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EFFECT OF DIETARY *Spirulina platensis* SUPPLEMENTATION ON GROWTH, CARCASS COMPOSITION, AND HAEMATOLOGICAL PARAMETERS OF PABDA (*Ompok pabda*)

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ABSTRACT: The study was conducted to find out the effects and optimum level of dietary *Spirulina platensis* (Spirulina) supplementation on growth and haematological parameters of pabda (*Ompok pabda*). Five experimental diets were prepared containing 33% protein. Different levels of Spirulina (0% as control or S_0, $S_{2.5\%}$, $S_{5\%}$, $S_{7.5\%}$ and $S_{10\%}$) were supplemented at the expense of fish meal (FM), respectively. Up to 10% of the total dietary protein (33%) in the control diet was replaced by Spirulina protein in the experimental diets. The feeding experiment was carried out in five treatments with three replications for 10 weeks. The water quality parameters viz. ammonia, dissolve oxygen, pH, and temperature were within the suitable range for pabda culture. The best growth performance was observed in the fish fed with 10% Spirulina supplemented feed followed by 7.5%, 5% and 2.5% Spirulina supplemented diet ($P < 0.05$). Maximum survival rate (98%) and hepatosomatic index (1.37) of pabda was also found in $S_{10\%}$ Spirulina group. But the feed conversion ratio (FCR) was decreased significantly ($P < 0.05$) with the increasing level of *Spirulina* supplementation which indicated better feed utilization. In case of carcass composition of pabda, the highest percentage of crude protein and ash were observed in fish fed with 10% followed by 7.5%, 5%, 2.5% *S. platensis* supplemented diet ($P < 0.05$). Likewise, haematological condition in fish fed with 10% *Spirulina* supplemented diet resulted better in this study. Therefore, it could be concluded that dietary supplementation of 10% *S. platensis* protein with FM protein may significantly improve the growth and the haematological parameters of *O. pabda*.

Keywords: Feed supplement, Fish meal, Growth, Haematology, *Ompok pabda, Spirulina platensis.*

INTRODUCTION

Fish is an important dietary animal protein source in a healthy diet of human because of its high protein, low carbohydrate, and unsaturated fat (Mishra and Pradesh, 2020). Moreover, fisheries sector plays an important role in employment, nutrition, food security, and foreign exchange earnings in the global economy including Bangladesh which is a leading contributor in inland freshwater aquaculture. In 2022-23, the fisheries sector of Bangladesh contributed approximately 2.41% to the total gross domestic product (GDP) and around 21.47% to the agricultural GDP (DoF, 2023). However, the main constraints in this sector are increasing feed cost. Fish feed generally constitutes 60 –70% of the operational cost in intensive and semi-intensive aquaculture system of which 45% of the total feed cost is allocated to protein sources (Singh et al., 2006). Fish meal (FM) is an appropriate protein source for aquafeed because it contains a high crude protein content (65% to 72%) as well as an optimal percentage of all 10 essential amino acids (EAAs) required by all fish species (Gasco et al., 2018). One of the vital problems is the price of FM which is becoming consequently higher due to the increasing requirement of FM with the expansion and intensification of aquaculture (Hardy and Tacon, 2002).

To achieve sustainable aquaculture, novel alternative protein sources, such as cheaper plant or animal origin proteins, must be developed for sustained aqua feed production. It is proposed that increasing the use of plant protein in fish diets can minimize the cost of FM and feeds (Amer et al., 2020). But plant protein contains some factors such as cyanogenic glycosides, protease inhibitors, lectins, tannins, alkaloids, and saponins which suppress other nutrients and have negative effects in animal health (Khajali and Rafiei, 2024). Therefore, it's a crucial need to find out alternative protein sources from plant origin where anti-nutritional factors will not be a major concern like algal protein. Throughout the world, many algal species have been employed in aquaculture, mostly for nutritional purposes i.e. *Spirulina platensis, Arthrospira maxima, Chlorella vulgaris* etc. (Chen et al., 2022). Among them *Spirulina* is the most common and widely available species. It has no anti-nutritional factors, immunostimulant properties, and cheap protein source compared to FM (Amer, 2016).

Spirulina is a filamentous and multicellular blue-green microalga which belongs to two separate genera *Spirulina* and *Arthrospira*, and consists of about 15 species (El-Sheekh et al., 2014). *Spirulina* is the most common and widely

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distributed species. *Spirulina* contains high protein contents, lipids, bioactive components such as vitamins (especially vitamin A and B_{12}), minerals, polyunsaturated fatty acids, carotenes, and other pigments that have antioxidant activity (Spinola et al., 2024). Besides the nutritional properties, it also acts as a cleansing and detoxifying factor against toxic substances (Gargouri et al., 2020). *Spirulina* has no cellulose in its cell walls, being composed of soft muco-polysaccharides, thus, makes it easily digested and assimilated its 85 to 95% plant protein (Hanel et al., 2007). Muller et al. (2000) found that *Spirulina* might be used to partially or totally replace FM in formulated aqua diets. Nandeesha et al. (2001) reported that *Spirulina* may substitute up to 25% of FM, resulting in greater development in rohu (*Labeo rohita*).

Blood is a useful means of signaling and recognizing the impacts of stress, environment, and the health state of fish in a certain location (Asgah et al., 2015). The examination of haematological parameters aids in understanding the relationship between blood properties and habitat, as well as the species capacity to adapt to its surroundings. As a result, fish blood is crucial for correctly assessing species health (Celik, 2004). Including *Spirulina* in fish diets enhances haematological markers as well as immunological response, making farmed fish healthier and disease resistant.

