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EFFICACY OF MULTI-STRAIN PROBIOTICS ON GROWTH PERFORMANCE, FEED UTILIZATION, PROXIMATE COMPOSITION, AND HAEMATOLOGICAL PARAMETERS OF ASIAN CATFISH (*Clarias batrachus*) JUVENILES

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ABSTRACT

This study was conducted to determine the effects of different commercial probiotics on the growth performance, feed utilization, proximate composition, and haematological parameters of Asian catfish (Clarias batrachus) juveniles. The study design consists of a total of 300 fish (avg. wt. 11.14 ± 0.5 g) randomly distributed in twelve tanks with a stocking density of 25 fish/tank. Fish diets were formulated using 0.2% of three different commercial probiotics namely T1, CP-1; T2, CP-2; and T3, CP-3, and a control diet without probiotics. Fishes were fed at a rate of 3% body weight twice daily. The growth performance indicators i.e. final body weight (g), weight gain (g) and specific growth rate (%/day), were found to be highest in T3 (CP-3) and were significantly different from the others. Similarly, feed utilization (feed conversion ratio) was significantly improved in fish fed 0.2% probiotic diet. Among body indice parameters, intra peritoneal fat (%) reduced significantly in probiotic fed groups. Besides, highest percentage of protein (79.52 ± 0.61) and lowest lipid content (6.14 ± 0.25) were recorded in T3 (CP-3) supplemented diet. Haematological parameters especially percentage of packed cell volume and white blood cell were escalated in T1 (CP-1) and T3 (CP-3) whereas mean corpuscular volume found comparatively lower in control. Therefore, finally the results of principal component analysis concluded that the incorporation of probiotic T3 (CP-3) at 0.2% in diet proved more effective for the improvement of growth performance, proximate composition and haematological parameters for C. batrachus juvenile farming.

KEYWORDS: *Clarias batrachus*; growth; haematology; probiotic; and proximate composition

INTRODUCTION

Aquaculture has become one of the fast growing food producing sectors that consistently provides animal protein and ensures food security for mass people for a long time (FAO, 2020). Moreover, this sector is also responsible for creating diverse income sources through providing new employment opportunities, increasing profitability, eliminating poverty, and improving livelihood (Subasinghe *et al.*, 2009). In recent times, the evolution of aquaculture has introduced modern aquafarming techniques and replaced traditional fish culture to increase more productivity and/ or yield (Gaffar *et al.*, 2023; Mohapatra *et al.*, 2013). With the intensification of fish culture, this sector suffers from many problems like poor water quality, compromised growth, reduced survival, increased feed cost, and serious disease outbreaks (Elsabagh *et al.*, 2018; Rohani *et al.*, 2022). Besides, various chemicals like inorganic fertilizers and antibiotics, are used to increase productivity as well as to control fatal fish disease that sometimes negatively affects the overall sustainability of aquaculture. As a result, the need for environmentally friendly culture practices, along with the increased fish production, should be ensured (Akter *et al.*, 2019). The application of probiotics in aquaculture might prove to be an eco-friendly practice as several studies have documented that its usage not only increases fish production but also takes care of the environment in an intensified aquaculture system (Akter *et al.*, 2021; El-Saadony *et al.*, 2021).

Probiotics are the congregation of useful living microbes, mostly comprising different taxa of bacteria, algae, and fungi usually adapted or colonized in the intestine of host species (Hai, 2015; Martínez Cruz *et al.*, 2012). The supplementation of probiotics in feed increases the colonization of beneficial micro living beings in the intestine; subsequently re-

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placing or eliminating harmful microorganisms (Dawood *et al*., 2019). Besides, probiotics are considered growth promoters because their usage ensures better growth performance and feed utilization through facilitating diverse enzymatic activity (Hossain *et al*., 2022; Islam *et al*., 2021). According to Dawood (2021), probiotics are a useful feed supplement as well as an effective alternative to antibiotics that work competently against deadly pathogens. In addition, probiotic improves immunity, defense mechanisms and disease prevention capability of fish through improving different haematological parameters (Akter *et al*., 2021). However, a wide range of microbes, especially bacteria and yeast (either single or combined), are being used in aquaculture operations (El-Saadony *et al*., 2021).

The most common bacterial genera are as *Bacillus*, *Lactobacillus*, *Rhodococcus*, *Nitrobacter*, *Aspergillus*, *Micrococcus*, *Lactococcus* and *Streptococcus*, *Enterobacter* etc. (Hai, 2015). Recently, baker yeast (*Saccharomyces cerevisiae*) is also been found beneficial for improving growth, feed utilization, and gut morphology of cultured fish (Islam *et al*., 2021). In earlier, the usage of single strain of bacteria was very common whereas it is now believed that single species might not tackle all the problems effectively that arise during culture period; therefore, the application of multiple strains is being popular nowadays (El-Saadony *et al*., 2021; Mohammadi *et al*., 2021). Besides, commercial probiotics are also used extensively as these probiotics contain multi-species, are easy to use, and impart noteworthy benefits to fish in terms of growth, feed utilization, survival, intestinal-morphometry, and improve haematological parameters as well (Gaffar *et al*., 2023; Standen *et al*., 2016; EL-Haroun *et al*., 2006).

