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Efficacy of Probiotics Supplementation on Growth Performance, Carcass Composition and Hematological Parameters of *Cyprinus carpio* Fingerlings Fed Corn Gluten Meal-Based Diet

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HIGHLIGHTS

- Maximum improvement in growth parameters was observed in Cyprinus carpio fingerlings fed corn gluten meal based diet supplemented with 2 gkg-1 level of probiotics supplementation.
- C. carpio fingerlings fed with 2 gkg-1 level based diet showed higher values of RBCs, WBCs and platelets whereas 3 gkg-1 level showed second higher concentrations of blood parameters.
- Highest carcass composition was noted when fingerlings were fed with 2 gkg-1 level while the second higher level of these parameters were noted at 3 gkg-1 level based diet.

Abstract: Plant based protein sources are one of the best, cost effective and easily available protein sources being used in fish feed. But due to a lower number of micro-biota in fish gut plant meal based diets cannot be digested and absorbed well in fish body. Probiotics were supplemented at 0, 1, 2, 3, 4 and 5 gkg^{-1} levels in fish feed for formulating one control and five test diets. In this study, three replicates of each treatment

were used and number of fingerlings was 15 in each replicate. The *C. carpio* (common carp) fingerlings were fed at 5% of live wet weight on their prescribed diet twice daily. The results revealed that supplementation of probiotics in corn gluten meal based diets significantly (*p<0.05*) improved growth performance, carcass composition and hematological parameters. Most optimum values of growth performance parameters were noted at 2 gkg^{-1} level of probiotics supplemented diet. *C. carpio* fingerlings fed corn gluten meal based diet supplemented with 2 gkg^{-1} level of probiotics indicated significant (*p<0.05*) improvements in crude protein (17g) crude fat (9g) and gross energy (3 $kcalg^{-1}$) whereas higher red blood cells (RBCs), white blood cells (WBCs) and hemoglobin (Hb) was also recorded in fish blood when fed 2 gkg^{-1} probiotics level diet. From these results, it was concluded that 2 gkg^{-1} probiotics supplementation in corn gluten meal based diet is optimum for improving growth performance, body composition and hematology of *C. carpio* fingerlings.

Keywords: probiotics; body composition; hematology; common carp.

INTRODUCTION

Cyprinus carpio (Common carp) is locally cultured in Asia, Eastern Europe and all around the world. Being a top cultured member of Cyprinids, its annual production range is over 4 million metric tons [17]. It is a freshwater fish and can tolerate a broad range of temperature, pH and salinity. It is one of the most-willingly farmed species in the world and characterized by the fast growth rate and a good source of protein for humans. It is the third most culturable specie in the world representing 71.9% of the freshwater species [1]. Aquaculture industry is providing proteinaceous food to the developing and under-developed countries at the rate of >20 and 50%, respectively [6]. It is the fastest growing food producing sector to fulfill the requirements of protein [18]. Fishmeal is considered as a superior ingredient in fish feed because it has largest portion of protein content, beneficial lipids, essential amino acids, minerals, vitamins and several growth elements [11]. To provide such nutritious feed to the fish, it is favored by fish farmers, but there are inadequate FM resources [35]. To encounter this problem, fish nutritionists are in continuous struggle to find substitutes of animal protein with novel and low-cost plant proteins. Plant derived proteins fulfill nutritional supplies of fish and simultaneously increase fish culture worldwide [28].

One of the major alternative sources is corn gluten meal (CGM), which have high protein contents (60-70% on dry matter basis), very low anti-nutritional factors (ANFs) and high ratio of essential amino acids [27]. It is found highly digestible for many fishes [37,29,22]. Plant meal-based diets are difficult to digest and absorb in fishes as they have smaller number of micro-biota in their gut [15,49]. Probiotics are living microorganisms that may improve the overall fish performance by developing immunity [20] and enhancing digestibility of plant meal-based diets [26]. These micro-organisms help to metabolize the nutrients that cannot be digested by fish without them [23,45]. The microbial breakdown of starch, protein, lipids and cellulose helps in fish digestion process [33,39]. [51] reported that fish growth performance response depends on type of probiotics used in fish feed. Mostly used microbiota as probiotics in freshwater fish species include Plesiomonas, Aeromonas, members of the family Enterobacteriaceae and Fusobacterium [13]. So, this study was carried out to find the optimum level of probiotics supplementation in CGM based diet for improving the growth performance, hematological parameters and carcass contents of *C. carpio* fingerlings.

