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Effect of Stocking Density, Multispecies Probiotics, and Biofloc on Metabolic and Physiological Responses of *Puntius sophore* in Laboratory Conditions

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Abstract: A 60-day experimental trial was conducted to evaluate the effect of different stocking densities, probiotic supplementation, and a biofloc system on the growth and physiological responses of *Puntius sophore* in laboratory conditions. *P. sophore* (8.64 ± 1.24 g) was obtained from the Brahmaputra River, Mymensingh, and immediately transferred to a flow-through water system. In experiment 1, fish were subjected to three treatments (20 fish per 400 L as LD, 25 fish per 400 L as MD, and 30 fish per 400 L as HD), and similarly, in experiment 2, three different types of diets were provided (control diet (D1), biofloc (D2), and a probiotic-containing diet (D3)). Three replications were used in the completely randomized experimental design. Growth parameters, viz. TLG, BWG, FCR, FCE, SGR, RGR, and DGR, were significantly influenced by stocking density and probiotics ($p < 0.05$). The highest growth rate was observed in LD and in D3. The lowest FCR was also observed in LD and in D3, while highest was in HD (30 fish per 400 L) and in D2. Though stocking density does not impact the blood profile, with the exception of WBC, in the case of feeding regime, WBC, RBC, MCV, MCH, and PLT levels differed significantly ($p < 0.05$); however, all haematological measures were within the normal range in both experiments, and the fish's physiological conditions were better in LD and feeding with probiotic supplementation (D3). The results obtained from this study suggested that the welfare of *P. sophore* is adversely affected by high stocking density and increased growth and physiological conditions when cultured with LD and supplemented with probiotics.

Keywords: stocking density; probiotic; biofloc; growth; haematology

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Citation: Debi, S.; Salam, M.A.; Das, S.K.; Alam, M.S.; Rahman, M.L.; Hossain, M.S.; Mazumder, S.K. Effect of Stocking Density, Multispecies Probiotics, and Biofloc on Metabolic and Physiological Responses of *Puntius sophore* in Laboratory Conditions. *Water* **2024**, *16*, 820. https://doi.org/10.3390/w16060820

Academic Editor: Reynaldo Patiño

Received: 3 December 2023
Revised: 27 January 2024
Accepted: 31 January 2024
Published: 12 March 2024

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1. Introduction

The pool barb, also called the spotfin swamp barb or stigma barb (*Puntius sophore*), is a tropical freshwater fish species belonging to the Cyprinidae family. It is found in Asian nations and is indigenous to inland waters in Asia [1]. The market's need for both fresh and processed products, as well as their nutritional and decorative value, are driving up the demand for this widely distributed small indigenous fish species (<25 cm) in freshwater [1,2]. Though *P. sophore* has been classified as lower risk to near threatened in recent research, the wild populations are declining quickly because of intense fishing pressure [3]. There is fear that this fish may disappear unless proper steps are urgently

taken to protect the fish from extinction. Nevertheless, there are few studies in the literature regarding their growth and physiological performances in captivity.

Stocking density is an essential aspect in fish culture operations since it directly affects the growth and survival of the fish, which in turn affects production. According to Ahmed et al. [4], social interactions and poor water quality are where stocking densities have the greatest negative consequences on fish; thus, the appropriate stocking density varies depending on the species raising system, feed utilization, growth potential, management techniques, and best size at harvest [5]. Fish metabolism, development, and stress are significantly impacted by the stocking density, and this impact is frequently species-specific [6]. The severity, duration of the stressor, and physiological and behavioural changes that mobilize energy sources result in decreased performance and slower growth [7,8]. These effects may be brought on by physiological stress or a decline in water quality, such as a drop in dissolved oxygen levels and an increase in ammonia levels [8]; therefore, it is crucial to establish the proper stocking density for each fish species at each stage of their lives in all varieties of production systems [5].

Dietary supplements to enhance growth and disease resistance through nutrition have drawn a lot of interest. Probiotics are one of the more promising biological prevention and control methods among dietary supplements. Increased digestive enzyme activity, the production of inhibitory chemicals against various dangerous microbes, immune system regulation, and the prevention of gut pathogen colonization by competitive exclusion are some positive benefits of probiotic use [9]. Probiotics' effects in aquaculture deserve special attention in terms of economic evaluation, as they can help farmers make a profit by producing high-quality fish [10]. Aquaculture routinely employs Bacillus spp. as a probiotic [11,12]. They have been shown to improve fish immunity and protect them from a number of diseases and convert carbohydrates into lactic acid [13].

The alteration of the gut microbiota and establishment of beneficial microorganisms, as well as higher specific and total digestive enzyme activity in the brush border membrane, all of which promote nutritional digestibility and feed utilization, are all benefits of probiotics. In aquaculture, probiotics have been shown to have many benefits in aquaculture, including enhancing growth, immunity, disease resistance, and water quality [14,15]. Probiotics can be categorized as single-strain or multi-species probiotics based on the number of probiotic species or genera. A variety of single-strain probiotics are available. For instance, *Saccharomyces cerevisiae*, a probiotic yeast, has been effectively assessed and is well known for its capacity to strengthen defence mechanisms and increase immunity in a range of finfish species [16,17]. Numerous fish species have been shown to benefit from the probiotic properties of *Bacillus* species, particularly *B. subtilis* [18,19]. In aquaculture, lactic acid bacteria (LAB) have also been effectively used as possible probiotics [20]. Due to its growth-promoting and immunomodulatory properties, *Enterococcus faecium* probiotic applications have become quite popular in aquaculture [21,22]. Conversely, multispecies probiotics are generally more beneficial to the host than single- or separate-strain probiotics since they are a combination, blend, or cocktail of two or more probiotic species or genera [23,24].