Clarias batracus, commonly known as Asian walking catfish, is an air breathing freshwater catfish species that are native to Southeast Asian countries like Bangladesh, Indian, Myanmar, Thailand, Nepal, Bhutan, Indonesia, and Pakistan etc. and is widely distributed to the freshwater ponds, canals, plains, steams, tributaries, rivers and sometimes in slightly saline brackish waterbodies (Ng & Kottelat, 2008). This carnivorous catfish species has high consumer preference because of its great nutritional profile such as 14.87% edible protein, 7.9% poly unsaturated fatty acids (PUFA), cholesterol and minerals like calcium, phosphorus, sodium, and potassium etc. (Chetia Borah, 2020). Besides, due to its medicinal value, it is commonly used to cure weakness, anemia, and malnutrition of the birth givers (Gehring *et al*., 2011).

However, in recent times, compromised growth, poor nutrient digestibility, greater disease susceptibility and increased mortality of *C. batracus* have been observed due to high stocking density culture and farmers have failed to retrieve enough profit from culturing it (Akter *et al*., 2021). Although the use of antibiotics sometimes provide immediate good results whereas the application of antibiotics for a long time could produce serious implications, i.e. alterations of intestinal microbes and development of resistant bacterial population colonies in fish gut (Yang *et al*., 2024). Besides, usage of antibiotics is costly and needs expertise (Limbu, 2020), consequently, there is a need of ecofriendly culture practices that will able to enhance fish growth, nutrient utilization, hematological parameters and immunity against disease. Earlier, the supplementation of probiotics showed positive results in some catfish culture (Dey *et al*., 2016; Banerjee *et al*., 2015; Dahiya *et al*., 2012) whereas detailed work on the effects of probiotic on growth performance, feeding efficiency, nutritional profile and immunological parameters of *C. batracus* are yet to be conducted. Therefore, this study aimed to assess the effects of multi-strain commercial probiotics on the growth, feed utilization, body indices, proximate composition, and hematological parameters of *C. batracus* juveniles.

MATERIALS AND METHODS

Experimental Site and Design

Juvenile Asian catfishes (*C. batrachus*) with an average weight of 11.14 ± 0.50 g were procured from a local hatchery, kept in oxygenated plastic bags, and transported to the wet laboratory of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. The fish were acclimatized for 15 days before initiating the experiment and were fed with commercial feed up to satiation. Before starting the experiment, twelve (12) rectangular cemented tanks (size: 75 × 70 × 75 cm^3, water holding capacity: ~ 450 liters) were cleaned, disinfected, and filled with groundwater. A total of 300 fish were randomly stocked at 25 fish per tank with 3 replicates for a period of a 12-week feeding trial. Three different commercial probiotics treatments (T1, CP-1; T2, CP-2; and T3, CP-3) were incorporated at 0.2% in basal diet. The control treatment was without probiotic. Each treatment had three replicates. Based on manufacturer, the composition of probiotics was as follows; CP-1 (*Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *Nitrobacter*, *Rhodobacter*, *Nitrosomonus*); CP-2 (*B. subtillis*, *B. coagulans*, *B. amyloliquefaciens*, *B. licheniformis*, *B. polymyxa*, *B. pumilus*, *B. megaterium*, *Aspergillus oryzae*), CP-3 (*B. subtilis*, *B. licheniformis*, *B. megaterium*, *Lactobacillus acidophilus*, *L. plantarum*, and *Saccharomyces* *cerevisiae*). Fish were fed experimental diet at the rate of 3% of standing body weight and supplied two times a day i.e. early morning and late evening throughout the experimental period. Besides, water was exchanged every four days.

Diet Preparation

Three isonitrogenous diets were prepared, containing 0.2% each probiotic, and the amount of diet was adjusted with wheat flour in the control. The ingredients of the fish diet consist of protein sources (fish meal and soybean meal), lipid sources (soya bean oil and fish oil), and carbohydrate sources (corn starch and wheat flour) to ensure an equal energy level in each treatment, as stated by Akter *et al.* (2021). The list of feed ingredients used in this study is shown in Table 1. Each ingredient (except fish oil and soya bean oil) was estimated in grams and systematically mixed for a few minutes in a large plastic container. Thereafter, fish oil and soybean oil were added and mixed for an additional 10 minutes (Salaghi *et al.*, 2013). Enough water was added to prepare a dough, which was then extruded through a pelletizing machine to make 3 mm diameter pellets. The pellets were sun-dried until the moisture level reached below 10%, finally stored in plastic bags separately and preserved in a freezer at -20°C.

