Single or combined effects of safegut and mannanoligosaccharide on growth performance, proximate composition and haematological parameters of walking catfish (*Clarias batrachus*, Linnaeus, 1758) juveniles

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Abstract

This study was carried out to evaluate the safegut and mannan oligosaccharide (MOS) as feed additives and their single or combined effects on the growth performance, proximate composition and haematological parameters of juvenile walking catfish (*Clarias batrachus*, Linnaeus, 1758). Triplicate groups of juvenile walking catfish (initial weight 22.35±0.12g) were fed twice daily at a rate of 2.5% of body weight for 12 weeks, with 0 (control), 0.2% safegut as probiotic, 0.4% MOS as prebiotic and 0.2% safegut and 0.4% MOS as synbiotic. The results revealed that the growth performance and feed utilization parameters were significantly improved ($p<0.05$) in the groups fed with 0.2% probiotic and synbiotic than the control group with the highest value in the probiotic group. Among the body indices only viscera-somatic index showed a significant reduction in the synbiotic group compared to the control treatment. Results showed that the highest protein and lipid with the lowest moisture and ash content were observed in 0.2% probiotic fed group than the control. Haematological parameters such as haemoglobin concentration and packed cell volume were improved in the probiotic group compared to the control. On the other hand, number of leucocytes increased significantly in the prebiotic supplied diet. Synbiotic fed group showed a significant elevation of haemoglobin content and Mean Corpuscular Haemoglobin than the control diet. Based on the results of growth performance, proximate composition and haematological parameters, it can be concluded that single use of 0.2% safegut was more effective for *C. batrachus* juvenile.

Keywords: Probiotic, Prebiotic, Synbiotic, Innate immunity, *C. batrachus*.

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Introduction

The growth and sustainability of aquaculture has been affected by a number of disease outbreak especially the bacterial diseases, causing a peak level of stock mortality (Kurath, 2008). Antibiotics were used to prevent the possible outcome of disease outbreak which helps to increase aquaculture growth but their secured application has been doubted (Cabello, 2006; Yousefian and Amiri, 2009). These antibiotics kill both harmful and beneficial bacteria, which are present in the gastro-intestinal tract of animals. This is contradictory to our expectation of leading a healthy life as most of the people want to have 100% natural food (Sayes *et al*., 2018). By realizing the severity of these factors, the use of non-antibiotic components comes in considerations which are more suitable for overall culture system and animal health (Denev, 2008). In this case, feed additives such as probiotics and prebiotics can promote non-specific disease resistance capacity and helps in uplifting growth (Das *et al*., 2017).

Probiotics were first introduced in 1965 (Lilly and Stillwell, 1965) to describe "substances secreted by one microorganism that stimulate the growth of another". The total effects of probiotics can be summarized as disease controlling agents, growth enhancer, digestion improvement way, immune system development, supplies a source of nutrients, water quality improvement and promotes reproduction. Safegut is a non-antibiotic eco-friendly commercially available bio product premix of *Bacillus subtilis, B. licheniformes*, *Aspergillus niger*, *Saccharomyces boulardii,* vitamin and enzymes. Another beneficial non-digestible feed additive is prebiotic. Administration of prebiotic (a non-living substance) within feed leads to a better working environment for probiotic. Now-a-days there are many substances which act as prebiotic like some proteins, peptides, certain lipids, mannan-oligosaccharides (MOS), fructo-oligosaccharides, inulin or B-glucan (Das *et al*., 2017). Among all of them, MOS is widely used in feed for fish and crustaceans due to having an extra advantage over others. MOS is one kind of glucomannoprotein complex that is extracted from a cell wall of brewer's yeast *Saccharomyces cerevisiae* (Sang and Fotedar, 2010). Use of probiotics and prebiotics alone in feed is common. The result when they are administered at a time in feed is obviously positive but the information is not sufficient yet. This combination of probiotic and prebiotic is known as synbiotic. Gibson and Roberfroid (1995) first introduced the term of symbiotic.

