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# Improvement in Body Composition and Blood Parameters of *Catla catla* Fingerlings by Supplementing Rapeseed Meal Based Diet with Probiotics

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**Key words**
**Meal based diet, Probiotics, *Catla catla*, Hemato-immunity, Carcass composition**

## ABSTRACT

The current experiment was conducted to find the effect of probiotics on haemato-immunity and proximate composition of *Catla catla* fingerlings fed on rapeseed meal-based diet (RSM). Probiotics enhance the effectiveness of feed utilization, compete against pathogens, and provide resistance against diseases. Six experimental diets were formulated by utilizing an RSM-based meal supplemented with graded levels of probiotics such as 0, 1, 2, 3, 4, and 5 g/kg. For the experiment, triplicate tanks were utilized and in each tank 15 (avg. wt. = 6.76 g) fingerlings were stocked. Fish fingerlings were fed at the rate of 2 to 4% of live wet weight, the diet was given to the fingerlings twice a day for 10 weeks. The hematological profile of an animal is a reflection of its immunological status. Results showed significant improvement in the hematological indices and proximate composition of *catla* fingerlings. The highest carcass composition (CP 18.84%, CF 9%, GE 2.44 kcal/g) was found in fish fed on test diet III supplemented with 2g/kg of probiotics. Similarly, counts of RBCs (2.75 $10^6$ $mm^{-3}$), WBCs (7.87 $10^3$ $mm^{-3}$), Hb (8 g/100ml) as well as PLT (64.68) were highest in fish fed on the 2g/kg probiotics supplemented test diet. While the lowest carcass composition and blood parameters were observed in the fish fed on control and 5g/kg probiotics supplemented RSM-based diet. However immunological parameters (monocytes, neutrophil, lymphocyte and eosinophil) are higher in fish that were fed on control, 1, 3 and 5 g/kg of probiotics respectively. Based on these recordings it was concluded that probiotics supplementation at 2g/kg in an RSM-based diet was very helpful for maximum performance of *C. catla* fingerlings in contrast to the control and other test diets.

## INTRODUCTION

Aquaculture is a rapidly emerging food-producing sector and is becoming the main source of protein-rich food for humans (Msangi *et al*., 2013). Currently, aquaculture is facing a lot of problems, and feed is one of them, which limits profitability. Fish feed costs approximately 50-60% of total expenses in aquaculture production (Essa *et al*., 2004). Fish meal (FM) plays an essential role in the formulation of fish diet and is reported to have good nutrient digestibility, high protein contents, balanced essential amino acid but anti-nutritional factors (ANFs) in traces are also present (Daniel, 2018). But due to the dwindling supply of FM and the highest cost, it cannot cope up with the increasing demands of an ideal source of protein for feed formation, so we have to use alternatives to FM (Merrifield *et al*., 2010; Sheikhzadeh *et al*., 2012). Some researchers have shown that the replacement of FM with plant-based protein sources has proven to be beneficial when they are given under certain nutritionally balanced conditions (Daniel, 2018).

Plant-based proteins have positive effects on nourishment, utilization of nutrients, development, retention of protein, digestibility, nutrients bio-availability, variations in biochemical compositions, quality of flesh, resistance, and stress responses of fish (Li *et al*., 2016; Shahzad *et al*., 2020, 2021). Various plant-based ingredients are used as the alternative to fish meal on a trial basis. Among these ingredients, RSM, corn gluten,