MATERIALS AND METHODS

This experimental trial was conducted in the Fish Nutrition Laboratory, Department of Zoology, Government College University, Faisalabad. *C. carpio* fingerlings (average weight 7.145±0.045) were purchased for experimental trial from Government Fish Seed Hatchery, Faisalabad and kept in V-shaped water tanks (70 L) which are specifically designed for collection of feces. Fingerlings were acclimatized for two weeks to adjust with the experimental conditions and fed on basal diet [3]. Before starting the trial, fingerlings were treated with NaCl solution ($5gL^{-1}$), to ensure ecto-parasites and fungal free fingerlings [38]. Water quality parameters such as dissolved oxygen (DO), pH, temperature and conductivity were monitored through DO meter (Jenway 970), pH meter (Jenway 3510), thermometer and electrical conductivity (EC) meter (HANNA: HI. 8633) on daily basis. Water quality parameter ranges were maintained as pH 7.4–8.6 dissolved oxygen 5.8–7.3 mgL^{-1}, electrical conductivity 1.30–1.52 dSm^{-1} and temperature 24.9–28.7°C. Capillary system was used for providing (24 h) aeration to all experimental tanks.

Diet formulation and Experimental design

Corn gluten meal (CGM) having 30% protein was used to formulate diets by using varying levels of probiotics (Ecotec®). (Ecotec contains 2.0g of >8 billion CFU). The feed ingredients were procured from commercial feed mill and further analyzed for chemical composition ensuing standard methods [5] before formulating the experimental diet (Table 1). The feed ingredients were ground and then sieved to attain required particle size. Feed ingredients were blended as per formulation (Table 2) for 10 minutes to prepare basal diet and kept on adding fish oil gradually. Commercially prepared Ecotec TM (a multi-strain probiotic) was used in this trial that contains 4 billion CFU (4×10^9 $CFUg^{-1}$) in 1g having standardized freeze-dried cultures of *Lactobacillus delbrueckii* sp, *Streptococcus thermophilus* STY-31, *Bifidobacterium* BB-12 and *Bulgaricus* LBY-27. Probiotics were added at the level of 1, 2, 3, 4 and 5 gkg^{-1} in CGM based basal diet to prepare six test diets. Water (10 to 15%) was added for the formulation of suitable dough [32]. Lab Extruder (SYSLg30-IV Experimental Extruder) was used to prepare floating pellets (3mm). Prepared diets were dried in an oven and stored at 4°C throughout the trial. *C. carpio* fingerlings were fed with test diets in triplicate twice daily. Fifteen fingerlings were stocked in each replicate. Total 18 water tanks and 270 fingerlings were used to conduct this research work. After two hours of feeding time, the remaining uneaten diet was washed out from the tank and filled with fresh water again. Experimental duration was 70 days.

Chemical analysis of carcass

After 70 days, four fish were randomly selected for carcass analysis from each replicated tank, dried in oven at 60ºC, ground and were chemically analyzed. Standard methods were used for analyzing the proximate composition of whole-body samples [5]. The moisture content was analyzed by oven drying process (105°C for 12 hours), while micro kjeldahl apparatus was used for measuring crude protein (N×6.25). Soxhlet system was used for obtaining ether extracts from petroleum ether. Crude fiber was obtained as a loss on combustion of lipid-free dry residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH. Crude ash was determined by ignition in electric furnace (Eyela-TMF 3100) at 650ºC for 12 hours. Gross energy was calculated by an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, USA). Total amount of carbohydrates was calculated by following formula:

$$\text{Total carbohydrates}(\%) = 100 - (\text{crude protein}\% + \text{crude fat}\% + \text{crude fiber}\% + \text{ash}\%) \quad (1)$$