By creating microbial flocs from the addition of an external carbon source, such as molasses, rice bran, or wheat bran, among others, the biofloc system is a revolutionary culture technology that enables the growth of microalgae, protozoans, rotifers, and nematodes [25]. Fish can eat the floc biomass that forms in bodies of water as a source of additional food and as a water purifier [26]. Avnimelech [27] notes that fish are also exposed to microbial compounds through bioflocs, such as β-1, 3-glucan, peptidoglycan, lipopolysaccharides, etc., which activate and maintain the non-specific immune system; however, opportunistic and pathogenic microbes may be present in microbial communities [28]. What are the potential treatments for these potentially dangerous agents that could develop and multiply in the rearing water before eventually entering the host body through feeding and osmoregulation processes [29]?

The haematological value predicts the survival condition or physiological stress of organisms that respond to endogenous or exogenous changes [30], especially with environ-

mental status [31], simply acting as a biomarker in the different habitat of fish [32] as well as for changes of chemical and environmental stress, ecological condition, temperature, food habit, different periods of the reproductive cycle, and chemical exposure [33]. Haematological studies help to improve the aquaculture system because it is a vital tool to recognize the healthy individuals from diseased or stressed ones [34]. The current study was created with the following objectives in mind: (1) to observe the effect of different stocking densities on the growth and haematological responses of *P. sophore* and (2) to compare the growth performances and haematological profile of *P. sophore* diets using biofloc and probiotics as diet supplementation. These efforts will help to determine the optimal choice of stocking density and food for ensuring fish growth and physiological performance.

2. Materials and Methods

2.1. Fish Sampling and Experimental Site

The experiment was conducted at the Department of Genetics and Fish Breeding (GFB) laboratory at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh (Figure 1). Fish samples (9.82 ± 0.48 cm TL and 8.64 ± 1.24 g BW) were obtained from the Brahmaputra River, Mymensingh, and transported to the GFB laboratory.

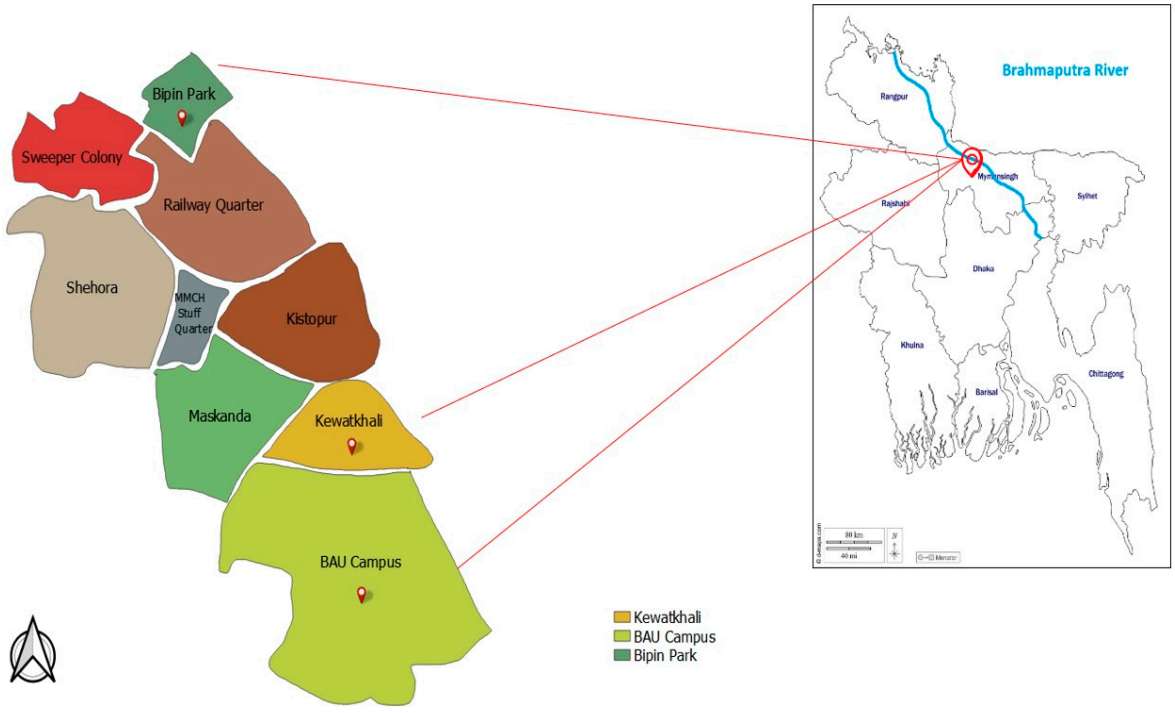

Figure 1. Map showing the sampling site.

2.2. Experimental Design

The fish were kept in stocking tanks of equal size (5000 L) with a good inlet and outlet system for up to 30 days at a constant ambient temperature (26 °C) and were fed a commercial feed (Table 1). The fish were moved to the experimental circular tank once they began to feed and expel excrement (400 L). To satisfy the oxygen requirement, each tank was supported by diffuser air stones linked to 35 W air compressors (70 L min^{-1}, >0.25 Mpa, Hailea Co., Ltd., Chaozhou, China). In the present study, two experiments were carried out:

(1) Stocking density experiment (SD): *P. sophore* were subjected to three treatments and three replicates each (low-density LD: 20 fish per 400 L; medium-density MD: 25 fish per 400 L; and high-density HD: 30 fish per 400 L). A total of 225 fish were used in the first experiment.

