

Original Article

Use of multi-strain probiotics in linseed meal based diet for *Labeo rohita* fingerlings

Uso de probióticos multi-cepas na dieta à base de farinha de linhaça para alevinos de *Labeo rohita*

M. M. Shahzad^{a*}, W. Butt^a, Z. Hussain^a, R. A. Rehman^b, M. K. A. Khan^c, M. Hussain^d, A. Noureen^d, S. Bashir^a, M. Tariq^a, M. T. Rafique^a, F. Khalid^a, H. Rashid^a, K. Akhtar^a and L. Tahir^a

^aUniversity of Education, Division of Science and Technology, Department of Zoology, Lahore, Pakistan

^bUniversity of Health Sciences Lahore, Department of Forensic Sciences, Lahore, Pakistan

^cUniversity of Okara, Department of Zoology, Okara, Pakistan

^dUniversity of Lahore, Institute of Molecular Biology and Biotechnology, Lahore, Pakistan

Abstract

Unavailability of probiotics in fish digestive system fingerlings is unable to digest and absorb their food properly. The current research was conducted to investigate the influence of probiotics added Linseed meal based (LMB) diet on hematology and carcass composition of *Labeo rohita* juveniles. Hematological parameters are essential diagnostics used to estimate the health status of fish. The usage of probiotics for fish health improvement is becoming common due to the higher demand for environment-friendly culture system in water. Linseed meal was used as a test ingredient to prepare six experimental test diets by adding probiotics (0, 1, 2, 3, 4 and 5 g/kg) and 1% indigestible chromic oxide for seventy days. According to their live wet weight, five percent feed was given to fingerlings twice a day. Fish blood and carcass samples (Whole body) were taken for hematological and carcass analysis at the end of the experiment. The highest carcass composition (crude protein; 18.72%, crude fat; 8.80% and gross energy; 2.31 kcal/g) was observed in fish fed with test diet II supplemented with probiotics (2 g/kg). Moreover, maximum RBCs number ($2.62 \times 10^6 \text{mm}^{-3}$), WBCs ($7.84 \times 10^3 \text{mm}^{-3}$), PCV (24.61), platelets (63.85) and hemoglobin (7.87) had also been reported in the fish fingerlings fed on 2 g/kg of probiotics supplemented diet. Results indicated that probiotics supplementation has a critical role in improvement of fingerlings' body composition and hematological indices. Present findings showed that probiotics supplementation at 2 g/kg level in linseed by-product-based diet was very useful for enhancing the overall performance of *L. rohita* fingerlings.

Keywords: LMB, hematology, probiotics, carcass composition.

Resumo

A indisponibilidade de probióticos em alevinos do sistema digestivo de peixes faz com que ele seja incapaz de digerir e absorver seus alimentos adequadamente. A presente pesquisa foi conduzida para investigar a influência de probióticos adicionados à dieta à base de farelo de linhaça (LMB) na hematologia e na composição da carcaça de juvenis de *Labeo rohita*. Os parâmetros hematológicos são diagnósticos essenciais usados para estimar o estado de saúde dos peixes. O uso de probióticos para a melhoria da saúde dos peixes está se tornando comum devido à maior demanda por sistemas de cultivo em água que não agredam o meio ambiente. Farinha de linhaça foi usada como ingrediente para preparar seis dietas de teste experimentais adicionando probióticos (0, 1, 2, 3, 4 e 5 g/kg) e 1% de óxido crômico indigestível por 70 dias. De acordo com seu peso úmido vivo, 5% de alimento eram dados aos alevinos duas vezes ao dia. Amostras de sangue e carcaça de peixes (corpo inteiro) foram coletadas para análise hematológica e de carcaça no final do experimento. A maior composição da carcaça (proteína bruta, 18,72%; gordura bruta, 8,80%; e energia bruta, 2,31 kcal/g) foi observada em peixes alimentados com a dieta teste II suplementada com probióticos (2 g/kg). Além disso, os números máximos de RBCs ($2,62 \times 10^6 \text{mm}^{-3}$), WBCs ($7,84 \times 10^3 \text{mm}^{-3}$), PCV (24,61), plaquetas (63,85) e hemoglobina (7,87) também foram relatados em alevinos alimentados com 2 g/kg de dieta suplementada com probióticos. Os resultados indicaram que a suplementação de probióticos tem um papel crítico na melhoria da composição corporal dos alevinos e índices hematológicos. As descobertas atuais mostraram que a suplementação de probióticos no nível de 2 g/kg em dieta à base de subproduto de linhaça foi muito útil para melhorar o desempenho geral de alevinos de *L. rohita*.

Palavras-chave: LMB, hematologia, probióticos, composição de carcaça.

*e-mail: drmudassarshahzad@gmail.com

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1. Introduction

Aquaculture is one of the prime food sectors worldwide that represents the novel source of animal protein for the constantly growing population (Dawood and Koshio, 2016). World output in 2012 included 91.3 million tons of commercial fishing in the wild and 158 million tons by aquaculture (FAO, 2015). World aquaculture fish production was 31.1% in 2004, 42.1% in 2012 and in 2014 was 44.1%, making aquaculture the world's fastest-growing food sector (FAO, 2016).

