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Fermos® prebiotic dietary supplementation enhances immune, antioxidative responses and growth performance of Nile tilapia *Oreochromis niloticus*

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ABSTRACT

The current study determined the influences of supplementing Nile tilapia (Oreochromis niloticus) basal diet with a prebiotic mixture on hematological, immune and biochemical parameters and growth performance. Two fish groups (Average weight 16.0 ± 0.5 g) were supplemented with prebiotic Fermos® at a rate of 0.5 and 1.0 g/kg diet and the third group which served as control received basal non-supplemented diet. The feeding trial continued for 6-weeks; and representative blood, serum as well as liver samples were collected from the three groups at the end of the third and sixth week. Differences in hematologic parameters, serum biochemical and oxidative indicators were determined. Results showed that Fermos® showed significant increase in hematological markers (mean corpuscular hemoglobin, packed cell volume concentrations, total leukocytic count, monocytes, and lymphocytes); and a significant decrease in (mean corpuscular hemoglobin concentration, basophils and heterophils counts). Fermos® increased serum albumin, globulin, and total protein. Biochemical parameters (AST and ALT levels) showed significant decrease at the end of the sixth week and third week, respectively in Fermos® groups, while glucose levels were significantly increased at the two sampling points for supplemented fish. All immune, antioxidants and growth parameters were improved in supplemented fish, compared to control group. Conceivably, our results demonstrated the beneficial effects of supplementing O. niloticus with Fermos® prebiotic on hematological parameters, immune and biochemical and growth profiles.

1. INTRODUCTION

The intensification of aquaculture production is usually challenged by infections including bacterial and parasitic burdens, which obligates the use of chemicals and antibiotics to control disease outbreaks (Martinez Cruz et al., 2012). The injudicious use of antibiotics inevitably led to development of resistance, mutagenic microbial strains and detrimental effects to fish and consumer health (Jahangiri and Esteban, 2018). Therefore, it is imperative to find alternative ecofriendly sources as prebiotics and probiotics which can improve fish health, performance, and immunity without side effects to the fish themselves or the consumer health (Dawood and Koshio, 2016; Song et al., 2014). Additionally, intensified fish farming and increased stocking densities are usually associated with elevated levels of stressors to fish and initiation of an adaptive stress response which conveys damaging effects on fish, with subsequent immune suppression as the stressors prolonged (Bittencourt et al., 2003) .

During the last decade, research has been targeted toward modulation of fish immune system as a preventive and sometimes a control strategy against fish diseases (Elkamel and Mosaad, 2012). Prebiotics are known as a group of non-digestible food ingredients which promote the growth of beneficial microorganisms in the gastro-intestinal tract. Like probiotics and symbiotic, inclusion of prebiotics as feed supplements into diets of Nile tilapia was found to be accompanied with immunomodulation and increased resistance of fish against serious bacterial agents as Aeromonas hydrophila infection (Cavalcante et al., 2020). The combination of probiotics and prebiotics may improve survival rates and modulation of intestinal microbiota (Gibson and Roberfroid, 1995), since the action of probiotic bacteria may be increased by prebiotics due to the contribution of this component for their growth metabolism and activation (Akhter et al., 2015) .

Fermos® principle active ingredient is mannan oligosaccharides (MOS) which is derived from yeast cell wall and block colonization of pathogens into fish intestine (Abraham and Beachey, 1985). Furthermore, MOS modify the intestinal morphology causing higher density of microvilli, reduced tight junction exposure and improved nutrient absorption capacity (Dimitroglou et al., 2010).

Thus, the current study was conducted to evaluate the potential beneficial effects of Fermos® (MOS-containing prebiotic) on hematobiochemical profiles, immunity, and antioxidant system in Nile tilapia, Oreochromis niloticus.