Clarias batrachus is an air breathing catfish found in inland waters (Więcaszek *et al*., 2010). This fish is medicinally significant compared to other fish species (Debnath, 2011). It contains easily digestible protein, ash and high-density lipoprotein that are considered as good cholesterol. Therefore, study on the various aspects of this species can improve the nutritional profile of our country people. Still there is no study related with single or combination use of safegut and MOS

on *C. batrachus* growth and haematology. Hence the present study works to find out an environmentally friendly way by which the growth of this species and nutrition as well as medicinal quality can be improved. In this case, administration of single or combination of probiotic safegut and prebiotic MOS can open a new door for this culture species.

Materials and methods

Experimental site

The study was conducted at the hatchery complex of Caritas, in Setabganj upazilla, under Dinajpur district. Twelve cemented rectangular sized tanks (150cm× 75cm×75cm) were used for this experiment. Water was supplied from underground by using an electric pump. Tanks were cleaned fortnightly to reduce the risk of accumulation of nitrogenous waste.

Experimental design and feeding trial

Juvenile walking catfish (average weight 15.00±1.00g) were procured from a commercial hatchery complex of Dinajpur district and transported to the experimental site in oxygenated plastic bags filled with freshwater. The fish were acclimatized for 15 days prior to the commencement of the study and were fed with a commercial feed. Then fishes with an average weight of 22.35±0.12g were randomly distributed in twelve tanks with a stocking density of 10 fishes per tank. For each treatment, three replicates were prepared. Fishes were fed with the respective experimental diets at 2.5% body weight two times in a day, early morning and late evening for a period of 12weeks.

Diet preparation

Four isonitrogenous diets were prepared to contain probiotic 0.2% safegut (Eskayef Bangladesh Limited, Agrovet division, Tongi, Bangladesh), prebiotic 0.4% MOS (International Food Grade, Laboratory of USA, Purity>90%), combination of both (0.2% safegut and 0.4% MOS), and un-supplemented diet considered as control. According to the manufacturer recommended dose 0.2% safegut was selected to use for this experiment. In a previous study by Akter *et al*. (2021), who reported 0.4% showed the best growth performance, feed utilization and haematological parameters in *C. batrachus* juveniles, that is the reason for choosing 0.4% MOS for this research experiment. Fish meal was used as a main source of protein added with soybean meal to keep the protein level at 35 %, while soybean oil and fish oil at a ratio of 1:1 were used to maintain lipid content at 10%. Corn starch and wheat flour were used as the carbohydrate source to confirm that the energy levels of all diets were the same (Phumee, 2011). Diets were made by systematically mixing feed ingredients (Table 1) for some times in a food container. Subsequently addition of fish and soybean oils, the feed components were mixed for an extra 10 minutes (Salaghi *et al*., 2013). Enough water was added to prepare dough which was then extruded through a pelletizing machine to make 3mm diameter pellets. The pellets were then sun dried until the

moisture level maintained as less than10 %. The resultant pellets were then filled separately in plastic bags and kept in a freezer at -20°C during the experimental period.

Table 1: Ingredients used for various experimental diets (g kg^{-1}).

Ingredients	Treatments			
	Control	Probiotic	Prebiotic	Synbiotic
Fish meal[1]	247.20	247.20	247.20	247.20
Soybean meal	280.10	280.10	280.10	280.10
Wheat flour	270.0	268.0	266.0	264.0
Corn starch	97.50	97.50	97.50	97.50
Fish oil	32.60	32.60	32.60	32.60
Soybean oil	32.60	32.60	32.60	32.60
Vitamin[2]	20.00	20.00	20.00	20.00
Mineral[3]	20.00	20.00	20.00	20.00
MOS[4]	0.00	0.0	4.0	4.0
Probiotic[5]	0.00	2.0	0.0	2.0
Total	1000	1000	1000	1000
Proximate analysis (g kg^{-1}, dry weight)				
Protein	351.90	352.60	350.50	351.20
Lipid	100.10	100.50	100.70	99.90
Ash	63.10	59.90	60.30	62.20
Fibre	9.80	10.10	9.9	10.3
NFE	475.1	476.9	478.6	476.4

[1]Danish fishmeal: crude protein, 720; crude lipid, 50.

[2] Vitamin mix kg^{-1} (Rovithai Ltd 700/437 Chonburi THAILAND): Vitamin A 50 MIU, Vitamin D3 10 MIU, Vitamin E 130g, Vitamin K3 10g, Vitamin B1 10g, Vitamin B2 25g, Vitamin B6 16g, Vitamin B12 100mg, Niacin 200g, Pantothenic Acid 56g, Folic Acid 8g, Biotin 500mg, Antioxidant 0.200g and Anticake 20g.