Table 1. Ingredients used for preparing experimental diets

Ingredients (g/kg)	Treatments			
	Control	T1 (CP-1)	T2 (CP-2)	T3 (CP-3)
Fish meal[1]	247.20	247.20	247.20	247.20
Soya bean meal	280.10	280.10	280.10	280.10
Wheat flour	270.00	268.00	268.00	268.00
Corn starch	97.50	97.50	97.50	97.50
Fish oil	32.60	32.60	32.60	32.60
Soya bean oil	32.60	32.60	32.60	32.60
Vitamin mix[2]	20.00	20.00	20.00	20.00
Mineral mix[3]	20.00	20.00	20.00	20.00
Probiotic	0.00	2.00	2.00	2.00
Total	1000	1000	1000	1000

[1]Danish fish meal: crude protein, 720 g/kg; crude lipid, 50 g/kg;

[2]Vitamin mix kg^{-1} (Rovithai Ltd 700/437 Chonburi Thailand): A 50 Million International Unit (MIU), E 130 g, K_3 10 g, B_1 10 g, B_2 25 g, B_6 16 g, B_{12} 100 mg, Niacin 200 g, Pantothenic acid 56 g, Folic acid 8 g, Biotin 500 mg, Antioxidant 0.200 g and Anticake 200 g;

[3]Mineral mix kg^{-1}: Calcium phosphate (monobasic) 397.5 g; calcium lactate 327 g; Ferrous sulphate 25 g; Magnesium sulphate 137 g; Potassium chloride 50 g; Sodium chloride 60 g; Potassium iodide 150 mg; Copper sulphate 780 mg; Manganese oxide 800 mg; Cobalt carbonate 100 mg; Zinc oxide 1.5 g and Sodium selenite 20 mg.

Water Quality Parameters Assessment

The water quality parameters of each tank were determined fortnightly. Water temperature (°C) and pH were measured by a mercury thermometer and a digital pH meter (Hanna; model: HI98103), respectively. Dissolved oxygen (mg/L), ammonia (mg/L), and hardness/$CaCO_3$ (mg/L) were measured by digital oxygen meter (Lutron; model: PDO-519 meter), API ammonia test kit and hardness test kit, for freshwater (Hanna; model: HI3812), respectively.

Sampling

Fish without swimming or movement are considered dead. Individual fish in each tank was weighed at the beginning and end of the feeding trial. Bulk weighing for each tank was done fortnightly in order to adjust the feed amount accordingly. At the end of the feeding trial, three fish from each replicate tank were randomly taken for the determination of the body proximate composition and other indices. Fish were starved for 24 hours before final sampling. On sampling day, fishes were euthanized after placing them in ice for 10 minutes, quickly weighed and dissected using a surgical knife with a ventral incision. The viscera, liver, and intraperitoneal fat were carefully separated, weighed, and recorded.

Determination of Growth, Feed Efficiency and Survival

Final average weight (g), weight gain (g), specific growth rate (SGR; %/day), feed conversion ratio (FCR), and survival rate (SR %) were determined following the empirical equation previously used by (Akter *et al.*, 2019).

- Final average weight (g) = [Total weight of fish (g)/ Number of fish]

- Weight gain (g) = [Final weight of fish (g) - initial weight of fish(g)]
- Specific growth rate (SGR; %/day) = [(Log_n final weight (g) - Log_n initial weight(g))/ Time duration × 100]
- Feed conversion ratio (FCR) = [Total dry feed given (g)/ Net weight gain (g)]
- Survival rate (SR %) = [(Final number of fish/ Initial number of fish) × 100]

Estimation of Body Indices

The weight of peritoneal fat, liver, and viscera were recorded to calculate the percentages of hepatosomatic index (HSI), intraperitoneal fat (IPF), and viscerosomatic index (VSI) incorporating the following formula -

- Intraperitoneal fat (IPF %) = [(Intraperitoneal fat weight (g)/ Body weight (g)) ×100]
- Hepatosomatic index (HSI %) = [(Liver weight (g)/ Body weight (g)) ×100]
- Viscerosomatic index (VSI %) = [(Viscera weight (g)/ Body weight (g)) ×100]

Determination of Whole-Body Proximate Composition

Proximate analysis is the measure of chemical components such as percentages of protein, fat, moisture, ash, and fiber, of which the body muscle is composed. The whole fish proximate composition was analyzed following the protocol given by the Association of Official Analytical Chemists (AOAC, 1997).