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linseed meal, moringa by-products, soybean meal, and guar meal are used widely for research purposes (Morales *et al*., 2013). Products in RSM like sinigrin and phenolic compounds which are involved in antioxidant activities have positive effects on the health of fish (Alashi *et al*., 2014; Mazumder *et al*., 2016). Anti-nutritional factors of RSM have some destructive effects such as reduced feed digestibility and retard growth (Wu and Muir, 2008). To increase its efficacy, we use it along with probiotics. Probiotics perform many functions, such as enhancing the effectiveness of fish feed, competing against pathogens, providing resistance against diseases, increasing growth performance, enhancing nutrient digestibility, reducing water pollution, and improving the quality of water (Tuan *et al*., 2013). Due to the fast growth and high demand of Indian major carps mainly *C. catla* is the utmost preferred farm fish in Asia (FAO, 2013). Among them, *C. catla* is a famous aquatic fish in Asia. It is most abundant in Pakistan and cultivated with other major Chinese carps (Lone *et al*., 2009). The current research was carried out to find the optimal level of probiotics supplemented RSM-based diets for improvement in hemato-immunity and carcass composition of *C. catla* fingerlings. The research was carried out to identify the influence of rapeseed meal by-product-based diet supplemented with probiotics on hemato-immunity and carcass composition of *C. catla* fingerlings.

## MATERIALS AND METHODS

Analysis for experimental work was performed in the Fish Nutrition Lab, Department of Zoology, University of Education, Lahore.

### *Fish and their maintenance*

*C. catla* or thaila fingerlings were acclimatized for two weeks prior to the beginning of the experiment. Fingerlings were kept in 70L water capacity V-shaped water tanks, were fed once a day on a basal diet during the acclimatization period. Throughout the experimental period, the air pump was utilized for supplying air through the capillary system. Before starting the trial, fish fingerlings were washed for 60-120 seconds with 0.5% brine solution to kill parasites, if they exist (Rowland and Ingram, 1991).

Fingerlings of *C. catla* were fed two times a day. The surplus feed was cleaned from all tanks, by opening dedicated valves in each of them. The tanks were washed entirely to clean the uneaten feed and replenished with tap water.

### *Formation of feed pellets*

Constituents of diet formulation of *C. catla* fingerlings (Table I) examined by following standard methods (AOAC, 1995) were homogenized by addition of 10-15% distilled water to form appropriate textured dough. A pellet machine was used to prepare fish-food pellets (Lovell, 1989). One control and five experimental diets with graded levels (0, 1, 2, 3, 4, and 5 g kg$^{-1}$) of probiotics (Table I) sprayed on each trial diet were prepared. The control diet was also sprayed with the same quantity of $H_2O$ (without probiotics supplementation) to preserve an equal amount of moisture. Until the time of utilization, all sprayed diets after drying were stored in air-tight jars and set aside at 4°C.

**Table I. Ingredients (%) of rapeseed meals based test diet for *Catla catla* fingerlings.**

| Ingredients | Test diets composition (%) | Chemical composition (%) of fish feed ingredients | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Dry matter (%) | Gross energy (Kcal/g) | Crude protein (%) | Ether extraction (%) | Crude fiber (%) | Ash (%) | Carbohydrates (%) |
| Rapseed meal | 34 | 92.87 | 3.73 | 33.67 | 6.72 | 2.64 | 6.39 | 50.58 |
| Wheat flour * | 12 | 91.63 | 2.69 | 9.78 | 2.18 | 2.79 | 3.32 | 81.93 |
| Soybean | 16 | 91.84 | 4.23 | 32.54 | 5.96 | 2.41 | 2.31 | 56.78 |
| Rice polish | 12 | 93.71 | 4.19 | 13.67 | 10.17 | 3.78 | 6.91 | 65.47 |
| Fish meal | 14 | 92.07 | 3.81 | 44.92 | 8.62 | 1.59 | 21.45 | 23.42 |
| Fish oil | 8 | - | | | | | | |
| Vitamin premix** | 1 | - | | | | | | |
| Chromic oxide | 1 | - | | | | | | |
| Mineral premix*** | 1 | - | | | | | | |
| Ascorbic acid | 1 | - | | | | | | |
| Probiotics**** | 0-5 g/kg | | | | | | | |

*Probiotics were used at the expense of wheat flour; ** Vitamin $D_3$ (3,000,000 IU), Vitamin E (30000 IU), Vitamin A (15,000,000 IU), Vitamin $B_1$ (3000 mg), Vitamin $B_2$ (7000 mg), Vitamin $B_6$ (4000 mg), Vitamin $B_{12}$ (40 mg), Vitamin C (15,000 mg), Vitamin $K_3$ (8000 mg), Nicotinic acid (60,000mg), Calcium pantothenate (12,000 mg) and Folic acid (1500 mg); *** P (135g), Na (45g), Fe (1000mg), Ca (155g), Mg (55g), Cu (600mg). **** *Lactobacillus bulgaricus*, *L. acidophilus*, *Bifidobacterium lactis*, *L. rhamnosus*, *B. bifidum*, *Bifidobacterium*, *Streptococcus thermophiles*.