In aquaculture, fish meal (FM) is a chief source of animal protein, enriched with nutrients i.e. amino acid, vitamins, essential fatty-acids, minerals etc. (Zhou et al., 2004). FM has been served as the significant protein source in aqua feed of carnivorous fish, which tends to possess higher dietary protein requirements (Hardy, 2010; Kumar et al., 2008; Wang et al., 2015). Increasing demands and decreased accessibility with higher cost (about 220 PKR/kg) of the FM pushed the idea to explore other protein sources in fish culture (Pham et al., 2008; Shahzad et al., 2021). Therefore, it is important to explore cost-effective and locally available alternative source of protein for fish feed (Saad, 1979).

In fish diet, plant by-products are the excellent replacer (Liu et al., 2013), and are more economical than FM. Therefore, the substitution of FM with plant by-products is often recommended. Due to the high protein value (Reigh and Ellis, 1992), soybean, cottonseed, Moringa and canola meal were often suggested as possible elective fish feed (Shahzad et al., 2020). In Egypt, the linseed widely called 'Ketan' is commonly grown and considered an oil plant. China is expected to produce approximately 22% of linseed oil (Li et al., 2016). After oil extraction, the by-product known as linseed meal (about 40 PKR/kg) is used particularly in rural areas to feed young cows and calves. There is less detail on the use of linseed as an origin of protein in feeds for aquatic animals. Therefore, this research is conducted to assess the viability of linseed meal as a potential partial and total substitute of FM protein in diets for *L. rohita* fingerlings.

L. rohita is generally called Rohu and is cultivated in Asia, mainly in the Indian subcontinent (Khan et al., 2004). It is an inhabitant of the Indo-Gangetic river and raised successfully across northern and central rivers in Pakistan (Reddy, 1999). Among seven fish species that are most prominent, delicious, have great development rate and higher growth on a prepared diet. It also reaches the mark-able size of 800–1000 g in one year. (Khan et al., 2008; Hussain et al., 2011). Due to its numerous great properties and market preference, this fish's cultivation has been increased in recent years (Khan et al., 2004).

Due to high market demand, the stocking culture of *L. rohita* leads to stress (Wang et al., 2015). Stress affects fish's health and contributes to immunosuppression and increased infectious diseases (Awad and Awaad, 2017; Aliko et al., 2018; Gobi et al., 2018). Pathogenic organisms are commonly responsible for disease outbreaks and belong to the genera; *Aeromonas*, *Vibrio*, *Edwardsiella*, *Pseudomonas* and *Streptococcus*. Infection more often occurs in the gastric tract of fish (Sugita et al., 1996;

Defoirdt et al., 2011). These pathogenic bacteria usually cause ulcerations of skin, gills, opercula, tail and blade spoil, dropsy and hemorrhagic septicemia in fish (Austin and Austin, 2007; Kaleeswaran et al., 2011). For the control of these infections, chemical substances and anti-microbials have been introduced (Guardiola et al., 2016; Aliko et al., 2018). However, anti-infection agents are very expensive, and these compounds' utilization also provokes numerous issues (Zhou et al., 2010).

Thus, to cope all these problems, probiotics have been used in aquaculture. It is characterized as "live microbial feed supplement which when administered in sufficient amount, advantageously influences the host by improving microbial balance" (Fuller Afrc, 1989). Extra advantages of probiotics in aquaculture include optimizing the quality of water, health of host species through the production of digestive enzymes, efficient removal of harmful pathogens, increased survival rates and disease resistance (Verschuere et al., 2000). Probiotics are, therefore a good strategy for managing microbial diseases with great potential by replacement of antibiotics. It has been noticed that probiotic mixtures are more efficient in disease control than a single culture (Aly et al., 2008; Ridha and Azad, 2016). Additionally, information about the impact of probiotics on rohu immune system morphology is sparsely present. Therefore, the present study was planned to assess the efficacy of probiotics supplements on the hematology and body composition of *L. rohita* fed on a linseed by-products-based diet.

2. Materials and Methods

2.1. Experimental conditions

Study was conducted under the rules and regulations issues by ethical committee of the university to estimate the impacts of probiotics-supplemented linseed by-product based diet on hematology and proximate composition of *L. rohita* fingerlings.

Fingerlings of *L. rohita* were purchased from Manawa Fish seed Hatchery, Lahore and were kept in fish tanks (V shape) with a 70 L water capacity. Before the start of the experiment, fingerlings were acclimatized to the laboratory condition for fourteen days. During the acclimatization period, fish were fed once a day on the basal diet (Rowland, 1991). Water quality parameters such as dissolved oxygen (DO), pH, and temperature were recorded daily. The oxygen pump was used throughout the study period to supply oxygen from the capillary system. Before the experimental research began, fingerlings were treated with 0.5% saline solution for 1 to 2 minutes to kill the pathogens (Rowland and Ingram, 1991).