Estimation of Haematological Parameters

Three fish (03) from each replicate tank were selected randomly and anesthetized using clove oil to assess the better flow of blood in live as well as less stressed conditions. Blood was collected through 1 ml syringe and subsequently moved into a heparinized tube to avoid clotting. Hematological parameters such as erythrocyte sedimentation rate (ESR; mm/h), packed cell volume (PCV %), haemoglobin (Hb; g/dL), red blood cell (RBC; $*10^6/mm^3$), mean corpuscular haemoglobin concentration (MCHC; g/dL), mean corpuscular haemoglobin (MCH; pg/cell), mean corpuscular volume (MCV; fL), and white blood cell (WBC; $*10^4/mm^3$) were calculated according Akter *et al.* (2023).

Statistical Analysis

The data was presented as mean ± standard deviation. The data obtained from different parameters were analyzed statistically using one-way analysis of variance (ANOVA). Duncan's test was used to evaluate mean differences among treatments with a significant level of $P < 0.05$. All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) software, version 22.

RESULTS AND DISCUSSION

Water Quality Parameters

The water quality parameters recorded in this study are presented in Table 2. Water temperature, pH, dissolved oxygen, NH_3, and hardness ($CaCO_3$) were monitored every two weeks and ranged between 16.46-16.56°C, 6.92-7.13, 6.74-6.94 mg/L, 0.31-0.41 mg/L, and 39-52 mg/L, respectively. The one-way ANOVA for the aforementioned water quality parameters found no significant differences ($P > 0.05$) among treatments.

The use of probiotics in culture water usually assists in maintaining water quality parameters by transforming ammonia and unused feed particles into usable nitrogenous compounds. It has been documented that the Gram-positive genus *Bacillus* is very effective converting organic matter into CO_2 (Martínez Cruz *et al.*, 2012) along with *Enterobacter*, *Nitrobacter*, and *Pseudomonas*, etc. in channel catfish culture (Verschuere *et al.*, 2000). Similarly, commercial probiotic (Biogen) containing *B. subtilis* and *B. licheniformis* altogether could maintain the pH, dissolved oxygen, and ammonia concentration in a 17-week trial for Nile tilapia (EL-Haroun *et al.*, 2006). In

Table 2. Water quality parameters of the Asian catfish (*Clarias batracus*) culture tanks

Parameters	Treatments			
	Control	T1 (CP-1)	T2 (CP-2)	T3 (CP-3)
Temperature (°C)	26.49 ± 0.04^a	26.49 ± 0.06^a	26.56 ± 0.09^a	26.46 ± 0.07^a
pH	7.12 ± 0.08^a	6.92 ± 0.15^a	7.08 ± 0.15^a	7.13 ± 0.24^a
DO (mg/L)	6.80 ± 0.10^a	6.77 ± 0.17^a	6.94 ± 0.18^a	6.74 ± 0.13^a
NH_3 (mg/L)	0.31 ± 0.08^a	0.34 ± 0.14^a	0.39 ± 0.10^a	0.41 ± 0.10^a
Hardness (mg/L)	45.00 ± 13.74^a	49.00 ± 1.73^a	52.00 ± 6.24^a	39.00 ± 7.93^a

All values are presented as mean ± SD. Data with different superscripts in the same row indicate a significant difference ($P < 0.05$).

this study, no significant ($P > 0.05$) difference was observed among treatments and control in terms of water quality parameters (Table 2). This finding seems to be similar to Yanbo & Zirong (2006) who found no noteworthy effect on water quality parameters when common carp (*Cyprinus carpio*) were fed the probiotic supplemented diet.

Growth Performance and Feed Utilization

Growth indices and feed utilization parameters of *C. batrachus* juveniles fed with probiotic-incorporated diets are given in Table 3. Growth indices such as the highest mean final weight (29.04 ± 2.28 g), SGR (1.11 ± 0.12 %/day), and weight gain (17.68 ± 2.51 g) were observed in T3 (CP-3), followed by T2 (CP-2). In addition, the growth parameters of T3 showed significantly ($P < 0.05$) higher over other probiotic supplemented groups. Besides, better feed utilization was observed in fish fed probiotic-supplemented diets. Improved FCR (1.63 ± 0.12) was also exhibited in CP-3 fed fishes that were significantly different ($P < 0.05$) from those of other fed groups. The survival rate did not show any significant difference ($P > 0.05$) among treatments.

Here, substantial improvement in weight gain (g), specific growth rate (SGR; %/day) feed utilization efficiency (FCR), intraperitoneal fat (%), proximate composition (protein and lipid content), and haematological parameters (PCV %, MCV %, Hb and WBC) of *Clarius batrachus* was observed due to the supplementation of multi-strain probiotic in diets.

