Intermittent Supply of Mannan Oligosaccharide Boosts the Nile Tilapia Immunity

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ARTICLE INFO

ABSTRACT

Aquaculture has been facing numerous obstacles, particularly diseases causing great financial loss. A twelve-week trial was conducted to assess a strategy of intermittent dietary of mannan oligosaccharides and β-glucan (active-MOS) on the immunity of the Nile tilapia. Five groups (G1–5) were fed on a supplemented diet for 12 weeks, interrupted with a one-week-free diet every 0, 1, 2, 3, and 12 weeks. Growth parameters FW, WG, DWG, RGR, and FCR were significantly enhanced after supplementing of dietary-MOS compared to the control group, with a superiority of G3 122g, 70.7g, 0.83g, 134.95 and 1.57, respectively. The activity of serum antibacterial, oxidative burst, and phagocytosis (innate immunity) was significantly boosted in supplemented fish regardless of the supplementation period. The gene expression of interleukin *(il)-1β*, Heat shock protein (Hsp)-70, and tumor necrosis factor *(tnf)-α* were increased in response to dietary-active-MOS supplementation. They were significantly higher in G3 and G4 fish, recording values of 4.87, 0.81, and 7.1; 3.7, 0.86, and 6.74, respectively, compared to the control group 0.68, 0.15, and 2.3 fold change, respectively. Dietary-active-MOS induced the gene expression of catalase and superoxide dismutase (antioxidant enzymes); fish of G4 had significantly high gene expression with 0.7- and 2.89-fold changes, respectively. On *the* challenge test with *Aeromonas hydrophila*, the Nile tilapia fed dietary-active-MOS had a low mortality rate (35%), providing a high relative level of protection (41.67%) in G3 and G4. Thus, dietary-active-MOS is recommended with a week interval of free additive diet to boost the immunity of the Nile tilapia and provide protection against bacterial infection.

INTRODUCTION

In Egypt, farmers tend to use the same diet formula during the production season; unfortunately, the long term of dietary immune stimulants resulted in immune system exhaustion. Development and intensification of aquaculture practices, especially fish culture have raised concerns over stress conditions of environmental, biological and

physiological nature that, in turn, raise the possibility of infectious diseases to become an obvious outbreak in fish farms **(Ye *et al.,* 2011).**

Bacterial adhesion, which is mediated by the interaction of bacteria with specific carbohydrate groups present on the cell surface via specific lectins, is a fundamental process to colonize and become pathogenic **(Bavington & Page, 2005).** The outbreak of fish diseases has been progressively widespread as a result of intensive aquaculture in the last decade **(Sundberg *et al.,* 2016).** Disease outbreak could impair body health, which is strongly linked to reducing immune organ functions **(Amoah *et al.,* 2016; Sherif *et al.,* 2020, 2021a; Abdelsalam *et al.,* 2022; Elgendy *et al.,* 2022; Sherif & Kassab, 2023).**

Several scientists stated that dietary-MOS (functional feed additives) improved growth performance, feed utilization, innate immunity, and intestinal wall integrity leading to decreased vulnerability of several fish species of the Mediterranean Sea such as gilthead seabream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*) to infectious fish diseases **(Gültepe *et al.,* 2012; Torrecillas *et al.,* 2015; Guerreiro *et al.,* 2018).** The potential mode of mannan oligosaccharides (MOS) action happens mainly via stimulating intestinal integrity and innate immunity; however, this action is controlled by many factors related to the MOS structure added, dose, and duration in addition to some concerning fish species and size besides the farming strategy **(Torrecillas *et al.,* 2018).** In teleost fish, the hematopoietic organs such as spleen and head-kidney can be generally considered as crucial immune organs **(Salze *et al.,* 2008)**. The immune function of fish spleen and head-kidney is mainly dependent on the production of antibacterial peptides **(Dong *et al.,* 2017; Sherif *et al.,* 2019; Sherif & Mahfouz 2019)**, such as increasing the phagocytic activity of leucocytes in the head-kidney and spleen of *D. labrax* **(Torrecillas *et al.,* 2007)** and enhancing the lysozyme (LZ) activity in the head-kidney of rainbow trout (*Oncorhynchus mykiss*) **(Ahmadi *et al.,* 2014).** Another mode of MOS action is to alleviate inflammatory responses as in rainbow trout **(Ahmadi *et al.,* 2014)** and greater amberjack (*Seriola dumerili*) **(Fernández-Montero *et al.,* 2019).** Several studies showed that dietary-MOS supplementations for the Nile tilapia could elevate the mRNA transcription of *tnf-α* and *il-1β* **(Abu-Elala *et al.,* 2019).** In addition, the involvement of MOS also up-regulated the expression of interferon *(ifn-γ),* and *il-17D* in greater amberjack head-kidney **(Fernández-Montero *et al.,* 2019).** Besides these studied cytokines, other cytokines such as *il-4, il-13, il-15,* and transforming growth factor *(tgf-β)* also engaged in the crucial immune regulation of fish too **(Dong *et al.,* 2017).**