2.2. Experimental design

Linseed was used as a test diet and major feed element. One control and five test diets supplemented with graded levels (1, 2, 3, 4 and 5 g/kg) of probiotics (1 g=2×10⁹ CFU) were prepared. Five probiotics supplemented and one control LMB diet was given to six groups of fish, stocked in triplicate water tanks, each with 15 fingerlings.

Total feeding trial duration was conducted following a completely randomized design (CRD) for seventy days period. Probiotics supplemented diets were compared with each other and control diet to analyze hematology and carcass composition of fish.

2.3. Pellet formation

Feed ingredients for the experimental diet were bought from the local market (Table 1). The chemical composition of the diet was tested by using standard methods (AOAC, 1995) before the experimental diet formulation. As an inert marker, chromic oxide (1%) was introduced. All feed constituents were blended for 10-15 (ten to fifteen) minutes and fish oil was added slowly. During this, 10-15% distilled H₂O was supplemented to prepare the required texture and feed pellet were prepared. All diets have been supplemented with the set percentages of probiotics, except control diet (with no probiotics supplementation) and stored at 4 °C until use after drying.

2.4. Analysis of hematological parameters

After 70 days of the experiment, three fish from each tank were selected to collect blood samples and anaesthetized with 150 mg/g methane-sulfonate solution (Wagner et al., 1997). Blood samples were obtained from the caudal vein by heparinized syringe and taken to the laboratory to analyze hematological indices. For the determination of hematocrit, micro-hematocrit technique was used (Brown, 1980). RBCs and WBCs were calculated on an appropriate chamber for Neubauer

counting haemocytometer (Blaxhall and Daisley, 1973). The hemoglobin (Hb) was estimated by a method used by Wedemeyer and Yasutake (1977). Whereas, MCV (mean cell volume), MCHC (mean corpuscular hemoglobin concentration) and MCH (mean corpuscular hemoglobin) were measured by using following Formula 1, 2 and 3:

$$MCV = PCV / RBC \times 10 \quad (1)$$

$$MCH = Hb / RBC \times 10 \quad (2)$$

$$MCHC = Hb / PCV \times 10 \quad (3)$$

2.5. Analysis of immunological parameters

For the examination of immunological parameters, blood samples were obtained without anticoagulant. For the determination of erythrocytes and leucocytes, blood sample smears were prepared. Lymphocytes, eosinophil, monocytes and neutrophils were counted with Neubauer differential counting method. The centrifugation method was used for the separation of serum samples, and these samples were stored in a freezer at -20 °C until analysis.

2.6. Chemical analysis of carcass composition

Fish (three) were randomly selected from each replicate and sacrificed using standard methods to analyze carcass composition (AOAC, 1995). With oxygen-bomb-calorimeter, gross energy in the whole body was assessed. The crude protein was analyzed by Micro Kjeldahl apparatus. The moisture content of the carcass was measured by

Table 1. Ingredients' composition (%) and chemical composition (%) of LMB test diet for *L. rohita* fingerlings.

| Ingredients | Composition of Test Diets | Chemicals Composition of Feed components (%) | | | | | | |
|------------------|---------------------------|--|-------------|--------|--------|--------|--------------|-------------------|
| | | Dry Matter (%) | GE (kcal/g) | CP (%) | EE (%) | CF (%) | Ash (kcal/g) | Carbohydrates (%) |
| Linseed meal | | 92.41 | 3.86 | 33.78 | 6.83 | 2.76 | 6.51 | 50.12 |
| Fish meal | 13 | 91.93 | 3.92 | 45.13 | 8.46 | 1.7 | 21.53 | 23.18 |
| Wheat flour | 13 | 91.81 | 2.8 | 9.69 | 2.27 | 2.68 | 3.49 | 81.87 |
| Sunflower | 14 | 92.03 | 4.11 | 32.68 | 5.74 | 2.54 | 2.43 | 56.61 |
| Rice polish | 12 | 93.59 | 4.08 | 13.59 | 10.09 | 3.9 | 6.83 | 65.59 |
| Fish oil | 9 | - | - | - | - | - | - | - |
| Mineral Premix* | 1 | - | - | - | - | - | - | - |
| Vitamin Premix** | 1 | - | - | - | - | - | - | - |
| Chromic oxide | 1 | - | - | - | - | - | - | - |
| Ascorbic acid | 1 | - | - | - | - | - | - | - |
| Probiotics*** | 0-5 g/kg | - | - | - | - | - | - | - |

****L. acidophilus*, *L. rhamnosus*, *B. lactis*, *B. bifidum*, *Bifidobacterium*, *S. thermophiles*, *L. bulgaricus*; **Vitamin B₁ = 3000 mg; Vitamin E = 30000 IU; Vitamin B₂ = 7000 mg; Vitamin B₆ = 4000 mg; Vitamin B₁₂ = 40 mg; Vitamin D₃ = 3,000,000 IU; Vitamin C = 15,000 mg; Vitamin K₃ = 8000 mg; Vitamin A = 15,000,000 IU; Calcium pantothenate = 12,000 mg; Folic acid = 1500 mg; Nicotinic acid = 60,000 mg; *Manganese = 2000 mg; Sodium = 45 g; Zinc = 3000 mg; Calcium = 155 g; Copper = 600 mg; Iron = 1000 mg; Cobalt = 40 mg; Iodine = 40 mg; Phosphorus = 135 g; Magnesium = 55 g; Selenium = 3 mg. GE stands for Gross Energy, CP stands for Crude Protein, EE stands for Ether Extract and CF stands for Crude fiber.

