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RESEARCH ARTICLE

Enhancement of Nile tilapia' Immune Cells Phagocytic Activity, Nitric Oxide Production, and Upregulation of Immunoglobulin M and Cytokines Gene Expression Post-Feeding on Moringa oleifera Leaf and Seed Mixture

Asmaa A. M.A. Nassar, Ahlam A. Gharib, Sarah Y. AbdElgalil, Ashraf M.O. Abdelwahab, and Gamal A. Elmowalid*
Microbiology Department, Faculty of Veterinary Medicine, Zagazig University 44511, Zagazig, Egypt

*Corresponding author E.mail: gaabedallah@Vet.ZU.edu.eg, gelmowalid@yahoo.com

ABSTRACT

Herbal products have been used in aquafeed as a dietary supplement. In the current study, the immunomodulatory efficacy of nonfermented (MO) and/or fermented (FMO) *Moringa oleifera* combined leaf and seed mixture as feed additives for Nile tilapia, *Oreochromis niloticus*, was evaluated in terms of phagocytic capacity, spleen immune-related cytokines, immunoglobulin M (IgM) gene expression, as well as nitric oxide production, after a 30-day feeding trial. To conduct the experiment, a total of 180 *O. niloticus* fingerlings were randomly divided into five groups (36 fish each, in triplicate); the control (1st group) which fed on a basal diet, 2nd group and 3 rd group were fed on MO 5% and MO 10%, 4th group and 5 th group were fed on FMO 5% and FMO 10%. The results demonstrated that MO10%-supplemented diets significantly ($P < 0.005$) reduced immune cells' phagocytic capacity, serum nitrite level, immune-related cytokines (IL-1β, TNF-α, IL-10), and IgM gene expression. Meanwhile, those fed on FMO10%, FMO5%, and MO5%-supplemented diets exhibited a significant ($P < 0.05$) improvement in the same tested parameters, with the greatest effects on FMO 10%. In conclusion, dietary supplementation with FMO especially at 10% level increased nonspecific and humoral immune parameters, and the outcome of this study recommends that FMO is a promising immune-stimulatory feed-additive candidate for Nile tilapia.

Keywords: Moringa oleifera, Immunostimulant, Nile tilapia, Phagocytic capacity, Nitric oxide, and Feed-additives.

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most intensively cultured fish species that could provide high-quality protein at an affordable cost. This industry is changing fast into intensification and recently was associated with resource shortages and elevation of fish meal costs, as well as other protein sources and increasing exposure to pathogens. Additionally, the risk of infectious disease outbreaks, along with some environmental hazards [1, 2]. Those factors may result in serious economic losses and zoonotic threats [3, 4].

Moringa oleifera (*M. oleifera)* is one of the most significant of economic importance that grows extensively around the world for its nutritional and therapeutic qualities [5]. *M. oleifera* is also utilized as a biofuel resource, human food, animal feed, and an antimicrobial agent [6]. It is considered one of the best-known medicinal plants, and it has been used in aquaculture as a dietary supplement as it is rich in macronutrients (carbohydrates, proteins, and lipids), micronutrients (vitamins and minerals), antioxidants, and bioactive compounds [7].