Thus, this trial was conducted to clarify the potential influence of the intermittent feeding strategy of MOS plus β-glucan on innate immunity, gene expression of cytokines and antioxidant enzymes of *Oreochromis niloticus*.

MATERIALS AND METHODS

1. Work design

The Nile tilapia secimens were purchased from a private fish farm located at Tolompate village in the north of Egypt. On the fish farm, fish were tranquilized with MS-222 at a concentration of 40mg/ L (MS-222, SyncaineR, Syndel, Canada), and then fish were transferred live to the wet laboratory. On arrival, two hundred and twenty-five fish individuals were subjected to an iodine bath at a concentration of 20ppm (povidone-iodine 5%/) L. Betadine® was produced by the Nile Company for pharmaceuticals, Egypt, and then fish were stocked in fiberglass tank with dimensions of 1.5×1.5×1 m for fourteen days. After the accommodation period, fish restored normal feeding behavioral; the accommodation process was performed according to the recommendations of previous studies (**Eldessouki *et al.*, 2023; Sherif *et al.*, 2023a, b**). Active-MOS *Saccharomyces cerevisiae* cell wall extract 100% (β-glucan 20% mannan oligosaccharides 18%) produced by Biovet Company, Egypt under the license of Acucareira-Brazil. Fish weight (51.95±0.17g) were subdivided into five groups **G1:** fed on a dietary-active-MOS diet for 12 weeks; **G2:** fed on active dietary-active-MOS for one week following a free diet for one week; this cycle was repeated six times in 12 weeks; **G3:** fed on active dietary-active-MOS for two weeks following a free diet for one week; this cycle was repeated four times in 12 weeks; **G4:** fed on active dietary-active-MOS three weeks followed a free diet for one week, this cycle was repeated three times in 12 weeks, and **G5:** fed on active dietary-active-MOS for 12 weeks. Feed composition and analyses (Table 1) were performed following the recommendations of **NRC (2011).**

2. Growth parameters

The impacts of dietary-active-MOS on growth performance (total weight gain (TWG) feed conversion ratio (FCR)) and feed utilization (relative growth rate (RGR %) and protein efficiency ratio (PER)) were calculated using the following equations:

- TWG = FW (g) – IW. (g)
- WG % = TWG (g) / IW (g) × 100
- FCR = Feed intake (g) / TWG (g)
- RGR % = 100 (FW – IW) (g) / IW (g)
- PER = WG (g) / Protein intake (g)

3. Innate immunity

A. Serum antibacterial activity (SAA)

The activity of the Nile tilapia serum was tested against bacteria in fish that received dietary-active-MOS and the control using the method mentioned by **Kajita *et al.* (1990)**. Concisely, the Nile tilapia serum and a bacterial suspension of *A. hydrophila* (2×10^8 CFU) were combined in equal quantities (100µl) and incubated at 25°C for 1h. Furthermore, a control (blank) was formed by substituting the serum with a

sterile phosphate buffer saline (PBS). A dilution 1:10 was formed with sterile PBS. On blood agar, the 100µl of the serum-bacterial mixture was incubated at 27°C for 24h then viable bacteria were counted by determining the colonies that grew on the tryptic soy agar.