Hematological study

For hematological study, fingerlings were anesthetized by using clove oil (Sigma; 60 mg/L) by dissolving in ethanol was used for anesthetizing fingerlings [12,36]. A heparinized syringe was used for taking blood samples from the caudal vein of fish. For analyzing hematological indices, blood samples were sent to the Molcare Lab of Biochemistry department, University of Agriculture, Faisalabad, Pakistan. Hematocrit was determined with Micro-hematocrit technique by using capillary tubes [10]. White blood cells (WBCs) and red blood cells (RBCs) were counted with a hemocytometer and approved Neubauer counting chamber [9]. Haemoglobin (Hb) was determined by ensuing the method of [50]. To compute mean corpuscular hemoglobin concentration (MCHC); mean corpuscular hemoglobin (MCH) and mean cell volume (MCV) following formulae were used:

$$MCHC = Hb/PCV \times 100 \quad (2)$$

$$MCV = PCV/RBC \times 10 \quad (3)$$

$$MCH = Hb/RBC \times 10 \quad (4)$$

Growth study

Growth parameters (weight gain, weight gain%, FCR and SGR) of *C. carpio* fingerlings were calculated by using standard formulae:

$$\text{Weight gain } (\%) = \frac{(\text{Final weight} - \text{Initial weight}) \times 100}{\text{Initial weight}} \text{Feed conversion ratio (FCR)} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}} \text{Specific growth rate (SGR)} = \frac{(\ln \text{final weight} - \ln \text{initial weight}) \times 100}{\text{No.of days of experiment}} \quad (5)$$

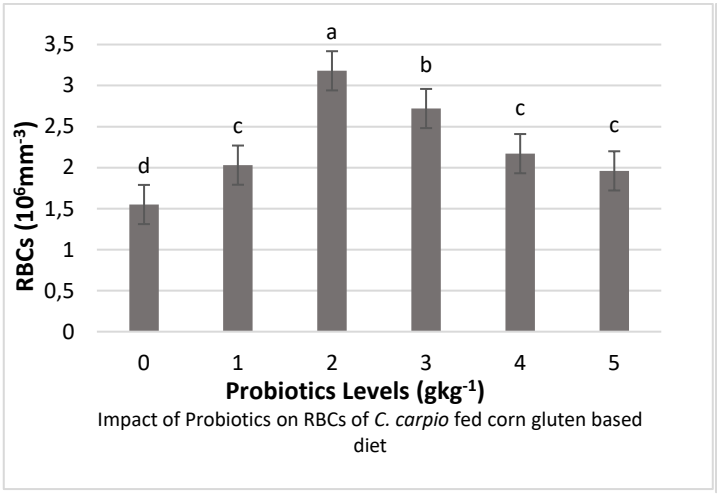
Statistical analysis

The data of growth, hematology and carcass composition were subjected to one-way analysis of variance ANOVA [43]. Differences among means were compared ensuing Tukey's honesty significant difference test and considered significant as $p<0.05$ [42]. For statistical evaluation, Co-Stat computer software (version 6.303, PMB 320 and Monterey, CA, 93940 USA) was used.

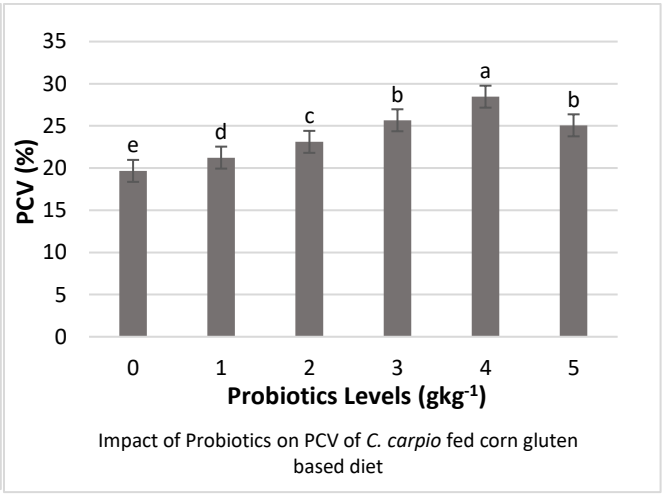
RESULTS

Highest protein, fat and gross energy contents were observed in fish fed 2 gkg^{-1} level of probiotics supplemented diet while the second highest level of these parameters were recorded at 3 gkg^{-1} in comparison to other groups (Table 3). The lowest protein, fat and gross energy values were noted in fish fed with 5 gkg^{-1} level based diet. Minimum ash contents were observed when fingerlings were fed at 2 gkg^{-1} level of probiotics diet and it was statistically alike to ash contents obtained at 3 gkg^{-1} level. The lowest moisture contents were recorded in fish fed 2 gkg^{-1} followed by 3 gkg^{-1} probiotics supplemented CGM based test diet. Whereas, highest values were found in fish fed 5 gkg^{-1} probiotics and it was statistically alike with the control group.

