides compared to other diets.

### 3.6. Digestive enzyme activity

The digestive enzyme activity of fish fed with control and experimental diets indicates the influence of watermelon rind inclusion in their diets. The digestive enzyme activities like amylase, protease, and trypsin were analyzed in the intestine and liver of the experimental fish fed with experimental diets (Fig. 3). On 30th day, both liver and intestine samples in fish fed with control diet exhibited slightly higher amylase activity compared to other diets. However, on the 60th day, fish fed with 50% FMR diet exhibited highest amylase activity than other diets fed fish in both liver and intestine. No significant variations were occurred in fish fed with various diets ($p>0.05$) compared to control. In protease activity, on 30th and 60th days of experimental trial, fish fed with 25% FMR diet showed increased activity in the intestine than other experimental diets, but no significant variations ($p>0.05$) were found in the liver of fish fed with all experimental diets. In trypsin activity, on 30th day, fish fed with control diet exhibited higher activity, then gradually reduced in the other diets, whereas in liver, fish fed with 25% FMR diet showed slightly higher than other diets fed fish. However, on the 60th day, the intestinal samples showed higher activity in fish fed with 25% FMR diet followed by 50%, control, 100% and 75% FMR; whereas, in liver samples, no significant variation was found among the fish fed with experimental diets.

### 3.7. Antioxidant activity

The presence of watermelon rind in the diet influences the antioxidant activities of the liver, muscle, and intestine of the *L. rohita* during the experimental period. Antioxidant enzyme activity was highest in range when fish fed with 100% FMR diet, whereas in fish fed with the control, 25% and 50% diets, exhibited lowest antioxidant activity. No significant variations were observed in all the diet fed fish with respect to the antioxidant enzymes ($P<0.05$). Lipid peroxidation activity was slightly improved in fish fed with 25% FMR, and 50% FMR diets intestine compared to liver and muscle, but in fish fed with control and 75% FMR diet, fish exhibited higher lipid peroxidation activity compared to other groups. Muscle showed higher lipid peroxidation activity in fish fed with 100% FMR diets compared to other diets. The intestine has the highest superoxide dismutase (SOD) activity, followed by the liver and muscle. SOD levels steadily increased from control diet to 100% FMR diet, although hepatic SOD activity was identical in all experimental diet fed fish. Muscle exhibited gradual decrease in SOD levels from control to 100% FMR diet fed fish. The catalase (CAT) activity was higher in the muscle followed by the liver and intestine. No significant variation in the catalase activity was observed in either liver or muscle of rohu fed with the experimental diets. The CAT activity was decreased in muscle from the control to the 100% FMR diet. The GSH and GPx activities also declined from control to 100% FMR diet. The liver had higher levels of GSH and GPx activity compared to intestine and muscle. The GSH activity of liver was lower in the 100% FMR diet and higher in the control diet. Similarly, the GSH activity of both intestine and muscle was highest in the control and lower in the 100% FMR diet. The GPx activity in the liver, and muscle was higher in the control and lower in the 100% FMR diet, whereas intestine exhibited no significant variation ($p<0.05$) in all the experimental diets (Fig. 4).

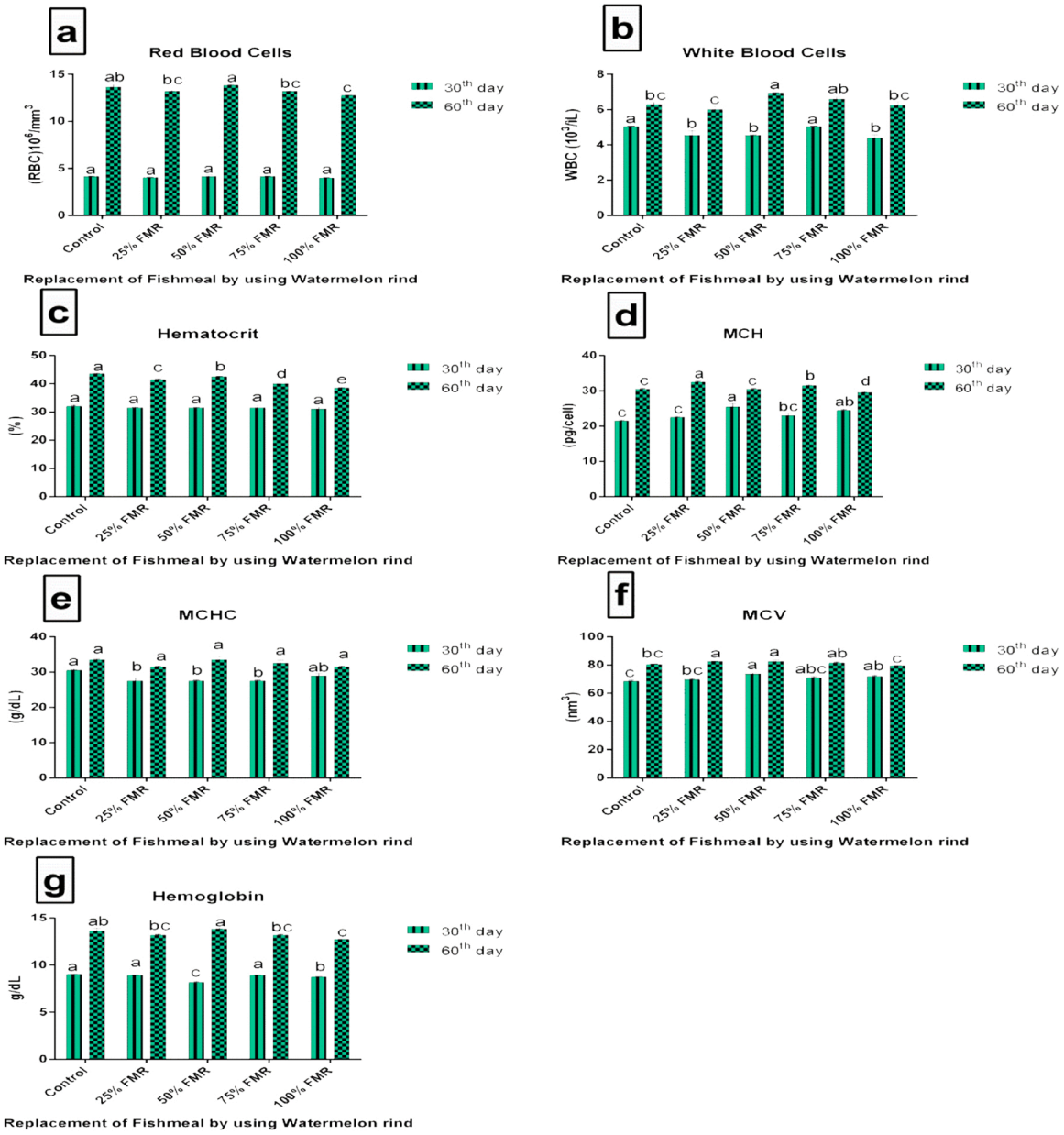


**Fig. 1.** Haematological parameters of *L. rohita* fed experimental diets sampled on 30th and 60th day: a) red blood cells; b) white blood cells; c) hematocrit; d) mean corpuscular haemoglobin (MCH); e) mean corpuscular haemoglobin concentration (MCHC); f) mean corpuscular volume (MCV); g) Hemoglobin. Each bar represents the mean±standard error of the mean (n=3). Different letters indicate significant difference between the groups ($p<0.05$) using one-way Analysis of variance (ANOVA) followed by Duncan's multiple range (DMRT) as post hoc test (SPSS).