(2) Diet supplementation experiment (DS): Similarly, fish were subjected to three treatments with triplicates each (D1: control diet; D2: biofloc; and D3: probiotic + commercial diet). For each tank, 20 fish were used (Figure 2). A commercial diet was used as a control diet. In this experiment, a total of 180 fish were used.

Table 1. Proximate composition (% dry matter) of the commercial diet used in the experiment.

Proximate Composition	Percentage (%)
Moisture	12.98 ± 0.41
Protein	31.20 ± 0.34
Lipid	6.72 ± 0.80
Ash	13.22 ± 0.61
Fibre	11.58
NFE *	24.30

Note(s): * Nitrogen-free extract (NFE) calculated as NFE = 100 − %(moisture + crude protein + crude lipid + ash + crude fibre).

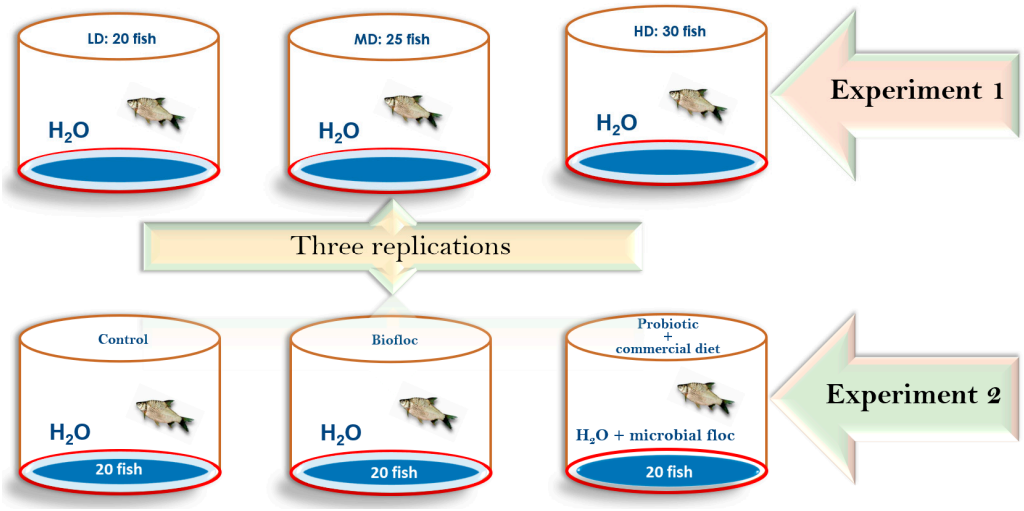

Figure 2. Experimental design for this study.

For both experiments, every tank was prepared with a good inlet and outlet system to allow for a daily water exchange of at least 20%, except for the biofloc group. Fish were fed until satiation twice daily (9.00 and 16.00 h) at a rate of 2–3% of their body weight. The experiments were conducted for 60 days. A 12:12 h light–dark cycle was used to maintain the photoperiod (with an artificial luminance of about 600 lx). Every 15 days, the fish were sampled and the data were recorded.

2.3. Experimental Feeds and Biofloc

In the biofloc treatments, fish were fed with commercial feed (Table 1), and molasses and rice powder were added as an external carbon source to promote the development of flocs. To facilitate the growth of biofloc, 500 mL of biofloc that was produced from a different experimental BFT balanced system (500 L) was added to each experimental unit seven days before the trial started. A C:N ratio of approximately 20:1 was maintained during the study to optimize heterotrophic bacterial growth. The adjustments to the molasses and C:N ratio were recommended according to Emerenciano [35]. The water was not changed in these tubs, and constant aeration was maintained to avoid the sedimentation of flocs.

2.4. Experimental Feeds with Probiotic

This work made use of commercially available probiotics consisting of *Bacillus subtilis*, *Lactobacillus plantarum*, and *B. megaterium*, which was previously isolated from the intestine of *Oncorgynchus mykiss* rainbow trout (provided by Zistyar Varna Co., Superzist, Iran).

To achieve the final conc. of 2 × 108 CFU kg^{-1} of feed (0.2% per 100 g feed), sterile physiological serum (0.9% *w*/*v* of NaCl) was mixed in 2 g of the probiotic (containing 108 CFU per 1 g of cell weight) and sprayed on 1 kg of a commercial dry feed [36]. The feed was dried at ambient temperature before being stored in a refrigerator (4 °C) until use.

2.5. Water Quality Parameters

Throughout the experimental period, the total ammonia nitrogen (NH3-N) and total hardness were determined on a weekly basis during the study period, whereas the temperature, dissolved oxygen (DO), and pH of the water were examined daily. At 9.00 h, all measurements were obtained. With the help of a YSI 59 multiparameter water quality probe, temperature, DO, and pH were measured (Yellow Spring Instrument Company, Brannum Lane, OH, USA). Utilizing an API Freshwater Master Test Kit, NH3-N and total hardness were measured (SKU: 35647).