In this experiment, the growth performance indicators, i.e., weight gain (g) and SGR (%/day) to yield better results in all probiotic supplemented groups, where the weight gain of CP-3 treated fish was 80% higher than that of the control (Table 3). Likewise, better feed utilization (indicated by lower FCR) was also recorded in T3 (CP-3). The probiotics composition of CP-1 (*Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *Nitrobacter*, *Rhodobacter*, *Nitrosomonus*); CP-2 (*B. subtillis*, *B. coagulans*, *B. amyloliquefaciens*, *B. licheniformis*, *B. polymyxa*, *B. pumilus*, *B. megaterium*, *Aspergillus oryzae*) and CP-3 (*B. subtilis*, *B. licheniformis*, *B. megaterium*, *Lactobacillus acidophilus*, *L. plantarum*, and *Saccharomyces cerevisiae*) diets were might be the reason of improving growth performance and feed utilization of *C. batrachus* juveniles. In general, probiotic supplementation either in water or diet supports beneficial microbial load in fish intestine; ensures better nutrient digestibility and absorption that ultimately facilitates higher feed conversion efficiency (FCR) as well as growth (weight gain) of aquatic organisms (Mohammadi *et al*., 2021; Dawood *et al*., 2019). More explicitly, the colony of microorganisms stimulates appetite and assists in better feed utilization through secreting extracellular hydrolytic enzymes such as bacterial protease, amylase, chitinase, and lipase that digest complex food particles, releasing additional nutrients for fishes (El-Saadony *et al*., 2021; Ray *et al*., 2012). Furthermore, probiotics stimulate growth by providing nutrient supplements like essential amino acids, fatty acids, and vitamins (B7 (biotin), B12 (cobalamin), and K) (Balcázar *et al*., 2006). Besides, several scientific studies reported that the inoculation of multi-strain bacteria or yeast found helpful to improve fish growth and feed conversion efficiency, such as *B. subtilis*, *L. buchneri*, and *L. plantarum* altogether enhanced the weight gain and reduced FCR of Gangetic mystus, *Mystus cavasius* (Gaffar *et al*., 2023). Similarly, the growth and feeding efficiency of African catfish (*Clarias gariepinus*) were escalated when diets were supplemented with *L. acidophilus* (Al-Dohail *et al*., 2011). The incorporation of *Bacillus* spp. and baker yeast (*Saccharomyces cerevisiae*) improved the growth performance and feed conversion ratio of channel catfish (*Ictalurus* sp.) and striped catfish (*Pangasianodon hypophthalmus*) (Boonanuntanasarn *et al*., 2019; Queiroz & Boyd, 1998). It has been assumed

Table 3. Growth performance and feed utilization of *C. batrachus* reared with probiotic containing diet

Parameters	Treatments			
	Control	T1 (CP-1)	T2 (CP-2)	T3 (CP-3)
Initial Av. Wt. (g)	11.14 ± 0.50^{a}	11.62 ± 0.44^{a}	11.33 ± 0.09^{a}	11.36 ± 0.23^{a}
Final Av. Wt. (g)	20.94 ± 0.38^{a}	24.36 ± 0.92^{b}	24.83 ± 0.75^{b}	29.04 ± 2.28^{c}
Wt. gain (g)	9.80 ± 0.85^{a}	12.74 ± 1.15^{b}	13.50 ± 0.69^{b}	17.68 ± 2.51^{c}
SGR (%/day)	0.75 ± 0.07^{a}	0.88 ± 0.07^{ab}	0.93 ± 0.03^{b}	1.11 ± 0.12^{c}
FCR	2.68 ± 0.34^{c}	2.15 ± 0.26^{b}	1.96 ± 0.09^{ab}	1.63 ± 0.12^{a}
Survival rate (%)	93.33 ± 6.66^{a}	97.77 ± 3.85^{a}	93.33 ± 6.66^{a}	95.55 ± 7.97^{a}

All values are presented in mean ± SD obtained from three replicate groups (n = 3); data with different superscripts (a, b, c) in the same row indicate significant difference ($P < 0.05$); Av = average, Wt. = weight, SGR = Specific growth rate, FCR = Food conversion ratio.

that the special microbial composition (*Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *Lactobacillus acidophilus*, *L. plantarum* and *Saccharomyces cerevisiae*) of this commercial probiotic (CP-3) proved its efficacy as a feed additive for Asian catfish (*C. batrachus*) juvenile culture. However, here, survival of Asian catfish was comparatively higher in probiotic-fed groups, although no significant variation (P > 0.05) was observed among treatments (Table 3).

Body Indices and Proximate Composition of Whole Body

The body indices of *C. batrachus* juveniles fed with different probiotic-containing diets are exhibited in Table 4. Among the body indices, only IPF percentage showed a significant different (P < 0.05) among the treatment groups. The percentage of IPF was found to be lowest in T3 (0.52 ± 0.06) and highest in control (0.71 ± 0.13). The percentage of IPF in T1 (CP-1) (0.56 ± 0.14) and T2 (CP2) (0.58 ± 0.05) showed no significant difference (P > 0.05) compared to those fish fed with the control diet. Moreover, T3 (CP-3) showed the highest percentages of HSI (1.12 ± 0.05) and lowest percentages of VSI (3.67 ± 0.55), respectively.