*Experimental design*

Rapeseed meal (RSM) was used as the chief protein source in the test diets for *C. catla* fingerlings. The trial diet was distributed into six sets: one control diet and five trial diets supplemented with graded levels (0, 1, 2, 3, 4, and 5g $kg^{-1}$) of probiotics. Triplicate tanks were used for each treatment. An average weight (6.76±0.18 g) of *C. catla* fingerlings was kept in each triplicate tank. *C. catla* fingerlings were fed on the proportion of 4% of live wet body mass for about 10 weeks. Feeding trial was conducted using completely randomized design (CRD) to compare the fish haemato-immunological indices and carcass composition of control group with other treatments.

*Chemical analysis of the whole body*

After completion of the trial period, blood was taken from three fish from each tank. Later they were sacrificed and desiccated at room temperature. After incubation of homogenized samples at 105°C for 12 h, moisture contents of the experimental carcass were determined. Crude protein (N × 6.25) was analyzed by using the Micro Kjeldahl Apparatus (InKjel M behr Labor Technik GmbH D-40599 Dusseldorf), while by following petroleum ether extraction (EE) method and using Soxhlet system (Soxhlet Extraction Heating Mantels, 250 ml 53868601) the amount of crude fat was determined. Ignition method after digestion of samples was used for the determination of crude fiber contents, while with the help of electric furnace for 12 h at 650°C ash was determined. Total amount of carbohydrates (N-free extract) was found out by difference, i.e., Total carbohydrate (%) =100- (EE % + CP % + Ash % + CF %). For calculating the gross energy oxygen bomb calorimeter was used.

*Haematological study*

Blood of anesthetized fish was collected from the caudal vein with the help of a heparinized syringe. For analysis of the haematological indices samples of blood were taken to the laboratory. Hematocrit was checked using the micro-hematocrit technique capillary tubes (Brown, 1980). For counting RBC and WBC haemocytometer was used with an approved Neubauer counting chamber (Blaxhall and Daisley, 1973). Description by Wedemeyer and Yastuke (1977) was used for determining the Hb (Hemoglobin) concentration. Using the following equations, MCHC, MCH, and MCV were calculated:

MCHC = Hb/PCV × 100

MCV = PCV/RBC × 10

MCH = Hb/RBC × 10

*Immunological study*

For the analysis of immunological parameters, blood samples were collected without anticoagulants. The counts of leukocytes and erythrocytes were determined by making smears of blood samples. Using the Neubauer differential counting method, the counts for lymphocytes, eosinophils, monocytes, and neutrophils were determined. Using the centrifugation method, samples of serum were separated and frozen at -20 °C till analyzed.

*Statistical analysis*

Finally, data of haemato-immunity and carcass composition of fish were subjected to one-way ANOVA by using the CoStat Computer Package. For comparison amongst all treatments, Tukey's HSD test was used and considered significant at P<0.05 (Snedecor and Cochran, 1991).