### 3.8. Muscle amino acid profile

Retention of amino acids in the muscle of fish fed with various experimental diets containing watermelon rind was significantly different (Table 6). Fish fed with 50% FMR diet had significantly ($p<0.05$) higher concentrations of alanine, aspartic acid, arginine, asparagine, glycine, histidine, leucine, lysine, methionine, proline and valine compared to fish fed with other diets

### 3.9. Muscle fatty acid profile

The fatty acid profile of *L. rohita* muscle fed with experimental diets is represented in Table7. Palmitic acid, moronic acid, stearic acid and margaric acid were significantly increased ($p<0.05$) by the admittance of WMR in diets. Concentrations were higher in fish fed with 100% FMR diet. Oleic acid was similar in fish fed with different FMR diets, whereas fish fed the control diet exhibited lower concentrations. Alpha linolenic acid level was lower in fish fed with 25% FMR diet, but similar in fish fed with other diets. Linolenic acid concentrations in fish fed with control and 50% FMR diets were similar whereas, concentrations in fish fed with 25% FMR, 75% FMR and 100% FMR diets showed equivalent values.

### 3.10. Histoarchitectural analysis

The histology of the liver, intestine, and muscle were evaluated to identify structural modifications. No significant alterations were observed in the tissues of the *L. rohita* fed with experimental diets. The hepatocytes in the liver of fish fed with all experimental diets showed normal organization. On the 30th day, fish fed with 75% and 100% diet displayed vacuole formation and on the 60th day, the vacuolization was reduced. Fish fed with 50% FMR diet displayed well-organized hepatocytes compared to the other diets (Fig. 5 & 6).

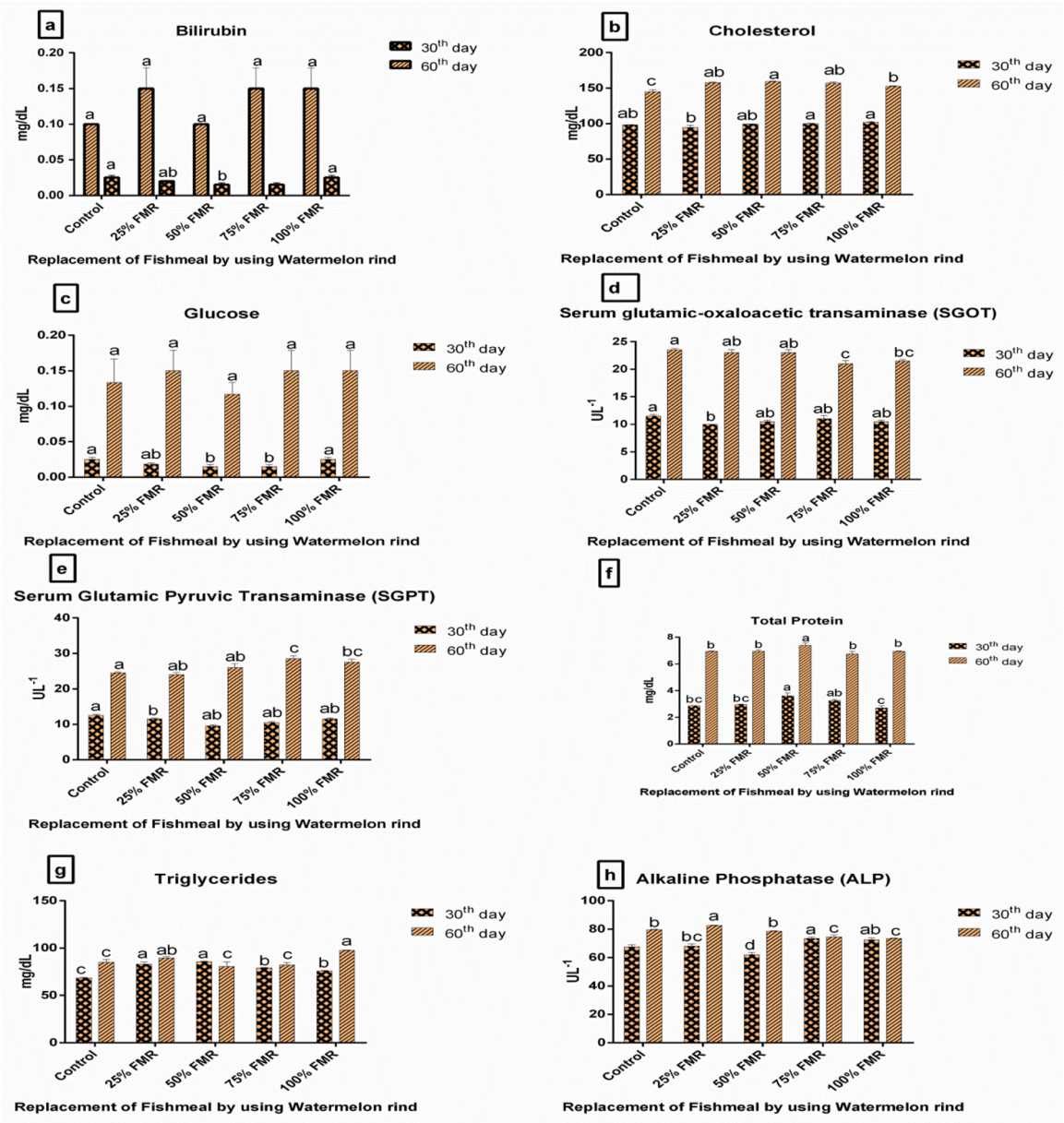


**Fig. 2.** Serum biochemical parameters of *L. rohita* fed the experimental diets on 30th and 60th days: a) bilirubin; b) cholesterol; c) glucose; d) serum glutamic oxaloacetic transaminase; e) serum glutamic pyruvic transaminase; f) total protein; g) triglycerides; h) alkaline phosphatase. Each value represents the mean ±standard error of the mean (n=3) and different letters indicate significant difference between the groups ($p<0.05$) using one-way Analysis of variance (ANOVA) followed by Duncan's multiple range (DMRT) as post hoc test (SPSS).

The villi were typically arranged in the intestine of all fish. The increased villus number and broad-area were found with a large surface area, which improves the efficiency of absorption of nutrients and minerals from the feed. The length of villi was higher in fish fed with control, 25% and 50% FMR diet (Fig. 7 & 8). The muscle bundles were regularly arranged in the muscle of all fish fed with various FMR diets containing watermelon rind (Fig. 9 & 10). These results suggest better quality of flesh in all the experimental diet-fed fish. Up to 100% replacement of fishmeal by watermelon rind did not alter the normal structural integrity of the liver, intestine, or muscle.

## 4. Discussion

The dried form of watermelon rind powder shows higher primary phytochemical compositions with respect to solvent's polarity during extraction. These primary phytochemicals play an essential role in antibacterial and antifungal activities (Osinubi et al., 2020). The bioactive compounds of watermelon rind provide improved antioxidant, anti-inflammatory, and free radical scavenging activities (Arumugam et al., 2022b). Although screening key phytochemicals assists in anti-oxidant and antibacterial capabilities, it also gives information about anti-nutritional substances that limit fish development and generate negative effects when constructing the experimental diet preparation (Varghese et al., 2013). The proximate composition of control and experimental diets shows good nutritional quality parameters compared with the commercially available feed. The optimum level of moisture content of the commercial aqua feed is 8–12% (Hou et al., 2010). The prepared diets in the current investigation, with the exception of the 100% FMR diet (12.11%), showed moisture contents in the range of >12%, which contributes to feed safety, freshness, and shelf life. Therefore, feeding at the ideal moisture level helps to avoid the growth of mold and other fungi in the feed (Mishra et al., 2022). The crude