[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.

[4] MOS (Mannan Oligosaccharide, International Food Grade, Laboratory of USA, Purity > 90%).

[5] Probiotic (Safegut).

Sample collection

Survival of the experimental fishes was recorded during the feeding experiment. Individual fish in each tank were weighed at the beginning and finishing of the feeding experiment, whereas bulk weighing was done fortnightly during the feeding trial to monitor growth and to correct the total feed to be given. After 12 weeks of accomplishment of the experiment, fishes were starved for 24 hours. From each replicate tank, three fishes were taken randomly for the determination of the proximate composition of whole body and body indices. Fishes were killed by placing them in ice for some times and quickly balanced then dissected. For the analyzing of proximate composition, the fishes were kept in freezer at -20℃. The weight of whole fish, liver, intraperitoneal fat and viscera were recorded to analyze the hepatosomatic index (HSI), intraperitoneal fat (IPF) and viscerosomatic index (VSI).

Determination of growth, survival and body Indices

Final weight, weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and survival rate were determined at the end of the feeding trial by using the following formula as previously used by Akter *et al*. (2016, 2019b):

Average final weight = Total weight of fish / number of fish
Total weight gain (WG) g = final weight - initial weight
Specific growth rate (SGR%) = [(lnWf - lnWi) / T}×100
Where, Wf = final weight, Wi=initial weight, T=duration of culture

Feed conversion ratio (FCR) = total dry feed intake (g) / total wet weight gain (g)
Protein efficiency ratio (PER) = Wet weight gain (g) / total protein intake (g)
Survival rate (%)=(final number of fish/ initial number of fish) × 100

Body indices were measured by the following formula:
Hepatosomatic index (HSI)% = {liver weight (g) / body weight (g)}×100
Intraperitoneal fat (IPF)%={intraperitoneal fat weight (g) / body weight (g)}×100
Viscerosomatic index (VSI) %={viscera weight (g) / body weight (g)}×00

Whole body proximate composition analysis

The whole body proximate composition of walking catfish after feeding with various supplemented diets was analyzed using standard reference methods (AOAC, 1997).

Haematological parameters

The experimental fishes were starved for 24 hours in order to the determination of the hematological parameters. The three fishes from each replicate tank were selected randomly and anesthetized by using clove oil. To have a better flow of blood, they should remain alive and in less stressed condition. A 21-guage needle was inserted into the muscle at the end of anal fin until it reached to the back bone. Blood was collected through 1ml syringe and moved into a heparinized tube to avoid the clotting of blood sample.

Erythrocyte sedimentation rate (mm /h)

The erythrocyte sedimentation rate was measured by Westergren's method (Britton, 1993). This is the rate of red blood cell sedimentation within one hour. This method requires anticoagulated blood within the microhematocrit tube. The lower end of tube was closed by using critoseal and remains in an uplift position on a rack for 1 hour at room temperature. The RBC was descended within the tube and the height of red part was reduced. The rate at which this RBC is sedimented within the tube is called erythrocyte sedimentation rate. The height of the plasma was measured and the ESR was presented as mm^{-h}.

ESR={(RBC/(RBC+plasma cell)}× 100

Packed cell volume (PCV)

PCV was measured according to the method described by Schäperclaus *et al*. (1992) and Akter *et al*. (2019a). To obtain this, blood samples were placed into the standard microhematocrit tube until three quarters part were filled up and centrifuged quickly for 4 min at 10,000 g using a microhematocrit centrifuge machine (Hawksley, England). The red part was packed in the lower area of the tube and the upper part remains colourless. The height of packed cell and plasma cell were measured in mm which was used for PCV measurement by using the following formula:

PCV={Height of packed cells (mm)/Height of RBC and plasma cells (mm)}×100

Haemoglobin (Hb) concentration

Sahil's method was used for Hb concentration measurement. At first, 2 mL of 10 N HCL solution was taken into a tube. After that the tube was placed beside the glass comparator. About 20 µL blood sample was taken using a pipette and placed into the tube. The solution was mixed up with a stirrer. This step was followed by addition of water drop by drop into the tube until the solution colour matches with the glass comparator. When the colour matches, the reading was recorded and expressed as g/dl. The same process was followed in every sample for Hb determination. The following equations were used to determine MCH, MCHC and MCV measurement:

Mean corpuscular haemoglobin (MCH) pg /cell=[Haemoglobing%/ RBC (millions mm^{-3})×10]
Mean corpuscular haemoglobin concentration (MCHC) g/dl=(Haemoglobin%/ PCV%)×100
Mean corpuscular volume (MCV) μm^3 = [PCV% / RBC (millions mm^{-3})×10]

Red blood cell count (millions mm^{-3})

The total red blood cell was counted by using the methods of Sado *et al*. (2008), Al-Dohail *et al*. (2009), Sirimanapong *et al.* (2014) and Akter *et al*. (2019a). At first by using Natt and Herrick (1952) solution, the blood sample was diluted for 200 times. The diluted blood sample was then placed into a haemocytometer chamber by using a pipette and allowed to set down for 3 minutes. RBC was counted in the five chambers (1/25) in the central squares of haemocytometer under microscope. The RBC count was expressed as millions of cells per cubic millimetre. The total number of RBC was counted by using the following formula:

RBC (mm^{-3})=(N×5×10×200)

Where, N=the number of RBC in five chambers; 5=multiplication factor provides the number of cells in $1mm^2$; 200=dilution factor; 10=the depth of the chamber from 0.1mm to 1mm

Total white blood cell or WBC count (WBC ×$10^4 mm^{-3}$)

The total amount of WBC count was performed by using the method of Al-Dohail *et al*. (2009) and Akter *et al*. (2019a). Natt and Herrick (1952) solution was used as a diluents while a Neubauer haemocytometer was used for counting. The result was expressed as thousands of cells per cubic millimeter. This WBC was measured by the following formula:

WBC (mm^{-3}) = LC×500

Where, LC=number of cells in four squares; 500=the dilution and volume correction factor.

Statistical analysis

The results were analyzed by an analysis of variance (ANOVA). Duncan's multiple range test (Duncan, 1955) was used to evaluate mean differences among the different treatments with a significance level of $p<0.05$. All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) software, version 22 for windows. The data were presented as mean±standard error.

Results

Growth performance, survival and feed utilization parameters

The growth performance, survival and feed utilization parameters of *C. batrachus,* fed with various experimental diets are given in Table 2. Significantly increased ($p<0.05$) SGR (1.01±0.02) and weight gain (30.06±1.16) were found in probiotic fed group compared to the fish fed with the control diet (0.73±0.02 SGR and 19.02±0.59 wt. gain). Significantly improved feed utilization parameters, such as FCR (1.58±0.06) and PER (1.76±0.06) were also observed in the fish fed with probiotic supplemented diet compared to those fish fed with other remaining treatment groups. Survival of walking catfish was not influenced ($p>0.05$) after feeding with probiotic and prebiotic diets.

Table 2: Single and combined effects of probiotic and prebiotic on the growth performances and feed utilization of juveniles walking catfish, *C. batrachus,* for 12 weeks

Parameters	Treatments			
	Control	Probiotic	Prebiotic	Synbiotic
Av. Initial Wt.	22.35±0.12	22.57±0.12	22.59±0.11	22.39±0.07
Av. Final Wt.	41.37±0.66^a	52.63±1.25^c	43.78±0.78^{ab}	45.21±0.44^b
Wt. Gain	19.02±0.59^a	30.06±1.16^c	21.19±0.79^{ab}	22.82±0.67^b
SGR (%)	0.73±0.02^a	1.01±0.02^c	0.79±0.02^{ab}	0.84±0.02^b
FCR	2.47±0.07^c	1.58±0.06^a	2.24±0.08^{bc}	2.07±0.08^b
PER	1.12±0.03^a	1.76±0.06^c	1.24±0.05^{ab}	1.35±0.05^b
Survival (%)	100±0.0	100±0.0	100±0.0	100±0.0

All values are mean±SE obtained from three replicate groups, (n=3); Data with different superscripts in the same row indicate significant differences (p<0.05); Av= average, Wt.=weight, SGR=Specific Growth Rate, FCR=Food Conversion Ratio, PER=Protein Efficiency Ratio.