The pabda (*Ompok pabda*) is an indigenous, freshwater small catfish belonging to the family Silurideae of the order Siluriformes. Pabda is considered as small indigenous species (SIS) and favorite food fish eagerly devoured by the consumers because of its delicious taste, palatability, high protein and minerals (Hossain, 2008). Due to overexploitation this species is declining significantly and included in the red list of IUCN (International Union for Conservation of Nature) (IUCN, 2003). Thus, natural production of pabda is decreasing day by day. In this context, pabda is a potential fish for aquaculture as it fetches very high market value which is 3-5 times higher than those of fishes having low price i.e. pangas (*Pangasius pangasius*) (Kohinoor et al., 2018). Considering its higher market price, consumer demand fish, availability of seed, cultivable in small seasonal ponds, attain marketable size with in short period, now-a-days farmers are showing considerable interest in pabda culture (Islam et al., 2021). Bangladesh has a great prospect of culture pabda in future but feed price is one of the main obstacles for its aquaculture propagation as it contains 50 to 70% of total operation cost (Hossain, 2008).

In this circumstance, the *Spirulina* can be supplemented with FM in order to enhance growth performance of pabda and make the culture more profitable for the farmers. Therefore, the efficient use of *Spirulina* should be started as a better nutrient supplement in fish culture. Though many works have been done as a replacement of FM in fishes but as far we are concerned no work has done on supplementation of *Spirulina* in pabda culture. Therefore, this study was conducted to find out the effects of dietary *Spirulina* supplementation on growth, biochemical composition, and haematological parameters of pabda.

MATERIALS AND METHODS

Experimental design

The experiment was conducted by using a completely randomized design (CRD) for a period of 10 weeks. The fingerlings of pabda were obtained from a commercial fish hatchery. Fish were acclimatized in a 300 L circular tanks with proper aeration for one week prior to the feeding trial. During their acclimatization period the fish were fed with control diet. A total 600 healthy juvenile fish (mean body weight of 0.50 ± 0.01 g) after a 1% salt solution treatment were equally divided into experimental pits; each sized 0.64 m^3 (length 1.00 m, width 0.8 m, height 0.8 m) with a water volume of 640 L. Fish in triplicate groups were provided with each experimental diet twice a day until they reached satiation. The water level in the experimental pits was maintained using water from a deep tube well. Additionally, a surface exit was installed in each pit to stop overflow in the case of rain. The experiment followed the natural photoperiod (about 10:14 light: dark) condition.

Diets preparation and fish rearing

FM, soybean meal, rice bran, wheat bran, mustard oil cake, and wheat flour were collected from the local feed market. The quality was considered during purchasing of the feed ingredients. *Spirulina* was cultured at a large scale for 3 weeks. Then it was collected, sundried, and grinded for formulating fish feed. After analyzing the proximate composition of all the feed ingredients experimental diets were formulated (Table 1).

Five experimental diets were prepared containing 33% protein (Table 2). Control diet contains fish meal and no Spirulina supplementation (S_0). In the experimental diets, up to 10% of the total dietary protein (i.e., 10% of 33%) was replaced by Spirulina protein at the expense of fish meal protein. Different levels (2.5%, 5%, 7.5% and 10%) of *Spirulina* were supplemented at the expense of FM protein and referred to as $S_{2.5}$, S_5, $S_{7.5}$, and S_{10} diets, respectively. Dietary feed ingredients were grinded using a laboratory mix grinder (Panasonic MX- AC300) and blended with water to prepare a dough. Then the dough was passed through a manual pellet machine to make 2 mm diameter pellets. After that the pellets were dried under the sun light for 2 days. The pellets were then stored in plastic containers and kept in refrigerator at – 18 °C for further use.

Table 1 - Proximate composition (%) of different feed ingredients used to prepare experimental diets.

Ingredient	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Mastard oil cake	35.26	10.73	9.10	11.99
Soyabean meal	44.03	5.90	6.87	15.65
Spirulina platensis	61.17	9.87	13.39	18.67
Fish meal	55.47	13.78	18.30	13.23
Wheat bran	13.55	2.11	2.04	10.64
Rice bran	12.74	13.28	7.59	11.39
Wheat flour	5.00	2.95	2.50	8.50

Table 2 - Composition of different feed ingredients and proximate composition (%) for the formulation of experimental diets.

Experimental diet composition (%) / Feed ingredients	S_0	$S_{2.5}$	S_5	$S_{7.5}$	S_{10}
Fish meal (FM)	30.10	29.40	28.70	28.00	27.40
Spirulina platensis	0.00	0.70	1.40	2.10	2.70
Soybean meal	18.00	18.10	18.10	18.15	18.10
Mustard oil cake	8.20	8.20	8.10	8.15	8.09
Rice bran	23.30	23.30	23.30	23.14	23.23
Wheat bran	17.80	17.70	17.70	17.70	17.70
Wheat flour	2.50	2.50	2.60	2.70	2.70
Total	100.00	100.00	100.00	100.00	100.00
Proximate composition (%)					
Protein	32.76 ± 0.33	32.83 ± 0.12	32.95 ± 0.35	33.01 ± 0.22	33.07 ± 0.15
lipid	5.52 ± 0.17	5.74 ± 0.17	6.43 ± 0.32	5.50 ± 0.09	5.13 ± 0.21
Ash	16.35 ± 0.20	13.22 ± 0.16	15.36 ± 0.25	14.54 ± 0.23	13.7 ± 0.40
Moisture	11.81 ± 0.66	11.67 ± 0.50	10.74 ± 0.38	10.45 ± 0.36	10.11 ± 0.20
Fiber	5.95 ± 0.05	5.83 ± 0.06	6.33 ± 0.06	6.49 ± 0.10	6.87 ± 0.06