Table 1. Feed composition and analyses

Nutrient 100kg	Control %	Active-MOS
Corn 7%	15	15
Soy 44%	30	30
Fish meal 65%	25	25
Corn gluten	15	15
Wheat flour	6	6
Soy oil	1.3	1.3
DDGs[1]	5	5
Salt	0.2	0.2
MCP	1	1
Vitamins mix[2]	0.15	0.15
Minerals mix[3]	0.15	0.15
D.L. methionine	0.14	0.14
Choline chloride	0.06	0.06
Carboxy methyl cellulose	1	0.8
Manan	0	0.2
Chemical composition analyses	%	
Moisture %	11.1	
CP[4] %	42.72	
DE[5] (Kcal/Kg)	2955.62	
Ether extract %	5.74	
Crude fiber	2.6	
NFE[6]	35.3	
Ash%	7.4	

Note: DDGs; Dried distilled grains. Vitamin premix contains vitamin A 12000000 IU, vitamin K3 2g, vitamin C 250g, vitamin E 10g, vitamin D3 2200000 IU, vitamin B1 1g, vitamin B2 5g, vitamin B6 1.5g, vitamin B12 0.01g, Biotin 0.050g, Niacin 30g, Folic acid 1g and Pantothenic acid 10g and carrier to 1000g. [3]Mineral premix contains Copper 4g, Manganese 60g, Zinc 50g, iron 80g, Iodine 1g, Cobalt 0.1g, Selenium 0.1g with calcium carbonate (CaCO3) carrier to 1000g. CP; crude protein. DE; Digestible energy, Kcal/kg, NFE; Nitrogen free extract.

B. Oxidative burst activity (OBA)

Following the method of **Anderson** *et al.* **(1992),** oxidative burst activity (OBA) of experimental fish heterophils was determined using a nitroblue tetrazolium (NBT) assay; briefly, within 15 minutes following the blood samples collection a drop was put on a coverslip, then the coverslips were incubated in humid chambers for 30 minutes at room temperature (25 °C). Meanwhile, the heterophils were left to adhere to coverslip then gently washed with PBS (pH 7.4). Afterwards, the adhered heterophils were stained

using a 50μl of filtered solution NBT (0.2%),   manufactured by Fluka Buchs, Switzerland. The number of stained cells (dark-blue) were counted as positive.

C. Phagocytosis activity

Firstly, blood cells heterophiles were isolated **(Faulmann *et al.,* 1983),** then a 24-h-old culture *Candida albicans* with a dose of 1×10^6 cells/ml and heterophile count was adjusted at 2.5×10^6 viable cells/ ml; 1 ml of blood was added to a suspension of the *C. albicans* (1 ml), and then the mixture (blood/ *C. albicans*) was incubated at 27°C for 1h under 5– 10% CO_2. The mixtures were smeared and stained with Giemsa. A minimum of 100 cells were counted in different fields under a microscope at 1000× magnification **(Kawahara *et al.,* 1991)**; the phagocytic assay PA and the phagocytic index (PI) were calculated using the following equations:

PA = (No. of ingesting phagocytes / total No. of phagocytes) × 100

PI = No. of ingested *C. albicans* cells / No. of ingesting phagocytes

4.   Antioxidant and cytokines gene expressions

The impacts of intermittent dietary-active-MOS were assessed on the expression of cytokines (interleukin *il-1β,* heat shock protein70 (Hsp) & tumor necrosis factor *(tnf)-α)* and antioxidants enzymes (catalase (CAT) and superoxide dismutase enzyme (SOD)). Firstly, three Nile tilapia were used to extract total RNA from the tissues of head kidney at the end of the experimental process, the reagent Trizol, which was produced by iNtRON Biotechnology Inc., Korea, was used in the extraction process. By Nanodrop D-1000 spectrophotometer, the harvested RNA was evaluated for quantity and quality, spectrophotometer manufactured by NanoDrop Technologies Inc., USA. The quantitative real-time polymerase chain reaction (PCR) was used to assess the impacts of active-MOS on the Nile tilapia's health. The template of the resulting cDNA and β-actin was used as the housekeeping gene, and the gene expression was determined by the Equation $2^{-\Delta\Delta CT}$ **(Livak & Schmittgen, 2001).**

Table 2. The primers sequences and genbank numbers

Gene	primers (5′–3′)	Genbank
β-actin	F: CAGGGAGAAGATGACCCAGA R: CAGGGCATAACCCTAGTAGA	EU887951.1
il-1β	F: GACAGCCAAAAGAGGGAGC R: TATCAGCGATGGGTGTAG	KF747686.1
Hsp-70	F: GCTCTGAACCCCAGCAACACT R: TTGTCCTCCCCTTTGTACTCCA	FJ213839.1
tnf-α	F: TAGAAGGCAGCGACTCAA R: CCTGGCTGTAGACGAAGT	NM_001279533.1
SOD	F: CATGCCTTCGGAGACAACAC R: ACCTTCTCGTGGATCACCAT	AY491056.1
CAT	F: AGCTCTTCATCCAGAAACGC R: GACGTCAGGCGTCACATCTT	JF801726.1

Note: *il*, interleukin, Hsp heat shock protein, *tnf* tumor necrosis factor SOD superoxide dismutase enzyme, CAT catalase.