oven drying the sample at 105 °C for 12 hours. Petroleum ether extraction method through Soxtec system was used to analyze the crude fat while, crude fibre was determined after digestion with 1.25% H₂SO₄ and 1.25% NaOH, as a loss on ignition of dried lipid-free residues. Ash was determined at 650 °C for 12 hours at constant heat in the electric furnace (Eyela-TMF 3100) Total carbohydrates were calculated using the following Formula 4.

$$\text{Total carbohydrates (\%)} = \left(\frac{100 - \text{moisture} + \text{protein \%} + \text{fat \%} + \text{crude fibre \%} + \text{Ash}}{\text{fat \%} + \text{crude fibre \%} + \text{Ash}} \right) \quad (4)$$

2.7. Statistical analysis

Data on the composition of carcass and fish hematology was subjected to one-way ANOVA. Variations between means were compared using the Honesty Significant Difference Test by Tukey and were considered significant at *p*<0.05 (Snedecor and Cochran, 1991). For statistical analysis, the CoStat computer software (Version 6.303, PMB 320, and Monterey, CA, 93940 USA) was used.

3. Results

The hematological parameters of *L. rohita* fed on probiotics supplemented linseed by-product based diet are summarized in Table 2. Analysis for hematological parameters revealed variations in fish fed on probiotics

supplemented test diets and control diet. From the results, it was found that when fingerlings fed with test diet 2 (2 g/kg of probiotics) had the highest RBCs (2.62×10⁶mm⁻³), hemoglobin (7.87 g/100 mL), PCV (24.61%) and PLTs count (63.85). These values are statistically different from control and other test diets. Lowest RBCs count (1.12×10⁶mm⁻³), Hb (5.70 g/100 mL) and PCV (21.13%) were observed in fish fed at test diet 5 (5 g/kg of probiotics level), whereas lowest PLTs count (52.64) was observed in fish fed on control diet (0 g/kg of probiotics). MCHC (33.90%) and Ht (31.31%) were highest in fish fed at test diet 3 (3 g/kg of probiotics), while the lowest MCHC (29.59%) and Ht (24.69%) were found in fish fed on test diet 5 (5 g/kg probiotics) and control diet respectively. Maximum values of MCH (48.64pg) and MCV (167.67fl) were noted in fingerlings when fed at 4 g kg⁻¹ of probiotics. On the other hand, minimum values of MCH (37.85%) and MCV (93.50fl) were observed when fingerlings were fed with test diet 1 (1 g/kg probiotics level) while, second-lowest values of MCH (40.31) and MCV (99.60) were observed at control diet. These results were significantly dissimilar from each other (Figure 1). These results showed that *L. rohita* fingerlings' hematological parameters improved with the addition of probiotics in linseed by-product-based diet. Hematological parameters improved with increased level of probiotics up to 2 g/kg of probiotics and decreased after it, as shown in Table 2. Hence it was concluded that 2 g/kg was the optimum value of probiotics that significantly increases the hemato-immunity of *L. rohita* fingerlings.

Table 2. Hematological parameters of *L. rohita* fingerlings fed with probiotics supplemented linseed by-product based diet.

| Test Diets | Probiotics Level (g/kg) | RBC (10 ⁶ mm ⁻³) | Hb (g/100 mL) | PCV (%) | MCHC (%) | MCH (pg) | MCV (fl) | Ht % | PLT |
|--------------|-------------------------|---|-------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|-------------------------|
| Control diet | 0 | 1.30±0.16 ^c | 5.96±0.76 ^b | 24.02±0.45 ^{ab} | 31.29±0.89 ^{bc} | 40.31±0.43 ^c | 99.60±0.92 ^c | 24.69±0.60 ^c | 52.64±0.94 ^c |
| Test diet 1 | 1 | 2.07±0.14 ^b | 7.00±0.39 ^{ab} | 24.05±0.32 ^{ab} | 31.99±0.52 ^b | 37.85±0.56 ^d | 93.50±0.62 ^f | 30.98±0.81 ^a | 61.56±0.74 ^a |
| Test diet 2 | 2 | 2.62±0.22 ^a | 7.87±0.40 ^a | 24.61±0.92 ^a | 32.89±0.65 ^{ab} | 44.29±0.60 ^b | 144.89±0.58 ^d | 26.15±0.73 ^{bc} | 63.85±0.92 ^a |
| Test diet 3 | 3 | 2.40±0.24 ^{ab} | 7.35±0.36 ^a | 23.66±0.46 ^{ab} | 33.90±0.55 ^a | 47.55±0.79 ^a | 165.47±0.82 ^b | 31.31±0.97 ^a | 62.53±1.18 ^a |
| Test diet 4 | 4 | 2.04±0.21 ^b | 6.58±0.40 ^{ab} | 22.91±0.35 ^b | 32.64±0.44 ^{ab} | 48.64±0.94 ^a | 167.67±0.94 ^a | 26.76±0.40 ^b | 58.24±0.80 ^b |
| Test diet 5 | 5 | 1.12±0.18 ^c | 5.70±0.56 ^b | 21.13±0.68 ^c | 29.59±0.77 ^c | 45.29±0.53 ^b | 148.18±0.82 ^c | 26.68±0.78 ^c | 57.14±0.91 ^b |