## RESULTS

It was observed that fish fed on a 2g/kg probiotics supplemented RSM-based diet showed improvement in hematological parameters (Table II). While fish fed on control diet and 5g/kg (Test diet VI) probiotics supplemented diet had the lowest improvement in hematological indices. Results showed that fish fed on a 2g/kg probiotics supplemented diet showed the highest values of red blood cells ($2.75\times10^6mm^{-3}$), white blood cells ($7.87\times10^3mm^{-3}$), platelets (64.68), and hemoglobin (7.99 g/100ml), these were partially different from the values of fish fed with test diet II and significantly different from control and 5g/kg probiotics supplemented diet. Second higher values of RBCs ($2.46\times10^6mm^{-3}$), PLT (62.62), and Hb (7.37g/100ml) were observed in fish fed on 1g/kg probiotics supplemented diet, but WBCs ($7.59\times10^3mm^{-3}$) were found in fish fed with 1g/kg of probiotics (Fig. 1). On the other hand, the lowest numbers of RBCs ($1.12\times10^6mm^{-3}$), WBCs ($5.65\times10^3mm^{-3}$), PLT (51.57), and Hb (5.52g/100ml) were analyzed in fish fed on a diet supplemented with the highest level of probiotics (5g/kg) and these were partially similar with fish fed on control diet (RBCs $1.27\times10^6mm^{-3}$, WBCs $6.51\times10^3mm^{-3}$, and Hb 6.18 g/100ml). The highest values of PCV (25.46%) and Hct (31.63%) were found in fish fed on 4g/kg probiotics supplemented diet following (24.92% and 30.40%) by fish fed on 3g/kg probiotics supplemented diet that were significantly similar with the highest values found in 4g/kg probiotics supplemented fish as shown in Figure 1. Results of MCHC and MCV disclosed that fish fed on test diet IV (3g/kg of probiotics) had the highest values (34.56%) and (155.58%) respectively. On the other hand, fish fed on test diet VI (5g/kg of probiotics) showed maximum value (46.72 pg) of MCH followed by (45.60 pg) in fish fed on 4g/kg of probiotics supplemented diet.

**Table II. Haematological parameters of *C. catla* fingerlings fed on probiotics-supplemented RSM based diet.**

| Diets | TD-I (Control diet) | TD-II | TD-III | TD-IV | TD-V | TD-VI |
|---|---|---|---|---|---|---|
| Probiotic level (g/kg) | 0 | 1 | 2 | 3 | 4 | 5 |
| RBC ($10^6$ mm$^{-3}$) | 1.27±0.21$^{d}$ | 2.46±0.28$^{ab}$ | 2.75±0.23$^{a}$ | 2.19±0.15$^{bc}$ | 1.66±0.16$^{cd}$ | 1.12±0.13$^{d}$ |
| WBC ($10^3$ mm$^{-3}$) | 6.51±0.52$^{bcd}$ | 7.59±0.29$^{ab}$ | 7.87±0.32$^{a}$ | 6.94±0.24$^{abc}$ | 6.07±0.53$^{cd}$ | 5.65±0.40$^{d}$ |
| PLT | 60.58±0.83$^{b}$ | 62.62±0.93$^{ab}$ | 64.68±0.82$^{a}$ | 61.57±0.88$^{b}$ | 57.71±0.80$^{c}$ | 51.57±0.66$^{d}$ |
| Hb (g/100ml) | 6.18±0.23$^{cd}$ | 7.37±0.62$^{ab}$ | 7.99±0.26$^{a}$ | 6.88±0.38$^{bc}$ | 6.37±0.34$^{bcd}$ | 5.52±0.46$^{d}$ |
| PCV (%) | 23.77±0.81$^{ab}$ | 21.94±0.46$^{bc}$ | 23.97±0.56$^{a}$ | 24.92±0.73$^{a}$ | 25.46±0.63$^{a}$ | 21.51±0.81$^{c}$ |
| MCHC (%) | 32.22±0.77$^{b}$ | 31.26±0.71$^{bc}$ | 32.48±0.81$^{b}$ | 34.56±0.88$^{a}$ | 31.83±0.80$^{bc}$ | 29.81±0.37$^{c}$ |
| MCH (pg) | 42.21±0.64$^{cd}$ | 43.47±0.70$^{bc}$ | 40.49±0.81$^{d}$ | 42.32±0.87$^{cd}$ | 45.60±0.85$^{ab}$ | 46.72±0.95$^{a}$ |
| MCV (fl) | 121.37±0.89$^{e}$ | 114.67±0.72$^{f}$ | 130.25±0.87$^{d}$ | 155.58±0.98$^{a}$ | 145.00±0.68$^{b}$ | 136.44±0.72$^{c}$ |
| Hct (%) | 24.19±0.36$^{c}$ | 28.45±0.78$^{b}$ | 28.67±0.91$^{b}$ | 30.40±0.68$^{ab}$ | 31.63±0.74$^{a}$ | 25.45±0.69$^{c}$ |