Body indices

The body indices of *C. batrachus* fed with different experimental diets are summarized in Table 3. Among all of the body indices, only VSI showed a significant variation within the treatment groups. VSI was found to be significantly *(p*<0.05*)* lowest in synbiotic group (4.07±0.09) compared to those fish fed with control diet (8.64±1.75).

Table 3: Body indices of juvenile's walking catfish, *C. batrachus,* fed with various experimental diets for 12 weeks.

Treatments	Parameters		
	HSI%	IPF%	VSI%(WI)
Control	0.97±0.21	0.03±0.00	8.64±1.75^{b}
Probiotic	1.10±0.27	0.07±0.03	5.18±0.95^{ab}
Prebiotic	0.98±0.22	0.03±0.00	7.75±1.37^{ab}
Synbiotic	0.94±0.23	0.03±0.00	4.07±0.09^{a}

All values are mean±SE obtained from three replicate groups, (n=3); Data with different superscripts in the same column indicate significant differences (*p*<0.05). HSI=Hepatosomatic Index, IPF=Intra-peritoneal Fat, VSI=Viscera-somatic Index (Whole Intestine).

Whole body proximate composition

Proximate composition of *C. batrachus* after feeding with experimental diets is shown in Table 4. Significantly higher *(p*<0.05*)* body protein (19.53±0.02) and lipid (2.56±0.01) content and lowest ash content (6.88±0.01) were observed in fish treated with probiotic diet compared to the control group (17.66±0.04; 2.47±0.00; 8.01±0.02 respectively). Whereas, significantly lowest *(p*<0.05*)* lipid content (1.27±0.00) and highest (*p*<0.05) ash content (8.33±0.01) were observed in fish fed with prebiotic diet when compared to all the remaining treatments. Control group showed a significantly high (*p*<0.05) level of moisture among all other groups.

Table 4: Proximate composition of juvenile's walking catfish, *C. batrachus,* fed with various experimental diets for 12 weeks (wet basis).

Proximate	Parameters			
Treatments	Moisture %	Protein %	Lipid %	Ash %
Control	71.86±0.06^{c}	17.66±0.04^{a}	2.47±0.00^{c}	8.01±0.02^{c}
Probiotic	71.04±0.04^{a}	19.53±0.02^{d}	2.56±0.01^{d}	6.88±0.01^{a}
Prebiotic	71.10±0.02^{a}	19.30±0.01^{c}	1.27±0.00^{a}	8.33±0.01^{d}
Synbiotic	71.65±0.02^{b}	18.33±0.01^{b}	2.20±0.01^{b}	7.82±0.00^{b}

Data presented as mean±SE, (n=3).Data with different superscripts in the same column indicate significant differences (*p*<0.05).

Haematological parameters

The influence of dietary probiotic, prebiotic supplementation on *C. batrachus* blood profile is given in Table 5. Fishes fed with probiotic diet showed a significant increase *(p*<0.05*)* in PCV (45.73±4.57) in relation to the control group (34.73±1.76), which was not significant when compared with prebiotic (37.98±1.75) and synbiotic diets (44.07±3.26). The haemoglobin level was found significantly high *(p*<0.05*)* in probiotic (8.25±0.45) and synbiotic (8.77±0.20) supplemented

diets compared to the control group (6.92±0.27). WBC count was significantly high *(p*<0.05*)* in fishes fed with prebiotic diet (8.80±0.80) compared to the fishes fed with control diet (6.72±0.63), which was not significantly differ compared to those fishes fed with probiotic (7.80±0.27) and symbiotic (8.35±0.43) diets. Significantly highest *(p*<0.05*)* level of MCH was found in symbiotic group (27.10±2.76) compared to prebiotic diet (19.71±1.06). Significantly lowest total platelet count (127.74±13.07) was observed in probiotic fed group compared to all the remaining treatments. However, haematological parameters showed no marked variation in regard to RBC, ESR, MCHC and MCV among all the treatments.