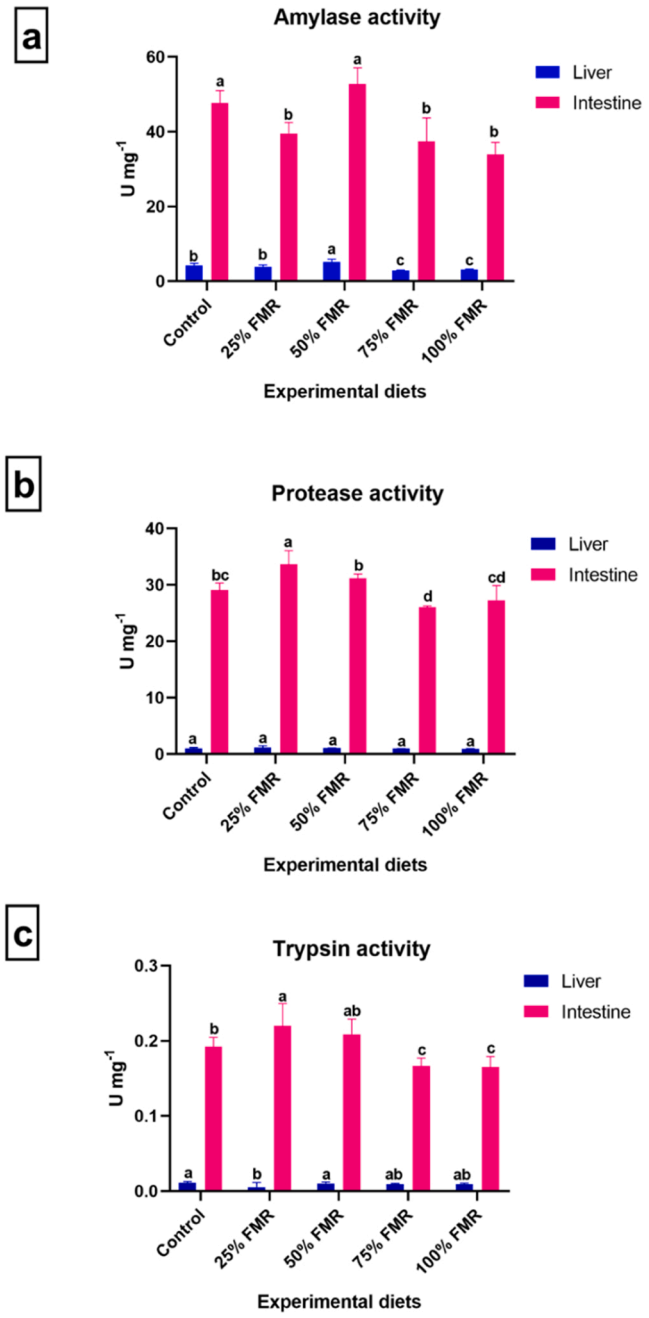


**Fig. 3.** Digestive enzymes activities of liver and intestinal samples of *L. rohita* fed experimental diets on 60th day: a) amylase activity; b) protease activity; c) trypsin activity. Each value represents the mean±standard error of the mean (n=3) and different letters indicate significant difference between the groups ($p<0.05$) using one-way Analysis of variance (ANOVA) followed by Duncan's multiple range (DMRT) as post hoc test (SPSS).

protein level in the prepared diets was 34%, in the case of omnivorous fish the optimum requirement should be in the range of 30–38% (Van Anrooy et al., 2022). The dry matter digestibility of the fish depends on the protein levels in the diet (Rubbani et al., 2011). The ash content of the feed shows the occurrence of minerals such as calcium, phosphorus, magnesium, and potassium. The normal level of ash in the fish feed is about 4–8%. In the present study, the prepared diets showed 5–6% total ash (Ombugadu et al., 2021). Moreover, watermelon is rich in vitamin C and vitamin A that stimulates the immunological responses by exhibiting leucocytes' phagocytic activity, natural killer cell activity, complement activity, lysozyme levels, cell proliferation, antibody concentration and cytokine production (Ibrahim et al., 2020). Similarly, Vitamin-C in the moringa leaf enhanced the *L. rohita* growth (Faisal et al., 2022).

The highest specific growth rate (SGR) and lowest feed conversion ratio (FCR) indicate better growth and feed utilization efficiency of the diet. The watermelon rind powder inclusion at 40 g $kg^{-1}$ level enhances both the development and health condition of the diet of Nile tilapia (Van Doan et al., 2020). In the present study, the growth declined beyond the inclusion level of 50% FMR diet due to the occurrence of high fibre content in 75% and 100% FMR diets that agree with the findings of Oladipupo and Salami. (2020), and they reported that above the 2.0 g/kg level of watermelon bark powder in the diet of *Clarias gariepinus* reduced the growth rate. According to Samuel et al. (2022), watermelon bark replaced the soybean in feed produced prominent growth efficiency in catfish. The 50% FMR diet shows better growth activity and a lower feed conversion ratio. The overall growth parameters indicate that the 50% FMR diet replaced by watermelon rind could be a suitable diet for the enhancement of the growth of *L. rohita*; it is worthwhile in the field of aquaculture to reduce the price of fish feed and enhance the health condition of fish. Similarly, the utilization of pineapple waste with 30% inclusion improves the growth parameters and reduces the feed conversion ratio of Nile tilapia (Sukri et al., 2022).

The inclusion of WMR in the diet influences the hematological parameters in rohu. The MCH and MCHC were in the similar range in the control and 50% FMR diet to those reported for Nile tilapia, where hematological indices like RBC, hematocrit, and Hb were improved (Hassaan et al., 2019). The increased RBC count provides better health conditions in the terms of better oxygen supply and, maintains the homeostasis of the body (Abdel-Tawwab et al., 2019). RBC's level was decreased when moringa leaf meal inclusion was increased in the diets of *Cirrhinus mrigala*, due to the presence of tannins (Tabassum et al., 2021). The MCV was approximately in similar levels in all the experimental diets and it indicates the formation of blood cells (hematopoiesis). The inclusion of watermelon rind in the diet helped to improve the production of blood cells confirming that the non-toxic nature of the diet (Yaqub et al., 2023). In an additional investigation, researchers found that including *Malvae sylvestris*, *Origanum vulgare*, and *Allium hirtifolium* in the diet of common carp improved the hematological parameters such as RBC, hematocrit, MCV, and hemoglobin (Ghafarifarsani, et al., 2021).

Serum biochemical analysis is one of the reliable indicators that can properly signify physiological condition of an animal. Bilirubin is a major endogenous and non-enzymatic antioxidant present in fishes (Sakai et al., 1998). Additionally, fish produce bilirubin as a byproduct of their daily red blood cell destruction (Chaklader et al., 2020). The typical range for bilirubin is 0.1–1 mg/dl (Knowles et al., 2006). Furthermore, the study concluded that the experimental diet did not imposes stress on the fish's usual metabolic functions.

The blood cholesterol level in all experimental diets shows an approximately equal range and little higher in fish fed with 50% FMR diet. A lipid molecule called cholesterol is generated mostly in the liver that is used as a building block for the production of steroid hormones (Tocher. 2003). It is also a component of the cell membrane. The level of cholesterol in the blood indicates the activity level and hepatic activity (Lopes et al., 2013). The significance of diet influences the levels of cholesterol in the blood. The glucose level in the blood serum indicates the various environmental stress and feed formulation. Glucose is one of the main energy sources in fish along with cholesterol and triglycerides (Borgeson et al., 2006). In the present study, glucose level was lower in fish fed with 50% FMR diet. It can be concluded that up to 50% inclusion of watermelon rind does not create any stress to the experimental fish.