RBC, red blood cell; WBC, white blood cell; PLT, platelets; Hb, hemoglobin concentration; PCV, packed cell volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; Hct, Hematocrit, TD, test diet. $^{a-e}$ Means within rows having dissimilar superscripts are quietly different at *p<0.05*. Data are means of three replicates with fifteen fingerlings in each.

**Table III. Immunological parameters of *C. catla* fingerlings fed probiotics-supplemented RSM based diet.**

| Diets | TD-I (Control diet) | TD-II | TD-III | TD-IV | TD-V | TD-VI |
|---|---|---|---|---|---|---|
| Probiotic level (g/kg) | 0 | 1 | 2 | 3 | 4 | 5 |
| Lymphocyte % | 16.53±0.68$^{de}$ | 15.72±0.94$^{e}$ | 18.69±0.85$^{d}$ | 23.68±0.75$^{b}$ | 26.69±0.87$^{a}$ | 21.32±0.74$^{c}$ |
| Eosinophil % | 1.47±0.15$^{ab}$ | 1.27±0.13$^{ab}$ | 1.15±0.14$^{ab}$ | 1.38±0.14$^{ab}$ | 1.51±0.18$^{ab}$ | 1.60±0.14$^{a}$ |
| Monocytes % | 3.18±0.31$^{a}$ | 2.71±0.30$^{ab}$ | 2.28±0.14$^{b}$ | 2.06±0.24$^{b}$ | 2.70±0.13$^{ab}$ | 3.05±0.25$^{a}$ |
| Neutrophil % | 78.82±0.74$^{ab}$ | 80.30±1.28$^{a}$ | 77.88±0.96$^{b}$ | 72.88±0.57$^{c}$ | 69.10±0.85$^{d}$ | 74.03±0.58$^{c}$ |

$^{a-e}$ Means within rows having dissimilar superscripts are quietly different at *p<0.05.* Data are means of three replicates with fifteen fingerlings in each.

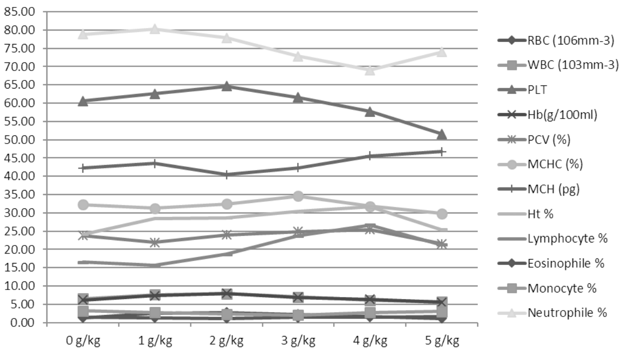


Fig. 1. Cumulative haemato-immunological parameters of *C. catla* fingerlings fed with probiotics in RSM-based diet.