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2. MATERIAL AND METHODS

2.1. Fish and Experimental conditions

180 fish (Average weight 16.0 ± 0.5 g) we procured and transported in polyethylene bags to (140 cm height × 140 cm diameter) fiberglass tanks at the fish production unit (1), Egyptian military veterinary administration, where the experiment was carried out. Water was provided from clean de-chlorinated tap-water and was exchanged twice daily, and fish were acclimatized for additional 10 days. Water temperature was adjusted at 25.0 ± 0.5°C and DO at 6.0±0.4 mg/L. Mortality and fish health status were also checked daily throughout the experiment. All the experimental procedures were carried out following the guidelines of the Research Ethics Board, Fac. of Vet. Med., Benha University BUFVTM 08-02-21.

2.2. Fermos® prebiotic and diets preparation

Fermos® was obtained as commercial product from Micron biosystems, UK (*saccharomyces servisiae* cell wall 500 g, Beta glucan 112.5 g, mannan 112.5 g and carried on silicate up to 1 kg). The control basal diet (Dry matter 67.15, crude protein 30.07, crude fiber 8.29 and lipid 5.83), and tested diets were prepared by the feed manufacture factory at the Food industries and packing complex (Fipco), affiliated to logistic authority of Egyptian armed forces. Fermos® at a rate of 0.5 and 1.0 g/kg was added to the raw materials at the mixing stage for 15 minutes. then, cooking line for 40 seconds at temperature of 85°C, then pressing, cooling and disintegration, and finally receiving the final product.

2.3. Feeding trial

After acclimatization, fish were divided into three equal groups (ninety fish per group, each in three replicates). The first group received the basal diet without any supplementation (control), the second and third groups were supplemented with 0.5 and 1.0 g Fermos® prebiotic/ kg diet, respectively for six-weeks feeding trial.

2.4. Sampling

Sampling included two sampling points at the end of third and sixth weeks from the start of the feeding trial. Blood samples (nine samples/group, 3 samples/ replicate) were collected through the caudal blood vessels using a plastic syringe with EDTA to evaluate the hematological picture (RBCs and WBCs counts, differential leucocytic count, Packed Cell Volume (PCV), Hemoglobin (Hb) and Corpuscular hemoglobin (MCH, MCV and MCHC) concentrations; and immunological parameters (phagocytic index and activity). Serum was separated from collected blood samples without anticoagulant, allowed to clot, and then centrifuged at 3000 rpm for 15 minutes. The obtained serum samples were kept at -20°C till being assayed for measuring immune parameters (lysozyme and nitric oxide (NO)), serum protein profiles (albumen, globulin, and total protein) and biochemical parameters (AST, ALT, glucose, and cortisol levels).

Organ collection: fish were carefully dissected to collect liver samples, which were homogenized in phosphate buffer saline (PH 7.2), centrifuged at 3000 rpm for 15 minutes, aspirated homogenate was preserved at -20°C till analysis of antioxidants Malondialdehyde (MDA), glutathione peroxidase (Gpx), Superoxide dismutase (SOD), and catalase (CAT) .

2.5. Hematological analysis

Total number of RBCs, WBCs and PCV were evaluated following Stoskopf (1993); differential leukocyte count were measured according to method described by Hrubec and Smith (1998). Hemoglobin (Hb) and Corpuscular hemoglobin indices (MCV, MCH and MCHC) were calculated according to Dacie and lewis (1957).

2.6. Immune parameters

Phagocytic index and activity were measured following methods described by Silva *et al.* (2002) and Dias *et al.* (2012)., where 0.5 ml of blood was shake mixed with 0.25 ml of 1×10^6 *Aeromonas hydrophila* suspension and blood smears were prepared. Number of active engulfing percentages were counted in relation to total leukocyte number and PI = total number of yeasts inside phagocytes/number of phagocytizing cells.