2.6. Growth and Survival

Fish from each experimental tank were individually weighed at the beginning of the experiment and every two weeks after that. Clove powder (99% purity, Talya Bitkisel Urunler Ind. Co., Ltd., Antalya, Turkey; 200 mg L^{-1}) was used to lightly sedate fish prior to sampling. Using a measuring board, the total length of the fish were recorded to the nearest 1.0 cm, and they were weighed using an electronic balance (Model: KD-300KC) to the nearest 0.01 g [37]. The same protocol was followed for both experiments. The following formulas were used to calculate the food consumption and growth indices:

$$\text{Body weight gain (BWG, g)} = (W2 - W1) \times n \quad (1)$$

$$\text{Food consumption (FC, g/d)} = ((\text{g food consumed})/\text{day}) \quad (2)$$

$$\text{Food conversion ratio (FCR)} = (F/(W2 - W1)) \quad (3)$$

$$\text{Food conversion efficiency (FCE)} = (\text{gain in fish mass/g food consumed}) \quad (4)$$

$$\text{Specific growth rate (SGR, \%day}^{-1}\text{)} = 100 \times ((\text{LnW2} - \text{LnW1})/t) \quad (5)$$

$$\text{Relative growth rate (RGR, \%)} = 100 \times ((W2 - W1)/W1) \quad (6)$$

$$\text{Daily growth rate (DGR, \%)} = 100 \times ((W2 - W1)/t) \quad (7)$$

$$\text{Survival rate (\%)} = 100 \times (\text{Final no. of fish/initial no. of fish}) \quad (8)$$

Here, F represents total amount of food consumed during the experiment, W1 and W2 represent the starting (0 day) and the final (60 day) mean individual weights for each treatment, *n* represents the total no. of fish, and t represents length of the experiment.

2.7. Surgical Protocol and Haematological Analysis

Five fish were randomly selected from each tank at the end of the experiment to assess various haematological factors. Fish were anaesthetized with clove oil (99% purity, Talya Bitkisel Urunler Ind. Co., Ltd., Antalya, Turkey; 200 mg L^{-1}) until ventilator movements stopped in order to lessen handling stress [38]. Fish were rapidly caught, and blood samples were collected from the caudal vein using a heparinized syringe and transferred to tubes that contained the anticoagulant substance EDTA. The haematological profile was determined using automated haematology analysis (HeCo Vet C; SEAC, Florence, Italy) and a special fish lysing reagent (SEAC, Code 71010460) previously used for other fish species [39]. The determination of the white blood cell count (WBC), red blood cell count (RBC), lymphocyte (LYM), monocyte (MON), haemoglobin conc. (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin conc. (MCHC), and platelets (PLT) were all

included in the evaluation of the haematological parameters. The animal ethics committee of BSMRAU approved the protocol with the code BSMRAU/Research Wing/Innovation Project/128/2019/903.

2.8. Statistical Analysis

Prior to statistical analysis, the Kolmogorov–Smirnov test was used to determine whether all data had a normal distribution, and Bartlett's test was used to check whether the variances were homogeneous [40]. A one-way ANOVA analysis was performed on the data to determine the impact of the stocking density and feeding experiment on the growth and haematological responses. If ANOVA indicates that there are significant differences in mean scores among the groups, then Tukey post hoc tests were performed. A significance level of $p < 0.05$ was used for each analysis. Every outcome was presented as mean ± S.E. The software programs OriginTM v 2016 and Minitab v 17 were used for all statistical analyses.

3. Results

3.1. Water Quality Parameters

Table 2 displays the values (means ± S.E.) of the physicochemical parameters measured for the experimental tanks during this study. None of the metrics showed a significant difference ($p > 0.05$), and they remained stable at the minimal treatment doses.

Table 2. Physicochemical parameters that were measured throughout the period of the experiment.

Parameters	D1	D2	D3
Temperature (°C)	26 ± 0.64	26 ± 0.14	27 ± 0.15
DO (mg L^{-1})	5.9 ± 0.20	5.8 ± 0.30	5.9 ± 0.30
TH (mg L^{-1})	119.33 ± 22.33	108.07 ± 18.41	125.4 ± 16.49
NH_3-N (mg L^{-1})	0.25 ± 0.20	0.24 ± 0.34	0.33 ± 0.24
pH	7.47 ± 0.09	7.07 ± 0.31	7.27 ± 0.17

Notes: DO: dissolved oxygen; TH: total hardness; and NH_3-N: ammoniacal nitrogen.

3.1.1. Growth Performances

After 60 days, the mean TLs did not differ substantially ($p > 0.05$) among the treatments. The stocking density with 20 individuals containing treatment showed the best performance in the case of mean weight gain, which was a significantly higher ($p < 0.05$) value than the stocking densities containing 25 and 30 fish per tank. The stocking densities of 25 and 30 individuals did not substantially ($p > 0.05$) affect the mean weight (Figure 3).

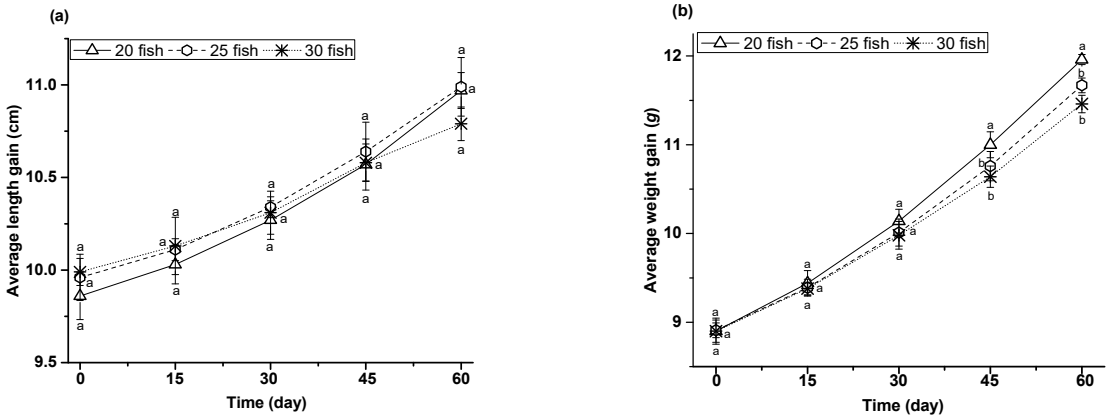

Figure 3. Changes in the average (**a**) length gain, and (**b**) weight gain of *P. sophore* after 60 days of rearing at three different stocking densities. The means with S.E. values on the same date that do not share the same letter differ significantly ($p < 0.05$).