5. Bacterial challenge

At the end of the experiment, a number of ten Nile tilapia were randomly obtained from different fish groups, and injected intraperitoneally (IP) with LD50 (2.4×105 CFU) of an *A. hydrophila* AHRAS2 pathogenic strain (accession number MW092007) **(Sherif & AbuLeila, 2022)**. In addition, 10 fish from the control group were injected with pure saline solution (0.65%) and were considered negative controls **(Boijink *et al.,* 2001)**. For fourteen days, the infected Nile tilapia were observed for deaths, and the mortality rate (MR) was determined with following equation:

$$\textbf{MR (\%) = (number of dead fish}\,/\,\textbf{total population during the experimental period)} \times \textbf{100.}$$

In addition, the RLP was verified among the experimentally infected fish according to following equation:

$$\textbf{RLP\% = 100} \times \textbf{[1– (\%deaths in the treated fish / \%deaths in the control fish)]}$$

6. Biosafety

The biosafety measures were followed in this work, the recommendations concerning the use and elimination the infectious pathogen safety data sheets *A. hydrophila* and *C. albicans,* following Pathogen Regulation Directorate step by step **(Public Health Agency of Canada, 2011)**.

7. Statistics

The obtained data concerning the supplementation of intermittent dietary immunostimulants to Nile tilapia were statistically analyzed using a one-way ANOVA test. The SPSS software package was used to calculate all statistics **(SPSS, 2004).** Duncan's various range was used to detect differences between treatments at a significance threshold of 0.05 **(Duncan, 1955)**, and the data are presented as the mean $\pm$ standard error (SE).

RESULTS

1. Feed utilization and growth performance

In Table (3), dietary-active-MOS (MOS plus β-glucan) supplementation affected the growth behavior of the Nile tilapia significantly. Regardless of the period, supplemented fish had significantly high FW, WG, DWG, and low FCR compared to the control fish. Over two weeks of supplementation, the Nile tilapia (G3) recorded a significant high growth performance (FW, WG, DWG, RGR, and FCR); fish of G3 were fed dietary-active-MOS for two weeks alternated with one-week free-diet, compared to the control fish (122g), recorded values of 70.7g, 0.83g, 134.95 and 1.57; 79.33g, 26.7g, 50.75 and

2.1, respectively. Feed utilization (FI and PER) had the same trend of growth performance (Table 3).

Table 3. Growth parameters and feed utilization

Item	IW	FW	WG	DWG	FCR	RGR	FI	PER
G1	52.63 ±0.09	79.33[C] ±1.45	26.7[C] ±1.54	0.32[C] ±0.02	2.1[A] ±0.08	50.75[C] ±3	55.79[C] ±2.02	1.6[C] ±0.06
G2	51.87 ±.09	88.4[B] ±3.45	36.53[B] ±3.52	0.44[B] ±0.04	1.96[AB] ±0.03	70.45[B] ±6.86	71.54[B] ±6.67	1.7[B] ±0.02
G3	51.93 ±0.55	122[A] ±1.39	70.07[A] ±1.3	0.83[A] ±0.02	1.57[D] ±0.05	134.95[A] ±2.94	110[A] ±3.85	2.13[A] ±0.07
G4	51.4 ±0.51	118.4[A] ±2.83	67[A] ±2.61	0.79[A] ±0.03	1.73[CD] ±0.05	130.37[A] ±4.8	115.5[A] ±3.37	1.93[A] ±0.06
G5	51.93 ±0.33	117[A] ±1	65[A] ±1.33	0.77[A] ±0.01	1.8[BC] ±0.02	125.27[A] ±3.37	117.3[A] ±3.15	1.85[A] ±0.02

Note: G1, fed control diet; G2, fed dietary-active-MOS alternated with control on week basis; G3, fed dietary-active-MOS for two weeks alternated with control for one week; G4, fed on dietary-active-MOS three weeks followed free diet for one week; G5; fed on dietary-active-MOS 12 weeks. IW, initial weight; FW, final weight; WG, weight gain; DWG, daily weight gain; FCR, feed conversion rate; RGR, relative growth rate; FI, feed intake; PER, protein Efficiency ratio. Different superscript letters within the same column indicate that values are significant differences at $P \leq 0.05$.