Lysozyme activity was assayed according to Demers and Bayne (1997) through adding 25µl of the undiluted serum to 175 µl of the substrate solution (*Micrococcus lysodeikticus* lyophilized cells, Inova Biotechnology, China). Alterations in turbidity was recorded every 30 sec. for 5 min. at 450 nm using the microplate ELISA reader and serum lysozyme (µg/ml) was obtained by matching with the standard curve made with lyophilized hen egg white lysozyme (Inova Biotechnology, China). Nitric oxide (NO) was determined using Nitric Oxide Assay Kit (ab211083, abcam, USA) at 450 nm.

2.7. Serum proteins profile

Serum globulins, albumins and total protein were determined according to the manufacturer's instructions (RA-50 chemistry analyzer (Bayer) using readymade chemicals (kits) supplied by Spinreact Co. Spain).

2.8. Biochemical parameters

All the parameters were measured in triplicates. Liver function enzymes ALT and AST were analyzed spectrophotometrically according to Klin (1970). Serum glucose and cortisol concentrations were assayed at 450 nm using available commercial kit (BioMed, Egypt).

2.9. Antioxidant profile

MDA was assayed based on thiobarbituric acid method at 540 nm and Gpx was measured at 534 nm according to Satoh (1978). SOD was estimated at 560 nm using nitro blue tetrazolium dye reduction method and CAT was measured at 510 nm according to Fossati *et al.* (1980).

2.10. Growth performance

All experimental fish were weighted at the start point (Zero-day); and at the end of the third and sixth week of the feeding trial. 30 fish from each group (10 samples /replicate) were used for the following measurements: Body Mass Gain % = 100 ×(final body mass - initial body mass)/(initial body mass); Specific Growth Rate % day^{-1} = (ln final body mass- ln initial body mass)/(number of days) X 100); Length gain rate % =100 × (Average terminal body length–Average initial body length) /(Average initial body length) X 100]; Feed Conversion Ratio = F/(Wf - Wi; Hepatosomatic Index = (weight of liver /total body weight) ×100; Spleen-somatic index = (weight of spleen/total body weight)×100 and Intestine somatic index = (weight of intestine/total body weight) ×100.

2.11. Statistical analysis

Data were analyzed using ANOVA with Duncan's multiple range tests using SPSS statistical software (v. 22.0). Means were considered significant when p value < 0.05.

3. RESULTS

3.1. Hematological profile

As shown in Table 1, Fermos® supplementation significantly increased ($P < 0.05$) total RBCs count, Hb concentration, MCHC and PCV with most significant increase for 1.0 g Fermos®/kg at both the 3rd and 6th weeks. While Fermos® 1.0 g/kg showed a significant decrease ($P < 0.05$) in both MCH and MCV and Fermos® 0.5 g/kg did not show a significant difference ($P > 0.05$) in neither MCV nor MCH values over the control group throughout the entire experiment. Fermos® 1.0 g/kg showed a significant increase ($P < 0.05$) in MCHC at the 6th week from the start of the experiment.

A clear variation of the leukogram has been observed among groups. Through the experimental period, percentage basophils and heterophils counts were lower in supplemented groups, as compared to control group. On contrary, percentage of total leukocytic count, monocytes, and lymphocytes ($P < 0.05$) significantly increased in supplemented groups than in control group (Table 2).

Table 1 Effects of 0.5 and 1.0 g/kg diet Fermos® prebiotic on hematological picture of Nile tilapia (*Oreochromis niloticus*) at 3 and 6 weeks

Fermos® (g/kg diet)	RBCs (x10/mm^6)	Hb (g/100ml)	PCV	MCH	MCV	MCHC
			3 weeks			
Control	2.8±0.01^c	8.4±0.01^c	27.0±0.01^c	30.5±0.05^a	98.1±0.01^a	31.2±0.05^c
Fermos 0.5	2.9±0.01^b	8.9±0.01^b	28.0±0.01^b	30.8±0.05^a	97.0±0.01^a	31.7±0.05^a
Fermos 1.0	3.1±0.01^a	9.2±0.01^a	29.0±0.01^a	30.0±0.05^b	95.2±0.01^b	31.5±0.05^b
			6 weeks			
Control	2.8±0.02^c	8.5±0.01^c	28±0.01^c	30.4±0.05^a	99.1±0.01^a	30.6±0.05^b
Fermos 0.5	2.9±0.01^b	9.0±0.05^b	29±0.01^b	30.2±0.05^b	96.9±0.05^c	31.2±0.01^a
Fermos 1.0	3.4±0.02^a	10.4±0.02^a	33.3±0.02^a	30.6±0.05^a	97.8±0.01^b	31.2±0.01^a