The overall growth performance of *P. sophore* at the three stocking densities is represented in Table 3. In the LD group, the TLG (1.11 ± 0.10 cm), BWG (3.06 ± 0.53 g), FCE (0.57 ± 0.10), SGR (0.49 ± 0.05% day^{-1}), RGR (34.34 ± 3.87%), and DGR (30.60 ± 5.33% day^{-1}) were significantly higher. Nevertheless, no significant difference in FCR was reported in the LD (1.78 ± 0.04) and MD (1.82 ± 0.05), but it was higher significantly in the HD (2.07 ± 0.07, $p < 0.05$); however, no fish died, and the survivability was 100% for every treatment.

Table 3. Growth performance of *P. sophore* reared at three different stocking densities.

Variables	Stocking Density		
	LD	MD	HD
L_1 (cm)	9.86 ± 0.22 ^{a}	9.96 ± 0.41 ^{a}	9.99 ± 0.31 ^{a}
L_2 (cm)	10.97 ± 0.30 ^{a}	10.99 ± 0.52 ^{a}	10.79 ± 0.48 ^{a}
W_1 (g)	8.90 ± 1.04 ^{a}	8.91 ± 0.78 ^{a}	8.90 ± 0.73 ^{a}
W_2 (g)	11.96 ± 1.49 ^{a}	11.67 ± 0.93 ^{a}	11.46 ± 0.83 ^{a}
TLG (cm)	1.11 ± 0.10 ^{a}	1.03 ± 0.14 ^{a}	0.80 ± 0.27 ^{b}
BWG (g)	3.06 ± 0.53 ^{a}	2.76 ± 0.21 ^{ab}	2.56 ± 0.32 ^{b}
FCR	1.78 ± 0.04 ^{b}	1.82 ± 0.05 ^{b}	2.07 ± 0.07 ^{a}
FCE	0.57 ± 0.10 ^{a}	0.52 ± 0.04 ^{ab}	0.49 ± 0.06 ^{b}
SGR (% day^{-1})	0.49 ± 0.05 ^{a}	0.45 ± 0.03 ^{ab}	0.42 ± 0.05 ^{b}
RGR (%)	34.34 ± 3.87 ^{a}	31.07 ± 2.09 ^{ab}	28.93 ± 4.15 ^{b}
DGR (% day^{-1})	30.60 ± 5.33 ^{a}	27.60 ± 2.06 ^{ab}	25.60 ± 3.23 ^{b}
Survival (%)	100	100	100

Note: Significant differences exist between the values (mean ± SE) displayed on the same row that do not share the same letter ($p < 0.05$).

3.1.2. Haematological Responses

In this investigation, there were no significant differences in the haematological indices of LYM, MON, RBC, Hb, HCT, MCV, MCH, MCHC, and PLT among the rearing densities (Table 4). The lowest and highest values were recorded at LD and HD, respectively. Only WBC, however, was substantially impacted by stocking density ($p < 0.05$). When compared to the fish subjected to the other treatments, the fish in the LD (41.93 ± 1.21) and MD (47.47 ± 4.41) groups had significantly lower values than those in the HD (60.83 ± 3.94) group for WBC (Table 4).

Table 4. Effects of different stocking densities on the haematological values of *P. sophore*.

Parameter	Treatments			Analysis of Variance			
	LD	MD	HD	Adj SS	Adj MS	*F*	*p*
WBC (×10^{9} L^{-1})	41.93 ± 1.21 ^{b}	47.47 ± 4.41 ^{b}	60.83 ± 3.94 ^{a}	566.5	283.25	15.54	0.004
LYM (%)	65.20 ± 3.90 ^{a}	69.67 ± 3.19 ^{a}	73.37 ± 5.53 ^{a}	100.3	50.17	1.79	0.245
MON (%)	4.47 ± 0.61 ^{a}	4.30 ± 0.16 ^{a}	4.93 ± 0.29 ^{a}	0.6467	0.3233	1.33	0.331
RBC (×10^{6} mm^{-3})	3.15 ± 0.54 ^{a}	2.81 ± 0.93 ^{a}	2.61 ± 0.51 ^{a}	0.4482	0.2241	0.32	0.741
Hb (g dL^{-1})	13.00 ± 1.76 ^{a}	11.62 ± 1.82 ^{a}	11.50 ± 1.20 ^{a}	4.177	2.089	0.53	0.613
HCT (%)	42.80 ± 5.92 ^{a}	39.13 ± 1.16 ^{a}	38.23 ± 2.02 ^{a}	35.11	17.55	0.87	0.467
MCV (pg)	137.60 ± 14.8 ^{a}	154.25 ± 44.77 ^{a}	153.40 ± 36.16 ^{a}	527.9	263.9	0.15	0.864
MCH (fl)	43.69 ± 14.10 ^{a}	47.21 ± 18.02 ^{a}	46.55 ± 13.39 ^{a}	20.98	10.49	0.03	0.971
MCHC (%)	31.29 ± 7.88 ^{a}	29.59 ± 3.98 ^{a}	30.00 ± 1.82 ^{a}	4.683	2.341	0.06	0.945
PLT (×10^{3} mm^{-3})	37.40 ± 4.97 ^{a}	27.45 ± 5.50 ^{a}	32.13 ± 3.13 ^{a}	148.8	74.39	2.30	0.182

Notes: The data are displayed as mean ± SE. Values with the same superscript letter in a row are not different significantly ($p > 0.05$).