The SGPT and SGOT are considered as biomarkers of health. The slight damage in the cells or tissues can be identified with the level of SGPT and SGOT (Palanivelu et al., 2005). The results of this study indicate that the SGOT values were lower in fish fed with experimental diets compared to the control diet. It indicates that the replacement of fishmeal with watermelon rind does not create cellular damage in the

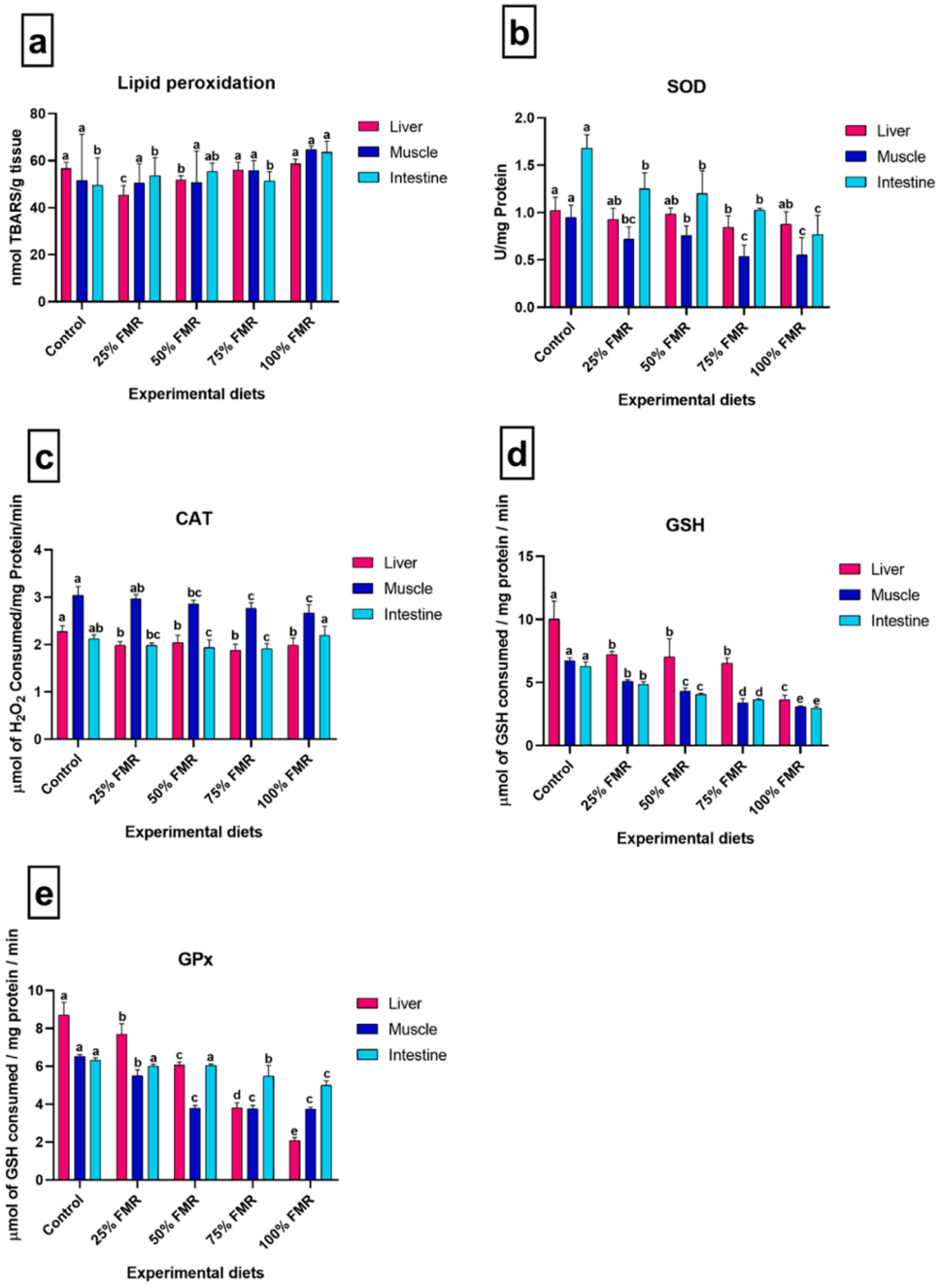


**Fig. 4.** Antioxidant activities of liver, muscle and intestine of *L. rohita* fed experimental diets on 60th day: a) lipid peroxidation activity; b) superoxide dismutase (SOD) activity; c) catalase (CAT) activity; d) reduced glutathione (GSH) activity; e) glutathione peroxidase (GPx) activity. Each value represents the mean±standard error of the mean (n=3) and different letters indicate significant difference between the groups ($p<0.05$) using one-way Analysis of variance (ANOVA) followed by Duncan's multiple range (DMRT) as post hoc test (SPSS).

experimental fish. Another report indicated that there was no negative effect on the serum parameters of *Paralichthys olivaceus* by the inclusion of fermented plant protein (Seong et al., 2018). Damages or oxidative stress in the hepatocytes leads to the leakage of SGOT from the liver cell to the fish's blood (Ghelichpour et al., 2020). In this study, dietary inclusion of watermelon rind did not produce any damage to the liver. The level of SGPT and SGOT were below the normal range in the present study. These findings help to conclude that the replacement of fishmeal with watermelon rind does not cause negative impact on the fish's physiology (Laltlanmawia et al., 2019).

In the present study, the total protein level was higher with 50% FMR diet than other experimental diet fed fish. Generally, in fish, increased concentration of total protein is associated with growth and development, which simultaneously increase the immunoglobulin levels (Kandemir et al., 2010). A similar result was obtained in juvenile spotted rose snapper by replacing corn gluten in diets (Hernández et al., 2021). The 50% replacement of fishmeal in the diet of rohu by watermelon rind improves growth and development. Increased total protein in serum on 60th day compared to 30th day of the experimental trial associates with the innate immunity of the fish (Ranjan et al., 2014).

The triglyceride concentration indicates the level of lipids present in the serum of fish and its increased range creates fatty acid deposition in the internal organs leading to steatosis in internal organs (Zhang et al., 2023). The increased carbohydrate consumption also increases the level

**Table 6**
Amino acid composition of *Labeo rohita* muscle fed experimental diets (g/100 g) in dry weight basis. Each value represents the mean±standard error of the mean (n=3) and different superscript letters indicate a significant difference between the groups ($p<0.05$) using one-way Analysis of variance (ANOVA) followed by Duncan's multiple range (DMRT) as post hoc test (SPSS).