RBCs = Red blood cells; Hb = Hemoglobin concentration; PCV = Packed Cell Volume; MCHC = Mean Corpuscular Hemoglobin Concentration; MCH = Mean Corpuscular hemoglobin; MCV = Mean corpuscular Volume; Ht = Hematocrit; PLT = Platelets. Means in columns having dissimilar superscripts are statistically different at *p* < 0.05. Data are means of triplicates.

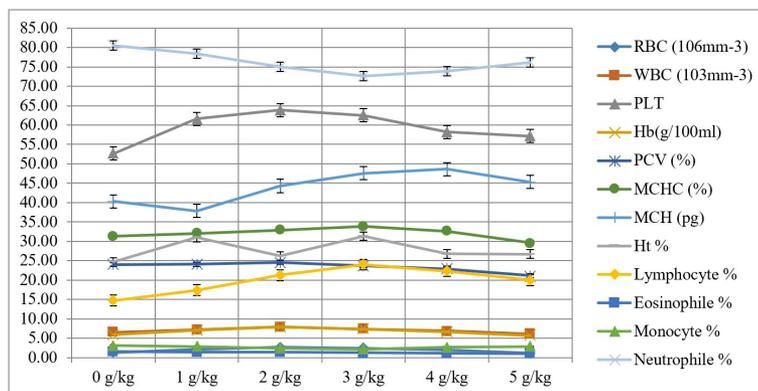


Figure 1. Cumulative hemato-immunological parameters of *L. rohita* fingerlings in different groups; T0 to T5.

Table 3 showed results of immunological parameters of *L. rohita* fingerlings, fed with probiotics supplemented linseed by-product based diet. Immunological parameters included lymphocytes, eosinophil, monocytes and neutrophils. Highest count of WBCs ($7.84 \times 10^3 \text{mm}^{-3}$) was observed in fish fed at test diet 2 (2 g/kg of probiotics), followed by second highest value ($7.34 \times 10^3 \text{mm}^{-3}$) at test diet 3 (3 g/kg of probiotics). Lowest value of WBCs ($6.10 \times 10^3 \text{mm}^{-3}$) was observed at test diet 5 (5 g/kg of probiotics). Second lowest value of WBCs ($6.53 \times 10^3 \text{mm}^{-3}$) was found in fish fed on the control diet. Fish fed on a control diet had the highest count of eosinophil (1.51%), neutrophils (80.58%) and Monocytes (3.18%). In comparison, the lowest (1.04%) value of eosinophil was noted in fish fed on test diet 5 (5 g/kg of probiotics). On the other hand, the lowest count of monocytes (2.16%) and neutrophils (72.63%) was found in fish, fed on test diet 3 (3 g/kg of probiotics). All these values were significantly different from each other. The highest count of lymphocytes (23.97%) was recorded in fish fed on test diet 3 (3 g/kg of probiotics), followed by second highest (22.28%) in fish fed on test diet 4 (4 g/kg of probiotics). Fish fed on control diet had lowest lymphocytes count (14.74%). These variations in different groups showed the usage of probiotics in fish gut and body as well. There was no significant improvement in the immunological parameters of fish due to supplementations of probiotics. Results showed that, the control diet (0 g/kg of probiotics) significantly increases the immunological parameters of *L. rohita* fingerlings as compared to other test diets.

Table 4 showed the carcass composition of *L. rohita* fed with probiotics supplemented linseed by-product based diet. As compared to control diet, probiotics