It was reported that Gilthead seabream (*Sparus aurata*) fish fed with varying amounts of *M. oleifera* leaves (MOL) showed improved systemic immunological function in terms of respiratory burst activity, peroxidase activity, and head kidney leucocyte phagocytosis. Furthermore, protease production, lysozyme activity, and serum immunoglobulin M (IgM) level were also increased, especially when moringa was included in the diet at 5% level. Additionally, feeding MOL resulted in an up-regulation of intestinal mucosal immunity genes (*lyso and c3*) and tight junction proteins (occludin and zo-1) [8]. In another study by Sanchooli *et al*.[9], it was observed that the gene expression level of head kidney immune-related cytokines, namely Interleukin-1β and tumor necrosis factor-α (IL-1β and TNF-1α) were significantly increased following *M. oleifera* leaf powder supplementation in rainbow trout (*Oncorhynchus mykiss*). Furthermore, the total mucus immunoglobulin and protein levels were also elevated in all *M. oleifera* supplemented groups particularly at 2.5 and 5% dietary inclusion levels. The immunostimulatory activity of *M. oleifera* seed extract was also assessed in Longfin yellowtail (*Seriola rivoliana*), and results showed an enhanced immune response in terms of respiratory burst activity, nitric oxide (NO) production, as well as, superoxide dismutase and catalase activities [10]. However, *M. oleifera* has anti-nutritional factors (ANFs), such as tannin and phytic acid, which severely affect the palatability and digestibility of the plant as well as disrupt some physiological functions in the treated fish [11]. Previous studies revealed that by increasing *M. oleifera* concentration in diets, the amounts of ANFs, such as tannins and phytate, were also increased. Adding moringa leaves at high levels elevated tannin from 0.5% to 2.0% and caused depression in growth of Bocourti's catfish (*Pangasius bocourti*); tannin was often lethal at levels above 5.0% [11]. High dietary phytic acid (2.5%) dramatically depressed the growth rate in salmon fish (*Oncorhynchus tshawytscha*) [12], and it has been reported that 0.5% to 0.6% can impair the growth of rainbow trout (*Salmo gairdneri*) [13], and common carp (*Cyprinus carpio L*.) [14]. The research into methods to reduce the toxic effects of the ANFs present in raw herbal plants has become necessary to safely utilize them as dietary stuffs. Fermentation studies have concentrated on establishing bioprocesses for biological detoxification of these toxic elements in order to eliminate or reduce them through fermentation, which has significantly improved the nutritional quality and bioavailability, as well as the bioactive composition of *M. oleifera* seeds [15]. A recent study also recommended multi-strain microbial fermentation of *M. oleifera* leaves, as it was found to improve its nutritional quality, making fermented moringa a functional feedstuff for use in livestock diets [16].

However, there is insufficient data about the efficacy of a fermented moringa leaf and seed mixture (FMO) on Nile tilapia immunity. Additionally, there were contradictory results and disadvantages to using leaf or seed alone at different doses as supplements in the different fish species. In our previous study, we reported that Nile tilapia-supplemented fermented *M. oleifera* were able to exhibit significant levels of phagocytic and lysozyme activities, as well as upregulation of cellular and humoral-related immune cytokines and protection against induced *Aeromonas hydrophila* infection (paper in process). In the current study, the efficacy of FMO and nonfermented *M. oleifera* (MO) leaf and seed mixtures, each of two different concentrations, was tested in a 30-day feeding trial on Nile tilapia, and the immunomodulatory activity of *M. oleifera* was evaluated through measurement of

different immune parameters, including immune cells' phagocytic capacity, nitric oxide production, spleen' immune-related cytokines, and IgM gene expression.

Materials and Methods

Preparation of the experimental diet

Moringa oleifera leaves and seeds were obtained from the Scientific Society of Moringa, Dokki, Giza, Egypt. The processing of *M. oleifera* leaves was done following the method of Mishra *et al*. [17], while the seeds were processed according to the method of Ijarotimi *et al*. [15], with minor modifications. The seeds were sorted, dehulled, and oven dried at 50°C for 10 h then it was ground using an electric grinder (Moulinex, Grenoble, France) and sieved through 60 mm sieve to obtain raw moringa seed powder. The seed powder was subsequently packed in plastic container and sealed with aluminum foil until it was used for diet formulation. Both *M. oleifera* leaf and seed powder were sterilized at 121°C for 20 min and cooled to room temperature. Thereafter, the powder fermentation was made by multi-strain microbial fermentation using probiotics (*Lactobacillus acidophilus*, *Bacillus subtilis*, and *Saccharomyces cerevisae*), obtained from the Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University. The strains were preserved in glycerol at -80°Cand revived by streaking samples of each culture on its corresponding media. *Lactobacillus acidophilus* was cultured in De Man-Rogosa-Sharpe (MRS) agar (Thermo Scientific™, USA) at 37ºC for 16 h using anaerobic jars containing Anaerocult® A gas packs. *Bacillus subtilis* was cultured in tryptic soy agar (TSA) with polymexin (Oxoid Australia Pty Limited ©) at 35ºC for 10 h. *Saccharomyces cerevisae* was inoculated in yeast extract-peptone-dextrose (YPD) broth containing conical tube, followed by overnight incubation in an orbital shaker at 30°C with agitation at 200 rpm. The grown yeast cells were then streaked on a petri dish containing 2% YPD agar (Sigma-Aldrich, USA) and incubated at 30°C until colonies showed growth. Culture suspensions of the three microbial strains were prepared and adjusted to a concentration of 10^8 CFU/mL with sterilized physiological saline solution. The three microbial strains were used at a ratio of 1:1:1 for the inoculation of moringa' leaf and seed powder. The optimized co-culture parameters were as follows: total inoculation size, 24%; temperature, 32 °C; fermentation time, 6 days; and initial water content, 60%. Fermentation of seeds and leaf was carried out separately by the method described by Shi *et al*. [16]. Following fermentation, fermented moringa leaf and seed samples were oven-dried at 60°C for 12 h. After that, oven-dried samples were further sieved, and then mixed at a ratio of 1:1 (w/w) in the form of fermented mixture (FMO). Raw powdered leaves and seeds were also mixed at a ratio of 1:1 (w/w) and referred to as non-fermented mixture (MO). Both MO and FMO were used for diet formulation, each in two different concentrations (5% and 10%).