Whole body proximate composition of *C. batrachus* juveniles under different treatments is shown in Table 5. Significantly higher body protein percentage was observed in fishes treated with CP-3 supplemented diet (79.52 ± 0.61%) compared to the other fed groups (P < 0.05). Whereas, the lipid percentage recorded significantly (P < 0.05) lower in T3 (CP-3) (6.14 ± 0.25%) compared to other groups. However, the experiment showed no marked differences in ash and fiber content among fed groups (P > 0.05).

Table 4. Body indices of *C. batrachus* juveniles reared with probiotic incorporated diets in a 12-week trial

Parameters	Treatments			
	Control	T1 (CP-1)	T2 (CP-2)	T3 (CP-3)
IPF (%)	0.71 ± 0.13^{b}	0.56 ± 0.14^{ab}	0.58 ± 0.05^{ab}	0.52 ± 0.06^{a}
HSI (%)	0.98 ± 0.22^{a}	0.94 ± 0.20^{a}	1.02 ± 0.24^{a}	1.12 ± 0.05^{a}
VSI (%)	4.48 ± 0.53^{a}	4.13 ± 0.66^{a}	3.81 ± 0.02^{a}	3.67 ± 0.55^{a}

All values are mean ± SD obtained from three replicate groups (n = 9); Data with different superscripts in the same row indicate significant difference (P < 0.05). HSI = Hepatosomatic Index, IPF = Intra-peritoneal Fat, VSI = Viscera-somatic Index.

Table 5. Whole body proximate composition of *C. batrachus* after 12 weeks of probiotic feeding trial (on dry basis)

Parameters	Treatments			
	Control	T1 (CP-1)	T2 (CP-2)	T3 (CP-3)
Protein (%)	69.08 ± 1.30^{a}	75.05 ± 1.01^{b}	75.89 ± 1.44^{b}	79.52 ± 0.61^{c}
Lipid (%)	8.61 ± 0.30^{c}	7.61 ± 0.23^{b}	7.95 ± 0.34^{b}	6.14 ± 0.25^{a}
Fiber (%)	0.25 ± 0.02^{a}	1.46 ± 1.93^{a}	0.31 ± 0.01^{a}	0.26 ± 0.02^{a}
Ash (%)	9.30 ± 0.47^{a}	10.64 ± 0.27^{a}	10.59 ± 1.40^{a}	9.51 ± 0.11^{a}

Data are presented as mean ± SD (n = 3). Data with different superscripts (a, b, c) in the same row indicate significant difference (P < 0.05).

However, the enhancement of growth parameters and nutrient utilization due to probiotic usage in the basal diet also reflected in enriched proximate composition and balanced nutritional profile of *C. batracus* in this experiment. Here, the intraperitoneal fat (IPF %) was found lowest in T3 (CP-3) which also indicated lower fat deposit and faster growth due to higher movement of fish during culture (Table 4). Besides, probiotic application significantly increased the protein percentages of Asian catfish juveniles, whereas the lipid content was significantly lowered (P < 0.05) in fishes fed with probiotic (Table 5). Similar findings were reported in other scientific reports, where the incorporation of commercial probiotics with feed reduced lipid content while increasing protein percentages in butter catfish (*Ompok pabda*) and Nile tilapia (*Oreochromis niloticus*) (Jinia *et al*., 2019; Biswas *et al*., 2018). In general, the increase of protein and decrease of lipid is the indicator of alteration of tissue structure build up and energy utilization, where protein usually involved in muscle build up and fat is being utilized as energy that may vary according to the age, size and growth rate of fishes (Abdel-Tawwab *et al*., 2006). Besides, the present experiment revealed that the uptake of probiotic supplementedprobiotic-supplemented diets did not influence the ash and fiber content.

Haematological Parameters

The influence of dietary probiotics supplementation on the haematological parameters of *C. batrachus* is presented in Table 6. The PCV was found significantly higher ($P < 0.05$) in T3 (32.10 ± 0.60%) and T1 (31.20 ± 4.10%) compared to the control (26.73 ± 0.68%). White blood cell (WBC) was recorded comparatively higher in fishes fed with CP-1 (6.07 ± 1.04 × 10^4 mm^{-3}) and CP-3 (5.96 ± 0.04 × 10^4 mm^{-3}). The dietary supplementation of CP-3 significantly improved haemoglobin (Hb; g/dL) concentration than that of other treatments ($P < 0.05$). MCV level was found higher in T3 (CP-3) followed by T2 (CP-2), and lowest in the control (156.20 ± 5.90 fL); T3 was significantly greater compared to the control group ($P < 0.05$). Whereas, the experiment showed no marked differences in RBC, Hb, ESR, MCH, and MCHC parameters among treatments and control ($P > 0.05$).