Highest values of RBCs and WBCs were noted when fish fed on 2 gkg-1 probiotic level diet, followed by 3 gkg-1 level that were statistically different ($p<0.05$) from the control group values as shown in figure 1. Lowest values of RBCs and WBCs were found in fingerlings fed at 0 gkg-1 probiotics level (control diet). Highest platelets (PLT) and Hb values were observed in fish fed on 2 gkg-1 probiotics level supplemented diet while the second best value was noted at 4 gkg-1 level (Table 4). These values were significantly ($p<0.05$) different in contrast to values found on other levels (0, 1, 3 and 5 gkg-1 probiotics). The highest values of packed cell volume (PCV) were found in fingerlings fed diet containing 4 gkg-1 probiotics level, while the second best level was 3 gkg-1 (Figure 1). The highest values for MCHC and MCV were recorded in fingerlings fed diet having 3 gkg-1 probiotics level and the lowest values were noted in fish fed at 1 gkg-1 probiotics level.



Impact of Probiotics on RBCs of *C. carpio* fed corn gluten based diet



Impact of Probiotics on PCV of *C. carpio* fed corn gluten based diet

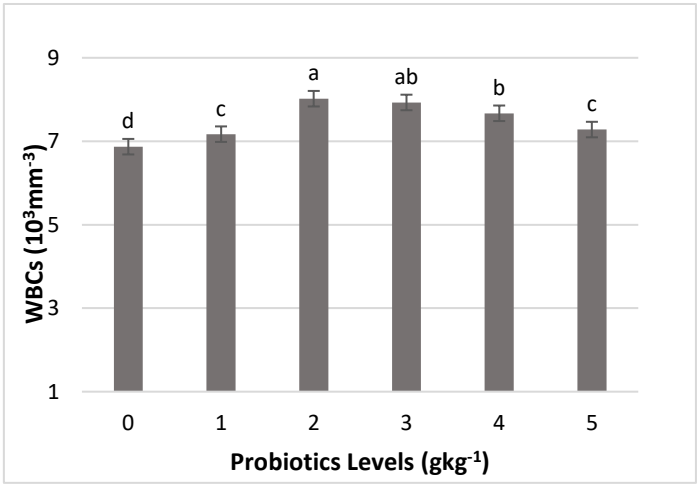


Figure 1. Relationship between probiotics and hematological parameters of *C. carpio* fingerlings fed corn gluten meal based diets

The maximum weight gain and SGR were recorded in fish fed at 2 gkg^{-1} level of probiotics supplemented CGM based diet followed by fish fed at 1 gkg^{-1} probiotics level (Table 5). These values were statistically different ($p<0.05$) from control and other experimental groups. Lowest weight gain, weight gain% and SGR values were recorded at 0 and 5 gkg^{-1} probiotic level. Optimum feed conversion ratio (FCR) and weight gain% of *C. carpio* fingerlings was observed at 2 gkg^{-1} probiotics supplementation level and it was significantly different ($p<0.05$) from remaining test and control diets as presented in Figure 2. Second best FCR value was found in fish fed 1 gkg^{-1} probiotics level whereas poor FCR was found in fish fed 5 gkg^{-1} probiotics supplemented diet and it was statistically alike with the values obtained at control diet.