Fish Management

The research was conducted experimentally using a completely randomized design of the fish groups in agreement with the standard procedures and policies approved by the Institutional Animal Care and Use Committee of Zagazig University in Egypt (approval number: ZU-IACUC/2/F/388/2023). A total of apparently healthy 180 *O. niloticus* fingerlings (30.3±0.5 g) were acquired from a local hatchery (Abbassa, Sharqiah, Egypt), and then they were transported alive to the Fish Research Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt, where the study experiment was conducted. Initially, the one hundred and eighty Nile tilapia fish were acclimatized for two weeks in

glass aquaria before starting the actual experiment, which lasted for 30 days. Each aquarium was filled with 60 liters of chlorine-free tap water. An aerator was used to provide air supply; the pH of the water was kept at 6.5–7.0; dissolved oxygen averaged 60.5 mg L^{-1}; ammonia averaged 0.01 mg L^{-1}; and nitrite was 0.20 mg L^{-1}. These parameters were measured routinely with a freshwater kit (La Motte®, Charlestown, MD, USA). Approximately 30% of the water was changed three times a week, and a constant water flow was maintained. During the acclimatization period, fish were fed a pelleted feed that was prepared to meet the basic nutrient requirements of Nile tilapia, according to the National Research Council (NRC) [18].

Experimental design

After the acclimatization period, the fish were randomly divided into five experimental groups (36 fish each, in triplicate), and then they were distributed in 15 aquaria. The experimental diets related to the five experimental groups were formulated and adjusted to meet the basic nutrient requirements of Nile tilapia, according to the NRC [18]. The five groups were designated as a control group which fed on a basal diet; MO5%, and MO10% were fed a basal diet containing nonfermented *M. oleifera* leaf and seed mixture at a concentration of 5% and 10%. In addition, FMO5% and FMO10% groups were fed on basal diets containing fermented *M. oleifera* leaf and seed mixture at a concentration of 5% and 10%. The approximate chemical composition of the experimental diets was presented in Supplement 1, which was determined following the protocols of the Association of Official Agricultural Chemists [19].

Samples Collection

Using sterile, disposable syringes rinsed with heparin, blood samples were drawn at random from the fish's caudal vein (4 samples per group) at the end of the feeding trial. The blood samples were divided into two main portions. One portion was transferred to tubes without an anticoagulant agent, followed by centrifuging at 3000 rpm for 10 min at 4°C, and then the serum (1st portion) was carefully separated, collected, and a portion was stored at − 80°C for analysis of nitrite production while the other serum portion was freshly used for the preparation of pooled homologous fish sera (used in phagocytosis assay). The other whole blood portion (2nd portion) was transferred to tubes containing the anticoagulant agent (EDTA-coated tubes) (Al-Nasr Pharmaceutical Chemicals Co., Egypt), and it was used to assess peripheral blood mononuclear cells (PBMCs)' phagocytic activity. For gene expression analysis, the spleen was randomly harvested from 3 fish per group after the 30-day feeding trial. The fish were euthanized by spinal cord severing, and the fish' spleen sample (0.5 gm) was immediately dissected and immersed in the TRIzol solution (Invitrogen, USA) for gene expression analysis of the immune-related cytokines.