Water quality parameters

The physicochemical parameters (water temperature, dissolved oxygen: DO, pH, and total ammonia) of the experimental pit water were monitored on a regular basis to monitor the overall cultural environment. The pH, DO, water temperature (°C), and total ammonia were measured using a digital pH meter (Hach Co., Colorado, USA), a digital DO meter (Hach Co., Loveland, Colorado, USA), a Celsius thermometer (Digi-thermo WT-2), and an ammonia measurement kit (HANNA instrument Test Kit), respectively.

Determination of fish growth, feed utilization, and biological indices

At the end of the 10-week feeding trial, fish from each experimental pit were collected, counted, and group-weighed. Growth performance and feed utilization metrics werecalculated according to the following equations:

1. Weight gain (g) = Mean final weight (g) - Mean initial weight (g)

2. $$\text{Percent (\%) of weight gain} = \frac{\text{Mean final fish weight} - \text{Mean initial fish weight}}{\text{Mean initial fish weight}} \times 100$$

3. $$\text{Specific growth rate (SGR \% day}^{-1}) = \frac{\ln W_2 - \ln W_1}{\text{Time}} \times 100$$

Where, W_1 = The initial live body weight (g) at time T_1 (day); W_2 = The final live body weight (g) at time T_2 (day).

4. $$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed consumption (g)}}{\text{Total body weight gain of fish (g)}}$$

5. $$\text{Survival rate (\%)} = \frac{\text{Final number of survived}}{\text{No. of actual fish stocked}} \times 100$$

6. Hepatosomatic index (HSI) = Liver weight (g) ×100 / Body weight (g)

Proximate composition analysis of fish

The proximate composition of fish samples was analyzed following the methods prescribed by the Association of Official Analytical Chemists (AOAC, 2007). After oven drying to a constant dry weight at 105 °C, the moisture content was measured. Petroleum ether extraction in a Soxhlet apparatus for 16 hours was used to estimate the total lipid content, while the micro-Kjeldahl apparatus was used to evaluate the crude protein content. The weight loss following the samples' 6-hour incineration at 550°C in a muffle furnace was used to determine the amount of ash present. The crude protein of *O. pabda* was measured by measuring its total nitrogen content using the conventional Micro-kjeldahl technique. The protein, lipid, ash and moisture content were calculated using the formulas shown below.

% Protein = % Nitrogen × 5.88 (conversion factor used for plant protein)

$$\text{Moisture content (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Weight of the sample (g)}} \times 100$$

$$\text{Ash content (\%)} = \frac{\text{Final weight (g)} - \text{Crucibal weight (g)}}{\text{Weight of the sample (g)}} \times 100$$

% Protein = % Nitrogen × 6.25 (for animal protein)

$$\text{Lipid (\%)} = \frac{\text{(Final weight of beaker+ Sample weight)} - \text{Initial weight of beaker}}{\text{Initial weight of the sample}} \times 100$$

Determination of haematological parameters

Hematological parameters like white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined by a fully automatic hematology analyzer (DYMIND, DH36, China).

Statistical analysis

Throughout the trial, all data were gathered, documented, and saved on a computer spreadsheet. The data were statistically analyzed using one-way ANOVA in Statistix 10 (2013), with significance assessed using the package's Least Significant Difference (LSD) option for comparing means. P < 0.05 was used to define statistical significance.

RESULTS

Water quality parameters

The physicochemical parameters monitored during the experiment are presented in Table 3. Throughout the trial, there were no significant variations (P > 0.05) in water temperature, DO, pH, or total ammonia across treatments.

Growth and feed utilization performance of fish

Table 4 displays the growth indicators of pabda catfish at the end of the feeding trial, expressed as final weight, weight gain (WG), WG%, and SGR values. In the present study, growth indicators revealed an increasing trend till the introduction of 10% *Spirulina*. The WG and WG% of pabda fed with *Spirulina* supplemented feed in S_{10} treatment was significantly higher than that of the other treatments. Significantly (P < 0.05) the higher SGR values were also found in treatment S_{10} (1.66 ± 0.02) where feed contained 10% *Spirulina* and lower value was found in control (1.23 ± 0.04) treatment. The mean values of FCR were observed as 2.22 ± 0.06, 2.11 ± 0.11, 1.65 ± 0.01, 1.78 ± 0.01 and 1.57 ± 0.03 in treatments S_0, $S_{2.5}$, S_5, $S_{7.5}$ and S_{10}, respectively. The means are significantly different (P < 0.05) among the treatments. In this study, significantly (P < 0.05) the lowest value of FCR (1.57 ± 0.03) was found in treatment S_{10}, where 10% *Spirulina* used. There were significant differences in survival rate (P < 0.05) among the treatments. The highest survivability was recorded in S_{10} where 10% *Spirulina* was used as supplement in fish feed. On the other hand, the lowest survival was found in the treatment S_0 where no *Spirulina* was supplemented. HSI values of pabda varied significantly and were 1.02, 1.35, 1.21, 1.12 and 1.37 in treatments S_0, $S_{2.5}$, S_5, $S_{7.5}$, and S_{10}, respectively (Figure 1). Hepatosomatic index of pabda fed with *Spirulina* supplemented feed in $S_{2.5}$, S_5, and S_{10} treatments were significantly (P < 0.05) higher compared to control and $S_{7.5}$ treatment.