Haemato-biochemical markers are those diagnostic tools that are being successfully used to represent the current health status or physiological well-being of aquatic organisms (Shahjahan *et al.*, 2022). Although haematological parameters could be influenced by many factors such as species, age of fish, type of stress, exposure time, and nutritional component of diet, etc. (Yousefzadeh & Khara, 2015), these parameters are able to exhibit the environmental stress or pathogen-induced disorders in fishes (Hoseinifar *et al.*, 2015; Dahiya *et al.*, 2012). In this study, the inclusion of multi-strain probiotic improved various significant blood parameters such as PCV (%), MCV (fL), Hb (g/dL), RBC (× 10^6/mm^3), and WBC (× 10^4/mm^3) that in turn could reflect escalated immune response in *C. batracus* (Table 6). The increased percentages of PCV (proportion of RBC in blood) and MCV (average volume of RBC in blood), along with RBC and Hb facilitated more iron molecules to bind with respiratory oxygen (O_2) to reduce stress (Akter *et al.*, 2023). Besides, the white blood cell (WBC) serves as a primary defense against disease-causing pathogens; its increase in blood acts as an immunostimulant (Liu *et al.*, 2017). Here, the amount of WBC was recorded high in all probiotic groups compared to the control (Table 6), implying enhanced immunity in probiotic-fed fish. Similarly, several studies previously reported that multi-species probiotics had significant effects on improving the haemato-chemical profile of fishes (Gaffar *et al.,* 2023; El-Saadony *et al.*, 2021; Salam *et al.*, 2021; Dahiya *et al.*, 2012; EL-Haroun *et al.*, 2006).

Table 6. Hematological parameters of *C. batrachus* juveniles fed with probiotic containing diets

Haematological Parameters	Treatments			
	Control	T1 (CP-1)	T2 (CP-2)	T3 (CP-3)
ESR (mm/h)	11.50 ± 3.50^a	15.00 ± 0.00^a	16.00 ± 2.00^a	15.00 ± 3.00^a
PCV (%)	26.73 ± 0.68^a	31.20 ± 4.10^b	28.95 ± 1.35^{ab}	32.10 ± 0.60^b
RBC (× 10^6/mm^3)	2.36 ± 0.04^a	2.80 ± 0.75^a	2.47 ± 0.46^a	2.43 ± 0.06^a
Hb (g/dL)	8.10 ± 0.30^a	9.05 ± 0.95^a	8.15 ± 0.55^a	9.20 ± 0.10^b
MCHC (g/dL)	22.50 ± 0.10^a	25.00 ± 5.20^a	21.80 ± 1.60^a	22.25 ± 0.25^a
MCH (pg/cell)	38.65 ± 0.35^a	40.25 ± 8.65^a	34.60 ± 3.00^a	37.70 ± 0.10^a
MCV (fL)	156.20 ± 5.90^a	158.45 ± 2.25^a	169.20 ± 2.30^b	171.40 ± 0.90^b
WBC (× 10^4/mm^3)	4.46 ± 0.40^a	6.07 ± 1.04^b	5.51 ± 0.39^{ab}	5.96 ± 0.04^b

Data are presented as mean ± SD (n = 9). Data with different superscripts (a, b) in the same row indicate a significant difference ($P < 0.05$). Erythrocyte sedimentation rate (ESR); Packed cell volume (PCV); Red blood cell (RBC); Haemoglobin (Hb); White blood cell (WBC); Mean corpuscular haemoglobin concentration (MCHC); Mean corpuscular haemoglobin (MCH); Mean corpuscular volume (MCV).

Principal Component Analysis

The diagrams of principal component analysis (PCA) are illustrated in Figure 1. The scree plot showed that the first three principal components (PC1, PC2 and PC3) were able to explain all the variance (Kaiser's Rule: Eigenvalue ≥ 1) among various components (estimated parameters) and treatments where PC1 (59.2 %) and PC2 (28.2 %) combined represented a total of 87.4 % of variance. The biplot diagram exhibited the graphical distribution of treatments based on the mean values of growth, feed utilization, survival, body indices, proximate composition, and haematological parameters. The biplot explained that the incorporation of 0.2% probiotic in the diet improved the growth, feed utilization, survival, body indices, proximate composition, and haematological parameters of *C. batracus* in a 12-week feeding trial. Most of the parameters were positively correlated with CP-1, CP-

2, and CP-3, whereas the negative eigenvector value of FCR, lipid (%), moisture (%), IPF (%), VSI (%), MCV (fL), and MCH (pg/cell) separated control from probiotic treatments. Furthermore, the distribution of CP-3 showed the best results in terms of all selected parameters.