Values are expressed as mean ± SE. ^{abc} Different superscript letters in the same column indicate significance ($P<0.05$).

Table 2 Effects of 0.5 and 1.0 g/kg diet Fermos® prebiotic on differential leukocyte count of Nile tilapia (*Oreochromis niloticus*) at 3 and 6 weeks.

Fermos® (g/kg diet)	WBCs (x10^3/mm^3)	Lymphocytes (%)	Heterophils (%)	Monocytes (%)	Basophils (%)	Eosinophils (%)
			3 weeks			
Control	20.2±0.01^c	74.3±0.01^c	17.3±0.01^a	7.0±0.01^c	0.7±0.01^a	0.7±0.05^a
Fermos 0.5	24.8±0.0^b	79.3±0.0^b	11.3±0.0^b	8.3±0.0^b	0.3±0.0^b	0.7±0.05^a
Fermos 1.0	30.1±0.01^a	81.3±0.01^a	9.3±0.01^c	8.7±0.01^a	0.3±0.0^b	0.3±0.0^b
			6 weeks			
Control	20.7±0.01^c	76.3±0.02^c	15.7±0.01^a	6.3±0.01^c	1.0±0.01^a	0.7±0.01^a
Fermos 0.5	25.4±0.02^b	81.3±0.01^b	10.3±0.05^b	7.3±0.05^b	0.3±0.0^b	0.7±0.02^a
Fermos 1.0	33.6±0.01^a	81.0±0.01^b	9.0±0.01^c	9.0±0.01^a	0.3±0.0^C	0.7±0.01^a

Values are expressed as mean ± SE. ^{abc} Different superscript letters in the same column indicate significance ($P<0.05$).

mean (n = 9) ± SEM. Mean values with asterisk (*) are different significantly ($P < 0.05$).

3.2. Immune parameters

The phagocytic activity and the phagocytic index were improved ($P < 0.05$) significantly at level of 0.5 g/kg Fermos® supplemented group, only at the 3rd week, and 1.0 g/kg Fermos® supplemented group at both time points, compared to the control (Figure 1 A). Lysozyme and Nitric oxide (NO) activities increased significantly ($P < 0.05$) in 1.0 g/kg Fermos® group at both the 3rd and 6th weeks compared to the control (Figures 1 B and C).

3.3. Serum proteins

Fermos® supplementation significantly increased ($P < 0.05$) globulin, albumin, and total protein levels in 1.0 g/kg Fermos® group at both time points compared to the control. The 0.5 g/kg Fermos® group showed a significant increase ($P < 0.05$) in albumin at both time pints, while only at the 6th week for globulin and total protein (Figure 2).

3.4. Biochemical parameters

AST and ALT levels were not significantly ($P > 0.05$) altered in Fermos® groups during the first three weeks, while showed a ($P < 0.05$) significant decrease at the end of the sixth week for AST level (Table 3). Serum glucose levels were increased in Fermos® groups, while serum cortisol levels did not show a significant ($P < 0.05$) difference in Fermos® groups over the experimental period compared to the control group (Table 3).

3.5. Antioxidants' status

MDA markedly decreased ($P < 0.05$) in Fermos® groups over the experimental period, with the highest decrease for 1.0 g/kg Fermos® compared to the control group (Figure 3). Gpx and CAT and SOD activities significantly increased in Fermos® groups with the highest ($P < 0.05$) significant increase for group receiving 1.0 g/kg Fermos® through the experimental period compared to the control (Figures 4-6).