Proximate composition of fish

By the end of the trial, the inclusion levels of *Spirulina* had a substantial (P < 0.05) impact on the proximate carcass composition of pabda catfish (Figure 2). The highest protein content (24.62 ± 0.14) % was found in S_{10}, followed by $S_{7.5}$, S_5, $S_{2.5}$, and S_0 treatments, respectively. There was an upward trend observed in the crude protein level with the increasing level of *Spirulina* supplementation (Figure 2a). In the present study, there were significant differences (P < 0.05) in moisture and S_0 treatment showed the highest moisture content compared to the other treatments (Figure 2b). Fish body moisture content (%) showed a downward trend in which represents that moisture content reduced with the increasing supplementation level of *Spirulina* during the 10 weeks of experimental period. There were significant differences in lipid content ($P < 0.05$) among different treatments. The highest lipid content was found in S_0, followed by $S_{2.5}$, S_5, $S_{7.5}$, and S_{10}, treatments, respectively (Figure 2c). There was a downward trend observed in the crude lipid curve with the increasing level of *Spirulina* supplementation. The values of ash content ranged from 1.87 to 3.21% and there were significantly different ($P < 0.05$) among the treatments. The highest ash content was found in the S_{10} (3.21%) followed by S_5, $S_{7.5}$, $S_{2.5}$, respectively (Figure 2d).

Analysis of Haematological parameters of fish

Haematological parameters of fish such as Haemoglobin (Hb), White blood cell (WBC), Red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC) are presented in Table 5. In this experiment, the RBC count of fish was 1.72×10^{12}, 1.90×10^{12}, 2.75×10^{12}, 2.35×10^{12} and 3.18×10^{12} cells L^{-1} in the treatment S_0, $S_{2.5}$, S_5, $S_{7.5}$ and S_{10}, respectively. Maximum RBC (3.18×10^{12}) was found in S_{10} where 10% *Spirulina* used as supplement. There was an upward trend of the RBC, Hb and HCTcontent observed in pabda with the increasing of *Spirulina* level in the treatments. The WBC count was significantly different ($P < 0.05$) among the treatments and the highest WBC count found in the S_{10} followed by $S_{7.5}$, S_5, $S_{2.5}$.. The levels of RBC, HCT, WBC, and Hb showed a dose-dependent rise, with the largest elevations recorded in fish fed 10% *Spirulina.* The MCV of pabda in this experiment were found 89.50, 92.50, 96.50, 125.50 and 148.50 (fl) in treatments S_0, $S_{2.5}$, S_5, $S_{7.5}$ and S_{10}, respectively. Maximum MCV, MCH and MCHC values were found in S_{10} where 10% *Spirulina* was included. On the other hand, the lowest MCV, MCH, MHC values were found in the treatment S_0 where no *Spirulina* was included.

Table 3 - Ranges of water quality indicators recorded during the trial period from different treatments

Treatments	Water temperature (°C)	pH	Dissolve oxygen (mg/L)	Total ammonia (mg/L)
S_0	27.20-29.60	6.13-7.58	6.40-6.95	0.99-1.08
$S_{2.5}$	26.96-30.93	6.21-7.56	6.45-6.97	0.98-1.07
S_5	27.83-30.16	6.50-7.57	6.41-7.00	0.99-1.07
$S_{7.5}$	27.86-30.15	6.61-7.55	6.59-6.89	0.96-1.04
S_{10}	27.96-31.09	6.36-7.51	5.72-6.98	0.98-1.02

Table 4 - Growth and feed utilization performance of pabda catfish feed different levels of *Spirulina* incorporated diets for 10 weeks.

Parameters \ Treatments	S_0	$S_{2.5}$	S_5	$S_{7.5}$	S_{10}
Initial weight (g)	2.48 ± 0.07	2.50 ± 0.02	2.53 ± 0.04	2.52 ± 0.02	2.46 ± 0.05
Final weight (g)	5.88 ± 0.02^c	6.08 ± 0.17^c	7.10 ± 0.04^b	7.03 ± 0.41^b	7.91 ± 0.07^a
Weight gain (g)	3.40 ± 0.09^c	3.58 ± 0.19^c	4.57 ± 0.19^b	4.51 ± 0.38^b	5.43 ± 0.09^a
Weight gain (%)	137.25 ± 5.98^c	143.30 ± 6.01^c	457.45 ± 9.35^b	451.50 ± 9.97^b	545.30 ± 11.38^a
SGR (%/day)	1.23 ±0.04^c	1.27 ± 0.05^c	1.47 ± 0.02^b	1.47 ± 0.07^b	1.66 ± 0.02^a
FCR	2.22 ± 0.06^a	2.11 ± 0.11^a	1.65 ± 0.01^{bc}	1.78 ± 0.01^b	1.57 ± 0.03^c
Fish Survival (%)	90.50 ± 2.11^c	92.25 ± 2.09^{bc}	96.25 ± 4.10^a	93.75 ± 3.12^b	97.50 ± 4.50^a

Values expressed as mean ± standard deviation (SD). Different lower-case letters denote a significant difference ($P < 0.05$) among the treatments. SGR: Specific growth rate, FCR: Feed conversion ratio.