Immunological parameters evaluation

1. Phagocytic capacity

The PBMCs' phagocytic activity of the fish in the different groups was evaluated post-feeding following the method described by Ainsworth and Chen [20]. The peripheral blood immune cells were isolated and their viability was determined by trypan blue exclusion assay [21]. *Candida albicans* yeast (Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University) was used as a phagocytosis microbial model and was prepared and heat inactivated according to the method described by Wood *et al*. [22]. The phagocytic activity was estimated in terms of phagocytic capacity which means the ability of the blood phagocyte to engulf more *C. albicans* cells [23].

2. NO evaluation

In this study, nitrite is used as an indicator of NO according to the method of Montgomery and Dymock [24]. The colorimetric method depends on the addition of Griess reagents [1% sulphanilamide, 0.1% 2-(1-Naphthylamino) ethylamine dihydrochloride (NEDA), and 2.5% hydrochloric acid] (Sigma-Aldrich, USA) to the tested serum sample, followed by incubation at 27ºC for 10 minutes. Nitrite in the serum sample was coupled with sulphanilamide in an acidic medium, and the formed nitrous acid di-azotise sulphanilamide was further coupled with NEDA, resulting in azo dye that had a bright reddish-purple color. The color intensity was spectrophotometrically measured (BM Co. 5010, Germany) at 540 nm, which was directly proportional to the nitrite concentration in the serum. The molar concentration of nitrite in the samples was determined from a standard curve generated using known concentrations of sodium nitrite (20–200 µM).

3. Immune-related cytokines and IgM mRNA expressions by Real-Time PCR (RT-qPCR)

The spleen samples were randomly collected from three fish per experimental group, then cleaned in cold PBS buffer (pH 7.2). The primer sequences of the selected immune cytokines (IL-1β, TNF-α, and IL-10) and IgM with the *β-actin* reference gene were designed according to NCBI Accession No. presented in Table 1. Total RNA was extracted from splenic tissues using the easy-RED kit (iNtRON Biotechnology), according to the manufacturer's instructions. The QuantiTect Reverse Transcription Kit (Qiagen, Germany) was used in accordance with the manufacturer's instructions to produce cDNA from a total of 1.0 µg of RNA, and cycling was done using an Applied Biosystems® 7500 Real-Time PCR (Thermo Fisher Scientific, USA). All gene tests were done in duplicate. PCR amplification was performed under standard conditions. As the housekeeping gene, *β-actin* was used as an internal control gene to normalize the expression of the tested target genes. After RT-qPCR and obtaining the threshold cycle (Ct) values of each sample, the relative mRNA expression levels of the tested cytokines and IgM genes were calculated using the $2^{-\Delta\Delta CT}$ method [25]. The actual results of each experimental group were compared to the control and expressed as fold changes, along with the corresponding values of the control group.

Table (1): Designated primer sequences and target genes for SYBR green RT-qPCR

Target gene	Primer sequence	Molecular size (bp)	NCBI reference sequence Accession no.
β- actin	F. GCAGGAGTACGATGAGTCCG	157	XM_003443127.5
	R. CTCTGCGCCTGAGTGTGTA		
TNF-α	F. CTGCTCCCTTCCACTCCTTG	74	XM_013266975.3
	R. CCGCTATCTGTGAGAGGCTG		
IL-1β	F. TCTTAGCGCTCCACTCCTTG	91	XM_019365844.2
	R. CTGCCTGACTGTCCTGACTC		
IL-10	F. ATGAGCAGAAGGCCTGTCAC	84	XM_013269189.3
	R. GCTCCCCAAATAGCCACACT		

IgM	F. GCGACAGTCACAGTTCCTCA	131	KC708223.1
	R. TGCTGCTACCATCACTGCAA		

F; forward, R; reverse; bp; base pair

Statistical analysis

The data analysis was performed using the SPSS software statistical package (SPSS version 17.0). Mean ± standard error (SE) was calculated for the data [26]. A one-way analysis of variance (ANOVA) was applied to detect statistically significant differences ($P < 0.05$) among experimental and control groups. Differences between means were assessed by Duncan's multiple range tests [27].