3.6. Growth performance

Fermos® supplementation revealed a significant improvement ($P < 0.05$) in growth performance parameters (BW, BMG, SGR and LGR) through the experimental period with the best performance for 1.0 g/kg Fermos® group, which also showed the most ($P < 0.05$) significant decrease in FCR compared to the control group. Gastrontestinal somatic indices did not reveal a ($P < 0.05$) significant difference over control, except for 1.0 g/kg Fermos® group that showed significant increase in both hepatosomatic and intestine somatic indexes at the sixth week from the start of the feeding trial (Table 4).

Table 3 AST, ALT, glucose, and cortisol of Nile tilapia (*Oreochromis niloticus*) supplemented with 0.5 and 1.0 g/kg diet Fermos® prebiotic at 3 and 6 weeks.

Fermos® g/kg diet	AST (U/l)	ALT (U/l)	Glucose (mg/dl)	Cortisol (ng/ml)
3 weeks				
Control	20.2±0.01^a	30.1±0.01^a	9.1±0.01^c	40.1±0.0^{ab}
Fermos 0.5	20.1±0.01^a	30.1±0.01^a	10.2±0.0^b	39.2±0.05^b
Fermos 1.0	20.0±0.01^a	29.8±0.0^b	12.1±0.05^a	39.1±0.05^b
6 weeks				
Control	21.0±0.05^a	29.8±0.01^a	9.1±0.0^b	40.2±0.01^{ab}
Fermos 0.5	20.0±0.2^b	29.9±0.01^a	12.0±0.01^{ab}	40.0±0.0^b
1.0	20.0±0.0^b	29.8±0.01^a	12.1±0.05^a	40.0±0.2^b

Values are expressed as mean ± SE. ^{ab}Different superscript letters in the same column indicate significance ($P<0.05$).

Table 4 Growth performance of Nile tilapia *(Oreochromis niloticus)* supplemented with 0.5 and 1.0 g/kg diet Fermos® prebiotic at 3 and 6 weeks.

Fermos® (g/kg feed)	Initial Wt. (g)	Final Wt. (g)	BMG (%)	SGR (%)	LGR (%)	FCR	HSI	SSI	ISI
3 weeks									
Control	16.0 ± 0.5	36.5± 0.5	128± 0.0	1.8±0.0	42.6±0.0	2.2± 0.0	2.91±0.02*	0.11±0.05*	4.03±0.0
0.5	16.0 ± 0.5	39.4± 0.7	146.5± 0.0*	2±0.0*	45.6±0.0*	1.9± 0.0	2.37±0.02	0.08±0.02	4.40±0.0
1.0	16.0 ± 0.5	39.8± 0.5*	149.1± 0.7	1.9± 0.0	44.1±0.0	1.8± 0.0*	2.11±0.05	0.07±0.02	4.40±0.05
6 weeks									
Control	16.0 ± 0.5	49.8± 0.5	211± 0.7	1±0.0	63.7±0.0	1.3±0.0	2.84±0.05	0.11±0.00	3.75±0.05
0.5	16.0 ± 0.5	53.6± 0.0	235.2± 0.5	1.2± 0.0*	83.0±0.0	1.2±0.0*	3.17±0.02	0.10±0.02	4.51±0.05
1.0	16.0 ± 0.5	54.8± 0.5*	243± 0.5*	1.2± 0.0*	97.7±0.0*	1.2±0.0*	3.40±0.00*	0.10±0.00	5.17±0.0*

Values are mean (n = 30) ± SEM. Mean values with asterisk (*) are different significantly (P < 0.05). BMG = Body Mass Gain, SGR = Specific growth rate, LGR=Length Gain Rate, FCR = Feed Conversion Ratio. LSI = Hepatosomatic index, SSI = Spleen somatic index and ISI= Intestine somatic index.