Table 5 - Hematological parameters of *O. pabda* in different treatments after feeding experimental diets for 10 weeks.

Blood parameters \ Treatments	S_0	$S_{2.5}$	S_5	$S_{7.5}$	S_{10}
RBC ($\times 10^{12}$ cells L^{-1})	1.72 ± 0.05d	1.90 ± 0.06^{cd}	2.75 ± 0.06^{ab}	2.35 ± 0.10^{bc}	3.18 ± 0.12^a
Hb ($gd.L^{-1}$)	9.65 ± 1.10^c	10.35 ± 1.22^c	11.45 ± 1.96^b	11.95 ± 1.96^{ab}	12.95 ± 2.01^a
WBC ($\times 10^3$cells mm^{-3})	18.34 ± 0.35^e	20.65 ± 0.66^d	23.05 ± 0.71^c	24.10 ± 0.71^b	26.67 ± 1.01^a
HCT(%)	24.93 ± 1.00^e	26.05± 1.01^d	28.43 ± 1.08^c	29.56 ± 1.10^b	32.75 ± 1.90^a
MCV (fl)	89.50 ± 3.79^e	92.50 ± 3.80^d	95.50 ± 4.47^c	125.50 ± 4.23^b	148.50 ± 4.77^a
MCH (pg)	42.45 ± 2.01^d	43.50 ± 2.00^d	45.20 ± 2.75^c	52.65 ± 2.80^b	58.05 ± 2.97^a
MCHC ($gd.L^{-1}$)	46.80 ± 2.77^d	47.85 ± 2.75^d	54.50 ± 2.81^c	58.75 ± 2.85^b	69.10 ± 3.02^a

Values are represented by the mean ± standard deviation (SD, n = 3). Different superscripted means within the same row differ considerably ($P < 0.05$). Hb: hemoglobin, RBC: red blood cell, WBC: white blood cell, HCT: hematocrit, PCT: platelet, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

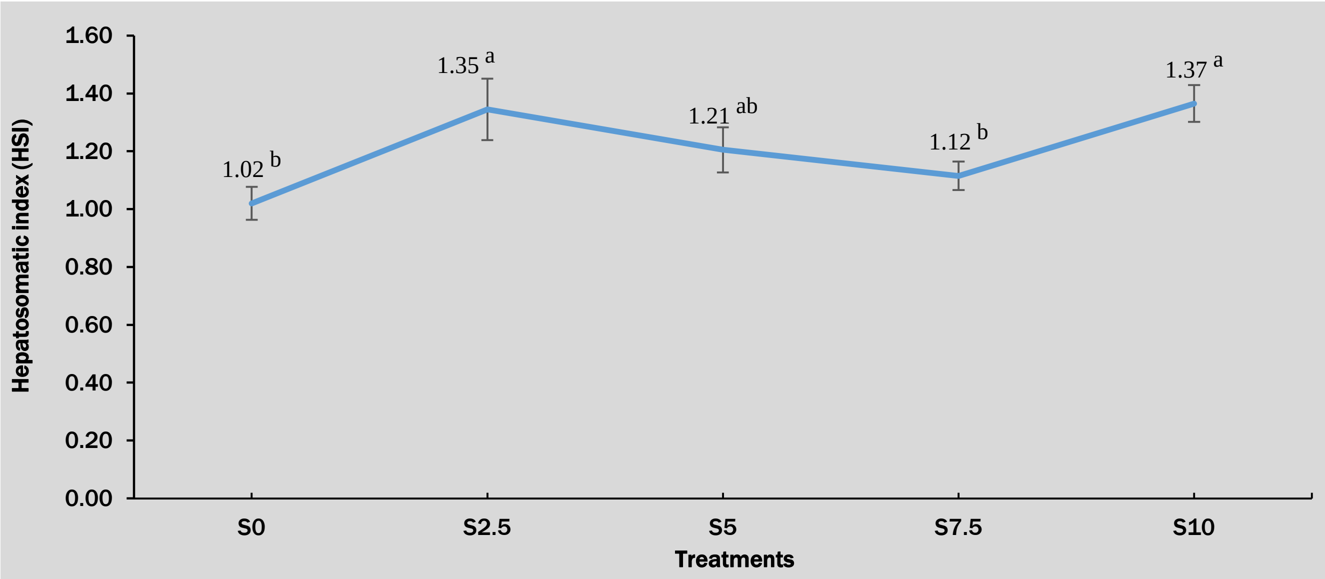

Figure 1 - Hepatosomatic index (HSI) of pabda. Different lower-case letters denote a significant difference (P < 0.05) among the treatments.