2. Innate immunity

The impacts of dietary-active-MOS on Nile tilapia immunity were assessed by determining the activity of serum SAA and immune cells (OBA, PA, and PI). The innate immunity was boosted in fish that received dietary-active-MOS, regardless of the supplementation period, where the fish of G3 and G4 had the superiority compared to the control group. The values of SAA, OBA, PA and PI were significantly higher in G3 and G4 recording values of 61.4, 7.7,48, and 2.67; 59.3, 7.33, 54.33 and 3.33, respectively, compared to the control group (45.7, 3.7, 34.3, and 0.67) (Table 4).

Table 4. Assessment of innate immunity of the Nile tilapia

Item	SAA %	OBA stained cell	PA %	PI
G1	45.7[C] ±1.09	3.7[C] ±0.33	34.3[C] ±1.2	0.67[C] ±0.3
G2	48.9[B] ±1.9	5.33[B] ±0.3	42.3[BC] ±1.45	1.33[BC] ±0.67
G3	61.4[A] ±0.56	7.7[A] ±0.3	48[AB] ±3.05	2.67[AB] ±0.3
G4	59.3[A] ±2.12	7.33[A] ±0.3	54.33[A] ±0.88	3.33[A] ±0.3
G5	53.73[B] ±1.77	5.67[B] ±0.3	45.67[AB] ±4.7	1.67[BC] ±0.7

Note: G1, fed control diet; G2, fed dietary-active-MOS alternated with control on week basis; G3, fed dietary-active-MOS for two weeks alternated with control for one week; G4, fed on dietary-active-MOS three weeks followed free diet for one week; G5; fed on dietary-active-MOS 12 weeks. SAA, serum antibacterial activity; OBA, oxidative burst activity; PA, phagocytic activity; PI, phagocytic index. Different superscript letters within the same column indicate that values are significant differences at $P \leq$ 0.05.

3. Gene expression cytokines and antioxidants

The cytokines gene expression (*il-1β,* Hsp-70, and *tnf-α*) was affected in the Nile tilapia that received dietary-active MOS. A prebiotic MOS plus β-glucan could trigger the immune systems in fish inducing high gene expression *il-1β,* Hsp-70, and *tnf-α* that were significantly higher in G3 and G4 (4.87, 0.81, and 7.1; 3.7, 0.86, and 6.74, respectivelym compared to the control group with 0.68, 0.15, and 2.3 fold change (Fig. 1).

In Fig. (2), antioxidant enzymes SOD and CAT gene expression had different trends compared with cytokines; fish of G4 had significantly higher gene expression of 0.7- and 2.89-fold change, whereas no difference was detected between G3 and G5 or between G2 and the control group.
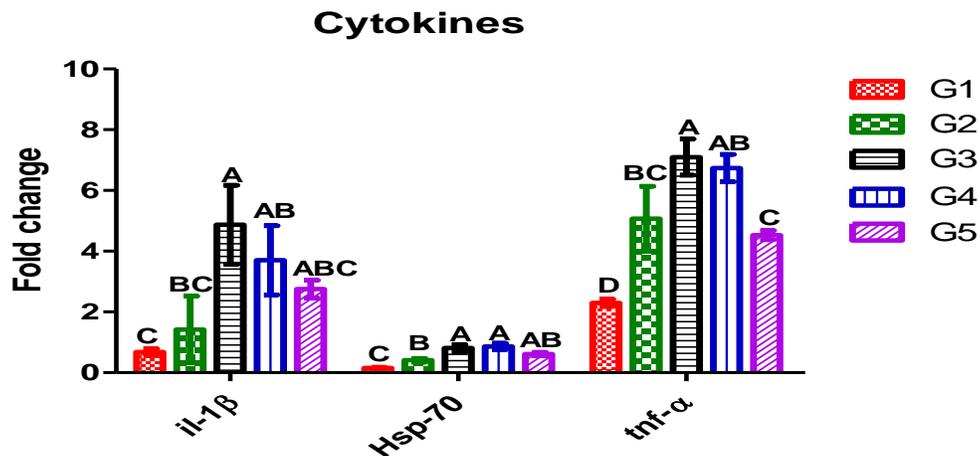