Table 5: Haematological parameters of juvenile's walking catfish, *C. batrachus,* fed various experimental diets for 12 weeks

Haematological parameters	Treatments			
	Control	Probiotic	prebiotic	Synbiotic
ESR (mm)	1.95±0.05	1.83±0.08	1.92±0.37	1.83±0.08
PCV (mm)	34.73±1.76^{a}	45.73±4.57^{b}	37.98±1.75^{ab}	44.07±3.26^{ab}
RBC (millions mm^{-3})	3.18±0.18	3.65±0.27	3.48±0.24	3.37±0.27
Hb(g/dl)	6.92±0.27^{a}	8.25±0.45^{b}	6.77±0.28^{a}	8.77±0.20^{b}
MCHC(g/dl)	20.13±1.11	19.03±2.18	17.90±0.71	20.64±2.08
MCH (pg /cell)	21.84±0.49^{ab}	23.29±2.44^{ab}	19.71±1.06^{a}	27.10±2.76^{b}
MCV/µm^{3}	110.14±6.54	127.96±14.95	111.12±7.52	131.38±4.80
WBC (c.mm)/1000	6.72±0.63^{a}	7.80±0.27^{ab}	8.80±0.80^{b}	8.35±0.43^{ab}
Total platelet Count (c.mm)	165.00±9.48^{b}	127.74±13.07^{a}	164.83±8.78^{b}	157.83±6.95^{b}

Data presented as mean±SE, (n=9; 3 fish per replicate tank). Data with superscripts in the same row indicate significant differences (p<0.05).

Discussion

The present study was conducted for the first time to demonstrate the single or combined effects of dietary probiotic safegut and prebiotic MOS on the growth performance and haematological parameters of *C. batrachus* over a period of 12 weeks. At the end of the experiment the highest growth was observed in those fishes which were fed with probiotic supplemented diet. The specific growth rate was also high in probiotic treated diet compared to the other treatments. This may be due to the capacity of probiotic safegut to improve the appetite of fish as well as bacterial colonization within the gut which helps to increase the growth performance and feed utilization (Gibson, 1998; Yanbo and Zirong, 2006). The observation of this experiment was expected and had been reported previously by some authors (Nozari and Shapoor, 2017; Dey *et al*., 2018; Jinia *et al*., 2019).

Although authors worked on different probiotics with various concentrations, however in all cases, growth was significantly influenced when compared to the control group. Besides, fishes which are treated with symbiotic diet also showed an increasing trend of growth and a significant reduction in VSI compared to those fishes fed with the control diet but the growth is comparatively inferior when compared the fish fed with only probiotic

supplemented diet. This result is alike with the outcome of the research of Mehrabi *et al*. (2012) who reported a significantly high growth and carcass protein content of rainbow trout fingerlings fed with symbiotic diet compared to the control. The decrease in VSI in the symbiotic fed group indicated that the fish had an increased amount of meat and, therefore, indicated a higher economic value (Akter *et al*., 2016) as a result of feeding a combination of probiotic and prebiotic diet.

A lower FCR and higher PER were also observed for probiotic fed group which is in line with the research of Mohapatra *et al*. (2012) where they reported a lower FCR and higher PER of *L. rohita* fingerling fed with probiotics supplemented diets compared to the control fed group. However in the current research, prebiotic MOS did not show any significant effect on the growth performance of walking catfish juveniles compared to the control group, which was also found in the Gulf of Mexico sturgeon (Pryor *et al*., 2003), channel catfish (Welker *et al*., 2007), Nile tilapia (Sado *et al*., 2008), Atlantic salmon (Grisdale-Helland *et al*., 2008), gilthead sea bream (Dimitroglou *et al*., 2010), giant sturgeon (Mansour *et al*., 2012) or sharpsnout seabream (Piccolo *et al*., 2013).

The improvement of growth observed in this study has attributed to the improved proximate composition and better nutrient enrichment of *C. batrachus*. Previous research reported that the supplementation of probiotics has been shown to improve protein and lipid content of *Ompok pabda* (Jinia *et al*., 2019) and *Nile tilapia* (Biswas *et al*., 2018). In the present study, proximate composition particularly protein and lipid were significantly high in fishes fed with probiotic diet compared to the control group. For the current experiment, ash and moisture content were not influenced by the intake of probiotic supplemented diets however the control group showed an increasing level of ash and moisture content. A similar observation was also reported by Jinia *et al*. (2019) where authors found the highest moisture and ash content in the control fed group. Synbiotic fed group showed a significant effect on all most all of the proximate composition of walking catfish compared to the control but these values were lower than the probiotic fed group. In contrast to the present study, Ye *et al*. (2011) and Mehrabi *et al*. (2012) reported significantly increased carcass protein content in symbiotic fed group.