supplemented diets played a significant role in fish body composition by the retention of useful nutrients. Results showed that body composition of *L. rohita* improved with the additions of probiotics in diet. Results showed that maximum value of crude protein (18.72%), crude fat (8.80%) and gross energy (2.31 kcal/g) were found in fish fed on test diet 2 (2 g/kg of probiotics supplementations). Second highest level of crude protein (17.89%) and crude fat (8.38%) were found in the fish fed on test diet 3 (3 g/kg of probiotics) and gross energy (2.20 kcal/g) in test diet 1 (1 g/kg of probiotics). On the other hand, lowest value of CP (12.66%) and crude fat (6.24%) were recorded in test diet 5 (5 g/kg of probiotics) while gross energy (1.14 kcal/g) in test diet 5 (5 g/kg of probiotics). Ash, crude fibre (CF) and carbohydrate concentrations were closely similar in all diets. But, the highest ash (5.94 kcal/g) and carbohydrates value (2.76%) were recorded in fish fed at test diet 3 (3 g/kg of probiotics). On the other hand, lowest level of ash (5.34 kcal/g) was noted in fish of test diet 4 (4 g/kg of probiotics) and carbohydrates (2.37%) in fish fed on test diet 5 (5 g/kg of probiotics). All these values of ash and carbohydrates were non-significant to other diets. Maximum amount of crude fibre (1.25%) was observed in fish fed with 3 g/kg probiotics-added diet. Lowest crude fibre concentration (1.02%) was found in fingerlings fed with test-diet 4 (4 g/kg of probiotics). Moisture content was highest (70.88%) in fish fed on test diet 5 (5 g/kg of probiotics) while minimum (61.01%) at test diet 2 (2 g/kg of probiotics). From the above mentioned results, it was concluded that 2 g/kg of probiotics is the optimum level for enhancing nutrient retention in the body and improving the carcass composition of *L. rohita* as shown in Figure 2.

Table 3. Immunological parameters of *L. rohita* fingerlings fed with probiotics supplemented linseed by-product based diet.

| Diets | Probiotics Level (g/kg ⁻¹) | WBC (10 ³ mm ⁻³) | Eosinophil % | Lymphocyte % | Monocyte % | Neutrophils% |
|--------------|--|---|-------------------------|--------------------------|-------------------------|--------------------------|
| Control diet | 0 | 6.53±0.45 ^{ab} | 1.51±0.29 ^a | 14.74±0.69 ^e | 3.18±0.24 ^a | 80.58±0.73 ^a |
| Test diet 1 | 1 | 7.14±0.73 ^{ab} | 1.45±0.21 ^{ab} | 17.38±0.61 ^d | 2.80±0.27 ^{ab} | 78.37±0.68 ^b |
| Test diet 2 | 2 | 7.84±0.40 ^a | 1.35±0.11 ^{ab} | 21.25±0.60 ^{bc} | 2.41±0.18 ^b | 74.99±0.45 ^{cd} |
| Test diet 3 | 3 | 7.34±0.44 ^{ab} | 1.23±0.16 ^{ab} | 23.97±0.86 ^a | 2.16±0.24 ^b | 72.63±0.93 ^e |
| Test diet 4 | 4 | 6.87±0.32 ^{ab} | 1.14±0.08 ^{ab} | 22.28±0.61 ^{ab} | 2.69±0.29 ^{ab} | 73.89±0.69 ^{de} |
| Test diet 5 | 5 | 6.10±0.64 ^b | 1.04±0.06 ^b | 20.00±0.62 ^c | 2.79±0.22 ^b | 76.18±0.35 ^c |

WBC = White Blood Cells. Means in columns having dissimilar superscripts are statistically dissimilar at $p < 0.05$. Data are means of triplicates.

Table 4. Carcass composition of *L. rohita* fingerlings fed with probiotics supplemented linseed by-product based diet.

| Diets | Probiotics Level (g/kg ⁻¹) | Crude Protein | Crude Fat | Gross Energy (kcal/g) | Ash | Crude Fibre | Carbohydrates | Moisture |
|--------------|--|--------------------------|--------------------------|-------------------------|------------------------|------------------------|------------------------|--------------------------|
| Control diet | 0 | 13.63±0.61 ^{cd} | 6.33±0.39 ^{bc} | 1.21±0.20 ^c | 5.43±0.23 ^a | 1.13±0.09 ^a | 2.37±0.19 ^a | 69.90±0.57 ^a |
| Test diet 1 | 1 | 17.01±0.75 ^{ab} | 7.43±0.63 ^{abc} | 2.20±0.26 ^a | 5.56±0.42 ^a | 1.20±0.13 ^a | 2.62±0.19 ^a | 63.97±1.82 ^c |
| Test diet 2 | 2 | 18.72±0.56 ^a | 8.80±0.57 ^a | 2.31±0.18 ^a | 5.56±0.42 ^a | 1.06±0.12 ^a | 2.70±0.11 ^a | 61.01±0.42 ^d |
| Test diet 3 | 3 | 17.89±0.56 ^a | 8.38±0.38 ^{abc} | 1.93±0.22 ^{ab} | 5.94±0.22 ^a | 1.25±0.07 ^a | 2.76±0.18 ^a | 61.85±0.37 ^{cd} |
| Test diet 4 | 4 | 15.33±0.72 ^{bc} | 7.73±0.50 ^{bc} | 1.57±0.27 ^{bc} | 5.34±0.38 ^a | 1.02±0.14 ^a | 2.51±0.18 ^a | 66.51±0.74 ^b |
| Test diet 5 | 5 | 12.66±0.60 ^d | 6.24±0.56 ^c | 1.14±0.16 ^c | 5.51±0.25 ^a | 1.20±0.08 ^a | 2.37±0.23 ^a | 70.88±0.54 ^a |

Means in columns having dissimilar superscripts are statistically dissimilar at $p < 0.05$. Data are means of triplicates.