Fig. 1. Cytokines gene expressions

Note: *: fold change, G1: fed control diet, G2: fed dietary-active-MOS alternated with control on week basis, G3: fed dietary-active-MOS for two weeks alternated with control for one week, G4: fed on dietary-active-MOS three weeks followed free diet for one week, and G5: fed on dietary-active-MOS 12 weeks. Interleukin *(il)-1β,* Heat shock protein (Hsp)-70, and tumor necrosis factor *tnf-α* were determined. Different superscript letters within the parameter indicate that values are significant differences at $P \leq 0.05$.
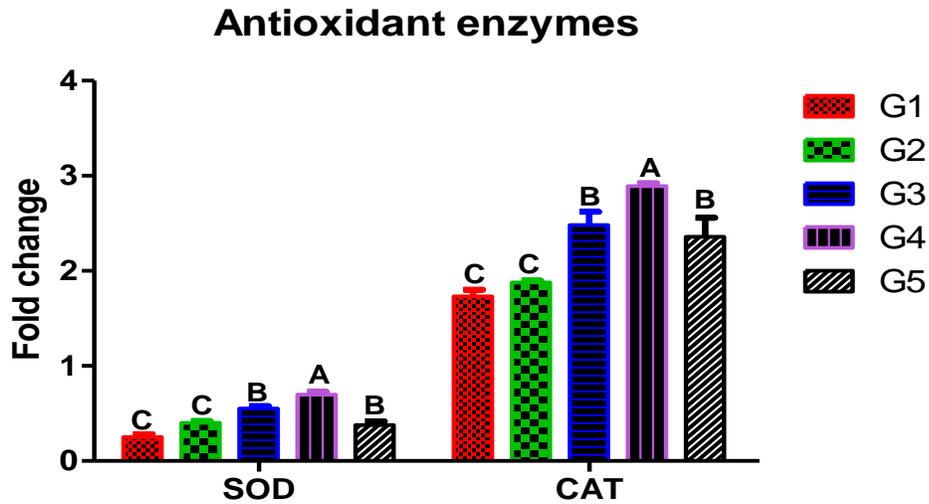
Antioxidant enzymes

Fig. 2. Antioxidants gene expressions

Note: *, fold change; Note: G1, fed control diet; G2, fed dietary-active-MOS alternated with control on week basis; G3, fed dietary-active-MOS for two weeks alternated with control for one week; G4, fed on dietary-active-MOS three weeks followed free diet for one week; G5; fed on dietary-active-MOS 12 weeks. SOD, Super oxide dismutase; CAT, Catalase. Different superscript letters within the parameter indicate that values are significant differences at $P \leq 0.05$.

4. Bacterial infection

Table (5) shows that, after the 12- week feeding trial, twenty Nile tilapia fish from each group were experimentally infected with *A. hydrophila*, then MR% and RLP% were determined along with re-isolation of the infective bacteria. Fish in G3 and G4 recorded significantly low MR% (35 and 35) and higher RLP% (41.67 and 41.67), respectively, compared to the other groups, and no RLP was recorded for G2. Fish in G4 had the lowest re-isolation rate 65%, whereas G5 and positive control had 100%.

Table 5. Mortality rate and relative protection level of the challenged the Nile tilapia

Item	Fish no.	Fish death	MR %	RLP %	No. re-isolated	Isolated%
NC	20	1	5	-	-	-
G1	20	12	60	-	20	100
G2	20	12	60	0	18	90
G3	20	7	35	41.67	14	70
G4	20	7	35	41.67	13	65
G5	20	10	50	16.67	20	100

Note: no, number; NC, negative control; G1, fed control diet; G2, fed dietary-active-MOS alternated with control on week basis; G3, fed dietary-active-MOS for two weeks alternated with control for one week; G4, fed on dietary-active-MOS three weeks followed free diet for one week; G5; fed on dietary-active-MOS 12 weeks.