3.1.3. Growth Performances

The graphs express the average TL and BW of *P. sophore* fed a commercial diet (control), biofloc as additional diet, and a probiotic-containing diet (Figure 4). After 60 days, the individuals given the probiotic-containing diet (D3) gained a significantly ($p < 0.05$) higher

mean total length value that was more than 11 cm. Similarly, the best performance in the case of average weight gain (>12 gm) was also observed in D3 which was significantly higher ($p < 0.05$) compared to D1 and D2. The average value of TL and BW were significantly lower ($p < 0.05$) in D2 than D1 (Figure 4).

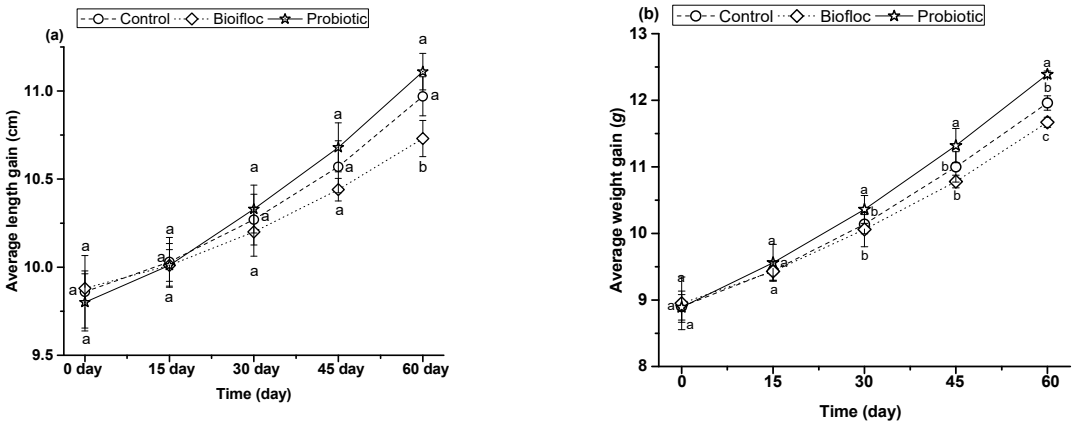

Figure 4. Changes in the mean (**a**) TL and (**b**) BW of *P. sophore* reared on three diets over 60 days. The means with S.E. values on the same date that do not share the same letter differ significantly ($p < 0.05$).

The overall growth performance of *P. sophore* reared on three different diets (D1, D2, and D3) is shown in Table 5. For fish fed with the dietary-probiotic-treated diets (D3), their TLG (1.31 ± 0.15 cm), FCE (0.67 ± 0.06), SGR (0.55 ± 0.04% day^{-1}), and RGR (39.48 ± 3.63%) values were significantly higher, and FCR (1.50 ± 0.14) was significantly lower than fishes fed with biofloc (D2) and control treatments (D1) ($p < 0.05$); however, the BWG (3.50 ± 0.32 g) and DGR (35.00 ± 3.25% day^{-1}) were increased significantly in D3 when compared with D2 ($p < 0.05$), but no significant difference was observed in D1. The survival rate (%) in the biofloc (72%) group was also lower than that of the other two treatment.

Table 5. Growth performance (mean ± SE) of *P. sophore* reared in different feeding systems.

Variables	Diet		
	D1	**D2**	**D3**
L_1 (cm)	9.86 ± 0.21	9.88 ± 0.31	9.80 ± 0.32
L_2 (cm)	10.97 ± 0.23	10.73 ± 0.40	11.11 ± 0.37
W_1 (g)	8.90 ± 1.00	8.95 ± 0.69	8.89 ± 0.61
W_2 (g)	11.96 ± 1.42	11.67 ± 0.92	12.39 ± 0.78
TLG (cm)	1.11 ± 0.10 ^{b}	0.85 ± 0.15 ^{c}	1.31 ± 0.15 ^{a}
BWG (g)	3.06 ± 0.51 ^{ab}	2.72 ± 0.37 ^{b}	3.50 ± 0.32 ^{a}
FCR	1.82 ± 0.29 ^{a}	1.88 ± 0.27 ^{a}	1.50 ± 0.14 ^{b}
FCE	0.57 ± 0.09 ^{b}	0.54 ± 0.07 ^{b}	0.67 ± 0.06 ^{a}
SGR (% day^{-1})	0.49 ± 0.05 ^{b}	0.44 ± 0.05 ^{b}	0.55 ± 0.04 ^{a}
RGR (%)	34.34 ± 3.69 ^{b}	30.44 ± 3.65 ^{b}	39.48 ± 3.63 ^{a}
DGR (% day^{-1})	30.60 ± 5.08 ^{ab}	27.20 ± 3.66 ^{b}	35.00 ± 3.25 ^{a}
Survival (%)	100	72	100

Note: Values shown in the same row sharing a different letter are significantly different ($p < 0.05$).