Table III show results of immunological parameters (included lymphocytes, eosinophil, monocytes, and neutrophils) of *C. catla* fingerlings fed on probiotic supplemented RSM-based diet. Fish fed on test diet IV (4g/kg of probiotics) had the highest count of lymphocyte (26.69%) in the blood following (23.68%) in fish fed on a diet supplemented with 3g/kg of probiotics. On the other hand, lowest count of lymphocytes (15.72%) was found in fish fed on test diet II (1g/kg of probiotics) followed by (16.53%) in the fish fed on the control diet that was significantly different from other test diets (Fig. 1). The count of eosinophil was highest (1.60%) in the fish that were fed with 5g/kg of probiotic supplemented diet in comparison to other test diets. The count of monocytes was highest (3.18%) in the fish that were fed on the control diet following (3.05%) by the fish fed with 5g/kg of probiotics supplementation that were significantly similar with each other. On the other side, the lowest (2.06%) and second-lowest (2.28%) counts of monocytes were found in fish fed with 3g/kg and 2g/kg of probiotics supplemented diets, respectively, and were also significantly similar to each other. Fish fed on 1 g/kg probiotics supplemented diet showed the highest count of neutrophil (80.30%) followed by (78.82%) in the fish fed on the control diet. 4 g/kg of probiotics had the lowest count (69.10%) of neutrophils following (72.88%) by the fish fed on test diet IV (3g/kg of probiotics). From these results, it was found that 2g/kg supplementation of probiotics is beneficial for the fish health when they were fed with RSM based diet (Fig. 1).

**Table IV. Proximate composition of *C. catla* fingerlings fed probiotic supplemented oilseed based diet.**

| Diets | Probiotic level (g/kg) | CP % | CF % | GE Kcal/g | Ash Kcal/g | Crude fiber % | Carbohydrates % | Moisture % |
|---|---|---|---|---|---|---|---|---|
| TD-I (Control diet) | 0 | 13.44±0.44[c] | 5.84±0.23[d] | 1.21±0.25[c] | 5.24±0.25 | 1.15±0.14 | 2.59±0.20 | 70.53±0.77[ab] |
| TD-II | 1 | 17.73±0.29[ab] | 8.30±0.77[ab] | 2.20±0.27[ab] | 5.64±0.40 | 1.20±0.11 | 2.46±0.15 | 62.48±0.97[d] |
| TD-III | 2 | 18.84±0.43[a] | 9.19±0.47[a] | 2.44±0.24[a] | 5.66±0.33 | 1.13±0.15 | 2.47±0.16 | 60.26±0.38[e] |
| TD-IV | 3 | 16.73±0.66[b] | 7.68±0.64[bc] | 1.92±0.31[ab] | 5.61±0.22 | 1.14±0.10 | 2.36±0.20 | 64.56±0.86[c] |
| TD-V | 4 | 13.73±0.38[c] | 6.38±0.53[cd] | 1.55±0.16[bc] | 5.20±0.26 | 1.20±0.13 | 2.68±0.19 | 69.27±0.40[b] |
| TD-VI | 5 | 11.36±0.62[d] | 6.09±0.23[d] | 1.11±0.16[c] | 5.44±0.26 | 1.18±0.13 | 2.48±0.25 | 72.33±0.15[a] |

[a-e] Means within column having dissimilar superscripts are quietly different at $p<0.05$. Data are means of three replicates with fifteen fingerlings in each.

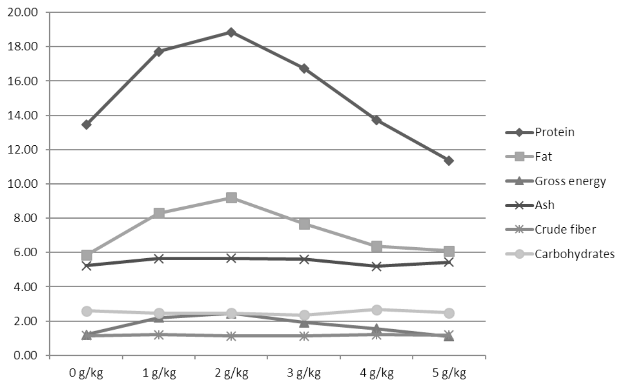


Fig. 2. Cumulative carcass composition of *C. catla* fingerlings fed with probiotics in RSM-based diet.