| S. No | Amino acid | **Control** | 25% FMR | 50% FMR | 75% FMR | 100% FMR |
|---|---|---|---|---|---|---|
| 1. | Alanine | 0.1088±0.003$^{b}$ | 0.1237±0.003$^{a}$ | 0.129±0.002$^{a}$ | 0.0921±0.001$^{c}$ | 0.069±0.008$^{d}$ |
| 2. | Arginine | 0.2167±0.001$^{a}$ | 0.1715±0.001$^{b}$ | 0.2168±0.004$^{a}$ | 0.1816±0.004$^{b}$ | 0.1378±0.004$^{c}$ |
| 3. | Asparagine | 0.2141±0.002$^{a}$ | 0.1654±0.004$^{b}$ | 0.2053±0.004$^{a}$ | 0.1709±0.004$^{b}$ | 0.1576±0.003$^{b}$ |
| 4. | Asparticacid | 0.2041±0.001$^{b}$ | 0.1903±0.004$^{c}$ | 0.3241±0.004$^{a}$ | 0.2074±0.002$^{b}$ | 0.2041±0.002$^{b}$ |
| 5. | Cysteine | 0.0776±0.003$^{d}$ | 0.2084±0.005$^{a}$ | 0.1358±0.004$^{c}$ | 0.1764±0.003$^{b}$ | 0.2076±0.003$^{a}$ |
| 6. | Glutamine | 0.1360±0.004$^{c}$ | 0.1792±0.003$^{b}$ | 0.1597±0.018b$^{c}$ | 0.1330±0.004$^{c}$ | 0.2104±0.004$^{a}$ |
| 7. | Glutamic acid | 0.1896±0.003$^{a}$ | 0.1378±0.004$^{c}$ | 0.1670±0.004$^{b}$ | 0.1819±0.004$^{a}$ | 0.1695±0.002$^{b}$ |
| 8. | Glycine | 0.3068±0.003$^{b}$ | 0.2741±0.016$^{c}$ | 0.4124±0.003$^{a}$ | 0.2878±0.005$^{bc}$ | 0.1877±0.003$^{d}$ |
| 9. | Histidine | 0.3162±0.005$^{b}$ | 0.2832±0.006$^{c}$ | 0.4171±0.012$^{a}$ | 0.2085±0.005$^{d}$ | 0.1888±0.002$^{d}$ |
| 10. | Isoleucine | 0.2208±0.003$^{a}$ | 0.2027±0.003$^{b}$ | 0.1767±0.003$^{c}$ | 0.1850±0.004$^{c}$ | 0.1789±0.002$^{c}$ |
| 11. | Leucine | 0.2319±0.011$^{b}$ | 0.2186±0.005$^{b}$ | 0.2777±0.003$^{a}$ | 0.0893±0.002$^{c}$ | 0.067±0.004$^{d}$ |
| 12. | Lysine | 0.3680±0.008$^{a}$ | 0.2674±0.003$^{c}$ | 0.3424±0.006$^{b}$ | 0.2103±0.003$^{d}$ | 0.184±0.006$^{e}$ |
| 13. | Methionine | 0.0743±0.004$^{b}$ | 0.0807±0.003$^{b}$ | 0.1266±0.001$^{a}$ | 0.079±0.002$^{b}$ | 0.0801±0.001$^{b}$ |
| 14. | Phenylalanine | 0.1214±0.001$^{d}$ | 0.1695±0.002$^{b}$ | 0.1415±0.001$^{c}$ | 0.1106±0.004$^{e}$ | 0.9786±0.003$^{a}$ |
| 15. | Proline | 0.0695±0.002$^{b}$ | 0.0464±0.009$^{b}$ | 0.1651±0.036$^{a}$ | 0.085±0.004$^{b}$ | 0.0787±0.003$^{b}$ |
| 16. | Threonine | 0.1822±0.003$^{a}$ | 0.1821±0.004$^{a}$ | 0.1292±0.003$^{c}$ | 0.1864±0.003$^{a}$ | 0.1657±0.004$^{b}$ |
| 17. | Tryptophan | 0.1991±0.008$^{a}$ | 0.1797±0.002$^{b}$ | 0.0848±0.007$^{d}$ | 0.1293±0.002$^{c}$ | 0.0811±0.001$^{d}$ |
| 18. | Tyrosine | 0.1819±0.001$^{a}$ | 0.1555±0.004$^{bc}$ | 0.1423±0.008$^{c}$ | 0.1675±0.003$^{ab}$ | 0.1113±0.003$^{d}$ |
| 19. | Serine | 0.2009±0.004$^{a}$ | 0.1148±0.001$^{a}$ | 0.1551±0.006$^{a}$ | 0.3577±0.264$^{a}$ | 0.0874±0.005$^{a}$ |
| 20. | Valine | 0.1830±0.005$^{c}$ | 0.1555±0.004$^{d}$ | 0.3003±0.009$^{a}$ | 0.2423±0.002$^{b}$ | 0.2250±0.004$^{b}$ |

**Table 7**
Fatty acid composition of *Labeo rohita* muscle fed experimental diets (g/100 g) in dry weight basis. Each value represents the mean±standard error of the mean (n=3) and different superscript letters indicate a significant difference between the groups ($p<0.05$) using one-way Analysis of variance (ANOVA) followed by Duncan's multiple range (DMRT) as post hoc test (SPSS).

| S. No | Fatty acids | **Control** | 25% FMR | 50% FMR | 75% FMR | 100% FMR |
|---|---|---|---|---|---|---|
| 1. | Palmitic acid | 0.098±0.0003$^{a}$ | 0.1094±0.0008$^{a}$ | 0.1235±0.0004$^{a}$ | 0.1599±0.0003$^{a}$ | 0.418±0.1048$^{a}$ |
| 2. | Margaric acid | 0.0318±0.0003$^{d}$ | 0.0388±0.0005$^{c}$ | 0.0379±0.0004$^{c}$ | 0.0427±0.0003$^{b}$ | 0.0553±0.0002$^{a}$ |
| 3. | Stearic acid | 0.1251±0.0006$^{e}$ | 0.1667±0.0006$^{d}$ | 0.2353±0.0002$^{c}$ | 0.2426±0.0004$^{b}$ | 0.3117±0.0003$^{a}$ |
| 4. | Oleic acid | 0.0945±0.0002$^{c}$ | 0.2471±0.0004$^{b}$ | 0.2501±0.0003$^{ab}$ | 0.2486±0.0004$^{c}$ | 0.254±0.0006$^{a}$ |
| 5. | Linolenic acid | 0.343±0.0007$^{c}$ | 0.407±0.0008$^{b}$ | 0.3365±0.0006$^{c}$ | 0.4002±0.0007$^{b}$ | 0.5707±0.0016$^{a}$ |
| 6. | Alpha linolenic acid | 0.8564±0.0023$^{a}$ | 0.6476±0.0014$^{b}$ | 0.8754±0.0036$^{a}$ | 0.8647±0.0033$^{a}$ | 0.8561±0.0026$^{a}$ |
| 7. | Moronic acid | 0.0104±0.0002$^{d}$ | 0.0669±0.0011$^{c}$ | 0.0734±0.0005$^{b}$ | 0.0847±0.0001$^{a}$ | 0.0864±0.0002$^{a}$ |

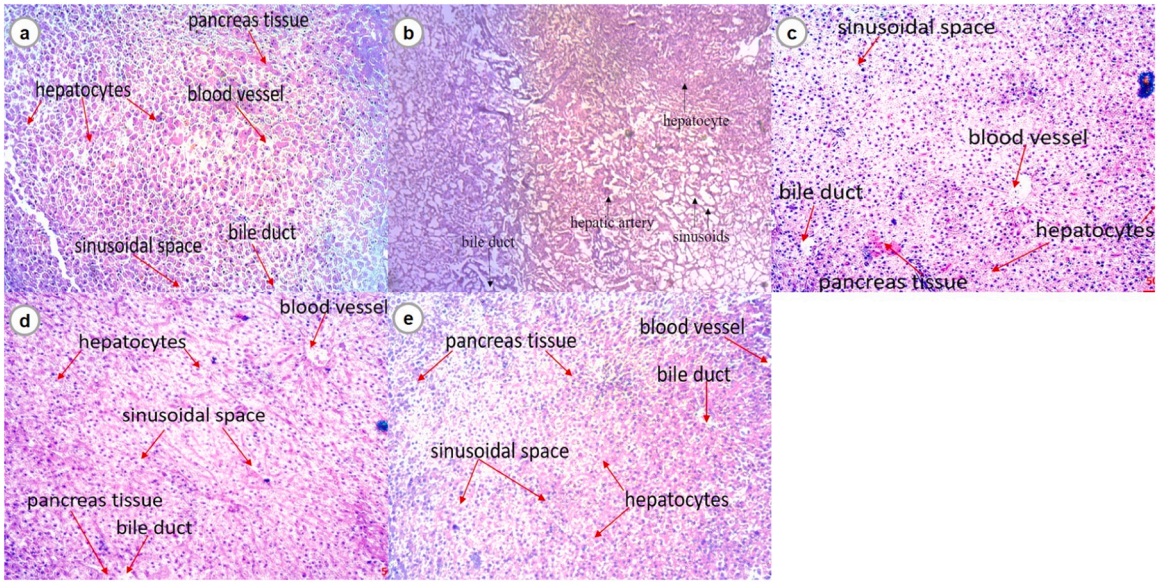


**Fig. 5.** Histoarchitectural analysis of liver of *L. rohita* fed experimental diets on 30th day: a) fish fed with control diet; b) fish fed with 25% FMR diet; c) fish fed with 50% FMR diet, d) fish fed with 75% FMR diet; and, e) fish fed with 100% FMR diet.

of triglycerides (Lin et al., 2018). In this study, fish fed with 100% FMR diet had higher triglyceride levels, which were lower than in fish fed with 50% FMR diet. Similar results were obtained by replacement of fishmeal by earthworm meal in Nile tilapia (Reynaldy et al., 2019). The occurrence of omega-3-fatty acids also decreases the triglycerides level. The related result was observed in hybrid tilapia after the inclusion of *Plukenetia volubilis* (sacha inchi) in their feed (Khieokhajonkh et al., 2021).