3.1.4. Haematological Responses

The levels of WBC, RBC, MCV, MCH, and PLT in three separate trials were substantially different among the treatments ($p < 0.05$, Table 6) despite the fact that all haematological parameters were within the normal range. Other factors were not substantially different among the treatments ($p > 0.05$). The WBC (65.53 ± 7.75), RBC (2.55 ± 0.11),

and PLT (34.07 ± 1.72) values of D3 differed significantly from those of D1. The level of MCH (48.35 ± 5.43) was significantly lower compared to D1 and D2 when the fish fed the probiotic-supplemented diet (D3). The MCV values were significantly decreased in D3 compared to D1 ($p < 0.05$) but did not significantly differ those in D2 ($p > 0.05$). Nevertheless, no significant differences in LYM, MON, Hb, HCT, and MCHC ($p < 0.05$, Table 6) were observed in any of the treatments.

Table 6. Effects of different feeding regimes on the haematological index values of *P. sophore*.

Parameter	Treatments			Analysis of Variance			
	D1	D2	D3	Adj SS	Adj MS	*F*	*p*
WBC ($\times 10^9$ L^{-1})	45.40 ± 1.10 ^{b}	54.57 ± 7.25 ^{ab}	65.53 ± 7.75 ^{a}	609.6	304.82	8.26	0.019
LYM (%)	72.00 ± 0.90 ^{a}	81.00 ± 8.16 ^{a}	69.33 ± 6.13 ^{a}	224.2	112.11	2.13	0.199
MON (%)	4.30 ± 0.16 ^{a}	5.10 ± 0.41 ^{a}	4.90 ± 0.16 ^{a}	1.04	0.52	4.73	0.059
RBC ($\times 10^6$ mm^{-3})	0.96 ± 0.12 ^{b}	1.94 ± 0.75 ^{a}	2.55 ± 0.11 ^{a}	3.85	1.92	6.48	0.032
Hb (g dL^{-1})	11.95 ± 1.41 ^{a}	11.30 ± 1.93 ^{a}	12.27 ± 2.21 ^{a}	1.46	0.73	0.14	0.874
HCT (%)	38.27 ± 1.51 ^{a}	38.97 ± 0.26 ^{a}	41.67 ± 1.17 ^{a}	19.29	9.64	5.19	0.049
MCV (pg)	407.21 ± 67.04 ^{a}	237.62 ± 100.01 ^{ab}	163.86 ± 6.70 ^{b}	93,424	46,712	6.43	0.032
MCH (fl)	128.00 ± 10.61 ^{a}	72.88 ± 5.13 ^{b}	48.35 ± 5.43 ^{c}	9984.1	4992.03	59.27	0.000
MCHC (%)	31.13 ± 2.45 ^{a}	29.01 ± 4.99 ^{a}	29.35 ± 4.63 ^{a}	7.76	3.89	0.15	0.865
PLT ($\times 10^3$ mm^{-3})	23.01 ± 0.91 ^{c}	29.00 ± 1.63 ^{b}	34.07 ± 1.72 ^{a}	183.69	91.84	28.50	0.001

Notes: The data are displayed as mean ± SE. Values with the same superscript letter in a row are not different significantly ($p > 0.05$).

4. Discussion

Water temperature (°C), pH, nitrate nitrogen (mg L^{-1}), and TH (mg L^{-1}) levels were all within the ideal range for the species during the 60-day experimentation period in all experimental groups [41]. As a result, the outcomes for fish welfare measures like growth, survival, and physiological conditions were unaffected by differences in the water quality parameters between the fish tanks.

Fish growth was examined, and the findings showed that the growth rate varied depending on the stocking density. Even though the same meal was given to all of the treatments equally, the growth of *P. sophore* was higher in the LD group than it was in the other treatments in terms of mean final length, final weight, body weight increase, FCR, FCE, SGR, RGR, and DGR. A slow growth rate appeared to be associated with HD and increased competition for food and space, with an inverse connection with stocking density, assuming that space had population-limiting effects [42]. The current findings are also consistent with those of Narejo et al. [43], who found that *Heteropneustes fossilis* farming performed best at lower stocking densities, as well as those of Chakraborty [44], who noted that *Bengala elonga* had a higher size and higher survival rate when there was less density. They also claimed that fish in a concrete cistern fed on formulated feed had a larger size and a higher survival rate because of the decreased density. Pouey et al. [45] also noted a negative correlation between the growth and high densities of young silver catfish reared in a closed water recirculation system and treated to three different SDs (3.75, 7.5, and 11.25 gL^{-1}, approximately). In the present study, the authors found that the lower SD produced the highest growth rate, yet mortality rates did not increase in high densities. An improper stocking density can lead to increased competition among individuals for food and housing space, thereby increasing individual growth variability, and potentially establishing a size hierarchy or social hierarchy [46].