DISCUSSION

Feeding practice is a fundamental stone of the fish industry that could determine gain or loss. With dietary-active-MOS (0.2%), the growth performance and feed utilization of the Nile tilapia were improved regardless of the period, especially with intermittent feed practice aligned with a week-free diet. Feeding two weeks supplemented diet interrupted with one-week free-diet (G3) had significantly enhanced FW, WG, DWG, RGR, and FCR compared to the control fish; values of 122g, 70.7g, 0.83g, 134.95 and 1.57; 79.33g, 26.7g, 50.75 and 2.1 were respectively recorded. Similarly, growth performance was improved when fed MOS at 0.4-0.6% for 3 weeks with respect to the Nile tilapia (**Samrongpan *et al.*, 2008; Selim & Reda, 2015; Sherif *et al.*, 2021b, c, d, 2022a**), common carp (*Cyprinus carpio*) fed 0.2% MOS for 30 days even under ammonia stress of 0.15mg/ L (**Mohammadian *et al.*, 2019; Xue *et al.*, 2022**), grass carp (*Ctenopharyngodon idella*) (**Lu *et al.*, 2020**). This was also evidenced in the case of the Caspian trout fish, salmon (*Trutta caspius*) (**Jami *et al.*, 2019**). In addition, the Japanese flounder fish (*Paralichthys olivaceus*) (**Ye *et al.*, 2011**) showed an improvement in the growth performance upon feeding the supplemented diet at different doses: 0.5% MOS for the Japanese flounder and 0.2% and 0.4% MOS for final weight gain. While, this supplementation decreased the liver lipid vacuolization in sea bass (**Torrecillas *et al.*, 2011**) and recorded the same decrease upon feeding a diet supplementation at a dose of 0.4% active-MOS for 46 days for the giant sturgeon juvenile (*Huso huso*) (**Razeghi Mansour *et al.*, 2012**) and a dose of 0.2% active-MOS for 70 days for the Atlantic salmon (**Refstie *et al.*, 2010**). Accordingly, low FCR (**Van Hai & Fotedar, 2009; Dimitroglou *et al.*, 2010**) and higher WG%, SGR% and PER (**Aramli *et al.*, 2015; Dash *et al.*, 2015**) were observed when immunostimulants such as MOS, β-glucan, and *L. plantarum* were individually used in fish and crustacean feeds. Different findings were observed by some authors; the growth performance of the Nile tilapia was not affected upon feeding for 45 days with MOS up to 1% (**Sado *et al.*, 2008**). In contrast, **Genc *et al.* (2007)** found that the hybrid tilapia (*Oreochromis niloticus×O. aureus*) fed on an excessive dietary MOS negatively impacted the growth rate; when supplemented with Bio-MOS at 0.4–1% for approximately 4 months, growth performance was not affected (**Grisdale-Helland *et al.*, 2008; Dimitroglou *et al.*, 2011**). On the contrary, the Nile tilapia, which received dietary-Bio-MOS up to 0.6% for 3 weeks showed low feed utilization (**Samrongpan *et al.*, 2008**). The same finding was evidenced in the case of the channel catfish fed 0.2% Bio-MOS for 4– 6 weeks (**Welker *et al.*, 2007; Peterson *et al.*, 2010**). In contrast, the FCR of juvenile western king prawns (*Penaeus latisulcatus*) did not alter by feeding dietary-symbiotic at 50:50 v/v (Bio-MOS and β-1, 3-D-glucan) and (*Pseudomonas synxantha* and *P. aeruginosa*; 105 CFU/mL), respectively (**Van Hai & Fotedar, 2009**). The responses of aquatic animals to dietary

MOS may differ according to fish species, fish age, environmental condition and feeding plan (**Xue *et al.,* 2022).**

In this study, the activity SAA, OBA, PA and PI of Nile tilapia fed on active-MOS in intermittent cycle showed a superiority, compared to fish of the other groups and the control. In accordance, dietary MOS could stimulate the innate immunity of *D. labrax,* and induce immune cells to migrate in the hind intestine (**Torrecillas *et al.,* 2015; Guerreiro *et al.,* 2018**). In this sense, *D. labrax* juveniles fed Bio-MOS up to 0.6% for 8 and 9 weeks presented a stimulated innate immunity in terms of enhanced plasma- and mucus-lysozyme activity, phagocytic index of head kidney leucocytes, and gut-associated lymphoid tissue (GALT) stimulation although no effect was detected on bactericidal activity of gut and skin mucus against *V. anguillarum* (**Torrecillas *et al.,* 2011).** On the contrary, no effect was found on the phagocytic index of head kidney leucocytes in adult *D. labrax* fed 0.3–0.5% Bio-MOS for 30 days, whereas Bio-MOS was an effective inducer of dicentracin (antimicrobial peptide) gene expression of cytokines in head kidney (**Terova *et al.,* 2009**). In the Japanese flounder, dietary Bio-MOS-supplementation at 0.5% for 8 weeks did not affect cellular and humoral immune (**Sun *et al.,* 2009**).