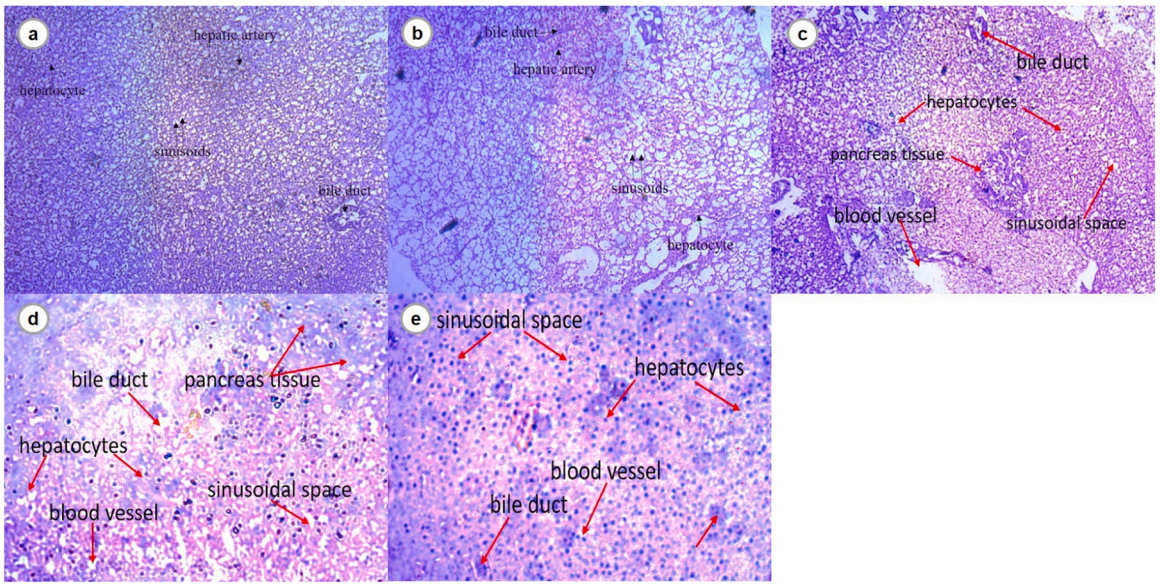


**Fig. 6.** Histoarchitectural analysis of liver of *L. rohita* fed experimental diets on 60th day: a) fish fed with control diet; b) fish fed with 25% FMR diet; c) fish fed with 50% FMR diet, d) fish fed with 75% FMR diet; e) fish fed with 100% FMR diet.

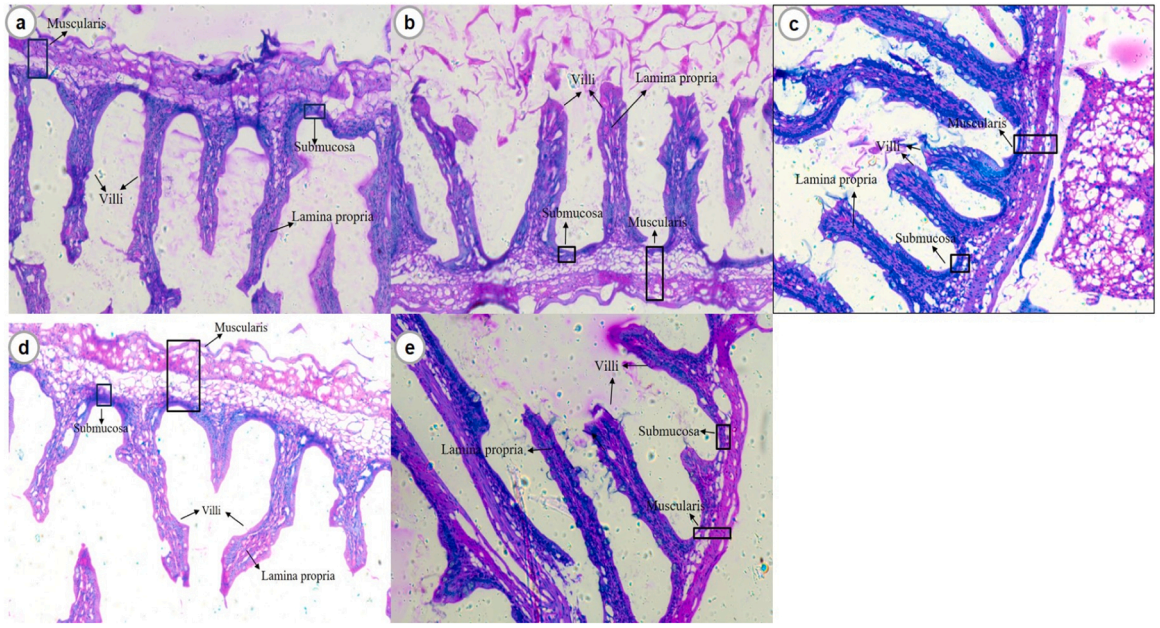


**Fig. 7.** Histoarchitectural analysis of intestine of *L. rohita* fed the experimental diets on 30th day: a) fish fed with control diet; b) fish fed with 25% FMR diet; c) fish fed with 50% FMR diet, d) fish fed with 75% FMR diet; and, e) fish fed with 100% FMR diet.

The alkaline phosphatase (ALP) present in the serum of experimental fish directly indicates the immunological activities (Lallès. 2019). It actively participates in the metabolism and hydrolysis of various phosphate groups (El-Houseiny et al., 2023). This finding indicates that the dietary inclusion of watermelon rind up to 50% improves the immunity of experimental fish. A study carried out in *L. vannamei* by replacing fishmeal with dried distiller's grains shows a similar pattern, in that within the level of 8% replacement showed an increased level of alkaline phosphatase activity; however, beyond that level, the ALP was reduced significantly (Gyan et al., 2022).

Analyzing the digestive enzyme activity helps to predict the acceptability of formulated fish feed (De et al., 2015). Amylase catabolizes carbohydrates present in the feed (Zambonino-Infante et al., 2019), whereas protease and trypsin enzymes break down protein into simple amino acids (Santos et al., 2020). In this study, the level of amylase was higher due to the high fibre content in the formulated diet. According to Maiti et al. (2019), *Hygrophila spinosa* leaf was used for the substitution of de-oiled rice bran in diets fed to rohu and reported higher amylase activity in fish fed experimental diets associated to fish fed with control diet. The inclusion of neem seed cake resulted in increased activity of amylase enzyme in *Labeo rohita* (Gopan et al., 2021). The protease activities in fish fed with experimental diets exhibited higher than the control. Partial replacement of fishmeal in the diet of *L. rohita* by aquatic weed *Pistia stratiotes* also resulted in increased activity of protease in the intestinal tract (Nisha and Geetha. 2017). Similarly, feeding the aquatic weed *Ipomoea aquatica* as an alternative to fishmeal in *L. rohita* resulted in increased protease activity (Ali and Kaviraj, 2018). Previous research conducted with *L. rohita* fed with macrophyte and