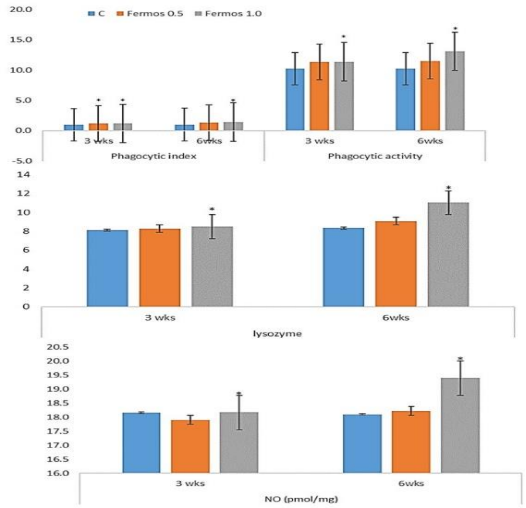

Fig.1 Phagocytic index and activity (A), lysosyme (B) and NO (C) of Nile tilapia *(Oreochromis niloticus)* supplemented with 0.5 and 1.0 g/kg diet Fermos® prebiotic at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Mean values with asterisk (*) are different significantly (P < 0.05).

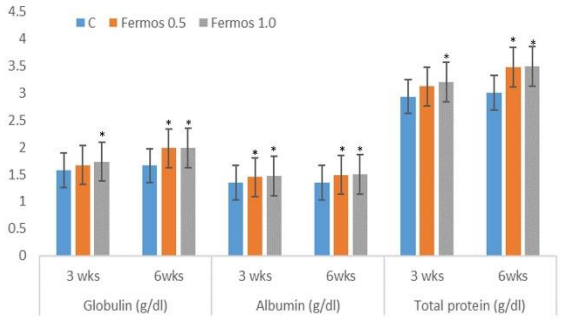

Fig 2. Serum albumin, globulin and total protein of Nile tilapia *(Oreochromis niloticus)* supplemented with 0.5 and 1.0 g/kg diet Fermos® prebiotic at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Mean values with asterisk (*) are different significantly (P < 0.05).

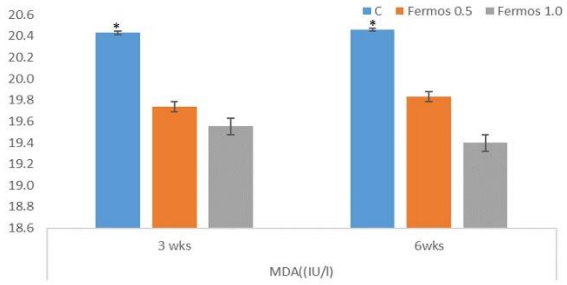

Fig 3 MDA of Nile tilapia *(Oreochromis niloticus)* supplemented with 0.5 and 1.0 g/kg diet Fermos® prebiotic at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Mean values with asterisk (*) are different significantly (P < 0.05).

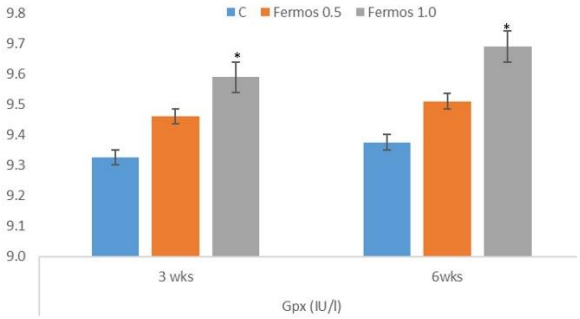

Fig. 4 Effect of 0.5 and 1.0 g/kg diet Fermos® prebiotic on GPx of Nile tilapia *(Oreochromis niloticus)* at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Mean values with asterisk (*) are different significantly (P < 0.05).