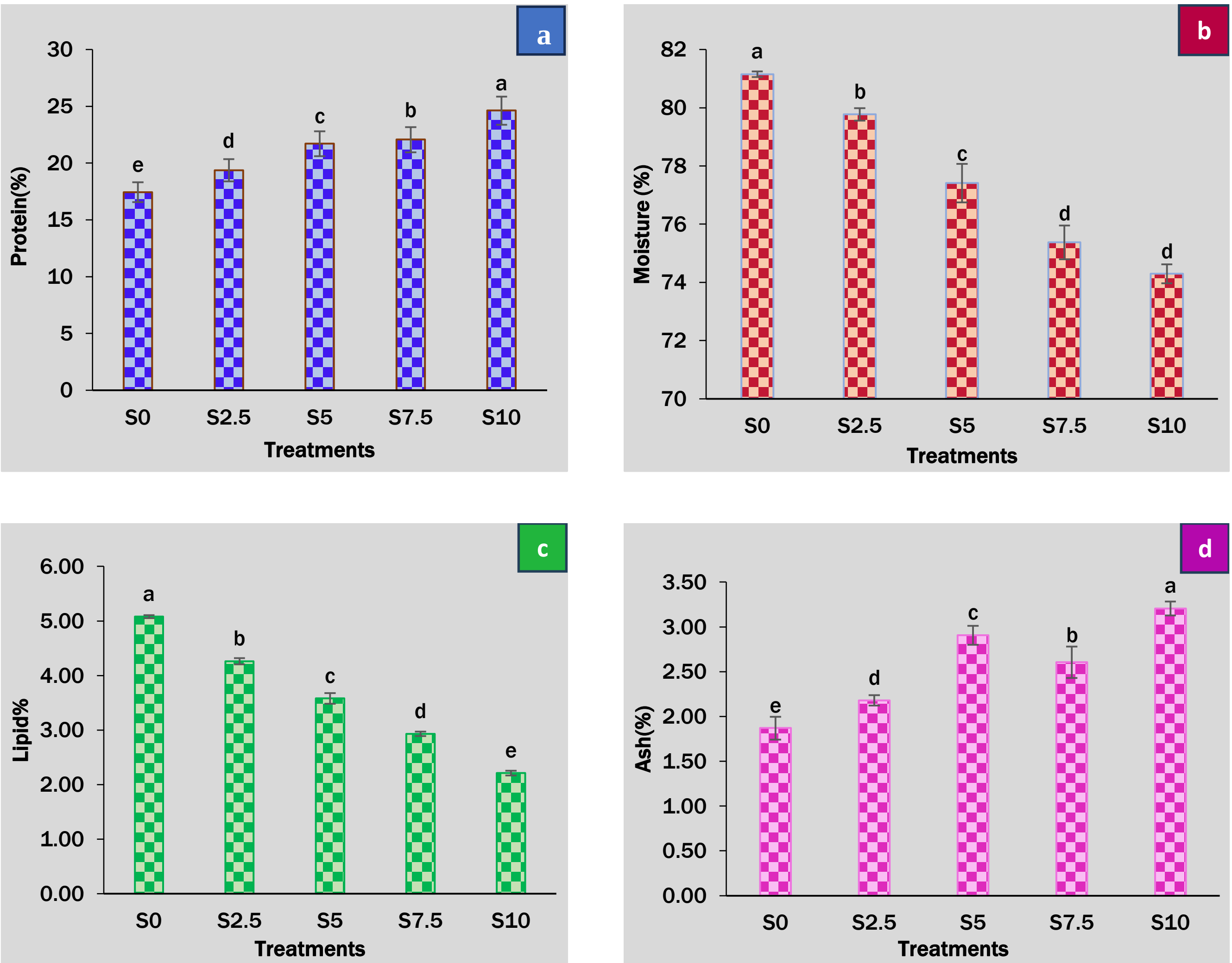

Figure 2 - Proximate carcass composition (% on fresh weight basis) of pabda (*O. pabda*) fed different level of *Spirulina* (*S. platensis*) incorporated diets for 10 weeks. Different lower-case letters denote a significant difference (P < 0.05) among the treatments.

DISCUSSION

Water temperature is an important physical characteristic that has a direct impact on the chemical, physical, and biological properties of a water body. Hossain (2008) reported that, the water temperature ranged from 28.9 to 32.8 °C is suitable for pabda culture. Nelson (2008) observed that fish do well in a range of 17 to 32 ^{0}C temperature depending on the species but better growth achieved at 26 °C or higher. The above statements imply that, water temperature in the present study was within the suitable range for pabda culture system. In this investigation, the pH levels varied from 6.13 to 7.58. According to Bhatnagar and Devi (2013), 6.5 to 8.5 is the ideal pH range for fish culture in ponds, while greater than 9.5 is not recommended. According to Banik et al. (2015) the optimal pH range for pabda in zooplankton-fed fish was 7.3 to 8.5. The previous studies indicate that the pH value in the current research was close to neutral, which is ideal for pabda fish rearing. Dissolve oxygenlevels in this research ranged from 6.40 to 7.0 mg.L^{-1}. According to Boyd and Tucker (2012), pond water with a Dissolve oxygenvalue of 5.0 to 7.0 mg.L^{-1} is favorable for fish production, whereas water with a DO level less than 3 mg.L^{-1} is unproductive. Malla et al. (2015) observed that the Dissolve oxygenlevels of 8.4 ± 0.28 mg.L^{-1} is suitable for pabda reared on live and artificial meals. In terms of total ammonia in the tanks' water, the primary source is fish excretion. Boyd and Tucker (2012) defined safe total ammonia levels as 1.0 mg.L^{-1}. This discussion clearly shows that the water quality parameters were within the acceptable limit for pabda catfish growth and survival. In other words, the addition of *Spirulina* to fish meals had no negative impact on water quality during fish rearing.