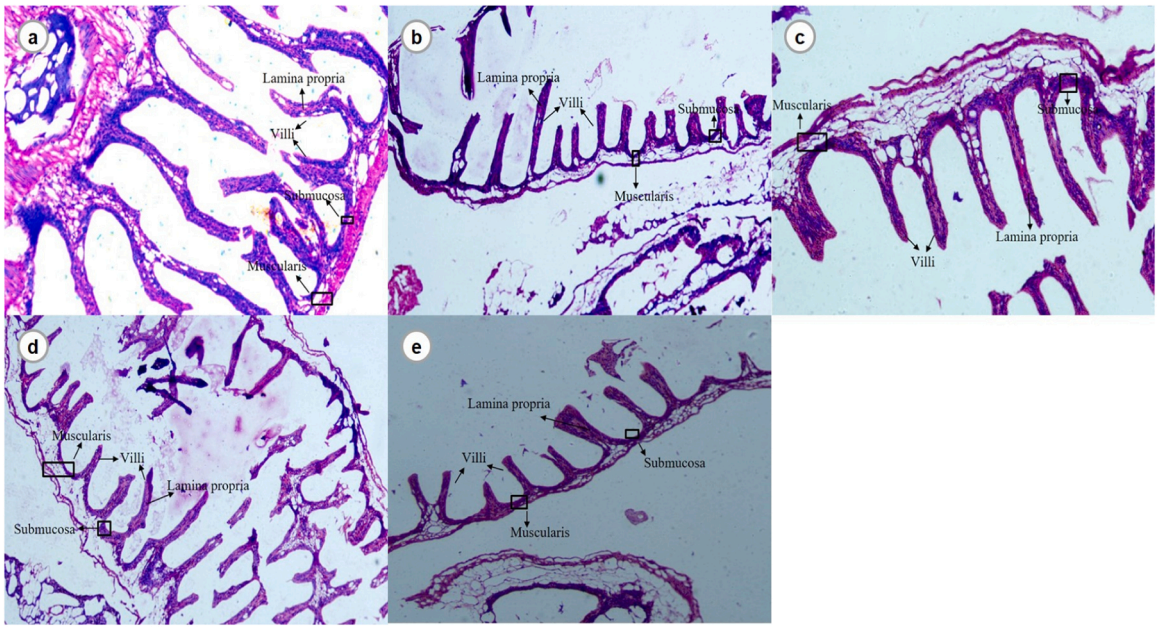


**Fig. 8.** Histoarchitectural analysis of intestine of *L. rohita* fed the experimental diets on 60th day: a) fish fed with control diet; b) fish fed with 25% FMR diet; c) fish fed with 50% FMR diet, d) fish fed with 75% FMR diet; and, e) fish fed with 100% FMR diet.

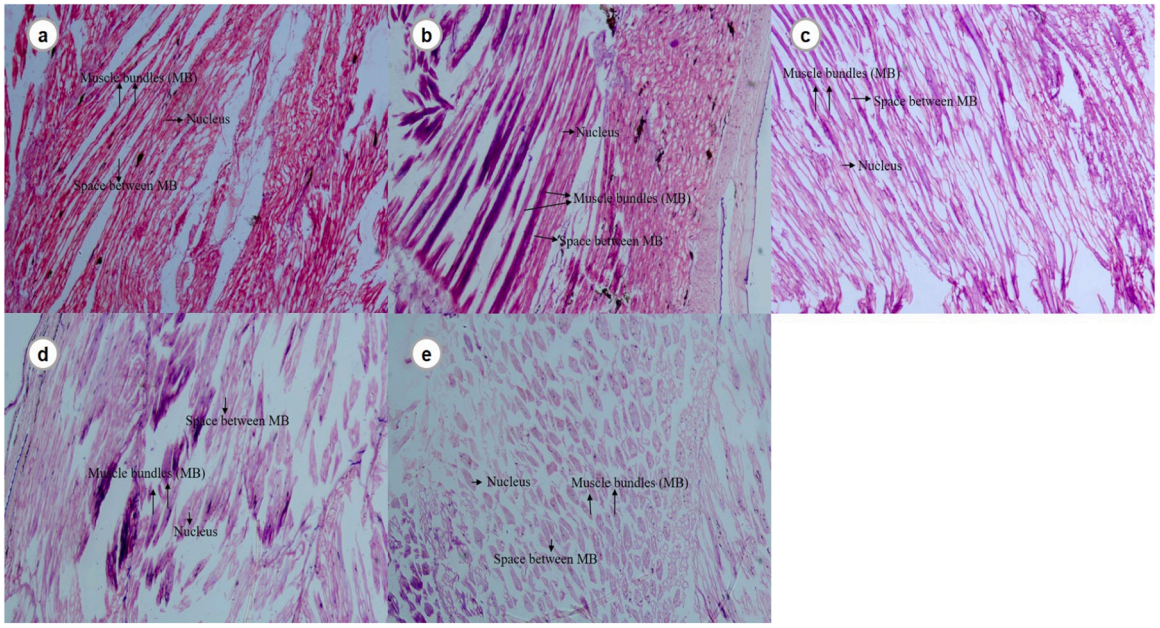


**Fig. 9.** Histoarchitectural analysis of muscle of *L. rohita* fed the experimental diets on 30th day: a) fish fed with control diet; b) fish fed with 25% FMR diet; c) fish fed with 50% FMR diet, d) fish fed with 75% FMR diet; and, e) fish fed with 100% FMR diet.

almond oil cake reported increased trypsin activity in diet containing macrophytes (duckweed) (Goswami et al., 2020).

The antioxidant activity of the fish indicates the scavenging ability of free radicals created during oxidative stress (Sandamalika et al., 2021). The SOD initially breaks down during the ROS elevation, and then CAT works to break down substances like hydrogen peroxide to generate inactive forms. Superoxide dismutase (SOD) acts on superoxide ($O^{2-}$), while others, like catalase (CAT), work on hydrogen peroxide ($H_2O_2$), and glutathione peroxidase (GPx), which scavenges both $H_2O_2$ and lipid hydroperoxides, are known for antioxidant defense mechanisms in fish (Reda et al., 2021). ROS are broken down into a variety of chemicals by the body's innate immune system (Kurhaluk et al., 2021). Another significant and widespread enzyme that is present in most of all living things is catalase (CAT). It acts as a catalyst, converting hydrogen peroxide to water and oxygen. It helps to protect cells from the oxidative damage caused by ROS. The cellular signaling mechanism requires an ideal number of molecules in the cells, in which hydrogen peroxide neutralization helps to maintain oxidative stress-free condition (Nandi et al., 2019). This study suggests that a higher inclusion of WMR content in FMR diets may result in a lower catalase enzyme production in all the experimental diets including control diet fed fish. It clearly indicating that formulated diet using WMR does not creates oxidative stress in experimental fish *L. rohita*.

Oxidative stress in fish is directly influenced by the lipid

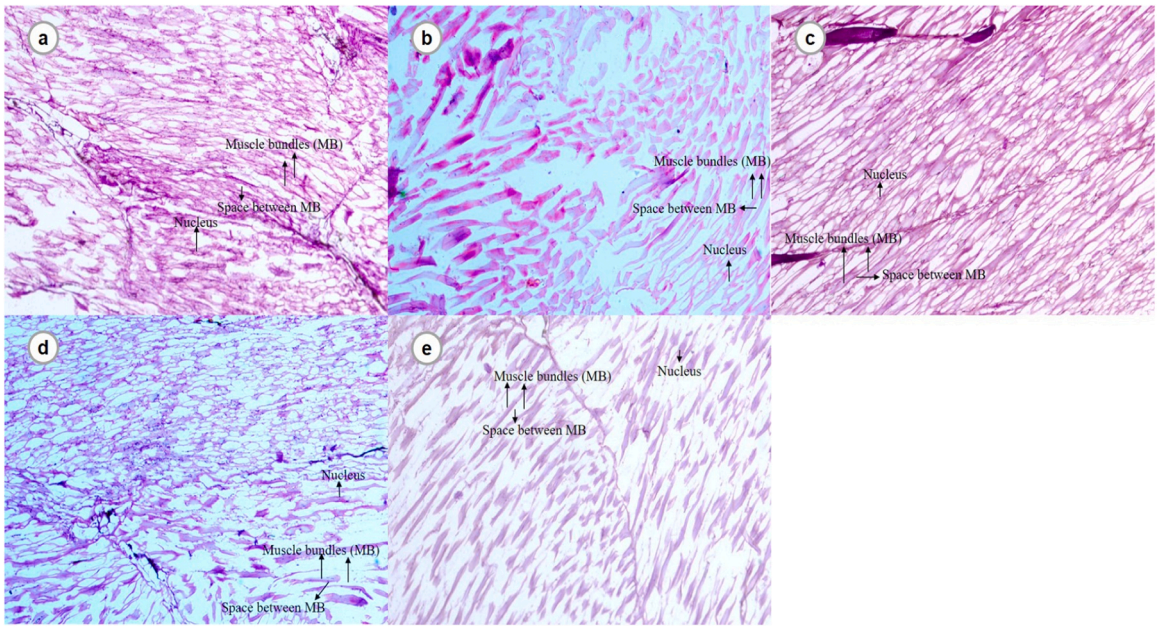


**Fig. 10.** Histoarchitectural analysis of muscle of *L. rohita* fed the experimental diets on 60th day: a) fish fed with control diet; b) fish fed with 25% FMR diet; c) fish fed with 50% FMR diet, d) fish fed with 75% FMR diet; and, e) fish fed with 100% FMR diet.