Probiotic diet supplementation in the current study improved *P. sophore*'s growth and feed consumption. The data for the current study showed that the probiotic-containing diet had considerably higher TLG, BWG, FCE, SGR, RGR, and DGR values, and survival rates than the other diets. It revealed that one of the explanations could be probiotics, which have been demonstrated to enhance feed digestion by releasing digestive enzymes or changing the environment in the stomach, leading to greater development [11]. They may adhere

for a short period, colonize the gastrointestinal tract, and raise antibody levels [47]. As a result, probiotics have been shown to have positive impacts on bacterial populations in the environment and immune system responses in aquatic organisms [48]. The growth of multiple fish species, including Nile tilapia [49], common carp (*Cyprinus carpio*) [50], and rainbow trout (*Oncorhynchus mykiss*), has been shown to be impacted by *Bacillus* spp. [51]. Giri et al. [52] found that the addition of probiotics using *Lactobacillus reuteri* P16 enhanced the total bacteria in intestine of *Cyprinus carpio* exposed to lead. Improving the bacterial population in fish guts can help improve nutrient absorption and metabolism which increase the immune system in the body of fish, leading to the disease resistance of the fish [11]. Fish species such as *Oreochromis mossambicus* [53], *Macrobrachium resenbergii* [54], *Litopenaeus vannamei*, and *P. conchonius* [55] have shown growth and better health when fed biofloc instead of conventional fish food [25]. However, in this study, for *P. sophore* reared on biofloc, their growth performances were significantly lower compared with other treatments. A 28% mortality was observed in the biofloc-containing treatment, whereas there was no mortality in the control or probiotic-containing diets. It revealed that there might be a reason why fish did not like to consume external carbon sources and microbial flocs as an additional protein source.

The physiological stress the fish are under and any factors that could have a negative impact on their health should be taken into account before making a choice to improve biomass production in aquaculture by increasing fish density. The haematological values recorded in this study were unfold the changes in health status in different stocking densities and dietary treatments for the better management of *P. sophore* when cultured in captivity, thus assuring sustainable aquaculture and the longevity of the species. All of the treatments were in the normal range, and there was no significant difference between them ($p > 0.05$), except between WBC and stocking densities [56–58]. These findings imply that *P. sophore* may be somewhat sensitive to high-density stress. The general impact of fish crowding that appeared as an increase in haemoglobin values was described by many authors as haemoconcentration [59,60]. Under conditions of high energy demand, such as prolonged stress, this reaction may be the reason why the blood's ability to carry oxygen increases. Additionally, as a result of increased energy needs for feed acquisition and the development of dominant–subordinate interactions, fish that are crowded have higher metabolic rates.

Likewise, the blood parameter data revealed no significant differences between the various experimental diets except RBC, MCV, MCH and PLT levels, but it was recorded that fish fed probiotic diets had slightly improved physiological functions and health status when compared to fish from the control and biofloc groups. Fish with higher levels of haemoglobin in their blood probably transported oxygen to their tissues more effectively, which led to better growth [61]. According to Jahan et al. [36], adding more yeast probiotics to fish diets may result in higher levels of Hb and Glu. Probiotic use considerably raised the RBC and Hb levels in *P. sophore* in the current investigation, possibly as a result of better dietary protein absorption. A positive effect was also found by Marzouk et al. [62], as shown by a notable increase in RBC count and Hb conc. in both fish groups fed probiotics-supplemented diets. Jäger et al. [63] recommended the supplementation of probiotics for facilitating the absorption of essential amino acids. When diets containing S. cerevisiae and probiotics were employed, Abdel-Tawwab et al. [16] saw similar outcomes in *O. niloticus*, Sharma et al. [64] in *Cirrhinus cirrhosis*, and Talpur and Ikhwanuddin [65] in Lates calcarifer. The current research implies that administering probiotic supplements to feed diets will improve fish health which supports the findings of Khattab et al. [66], who found that fish fed diets containing commercial probiotics composed of *Bacillius licheliformes* and *Bacillius subtilis* had blood haematological parameters that were significantly higher than those of the control group.

The treatments with biofloc had the highest LYM and MON levels despite no significant differences ($p > 0.05$) in any of the blood parameters. Other blood values showed a similar trend. Compared to the clear water condition represented by the control, it appears

that the biofloc condition had no detrimental effects on fish health. Similar findings were made by Long et al. [67], who hypothesized that biofloc had no appreciable impact on blood haematological measures, including RBC, WBC, Hb, and HTC. The same range of haematocrit values for tilapia under the biofloc treatment (BFT) or recalculating aquaculture system (RAS) was also suggested by Azim and Little [68]; however, in the current study, though the haematological parameters were not affected by an external carbon source, the growth and survival rate was lower compared to the other treatments.

5. Conclusions

Overall, low stocking density and probiotic supplementation have an impact on growth, metabolism, and haematological indices. A deeper comprehension of these phenomena and the development of a logical link between changes in these parameters and various densities could have a significant positive impact on many rearing stages of this species. *P. sophore* fisheries have historically relied on capture. Starting its captive culture is now necessary. The addition of a probiotic supplementation with the feed to fish reared in low densities improved the growth performance and health conditions of this species; however, as the species did not perform better in the biofloc condition, this suggested that culture using biofloc should be avoided. To determine the threshold densities that negatively impact growth and physiological conditions for different ages and size-classes of this species, more research is required. Additionally, processes by which probiotics affect host species, particularly their effect on enzymatic function and disease resistance, must also be clarified through research.

Author Contributions: Conceptualization, methodology, formal analysis: S.K.M. and M.A.S.; investigation and writing original draft: S.D.; writing—review and editing: S.K.D., M.A.S., M.S.A., M.L.R. and M.S.H.; project administration and supervision: S.K.M., M.A.S. and M.S.H.; funding acquisition: S.K.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Research Management Wing (RMW), Bangabandhu Sheikh Mujibur Rahman Agricultural University, through Innovation Research grant (BSMRAU/RMW/Innovation project/128/2019/902/10) of Bangladesh, which is gratefully acknowledged.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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