Spirulina includes high-quality protein as well as bioactive substances that contribute to growth acceleration. In the present study, weight gain of pabda increased with the increasing supplementation rate of *Spirulina* and all the values in different treatments are higher than control diet. Several prior researches have shown that using *Spirulina* in fish diets improves development and health of fish. Abdulrahman (2014) and Khanzadeh et al. (2016) found that common carp (*Cyprinus carpio*) and three-spot gourami (*Trichopodus trichopterus*) grew the fastest with a *Spirulina* inclusion level of 10%. The results of growth indices in our study were similar as found in previous studies. The present study showed that supplementation with *Spirulina* up to 10% did not have negative impacts on growth performance which is in line with the finding of Teimouri et al. (2013). It happened because of *Spirulina* contains a high-quality nutrient that might have an important role in growth rate compared to the algal-free diet (Spolaore et al., 2006). *Spirulina* contains various elements, including vitamins and minerals, which may aid in fish development. Nandeesha et al. (2001) found SGR values of 1.50-1.83% in experiments with Indian main carp catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets with varying levels of *Spirulina*. Promya and Chitmanat (2011) found SGR values between 2.14-2.53% on the experiment where *Spirulina* positively effect on growth, quality and immunity of catfish. This difference of SGR value could be due to the species variation as well as initial size of fish used and also may be due to the difference of culture season and different kinds of feed. The findings are consistent with those of other studies (Hirahashi et al., 2002; Hernandez et al., 2012; Palmegiano et al., 2008) who discovered that feeding *Spirulina* to fish enhanced survival and growth rates.

A low FCR value is an indication of better feed utilization efficiency of a formulated feed. Al-Deriny et al. (2020) showed that dietary *Spirulina* increased the FCR of Nile tilapia, which ascribed to the algae's involvement in improving intestinal morphometry indices and therefore increasing intestinal absorption capacity. Furthermore, adding *Spirulina* to the diet of the Oscar fish (*Astronotus ocellatus*) boosted growth performance and feed utilization efficiency (Mohammadiazarm et al., 2021). This accounts for the nutritional value of algal bioconstituents such as fatty acids, amino acids, vitamins, and minerals. Nandeesha et al. (2001) found FCR value (2.10-2.56) on their experiment with diet containing different level of *Spirulina* in Indian major carp catla and rohu. Kim et al. (2013) reported that partial substitution of FM with *Spirulina* in diets for parrot fish (*Oplegnathus fasciatus*) increased FCR from 1.98 to 2.27. The current study's findings are consistent with those of Ibrahem et al. (2013), who discovered that feeding with *Spirulina* powder increased the FCR and growth rates of striped jack (*Pseudocaranx dentex*) and *O. niloticus*.

An upward trend was observed with fluctuation in the survival rate with the use of high amount of *Spirulina* in fish feed as supplement. Dernekbasi et al. (2010) found that, in comparison to other commercial feeds, fish survival improved with an increase in the amount of *Spirulina* in the diet. *Spirulina* most likely increased growth rate and decreased death rate in the current research. According to research done by Youssef et al. (2023), *Spirulina*'s lack of cellulose makes it more readily digestible, which increases fish appetite, feed intake, and nutrient digestibility. These factors all contribute to the fish's improved health and increased resistance to infection by lowering stress levels. Hepatosomatic index (HSI) provides an indication on status of energy reserve of an animal. The present study collaborates with HIS value found by Peres et al. (2003). The present study proved that dietary supplementation of *Spirulina* enhanced fish growth. These results may possibly be due to the better digestibility and absorption of nutrient.

In present study crude protein content in fish body carcass was found better in Spirulina supplemented diet compared to non-supplemented diet. This conclusion is consistent with that of Mohammadiazarm et al. (2021) who found an increase in protease levels in fish fed with *Spirulina*, implying that it plays a role in enhancing protein utilization. According to El-Sheekh et al. (2014) crude protein content increased in tilapia with the feed increase of *Spirulina* protein percentage. Abdulrahman (2014) also found the same effect of increasing crude protein level (17.86-25.14%) with the increase of *Spirulina* protein (%) in case of common carp. Therefore, all the findings are similar to the result found in the present study. The protein level in pabda after feeding different level of *Spirulina* protein suggested that supplementation of *Spirulina* increases the protein content in fish body. Fish protein percentages may rise as a result of *Spirulina*'s high protein content and capacity to stimulate fish somatic growth and protein synthesis (Mohammadiazarm et al., 2021). The decrease of lipid content concomitant with an increased *Spirulina* supplementation level agrees with the results found by Abdulrahman (2014) and Khanzadeh et al. (2016). The current findings demonstrated a reduction in lipid content in those administered *Spirulina*-supplemented meals. Mohammadiazarm et al. (2021) found that the polyphenol content of algae, such as β-carotene or phycocyanin, can reduce lipid levels (Kim et al., 2013; Hassaan et al., 2021). Hossain et al. (1999) conducted an experiment on nutritional value of some small indigenous fish species (SIS) of Bangladesh and found lipid content 1.87% - 9.55% fresh matter basis. In this study, lipid content ranged from 2.21% - 5.08%. *Spirulina* known to decrease lipid deposition (Nandeesha et al., 2001). Kim et al. (2013) conducted an experiment on partial replacement of FM with *Spirulina* in diets for parrot fish and found that higher the inclusion the *Spirulina* the lower crude lipid percentage which is similar to the present study. According to Nandeesha et al. (2001), the effect of dietary *Spirulina* on whole-body lipid content varies with the type of *Spirulina* utilized. The effects of *Spirulina* on whole-body protein and lipid levels are connected with their synthesis and accumulation rates in muscle, as well as the organisms' growth rate (Soivio et al., 1989). In a study of Stansby (1954) moisture content for fresh water fish was reported to be in the range of 72.1-83.6%. The finding in case of ash content was agreed with the studies of Khanzadeh et al. (2016); El-Sheekh et al. (2014), and Abdulrahman (2014). These studies found that higher inclusion levels of *Spirulina* led to increased ash content.El-Sheekh et al. (2014) found ash content in red tilapia ranged from 10.1 to 10.8 % on dry matter biases by using *Spirulina* in feed. Olvera et al. (2008) found ash content of tilapia between 3.13% and 4.17 % and the ash content was higher in control diet than the FM replacement *Spirulina* diet. Nandeesha et al. (2001) used four experimental diets for common carp by replacing FM protein through the incorporation of *Spirulina* and found ash content from 2.04 to 3.09%. In this study the ash content was measured on the dry weight basis. Though there was a fluctuation in $S_{7.5}$ treatment otherwise the ash content was increased with the raising of *Spirulina* supplementation level in the present study.