Results

Phagocytic cells' capacity

Phagocytosis is considered one of the most effective innate cellular barriers against the invasion of microorganisms and is utilized as an indicator of an optimum innate immune response. In the current study, the increase in immune cells' phagocytic capacity was higher ($P < 0.05$) in fish groups supplemented with FMO10%, FMO 5%, and MO 5%, respectively. Meanwhile, fish that were fed on MO10% exhibited depressed phagocytic capacity for a small number of *C. albicans* cells compared to the control, as shown in Figure 1.

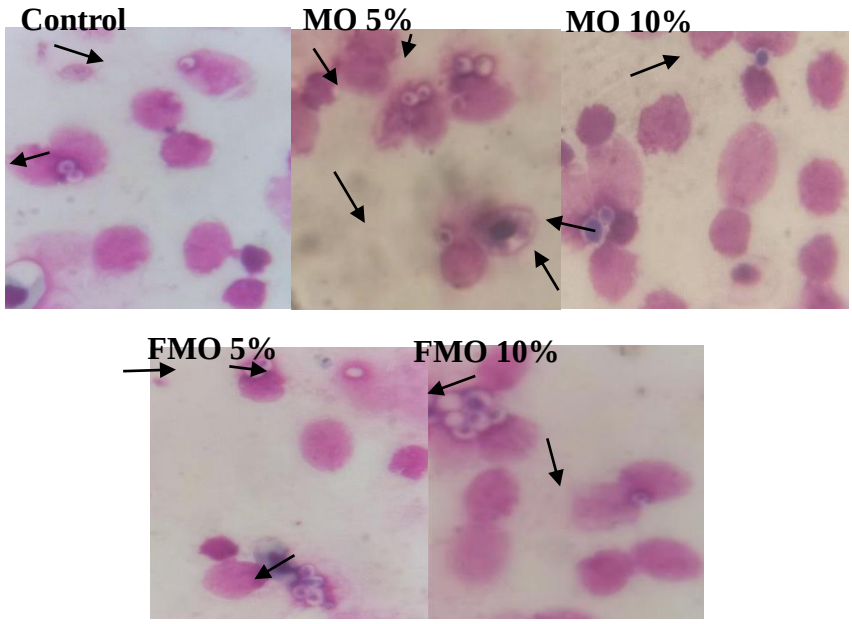

Figure (1): PBMC's cell phagocytic capacity in the different Nile tilapia groups post- *M. oleifera* dietary supplementation.

Professional phagocytic cells possess a wide variety of inducible cytotoxic molecules that are extremely capable of destroying invasive pathogens. NO production is one of the most significant antimicrobial mechanisms developed by fish phagocytes [28]. Our findings, as shown in Figure 2, indicated that

FMO10% supplementation markedly ($P < 0.05$) increased NO production, followed by FMO5% and MO5%. However, the lowest level of NO was detected in fish supplemented with MO10%, which was even lower than the control group (Figure 2)

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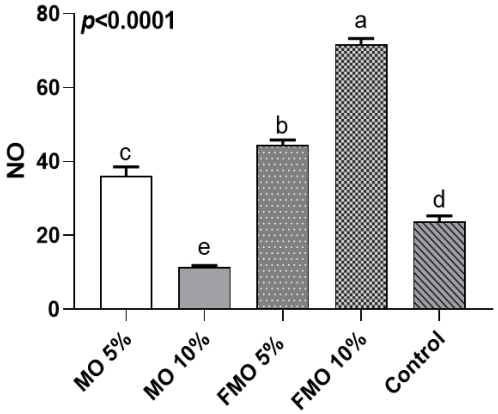

Figure (2): Nitrite production as an indicator of nitric oxide (NO) in the different fish groups fed on *M. oleifera* and the control. The values shown are means ± SE (n=4samples/group). Bars with different letters significantly differ from one another ($p < 0.05$).