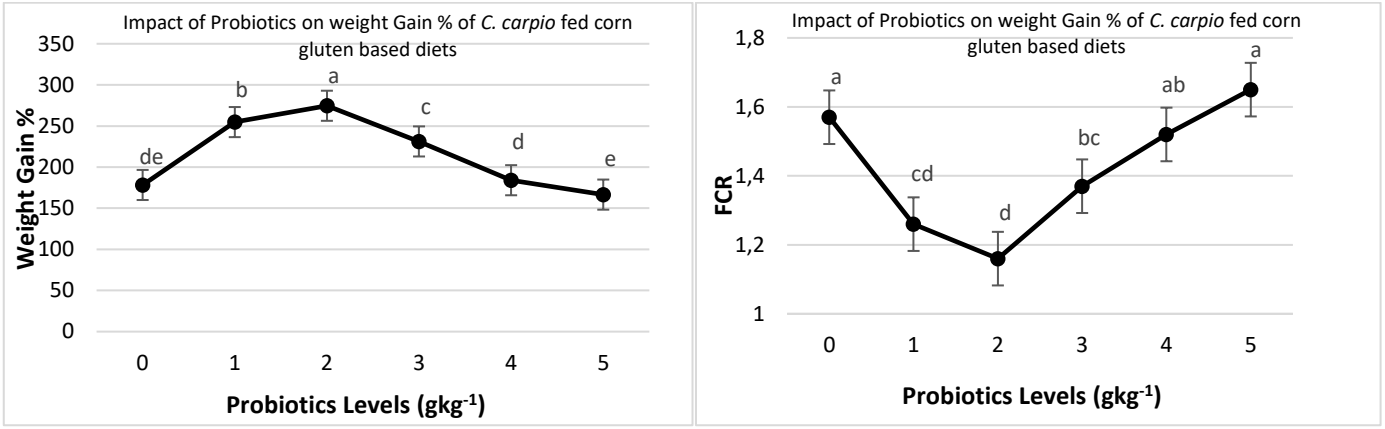


Figure 2. Relationship between probiotics and various growth parameters of *C. carpio* fingerlings fed corn gluten meal-based diets

Table 1. Ingredient composition (%) of CGM based diets supplemented with probiotics

Ingredients	Test Diet-I (Control)	Test Diet-II	Test Diet-III	Test Diet-IV	Test Diet-V	Test Diet-VI
Probiotics (g/kg)	0	1	2	3	4	5
Corn gluten (30%)	50	50	50	50	50	50
Fish meal	15	15	15	15	15	15
Wheat flour	11	10	9	8	7	6
Rice polish	13	13	13	13	13	13
Fish oil	7	7	7	7	7	7
Vitamin Premix*	1	1	1	1	1	1
Mineral Premix**	1	1	1	1	1	1
Ascorbic acid	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1

*Each 100g of Vitamin premix have

Vitamin A	2000,000 IU	Vitamin D	400,000 IU	Vitamin B_1	125 mg	Vitamin E	160 IU
Vitamin B_2	2000 mg	Vitamin K_3	900 mg				
Vitamin B_6	600 mg	Folic acid	200mg	Vitamin B_{12}	3,000 mcg		
Vitamin C	1,000mg	Nicotinic acid	10,000mg	Calcium pantothenate	3,000mg		

**Each Kg mineral granules have

Ca (Calcium)	155g	Mn (Manganese)	2000mg	P (Phosphorous)	135g		
Cu (Copper)	600mg	Mg (Magnesium)	55gm	Co (Cobalt)	40mg		
Fe (Iron)	1000 mg	I (Iodine)	40mg	Zn (Zinc)	3000 mg		
Se (Selenium)	3mg	Na (Sodium)	4mg				

Table 2. Chemical composition (%) of feed ingredients (Dry matter basis)

Ingredients	Dry matter (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)	Carbohydrates (%)	Gross Energy ($kcalg^{-1}$)
Fish meal	91.63	47.25	7.43	1.12	22.56	16.97	4.67
Corn gluten 30%	92.06	31.79	4.97	2.53	1.91	54.46	4.34
Wheat flour	93.04	11.18	2.24	3.09	3.16	77.08	3.25
Rice polish	94.86	12.62	9.98	12.87	11.29	49.65	3.59

Table 3. Carcass composition of *C. carpio* fingerlings fed CGM based test diets supplemented with probiotics

Diets	Probiotics Levels (gkg^{-1})	Protein (%)	Fat (%)	Ash (%)	Gross energy	Moisture (%)
Test Diet-I (Control)	0	11.77 ± 0.29^{d}	5.88 ± 0.15^{d}	7.51 ± 0.28^{a}	1.64 ± 0.08^{bc}	71.95 ± 0.70^{b}
Test Diet-II	1	13.54 ± 0.27^{c}	7.32 ± 0.30^{c}	6.98 ± 0.17^{a}	1.86 ± 0.11^{abc}	69.22 ± 0.19^{c}
Test Diet-III	2	16.55 ± 0.39^{a}	8.88 ± 0.23^{a}	4.93 ± 0.08^{c}	2.20 ± 0.18^{a}	66.31 ± 0.55^{d}
Test Diet-IV	3	15.46 ± 0.40^{b}	7.99 ± 0.22^{b}	5.13 ± 0.18^{c}	1.99 ± 0.19^{ab}	68.72 ± 0.39^{c}
Test Diet-V	4	12.93 ± 0.16^{c}	6.58 ± 0.18^{c}	5.85 ± 0.14^{b}	1.65 ± 0.12^{bc}	71.83 ± 0.35^{b}
Test Diet-VI	5	10.49 ± 0.21^{e}	5.55 ± 0.24^{e}	7.44 ± 0.27^{a}	1.51 ± 0.12^{c}	73.66 ± 0.54^{a}