On the contrary, no alterations in their immune responses were recorded in Nile tthe ilapia, which received dietary-Bio-MOS up to 1% for 45 days (**Sado *et al.,* 2008).** Moreover, no change was detected in the channel catfish receiving dietary-Bio-MOS at a dose of 0.2% for six weeks (**Welker *et al.,* 2007; Peterson *et al.,* 2010; Welker *et al.,* 2012**). Limited studies showed that MOS supplementation could increase the phagocytic activity of leucocytes in the head kidney and spleen of *D. labrax* (**Torrecillas *et al.,* 2007).** On the other hand, dietary MOS had beneficial effects on the immune function of juvenile grass carp head-kidney and spleen mainly, depending on antibacterial compounds and antibacterial peptides (**Dong *et al.,* 2017**).

Our results concerning immune-related cytokines showed that *il-1β,* Hsp-70, and *tnf-α* were significantly higher in fish that received active-MOS for two or three weeks interrupted with a one-week free diet for twelve weeks compared to control fish. In this work, the Nile tilapia fed intermittent cycle (3-supplemented weeks and one-week free diet), SOD and CAT gene expression was significantly higher than other groups and the control. Similarly, concerning gene expression of cytokines, fish received high doses of dietary-prebiotic showed an up-regulation of some cytokine genes, such as *tnf-α* and COX-2, compared to un-supplemented diet (the control) (**Torrecillas *et al.,* 2015**). Accordingly, individual treatment of MOS and β-glucan, respectively, induced the highest expression of *tnf-α1* and *il-1β* in the head kidney of the Caspian trout fish (**Jami *et al.,* 2019**). Some different findings were observed in various research works concerning the administration of beneficial probiotics and/or prebiotics induced up-regulation (**Guzmán-Villanueva *et al.,* 2014; Schmitt *et al.,* 2015; Sherif *et al.,* 2020,**

2022b). In addition to the aforementioned item concerned, the down-regulation of inflammation-related genes **(Skov** *et al.,* **2012, Gracia** *et al.,* **2014)** was differently postulated**.** Indeed, gene expression of *il-1β* and *il-8* were high in the posterior intestine of Atlantic cod (*Gadus morhua*) receiving dietary-MOS at a dose of 0.1% for 5 weeks after challenging via immersion route with *V. anguillarum* **(Lokesh** *et al.,* **2012).** In contrast, dietary MOS 0.5% also down-regulated the gene expression level of inflammation factors in the liver **(Wang** *et al.,* **2022).** Differently, supplementation of dietary MOS could relieve inflammatory response in grass carp as the supplementation resulted in the up-regulation of anti-inflammatory cytokines with down-regulation of pro-inflammatory ones **(Lu** *et al.,* **2020).**

Our results indicate that intermittent supplementation of active-MOS to the Nile tilapia diet could decrease MR (35%) after challenging against *A. hydrophila* providing high RLP% (41.67). In accordance, active-MOS supplementations could induce the cellular innate immune system to play an important role in pathogen survival after translocation across the mucosal barrier, since similarly to that occurring in mammals, and in fish, translocated bacteria will be mainly neutralized by the local immune system in the lamina propria **(Jutfelt** *et al.,* **2008)**. This stimulation denotes a higher immune response in the posterior gut of fish fed MOS when challenged against a potential pathogen with higher levels of gut leucocytes infiltrated **(Torrecillas** *et al.,* **2014)**. Similarly, **Wang** *et al.* **(2022)** stated that, *D. labrax* received two treatments of low dietary-prebiotic or -symbiotic for 90 days and had low MR% after challenging with *V. anguillarum*, recording no differences between treatments. Accordingly, **Lu** *et al.* **(2020)** conducted a 60-day feeding trial in which growing grass carp received different levels of MOS (0, 0.2, 0.4, 0.6, 0.8, and 1 %), fish were challenged against *A. hydrophila,* the MR% was decreased with supplemented fish and they added that dietary-MOS could trigger the production of antibacterial compound, immunoglobulin (Ig), up-regulate the gene expression of antimicrobial peptides, and regulating the gene expression of cytokines.

CONCLUSION

From the obtained results, intermittent feed strategy dietary-MOS improved growth behavior compared to control and continuous feeding, concomitantly innate immunity and gene expression of immune-related cytokines were boosted with feeding for two or three weeks interrupted with a one-week free diet protecting against *A. hydrophila* infection.

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