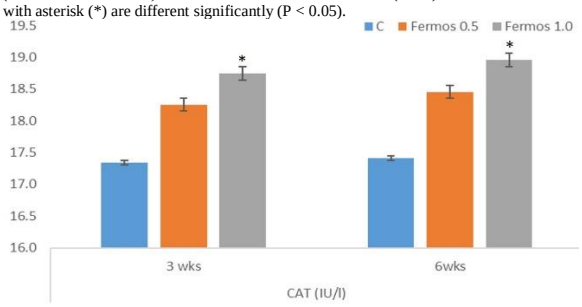

Fig.5. CAT of Nile tilapia *(Oreochromis niloticus)* fed on 0.5 and 1.0 g/kg diet Fermos® prebiotic at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Mean values with asterisk (*) are different significantly (P < 0.05).

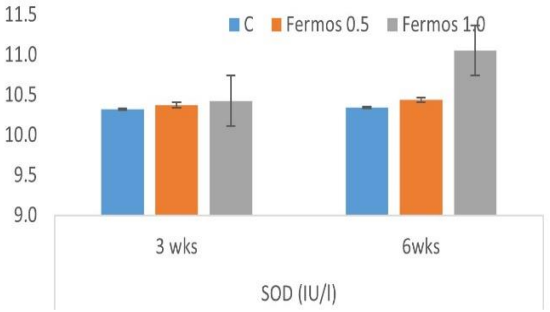

Fig.6. Effect of 0.5 and 1.0 g/kg diet Fermos® prebiotic on SOD of Nile tilapia *(Oreochromis niloticus)* at 3 and 6 weeks. Values are mean (n = 9) ± SEM. Mean values with asterisk (*) are different significantly (P < 0.05).

4. DISCUSSION

Inclusion of feed additives including prebiotics have been known for their growth-stimulating properties into Nile tilapia (*Oreochromis niloticus*) diets, which is an important cultured species worldwide (FAO, 2018). The present study aimed to evaluate the potential effects of Fermos® (MOS-containing prebiotic) dietary incorporation at levels of 0.5 and 1 g/Kg on hematological, immunological, serum proteins, biochemical responses, and growth performance in Nile tilapia (*Oreochromis niloticus*). Results revealed a significant (P < 0.05) alteration in hematology of fish in

response to two different levels of inclusion of the prebiotic Fermos®.

Collectively, those alterations were in favor of better erythrogram and leukogram in supplemented fish groups. Similarly, Sado *et al.* (2014) reported that dietary mannan oligosaccharides (Active MOS (R)-Biorigin) incorporation resulted in increased ($P < 0.05$) hematocrit, mean corpuscular volume, mean corpuscular hemoglobin values in pacu, *Piaractus mesopotamicus*. In addition, Abu-Elala *et al.* (2018) revealed that Immunowall® prebiotic that is composed mainly of yeast β-glucan and MOS significantly increased white blood cell count of Nile tilapia *Oreochromis niloticus*. However, Cavalcante *et al.* (2020) did not observe any significant effect of MO prebiotics or chitosan either alone or in combination with probiotic on hematologic parameters (erythrocyte, leucocyte and thrombocyte counts or erythrocyte indices in supplemented Nile tilapia. This might be because of the potential non-specific responses in fish (Abu-Elala *et al.,* 2018) as fish erythrocytes and leucocytes profiles might be affected by several intrinsic or extrinsic factors such as water contaminants (Grant, 2015, Carraschi *et al*., 2017).

Fermos® prebiotic supplementation significantly improved phagocytosis lysozyme and NO activities in supplemented Nile tilapia groups. Similarly, Elkamel and Mosaad (2012) found that *Nigella sativa* significantly increased the phagocytic index and activity were in Nile tilapia in response to supplemental feeding. In addition, Staykov *et al.* (2007) also reported immune status enhancement in MOS supplemented rainbow trout groups. This improvement in the immune response parameters could be due to the immune-stimulating effect of MOS (Abu-Elala *et al*., 2018) as they can bind to toll like receptors (TLRs) facilitating phagocytosis and non-specific opsonization of phagocytes and neutrophils (Lee *et al.,* 2014). Contradictory, Cavalcante *et al.* (2020) reported that phagocytic capacity and phagocytic index were not significantly altered by prebiotic, probiotic or symbiotic supplements in comparison to control group.