Table IV showed carcass composition of *C. catla* fed on probiotic supplemented RSM based diet. It showed that fish fed on test diet III had the highest carcass composition. Results showed the maximum values of crude protein (18.84%), crude fat (9.19%), gross energy (2.44 Kcal/g), and ash (5.66 Kcal/g) in the fish fed with 2g/kg of probiotics supplementation level followed by CP (17.73%), EE (8.30%), GE (2.20 Kcal/g) and ash (5.64 Kcal/g) in fish fed on Test diet II (1g/kg of probiotics). On the other hand, minimum values of CP (11.36%) and GE (1.11Kcal/g) were found in fish fed on a diet supplemented with 5g/kg of probiotics, but the lowest value of EE (5.84%) was observed in fish fed on the control diet. These were significantly different from values found on other test diets (Fig. 2). The lowest (1.13% and second-lowest 1.14%) values of crude fiber content were found in fish fed on 2g/kg and 3g/kg of probiotics supplemented diets, respectively. The highest value (2.68%) of carbohydrates was found in fish fed on test diet V (4g/kg of probiotics) following (2.59%) by the fish fed on the control diet. Moisture contents were highest (72.33%) in the fish fed on 5g/kg probiotics supplemented diet following (70.53%) by the fish fed on the control diet as shown in Figure 2. Based on these results, it was concluded that probiotics supplemented diets had a very crucial role in the retention of vital nutrients in the body of the fish. From these supplementary levels, 2g/kg is the most optimum level of probiotics for the maximum improvements in form of hematology and body composition of thaila fingerlings fed on RSM-based diets.

## DISCUSSION

Fish farming has been increased due to the increasing demand for fish as a cost-effective source of animal protein. However, lack of nutritionally balanced feed, due to the non-availability of information on dietary requirements, is a notable obstacle in fish species enhanced intensive cultivation. As a result of this intensive farming, fish are more susceptible to diseases. To cope with this problem, probiotics are added to the fish diet. Supplementation of probiotics in the feed of fish shows that probiotics have a significantly positive effect on the nutrient digestibility of fish as a result overall performance of the fish improves (Lara-Flores *et al*., 2013). It was revealed in many studies that fish fed on probiotics supplemented diet had comparatively better health, tissue composition, and resistance against diseases (El-Haroun *et al*., 2006; Lara-Flores *et al*., 2010, 2013).

Douglass and Janes (2010) verified that in the immune responses, WBC plays a very essential role by increasing animal immunity against pathogens. It was seen in the present study that a low level of probiotics (2g/kg) showed more value of RBCs ($2.75\times10^6$ mm$^{-3}$), WBCs ($7.87\times10^3$ mm$^{-3}$), and platelets (64.68) in the fingerlings of *C. catla*. Nearly similar results noticed by Hussain *et al*. (2018) for *O. niloticus* fed on 3g/kg of probiotic supplemented diet showed a maximum count of RBCs, WBCs, and platelets. Rajikkannu *et al*. (2015) recorded a substantial increase in RBCs ($4.48\times10^6$ µl$^{-1}$) of *L. rohita* and *C. carpio* fingerlings fed on $10^7$ CFUg$^{-1}$ of probiotics.

Putra *et al*. (2020) also observed the highest count of RBCs, WBCs, and activity of phagocytes in the catfish that fed on a probiotic supplemented diet (1x$10^9$ CFU/mL) in comparison to the control diet. Diet with probiotics supplementation enhances the immune system activity by increasing macrophages (Hoseinifar *et al*., 2018) and WBCs (Korkea-Aho *et al*., 2012) in the blood. Tilapia fed with *S. algalactiae* and *P. hypopthalmus* had an increased count of RBCs, WBCs, and activity of phagocytes (Agung *et al*., 2015; Tamamdusturi and Yuhana, 2016). The highest concentration of probiotics (5 g/kg) showed the lowest count of RBCs and WBCs. In contrast to these findings, Krishnaveni *et al*. (2013) observed more count of RBCs in *C. catla* fed with 3% (0.3g/kg) of probiotics. Probiotics at 1 x $10^{10}$ CFU/mL caused a decrease in the counts of RBCs and WBCs in catfish (Putra *et al*., 2020).