Immune-related gene relative expression post M. oleifera supplementation

Cytokines are the most effective immune mediators, as they are involved in cell-to-cell communication and immune response regulation. The relative fold change of pro-inflammatory cytokines, I IL-1β and TNF-α, anti-inflammatory cytokines (IL-10), and IgM in the different experimental groups post-*M. oleifera* feeding are presented in Figure 3. The levels of IL-1β, TNF-α, and IL-10 were significantly ($P < 0.05$) higher in the fish groups fed diets supplemented with FMO10%, followed by FMO5%, and MO5%, respectively, compared to those in the control and MO10% groups. The MO 10%-supplemented fish exhibited a significant reduction ($P < 0.05$) in the splenic expression of these cytokines, and the expression levels were lower than those of the control (Figure 3). Regarding the IgM mRNA expression, the highest level was expressed in the FMO10% treated group, while the lowest expression level was detected in the MO10% group compared to the control group.

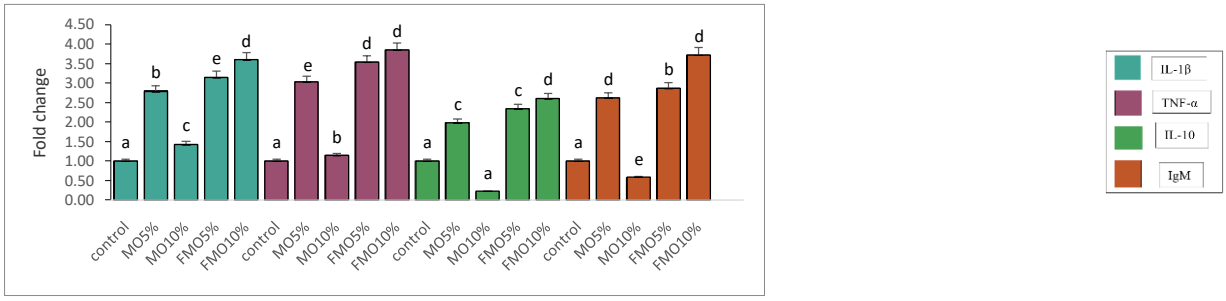

Figure (3): The relative mRNA expression level (3 fish/group) of Nile tilapia fed fermented and non-fermented *moringa* leaf and seed powder at 0, 5, and 10% dietary inclusion levels, post the 30-day-feeding trial. The values shown are means ± SE (n=3), ($p < 0.05$).

Discussion

Antibiotics were administered to control microbial infection and as growth promoters, but this was related to the emergency of antibiotic-resistant strains and the accumulation of antibiotic residues in the tissue [29]. So, the search for a new alternative that could be safely incorporated into the diets of aquatic animals has become necessary. The current study aimed to highlight the immunomodulatory potential of MO and FMO, each in two different concentrations.