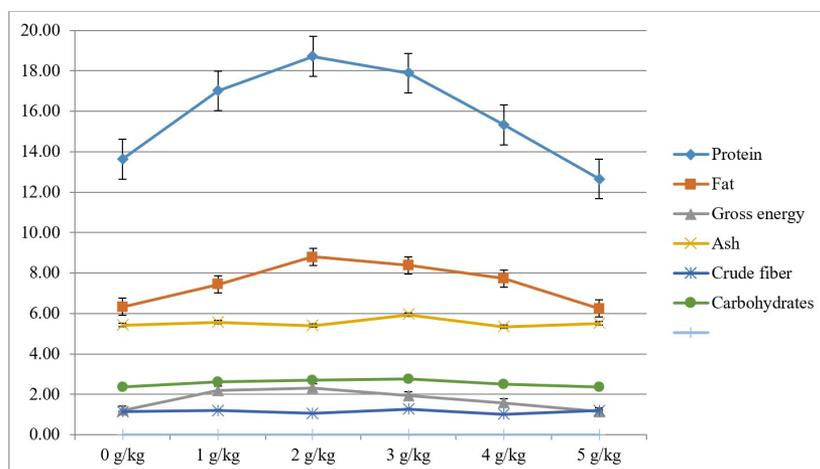


Figure 2. Cumulative carcass parameters of *L. rohita* fingerlings fed with linseed by-product based diet in different groups; T0 to T5.

4. Discussion

Fish meal (FM) is an important protein source and contains various nutrients, i.e. fatty acids, essential amino-acids and several attractants (Dawood et al., 2015). Higher cost of FM shifts attention towards the use of plant by-products protein sources. Due to easy availability throughout the year and low cost, plant by-products are going to be utilized as best alternate source of protein (Yuangsoi et al., 2014). It was revealed in many studies that fish fed with probiotics supplemented diet had a positive effect on health, body composition and disease resistance (Lara-Flores et al., 2003).

To monitor fish's health, hamato-immunological parameters can be used as an important indicator to track changes in fish physiology and pathology (Robertson et al., 2000). WBCs play a vital role in immunity and strengthen fish's efficacy to combat infectious diseases (Weiss and Wardrop, 2010). In this analysis, Fingerlings of *L. rohita* showed highest White ($7.87 \times 10^3 \text{ mm}^{-3}$) as well as Red Blood Cells ($2.62 \times 10^6 \text{ mm}^{-3}$), hemoglobin ($7.87 \text{ g} \cdot 100 \text{ mL}$) PCV (24.61%) and platelets (63.85), when fed with 2 g/kg of probiotics supplemented LSM based diet. These results showed that the fish hematological parameters improve with increased probiotics concentrations up to 2 g/kg of probiotics but decreased with further supplementations. Similar to our results, Hussain et al. (2018) showed that tilapia juveniles indicated the maximum number of Red ($3.17 \times 10^6 \text{ mm}^{-3}$) and White Blood Cells ($8.18 \times 10^3 \text{ mm}^{-3}$) count, fed on diet supplemented with 3 g/kg of probiotics. Rajikkannu et al. (2015) reported improvement in RBCs ($4.48 \times 10^6 \mu\text{l}^{-1}$) of common carp and raho fingerlings fed with soybean meal-based diet supplemented at 10^7 CFUg^{-1} level of probiotics. Soltani et al. (2019) also claimed that, an increase in RBCs was seen in fish fed with probiotics. Diet with probiotics supplementation enhances the immune system by increasing macrophage (Hoseinifar et al., 2018) and WBCs (Korkea-Aho et al., 2012) in the blood. *L. plantarum* supplemented diets for *C. gariepinus* increase the Hb, RBCs, PLT, and PCV compared with control diet (Falaye et al., 2017). Olalusi et al. (2014)

observe improvement in WBCs with higher concentrations of probiotics. Contrary to our results, Opiyo et al. (2019) noted that RBC and WBC levels were significantly higher in fish fed on test diet 5 (10 g/kg of probiotics).

In the present study, the highest concentration of MCHC (33.90%) and Ht (31.31%) were recorded in fish fed probiotics supplemented diet 3 (3 g/kg). Like our results, Martins et al. (2008) also analyzed improvement in numbers of thrombocytes, lymphocytes and hematocrit % in *O. niloticus* fingerlings after addition of probiotics. Similarly, Hussain et al. (2018) described that fingerlings fed with (2, 3, and 4 g/kg levels) probiotics addition had highest values of hemoglobin in contrast to fish that were fed with control diet. In *O. niloticus* fingerlings fed on diets having *Bacillus subtilis* and *B. cereus*, there was an increase in nutrient retention, resulting in improved body composition (Mello et al., 2013). While Salinas et al. (2008) documented that by using two specific strains of bacteria, such as *Lactis delbrueckii* and *B. subtilis*, had better influence on blood cell counts than a probiotic mono-strain and found higher PLT count when thaila fed on 3 g/kg of probiotics supplemented diet and lowest concentration of MCH (37.8pg) in fish was noted when fed on test diet 1 (1 g/kg probiotics level). Fish fed on control diet (without probiotics) had lowest Ht level (24.69%). Rajikkannu et al. (2015) reported significant improvement in Hb in *L. rohita* fed 10^7 CFUg^{-1} level of probiotics. Contrary to our results, higher hemoglobin was found in thaila fed with 3% (0.3 g/kg) of probiotics supplemented plant meal based diet (Krishnaveni et al., 2013). When fish juveniles fed on a diet having live *B. subtilis* as well as *Saccharomyces cerevisiae* and dead *S. cerevisiae*, showed significant ($P < 0.05$) improvement in the hematocrit level when compared to fish fed on the control diet (Firouzbakhsh et al., 2011). Rajikkannu et al. (2015) also discussed statistical improvements in Ht in raho fed 10^7 CFUg^{-1} level of probiotics. Contrary to our results, Dias et al. (2012) claimed that probiotics supplemented groups' hematocrit values showed a significant decrease compared with the control group. Our research indicated