In the current study, 3 and 5 g/kg of probiotic supplemented diets have high values of Hb, MCHC, and MCH in the *C. catla*. *G. candidum* ($10^9$ CFUg$^{-1}$) had a positive impact on the hematology of *L. rohita* (Amir *et al*., 2019). Cavalcante *et al*. (2020) recorded no difference in hematological parameters in tilapia fed on a probiotic supplemented diet. Similarly, not significant effects were found in *O. niloticus* fed on a diet supplemented with *B. subtilis* (Soltan and El-L, 2008) and *P. acidilactici* (Ferguson *et al*., 2010). The difference in results may be due to certain infections, dietary imbalance, etc. Diet formulation and replacement of FM with plant meal may also result in variation in hematological and immunological parameters.

For maintenance, growth, and reproduction the fish need a continuous supply of protein. In the current study, it was recorded that fish fed on 2 g/kg of probiotic supplemented diet had the highest possible values of carcass i.e. crude protein (18.84%), crude fat (9.19 %). Similar to our results, 2g/kg of G-pro (*S. cerevisiae*) showed a positive effect on the content of protein (19.43%) and fat (2.43%) in *C. carpio* (Kumar and Keshavanath, 2016). Abdel-Tawwab *et al*. (2008) and Mona *et al*. (2015) observed that the content of protein and lipids were significantly higher in Nile tilapia and African catfish fed on a diet supplemented with *S. cerevisiae*. Hussain *et al*. (2018) noted maximum values of crude protein (17%) and crude fat (10%) in probiotics supplemented diet at 3g/kg in *C. catla*. Nearly similar results were observed by Mazurkiewicz *et al*. (2005) that *C. carpio* fed on a 1g/kg probiotic supplemented diet had the highest protein (15%) and fat (3%). A low-level probiotic supplemented diet (0.5%) had enhanced values of protein (48%), fat (18%), and carbohydrates (23%) in the koi Carp (Dhanaraj *et al*., 2010). Bagheri *et al*. (2008) observed contrast results that control diet had maximum contents of lipids in rainbow trout in comparison to other test diets. Nile tilapia fed on the control diet had maximum content of fats in the body in comparison to test diets supplemented with *B. subtilis* (Hassaan *et al*., 2018). Similarly, Opiyo *et al*. (2019) also recorded a low level of lipids in tilapia fed with the control diet. Hassaan *et al*. (2018) and Merrifield *et al*. (2010) recorded that, probiotics supplemented diets did not affect protein and lipid contents.

In the present study, it was observed that the control and all test diets have a significantly positive effect on the ash, gross energy, crude fiber, and carbohydrates contents of *C. catla*. The highest ash content (5.66 Kcal/g) was present in fish fed on 2 g/kg of probiotics supplemented diet. Ullah *et al*. (2018) also recorded an increase in ash content in probiotics supplemented fed fish. In contrast to it, Bhatnagar and Lamba (2017) observed the lower ash content in fish fed on supplemented with 200 CFU/g of *B. cereus*. The level of ash was highest in the control diet than G-Pro containing experimental diets (Kumar and Keshavanath, 2016). Similarly, Hussain *et al*. (2018) also recorded maximum gross energy in 2g/kg probiotics supplemented diet. In contrast to our findings, Azarin *et al*. (2015) found maximum moisture in fish that were fed on the control diet instead of probiotics-based diets. Oliva-Teles and Goncalves (2001); El-Haroun *et al*. (2006); Saini *et al*. (2014) said that probiotics-based diets did not affect the moisture content of fish carcass. Variation in results may be due to the differences in fish type, probiotics type, and plant meal-type used for experiments.

## CONCLUSION

In summary, results disclosed that probiotics supplementation had a beneficial effect on the carcass and hemato-immunological parameters of fish. Fish that were fed on probiotics supplemented diet showed maximum nutrient retention and best values of hemato-immunological parameters in contrast to the control diet (0g/kg). It was concluded that 2g/kg of probiotics supplementation is the optimum level that significantly enhanced the hemato-immunological parameters and carcass composition of fish fed on RSM based diet.

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*Statement of conflict of interest*

The authors have declared no conflict of interests.

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