In the current study, supplemented Nile tilapia expressed significantly increased serum albumen, globulin, and total protein in comparison with the control group. Similarly, Kumar *et al.* (2018) found higher protein levels in *Cirrhinus mrigala* fingerlings supplemented with MOS prebiotic in conjunction with *B. subtlis* at a concentration of 4 g and 100 X 10^7 CFU/Kg diet. That positive results might be due to the immune-stimulating effect of MOS (Abu-Elala *et al*., 2018). Nevertheless, inclusion of MOS as a supplement for Nile tilapia at a rate of 4 g/Kg diet did not alter protein levels in a study conducted by Cavalcante *et al.* (2020). However, in that study, fish might have been exposed to handling which might have affected outcome variables investigated in that study.

In this study, The MOS-containing Fermos® prebiotic also helped to maintain the wellbeing of Nile tilapia through decreasing the level of AST that is linked with the normal liver function like Gelibolu *et al.* (2018) who recorded hepatoprotective effect in gilthead sea bream *(Sparus aurata)* fed a diet supplemented with MOS with different concentrations in comparison to control group. AST and ALT enzymes are indicators of liver damage and their increase in blood is indicative of liver tissue necrosis, tissue degeneration and reflect changes in protein metabolism (Bruslé and Anadon, 1996), thus our results assume the hepatoprotective action of MOS. Furthermore, our work revealed that serum glucose levels were increased in Fermos® groups, while serum cortisol levels did not show a significant ($P < 0.05$) difference in Fermos® groups, this may be due to the combination of Fermos active ingredients as MOS and beta glucan can alter the glucose and insulin levels and the best results are well-known to be gained from using beta-glucan separately (Brydges *et al.,* 2009; Ramsay et al., 2009; Jami *et al.,* 2019). On the same instance, MOS-prebiotic supplemented for rainbow trout at a concentration of 2g/Kg diet for 45 days revealed hepato-protection properties which might be attributed to the higher innate immune response and leucocyte counts in supplemented fish which may substantiate lowered stress indicators as cortisol (Yarahmadi *et al.,* 2016).

Antioxidant enzymes SOD, CAT and Gpx were increased in Nile tilapia because of supplementing the basal diet with the Fermos® MOS-prebiotic in the present investigation. As far as we know, limited research focused on the oxidative stress modulation through supplementing the basal diet of Nile tilapia with MOS-prebiotic. Dawood *et al.* (2020) found no effect of the MOS-prebiotic on the concentration of reactive oxygen metabolites in Red seas beam after supplementation with different concentrations from 0.5 to 2 g/Kg diet. Meanwhile, in that study, the biological antioxidant potential was significantly increased when Red sea beam was supplemented the prebiotic at 1g/Kg or more. This may be because of hepatoprotective effect of MOS (Yarahmadi *et al.,* 2016).

Regarding growth performance, Fermos® supplementation revealed ($P < 0.05$) significant improvement in growth performance parameters (BW, BMG, SGR and LGR) through the experimental period. Likewise, Torrecillas *et al.* (2014 and 2015) demonstrated that dietary inclusion of MOS significantly increased SGR of European Sea Bass (*Dicentrarchus labrax*). This improvement is a result of the fact that MOS has positive effects on fish growth through directly affecting absorption rates of nutrients via changing the intestinal pH and changing the peptides levels that control appetite (Terova *et al.* 2009).

5. CONCULOSION

In conclusion, the current investigation revealed the positive role of Fermos® MOS-containing prebiotic dietary incorporation in *Oreochromis niloticus diets* through improving hematologic, immune, antioxidants and growth profiles of supplemented fish.

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