Variation in the haematological parameter is an important tool for representing the fish immunity as well as the health status (Celik, 2004). The levels of RBC, HCT, WBC, and Hb showed a dose-dependent rise, with the largest elevations recorded in fish fed 10% *Spirulina* supplemented diet. Similar to our findings, including *Spirulina* into the diet of Great Sturgeon (*Huso huso*) dramatically raised RBC and Hb (Milad et al, 2016). *Spirulina* contains a lot of iron and has considerable effects on erythropoiesis in anaemic rats by boosting the amounts of RBCs and Hb (Kapoor and Mehta, 1992). Sayed and Fawzy (2014) reported that dietary *Spirulina* significantly affected the haemoglobin content of *C. gariepinus* and varied from 7.51 to 9.83 g.dl^{-1}. Ibrahem et al. (2013) found that haematocrit tended to increase with increasing dietary *Spirulina* levels than the control fish. In the present study after supplementation of *Spirulina* in the experimental diets the haemoglobin counts significantly increased in the treatment, S_{10} (12.95 g.dl^{-1}) than the control, S_0 (9.65 g.dl^{-1}). Thus, the increase of RBC and Hb in our study might be ascribed to the presence of iron element in *Spirulina*. Increase in red and white blood cells of fish resulted may be due to the presence of C-phycocyanin in *Spirulina*, which can help to increase the immunity stimulating capacity of fish (Eissa et al., 2024). RBC carries glucose from blood to different cells of the whole body. Higher value of RBC represents increasing haemoglobin content in blood (Pelster, 2001). Raising the haemoglobin level of fish is an excellent way to measure their ability to transport oxygen, which allows correlations to be established between the fish's health and the oxygen concentration in their environment. It is a good immunological sign for fish because it indicates more transportation of oxygen in the blood which will prevent fish from anemia (Esmaeili, 2021). Furthermore, in the current study, nutritional supplementation with *Spirulina* resulted in greater levels of WBC compared to control, which was consistent with the findings in yellow croaker (*Pseudosciaena crocea*) (Li et al., 2014) and common carp (Samah et al., 2017). Higher WBC levels in fish given *Spirulina* supplemented diets might be due to the existence of a polypeptide-phycocyanin, which was discovered to be a significant role in boosting WBC in mice (Zhang et al., 1994). The increased WBC cells in blood usually help fish body to fight against infections and some diseases. So, the higher value of WBC indicates better immunity of fish (Kori-Siakpere et al., 2006). MCHC depends on hemoglobin (Hb) synthesis. When Hb synthesis decreases the MCHC also reduces and causes anemia (Javed et al., 2016). If hemoglobin is increased, the MCHC value is able to diminish hypochromic anemia. The results of the current study are in accordance with Ibrahem et al. (2013) who found that the MCV, MCH, MCHC in fish were increased with increasing dietary *Spirulina* levels. In the present study, the values of these parameters gradually increased with the increasing supplementation of *Spirulina* and within in suitable range which indicated a better immunity of fish than control.

CONCLUSION

The current study showed a positive dietary effect of *Spirulina* on growth performance, body composition, and haematological parameters of pabda. Among the different supplementation levels, the best growth performance of pabda was obtained in the case of 10% *Spirulina* supplementation. In addition, all the haematological parameters also improved with the increasing of *Spirulina* supplementation level during the experiment which indicates better immunity of pabda. Among the different supplementation levels, 10% *Spirulina* supplementation showed the best value of haematological parameters. From this study, it can be concluded that, 10% *Spirulina* supplementation in pabda feed could help to get better production and health performance in the culture. However, further work is necessary to understand the effect of higher percentage of *Spirulina* supplementation, its chemical nature, mode of action and also in vivo and in vitro tests need to be conducted for better growth, quality and health of pabda.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval

The authors followed all applicable international, national, and institutional guidelines for the care and use of fish.

Authors' contribution

Conceptualization: T. Akter, M. Das; Data curation: S. Noman, M. Das, M.R. Islam; Formal analysis: S. Noman, F.S. Bristy; Investigation: S. Noman, M. Das, M.R. Islam, T. Akter; Methodology: S. Noman, T. Akter, M.R. Islam; Validation: S. Noman, M. Das, T. Akter; Writing – original draft: T. Akter, M. Das, S. Noman, R.R. Islam, F.S. Bristy; Writing – review & editing: T. Akter, M. Das, M.R. Islam.

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Consent to publish

All authors agree to the publication of this manuscript.

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Competing interests

The authors declare no competing interests in this research and publication.

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