All values of means within rows are different significantly ($p < 0.05$)
Data values are mean (Mean ± Standard deviation) of three replicates

Table 4. Hematological parameters of *C. carpio* fingerlings fed CGM based test diets supplemented with probiotics

Diets	Probiotics level (gkg^{-1})	PLT	Hb (g/100mL)	MCH (pg)	MCHC (%)	MCV (fl)
Test Diet-I (Control)	0	57.62 ± 0.31^{f}	6.99 ± 0.19^{c}	30.40 ± 0.27^{e}	27.51 ± 0.22^{c}	97.30 ± 0.40^{e}
Test Diet –II	1	60.27 ± 0.32^{e}	7.27 ± 0.21^{c}	31.01 ± 0.22^{e}	27.09 ± 0.20^{c}	101.22 ± 0.43^{d}
Test Diet –III	2	68.02 ± 0.18^{a}	8.85 ± 0.10^{a}	36.59 ± 0.17^{d}	31.59 ± 0.15^{b}	172.59 ± 0.23^{c}
Test Diet –IV	3	64.29 ± 0.29^{c}	8.36 ± 0.23^{ab}	43.48 ± 0.23^{c}	33.44 ± 0.24^{a}	179.69 ± 0.25^{a}
Test Diet –V	4	65.96 ± 0.29^{b}	8.03 ± 0.20^{b}	50.66 ± 0.34^{a}	30.79 ± 0.20^{b}	171.68 ± 0.29^{c}
Test Diet –VI	5	61.14 ± 0.26^{d}	7.50 ± 0.17^{c}	49.27 ± 0.36^{b}	33.03 ± 0.86^{a}	$177.38\pm0.63_{b}$

RBC (Red Blood Cell), WBC (White blood cell), PLT (Platelets), Hb (hemoglobin) (PCV = Packed cell volume, MCHC = Mean corpuscular hemoglobin concentration MCH = Mean corpuscular hemoglobin, MCV = Mean corpuscular volume)
All values of means within columns are different significantly ($p < 0.05$)
Data values are mean (Mean ± Standard deviation) of three replicates

Table 5. Growth performance of *C. carpio* fingerlings fed on CGM based test diets supplemented with probiotics

Diet levels	Probiotic Levels (gkg^{-1})	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain($fish^{-1}day^{-1}$) g	Feed Intake($fish^{-1}day^{-1}$) g	SGR
Test Diet-I (Control)	0	7.12±0.17	19.81±0.29^{e}	12.69±0.13^{d}	0.18±0.00^{d}	0.28±0.02^{b}	1.14±0.01^{d}
Test Diet-II	1	7.19±0.19a	25.49±0.38^{b}	18.30±0.20^{b}	0.26±0.00^{b}	0.33±0.01^{a}	1.41±0.01^{b}
Test Diet –III	2	7.08±0.20	26.51±0.29^{a}	19.43±0.10^{a}	0.28±0.00^{a}	0.32±0.02^{ab}	1.47±0.02^{a}
Test Diet –IV	3	7.13±0.12	23.63±0.27^{c}	16.49±0.21^{c}	0.24±0.00^{c}	0.32±0.02^{ab}	1.33±0.01^{c}
Test Diet –V	4	7.15±0.25	20.32±0.48^{d}	13.16±0.23^{d}	0.19±0.00^{d}	0.29±0.01^{b}	1.16±0.01^{d}
Test Diet –VI	5	7.20±0.17	19.20±0.33d^{e}	12.00±0.23^{e}	0.17±0.00^{e}	0.28±0.01^{b}	1.09±0.02^{e}

All values of means within rows are different significantly ($p < 0.05$)
Data values are mean (Mean ± Standard deviation) of three replicates