Besides the improvement of growth performance and feed utilization, intake of probiotic and prebiotic shown to improve the health status of fish. As the health status of fish being reflected through its haematological parameters. Therefore, an understanding of the haematological parameters used as an effective index in evaluating physiological and pathological abnormalities in fish to verify its health status (De Pedro *et al*., 2005). Evidence has shown that probiotics have a direct influence in both the innate as well as the acquired immune system (Galdeano and Perdigón, 2006; Merrifield *et al*., 2010).

The innate immune system (non-specific) is the fundamental defense mechanism of the host against infectious microorganisms (Lee and Söderhäll, 2002) which can be influenced by the haematological parameters. Among all the haematological parameters, leukocyte count is being considered to be the most important parameter for evaluation of the fish health status, as the nonspecific or innate immunity of fish is primarily depends on this parameter (Andrews *et al*., 2009; Sumathi *et al*., 2014). Improvement of several haematological parameters after 12 weeks feeding trial indicates the positive influence of probiotic safegut and prebiotic MOS on the health status of walking catfish juveniles. In the current research, a significantly better concentration of PCV and Hb concentration was observed in walking catfish juveniles maintained on the probiotic supplemented diet. These results are an indication of improved health of walking catfish, also supported by Kumar *et al*. (2006) when the *L. rohita* fed with probiotic *B. subtilis* added diets. Elevated PCV values were also noted in tilapia when fed two microbial feed additives such as *Bacillus subtilis* and *Lactobacillus acidophilus* (Aly *et al*., 2008). Similarly, several studies (Dhaiya *et al*., 2012; Sharma *et al*., 2013; Renuka *et al*., 2014) also reported that probiotic had a significant effect on the haematological parameters. Dhaiya *et al*. (2012) found that probiotics help to increase the haemoglobin level at 24% and packed cell volume at a rate of 20% compared to the control diet.

In the current study, the outcome of most of the haematological parameters including ESR, RBC, Hb, MCHC and MCV were not significantly influenced by the intake of MOS supplemented diet compared to the control diet. Similarly, many studies also reported that dietary MOS had no effect on the haematological parameters of channel catfish (Welker *et al*., 2007); Nile tilapia (Sado *et al*., 2008); giant sturgeon (Mansour *et al*., 2012) and striped catfish (Akter *et al*., 2019a). However, a significantly elevated level of WBC was reported in walking catfish fed with 0.4% MOS diet, which was also seen in *L. rohita* fed with 1% MOS diet (Andrews *et al*., 2009). These might be happened due to different sources of prebiotics, purity of MOS, variation in culture period and species (Pryor *et al*., 2003).

On the other hand, synbiotic diet provides a significantly higher concentration of Hb and MCH when compared to the control diet. A non-significantly higher concentration of PCV, RBC and WBC were also found in walking catfish fed with symbiotic diet which was also reported in Nile tilapia fed with symbiotic diet (Hassaan *et al*., 2014). A non-significantly lower value of ESR was obtained in probiotic and synbiotic groups compared to the remaining treatments. Since, ESR is the rate of RBC sedimentation per hour; the lower is the rate of sedimentation indicates the higher rate of RBC level (Rashid *et al*., 2005).

The results observed in this study clearly indicated that probiotic is a beneficiary dietary supplement which has a more positive influence on the enhancement of growth performance, body composition and haematology of *C. batrachus.* Since the probiotic application can improve the blood parameters, it can also improve the health condition of those fishes. The outcome of the present study revealed that probiotic supplementation appears to be the most effective feed additive compared to the non-supplemented diet.

Additionally, combined administration of probiotic and prebiotic is also beneficial for improving the growth and health status but the outcome is lower than the single administration of probiotic safegut. Therefore, this study confirms the farmers for sole application of probiotic at a rate of 0.2% for overall health and growth improvement of *C. batrachus* juvenile.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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