peroxidation activity (Zengin. 2021). Fish can experience a substantial amount of oxidative stress because of the enhanced lipid peroxidation activity (Teimouri et al., 2019). The oxidative breakdown of lipids found in the cell wall is indicated by the lipid peroxidation activity. The fish flesh quality and flavor deteriorate in an oxygen environment while being stored. Fish omega-3 fatty acids are damaged by increased lipid peroxidation activity, which lowers the meat's quality (Hematyar et al., 2019). Lipid peroxidation is caused by free radicals stripping electrons from the lipid membrane, which harms cells (Habotta et al., 2022). In the current study, it has been confirmed that 50% fishmeal replacement with watermelon rind did not produce oxidative stress to the fish. Lower lipid peroxidation activity suggests improved feed utilization and immunological health status of the fish. The inclusion of dried lemon peel in the diet of Asian sea bass beyond the level of 5% created an increase in the lipid peroxidation activity; up to 3% of lemon peel inclusion did not cause any stress on the fish (Zhuo et al., 2021). The other antioxidant parameters like SOD, CAT, GSH, and GPx were reduced in fish fed with 100% FMR diet compared to fish fed the control diet. These data indicate the experimental feed did not create oxidative stress on the fish.

Thus, the amino acids found in the muscle samples suggest that the major quantity of these amino acids might take part in the fish weight gain (Kabir et al., 2015). Thus, the amino acid concentrations were not to be influenced by dietary intake of watermelon rind. Apparently, the effect of amino acid did not associate or independent to each other**.** The present study agrees with Kari et al. (2023) in which 50% replacement of FMR resulted in better amino acid profile in muscle tissue of African catfish.

Fatty acids are lipids that make up biological membranes. These lipids have an impact on membrane characteristics including permeability, fluidity, the activities of membrane-bound enzymes, and integrity (Eder, 1995). The dietary nutrition profile significantly influences the fatty acid profile of farmed fish (Zhu et al., 2022b). Based on these data, WMR incorporation results in increased palmitic acid, which is a powerful source of saturated fatty acids in the form of triglycerides in *Labeo rohita* (PG et al., 2010). Palmitic acid is an important source of energy storage for swimming, for signal transmission, and pro-inflammatory actions (Korbecki and Bajdak-Rusinek. 2019). Additionally, it increases myostatin levels, which contribute to muscular development (Gao et al., 2023). By directly increasing the content of palmitic acid, the WMR feed promotes the development of the fish. Ingredients like soyabean and ground oil cake in the diet tends to increase the level of fatty acids in the *L. rohita* muscle (Qiu et al., 2017). Oleic acid is a key player, aids in ion transportation processes in the muscle fibres, and influences flavor of fish muscle (Alam et al., 2012). In addition to preventing muscle damage, alpha linolenic acid is also transformed to the healthier fats docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) which lower the risk of cardiovascular disease and support brain function in consumers (Wall et al., 2010; Afridi et al., 2023). Both EPA and DHA did not detected in the muscle samples of the experimental fish suggesting the excessive presence of saturated fatty acids in the diets (Li et al., 2015). Increased HSI and VSI with respect to the inclusion of WMR as the replacement for fishmeal, agrees with the lipid deposition in the respective samples (Siddiqua and Khan. 2022). This study also agrees that the fatty acid composition of the diet directly proportional to related muscle fatty acid composition of *L. rohita* (Afridi et al., 2019).

The liver shows better morphology by normal range of hepatocyte arrangement during the experimental trials. It also indicates that feed causes no pathological changes like damages in the organs leading mortality in the fish (Maftuch et al., 2018). The higher level of watermelon rind powder inclusion in the feed of *Labeo rohita* creates vacuolization. Similar results were obtained by Sulaiman et al. (2022) in hybrid red tilapia *Oreochromis sp*. and Malaysian mahseer, *Tor tambroides* (Bleeker, 1854) and in gilthead seabream indicated that the higher level of fishmeal replacement affects the normal structure of the liver and creates vacuolization.

Inclusion of plant-based ingredients in the fish feed creates certain changes in the basic structure of the intestine. Inclusion of guar meal in the *L. rohita* diet modified results in the presence of sloughed tissue debris (Iqbal et al., 2018), and infiltration of leucocytes into the lumen and lamina propria. An earlier study reported that replacement of soybean with discarded cashew nut in the diet of *Clarias gariepinus* did not create alterations in fish fed with control and 50% diet (Ogueji et al., 2020). There was no histological variation displayed at the muscle sample treated with the formulated diets suggesting that the fish provided with potential nutritional diet (Alami-Durante et al., 2018). Muscle histology revealed a bundle of striated skeletal muscle fibres

with a peripherally located nucleus (Jaffer et al., 2017). Muscle fibres, intramuscular fat and intramuscular connective tissue that play important roles in determining the nutritional value of fish flesh (Listrat et al., 2016). From the present study, the replacement of fishmeal with watermelon rind up to 50% does not create any basic structural alternation in the liver, intestine, or muscle.

## 5. Conclusion

The overall results of the current study revealed that replacement of fishmeal with watermelon rind shows better growth, hematological parameters, serum biochemical, digestive, and antioxidant activity in fish fed with 50% FMR diet. The consumption of fish and its related products play an integral role in food security and the nutritional needs of the population. Consequently, substituting FM with watermelon rind partially (50%) resulted in significant growth performance and composition of its body with improved cost efficiency. Fish fed with 50% FMR diet exhibited better results in all parameters during the experimental trial. Amino acid and fatty acids concentrations were higher in fish fed the FMR diets using WMR. Moreover, phytochemical screening of the watermelon rinds confirmed the presence of phenols, tannins, and flavonoids that may enhance the immunity of the fish. This is the first and most promising result of recycling watermelon rind as the potential dietary ingredient for the partial replacement of fishmeal in diets for rohu. Thus, the study concludes that watermelon rind could be a valuable nutritional source for aquatic feed with relatively low environmental impact.

## Ethical approval

This study was approved by Institutional Animal Ethical Committee (IAEC) Department of Animal Science, Bharathidasan University, Tiruchirappalli – 620 024, Tamil Nadu, India. Ref. No: BDU/IAEC/P18/2021.

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## CRediT authorship contribution statement

**Lekshmi Vijayan**: Investigation, Methodology, Formal analysis, Writing – original draft, Writing - review & editing. **Manikandan Arumugam**: Investigation, Resources, Data curation, Writing- review & editing. **Sivagaami Palaniyappan**: Resources & validation. **Sudharshini Jayaraman**: Resources & validation. **Paul B. Brown**: review & editing. **Zulhisyam Abdul Kari**: Review & editing. **Abdel-Wahab A. Abdel-Warith**: Review & editing. **Elsayed M. Younis**: review & editing. **Thirumurugan Ramasamy**: Conceptualization, Project administration, Supervision, Validation, Visualization, Writing – review & editing.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The data that has been used is confidential.

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