DISCUSSION

Probiotics are live microorganisms that may be used as dietary supplements to enhance fish performance and immunity. Probiotics impart several benefits to the fish, like improved growth performance, enhanced feed efficiency, boosted immunity status and flourished beneficial intestinal microflora [2,25,48,47]. Probiotics produce digestive enzymes that enhance feed digestibility and absorption. The present results showed the highest amount of gross energy (2%), crude fat (9%) and crude protein (17%) in whole body when fish fed on 2 g kg-1 probiotic supplemented corn gluten meal-based diet while second higher level on these parameters was observed at 3 g kg-1 probiotic supplementation. Much like present findings, [14] observed better carcass composition of C. carpio fingerlings fed 0.5% Saccharomyces cervisiae supplemented soybean meal-based diet. Bisht and coauthors [8] observed lower level of moisture (6.75%), while higher protein (30%) and lipid (8%) contents in the body of C. carpio fed at 4×108 cells 100-1g B. subtilis supplemented (4×106 cells 100-1 g) rice bran-based diet compared to control group. Suprayudi and coauthors [44] recorded higher protein and lipids absorption in the body of Nile tilapia upon feeding of diet supplemented with 0.25 and 0.5 g kg−1 of dietary probiotics. Similarly, Hoyoux and coauthors [23] reported that the diet containing 1×107 CFUg-1 probiotics for fish showed highest protein, fat and gross energy contents in Nile tilapia. Sahandi and coauthors [40] observed improved apparent digestibility coefficient of crude protein (68.33%), crude fat (9.55%) and gross energy (4516.80 cal/g) while using 1 × 107 of two probiotics strains (Bifidobacterium). Contrarily, Ayoola and coauthors [7] recorded highest values of moisture (8.20%), lipid (12.7%) and ash (4.34%) at control diet while higher crude protein (66%) in Clarias gariepinus group fed 1g commercially prepared probiotics (Lactobacillus and Bifidobacterium).

In aquaculture, fish hematology is used in monitoring fish health [24]. In our study fingerlings fed 2 gkg-1 probiotics showed greater value of RBCs (3.18×106mm-3), WBCs (8.02×103 mm-3) and PLT (68) whereas the second maximum value was recorded with 3 gkg-1 level of probiotic supplementation. It was unclear about the decrement of RBCs and WBCs values in fingerlings fed on higher levels of probiotics supplementation (3, 4 and 5 g/kg). RBCs numbers over 1.00×10-6 mm-3 in blood is considered high and show higher oxygen carrying capacity with increased metabolic activity [31]. Similar to present results, [30] observed high numbers of RBCs and WBCs at 3% probiotics supplementation in C. catla fingerlings.

Present results showed that PLT numbers improve with rising probiotics level up to 3 gkg-1. Suprayudi and coauthors [44] noticed significantly improved erythrocytes, leucocytes and phagocytic index while feeding O. niloticus upon 0.50 and 1.0 g of dietary probiotics. Similar to our results, Jafaryan and coauthors [27] studied that addition of probiotics (3×10-4 CFUg-1) in plant meal-based diet showed higher growth performance in Trichopodus trichopterus as compared to other fish groups. Probiotics supplementation at level of 108 and 1010 CFUg-1 in Labeo rohita diet improves the growth performance parameters such as growth, feed conversion ratio and SGR [21]. Tan and coauthors [46] reported significant change in weight gain, FCR and feed efficiency of Nile tilapia when fed on 106 CFU/g of probiotics supplemented diet. Furthermore, Feng and coauthors [19] found out striking increase in weight gain and SGR of common carp while feeding the fish with different strains of probiotics at (5 × 108 CFU/g) for 8 weeks. Munir and coauthors [34] ended up with the significant results in terms of FCR (1.24), SGR (1.44) and weight gain (400.45%) by employing probiotics like Lactobacillus acidophilus in Channa striata. Same findings were also reported by Aly and coauthors [4] in O. niloticus when fed with 10-6 g-1 of probiotics. However, Sha and coauthors observed that FCR and SGR values did not show any improvement when rainbow trout was fed probiotics supplemented diet compared to control group [41]. Similarly, Irianto and coauthors [26] observed non-significant differences in the growth performance of rainbow trout in response to probiotics supplemented plant meal-based diet.

CONCLUSION

Results suggested the usage of 2 gkg^{-1} probiotics as the optimal level for improving the carcass composition, growth performance and hematological parameters of *C. carpio* fingerlings fed CGM based diets.

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