that the highest MCV (167.67fl) was noticed in fish fed on test diet 4 (4 g/kg probiotics) as compared to other diets. Contrary to the present study, the highest MCV in fish fed at 3% (0.3 g/kg of probiotics) based diet was recorded (Krishnaveni et al., 2013). Therefore in the current study, enhancement in hematological indices of the fingerlings fed probiotic supplemented diets indicates the positive role of probiotics in fish body. Variations in these different studies are may be due to different probiotic strains, feed ingredients, lab conditions or feed preparation methods.

Results showed that the carcass composition improved with the addition of probiotics. In present study maximum crude protein (18.72%), crude fat (8.80%) and gross energy (2.31 kcal/g) contents were found in fish fed test diet 2, while minimum crude protein (12.66%), gross energy (1.14 kcal/g) and crude fat (6.24%) were analyzed in fingerlings fed with test diet 5 (5 g/kg of probiotics). Results close to present work were described by Hussain et al. (2018). They found highest level of gross energy (1.94 kcal/g) in fingerlings fed on diet supplemented with 2 g/kg probiotics, while maximum crude fat (10%) and protein (17%) were observed in the fingerlings fed on test diet 3 (3 g/kg probiotics). But there was a decline on higher levels in the absorption of nutrients in the fish body fed on canola meal based diets. In comparison, higher fat (30%) and protein contents (15%) were recorded in common carp fingerlings fed on 1 g/kg probiotic supplement (Mazurkiewicz et al., 2005). Improved body composition (18% lipid and 48% protein) of *C. carpio* fingerlings was discussed when they fed on probiotics (0.5 g/kg) supplemented diet (Dhanaraj et al., 2010). Azarin et al. (2015) described that all supplemented test diets groups had significantly higher crude protein at a 1.2×10^9 CFU/g-1 level of dietary *B. licheniformis* and *B. subtilis* compared to control. Highest lipid contents were found when tilapia fed a diet supplemented with probiotics at 10^7 CFU/g (Essa et al., 2004). Contrary to present results, fish fed on control diet had higher fat contents than fingerlings fed with an experimental diet (Bagheri et al., 2008).

In present study, maximum carbohydrates concentration (2.76%) was presented in fish fed on test diet 3 (3 g/kg of probiotics) and minimum (2.37%) in fish supplemented on the control diet. On contrary, Ibrar et al. (2017) described that the carbohydrate content of the control group was substantially higher ($P < 0.05$) than that of the probiotic *G. candidum* group. This variation in results could occur due to probiotics type and certain environmental conditions. In the present work, ash content was improved in all diets but was found highest (5.94Kcal/g) in fish fed on test diet 3 (3 g/kg of probiotics). Sahandi et al. (2017) described that probiotics supplementation increased the ash content and were highest at 3×10^7 CFU/g as compared to other diets. Contrary to present findings, Amir et al. (2018) observed the highest ash content in fish fed on control diet compared to probiotics supplemented diet. Ibrar et al. (2017) said that the overall ash content of fingerlings fed on control diet was substantially higher ($P < 0.05$) relative to the probiotic supplemented diet of fingerlings during experimental trial.

Moisture level was highest (70.88%) in fish fed on test diet 5 (5 g/kg of probiotics) in present study. Similar results

were also observed by Grisdale-Helland et al. (2008), who found improvement in moisture content due to probiotics supplementation. Contrary to our work, Amir et al. (2018) observed that moisture content was higher in fish fed on a control diet than probiotics supplemented diet. Mirghaed et al. (2018) claimed that fermentable fibre (Vitacel®), probiotic (PrimaLac®) and their combination does not affect on the moisture level of the fish body. Contradictions may occur due to certain environmental factors and differences in plant meals.

5. Conclusion

The present studies recommended that probiotics supplementation at 1 g kg^{-1} to 5 g kg^{-1} level incorporated in the plant-based diets caused changes in the hematology and carcass composition of fish. The outcome from this work demonstrated that 2 g/kg of probiotics supplemented LMB diet is suitable to significantly enhance the hemato-immunity and carcass composition of *L. rohita* fingerlings.

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