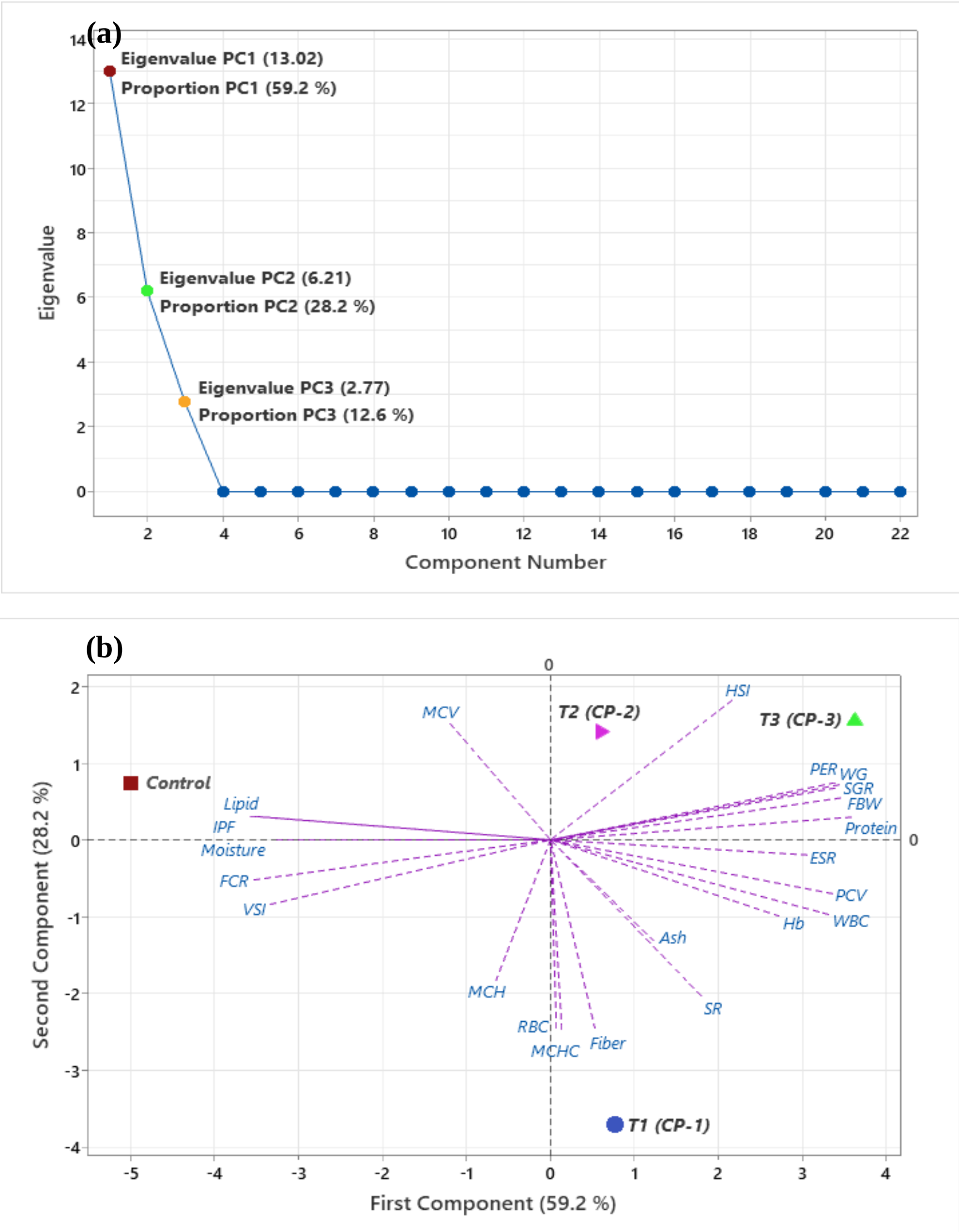

Figure 1. Principal component analysis (PCA) (a) scree plot and (b) biplot for growth, feed utilization, survival, body indices, proximate composition, and haematological parameters of *C. batracus* juveniles fed with probiotic-incorporated diet.

CONCLUSION

In conclusion, it is evinced that probiotic supplementation in diet has beneficial effects on growth, feed conversion efficiency, body indices, haematology, and proximate composition of Asian catfish. The outcome of principal component analysis (PCA) revealed that 0.2% inclusion of CP-3 probiotic in basal diet appears to be the most effective in influencing growth performance, feed utilization, proximate composition and haematological parameters of *C. batrachus* juveniles. Therefore, the microbial composition of this commercial probiotic (CP-3) proved potential as a feed supplement for Asian catfish culture.

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DECLARATION OF CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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