Phagocytic cells are responsible for trapping invading pathogens and communicating innate and acquired responses. Our findings revealed that dietary supplementation of FMO especially at a concentration of 10% (FMO 10%) markedly enhanced phagocytic cell capacity to engulf more *C. albicans* cells compared to control and MO groups. The increase in cell phagocytic capacity indicates the improvement of Nile tilapia innate immunity, as reported by Helmiati *et al*. [30], who found that moringa dietary supplementation increases leucocyte differentiation into functionally effective phagocytes. The immunomodulatory activity, as evidenced by the phagocytic-enhancing activity, could be attributed to dietary alkaloids found in *M. oleifera* leaf and seed mixture, which can enhance humoral and cellular immune responses by stimulating the phagocytic activity of macrophages with subsequent stimulation of respiratory burst activity and the production of NO in an oxygen-dependent manner in microbial killing [31]. It may also be correlated with the richness of *M. oleifera*, with several phytochemicals such as polyphenols, volatile oils, vitamins (E, A, and C), phenolic acids, and flavonoids, which exist at higher concentrations in the fermented moringa, as reported in previous studies [15, 32, 33]. Fermentation also results in an increase in isoflavones, which are disease-protective components, and total flavonoid aglycones that become more easily and rapidly absorbed in the intestine after enzymatic digestion and bacterial fermentation [34, 35]. Furthermore, it was proven that the content of crude protein, small peptides, and total amino acids was significantly increased post-multi-strain fermentation of *M. oleifera*, whereas the contents of crude fiber, tannin, and phytic acid (anti-nutritional factors) were significantly decreased, suggesting an improved nutritional quality of fermented vs. nonfermented moringa [16]. The increase in the concentration and bioavailability of the essential nutrients as well as the bioactive phytochemicals, in addition to the decrease in the content of the anti-nutritional factors; furthermore, the presence of antimicrobial plant-derived peptides post-enzymatic hydrolysis and multi-strain microbial fermentation of *M. oleifera* [36], could explain the significant improvement in the immune response and disease resistance observed in fish supplemented with FMO. In this study, the efficacy of MO 5% and MO10% dietary inclusions were studied on Nile tilapia's phagocyte activity under normal conditions. Results showed that higher *M. oleifera* concentrations were found to decrease phagocytic activity and other immune-related molecules [37]. This confirms our results, which revealed that increased levels of nonfermented *M. oleifera* beyond the 5% level can have a negative effect on the overall health status, producing an immunosuppressive effect on the host, as observed in the MO10% treated group. Our findings also agreed with those reported by Abidin *et al*. [38], who found that shrimp (*Penaeus vannamei*) fed moringa extract incorporated at 2.5 g/kg showed the highest phagocytic activity compared to those received moringa at a concentration of 5.0 g/kg. This could be attributed to the effect of the ANFs present in raw moringa

powder, especially when moringa was included at higher concentrations. Oxalates are one of these ANFs that was previously reported to exert an immunosuppressive effect on monocytes via impairment of their metabolism and chemoattractant protein (MCP-1) secretion, which is responsible for the recruitment of monocytes and macrophages to tissues in order to remove pathogens that could be a strong potential cause of the resultant immune suppression [39].

NO is mainly a product of macrophages, and its release is considered an indicator of immune activation. In the current study, a significant improvement in NO production was observed following FMO supplementation, followed by MO5%, respectively. On the contrary, feeding MO10% has significantly reduced NO production. This could be explained by the previously reported inhibitory effect of *M. oleifera* on arginase activity and its subsequent stimulatory effect on NO biosynthesis, as an elevated level of arginase was found to antagonize with NO synthase activity and *in-vivo* production of NO [40].

Inflammation is a defensive indigenous reaction against microbial invasion and tissue injury that aids in clearing harmful stimuli. Pro-inflammatory cytokines and acute-phase proteins mediate the inflammatory process along with anti-inflammatory cytokines, which act in synchrony to prevent further tissue damage and eventually lead to healing and restoration of normal tissue function [41]. The TNF-α and IL-1β are considered the most important pro-inflammatory cytokines. In this study, TNF-α, IL-1β, IL1-0, and IgM relative mRNA expression values were significantly up-regulated in Nile tilapia fed on FMO5%, FMO10%, and MO5% compared to the control group. MO10%-supplemented fish exhibited a modest upregulation of the pro-inflammatory cytokines with a correlated downregulation of the anti-inflammatory cytokine (IL-10) compared to the control group. The upregulation of the pro-inflammatory cytokines, this could be attributed to the high splenic infiltration of lymphocytes, which were further increased in number following *M. oleifera* supplementation [42]. Moreover, the high isothiocyanate content in moringa leaves [43], could be responsible for this up-regulatory effect, as isothiocyanates were found to induce a pro-inflammatory response in some species, which is mediated via Th1 activation [44]. This pro-inflammatory response may also be related to *M. oleifera*-based polysaccharides like MOP-1 and MOP-2, which were previously reported to have immuno-regulatory activity via activation of macrophages and secretion of various pro-inflammatory molecules, such as NO and TNF-α. These polysaccharides were further increased following fermentation [32], which may explain the higher mRNA expression values of these cytokines in FMO groups compared to MO and/or control groups. Interestingly, *M. oleifera* has also stimulated IL-10 release, which was significantly upregulated in FMO10%, FMO5%, and MO5%, respectively. The IL-10 not only functions as an anti-inflammatory cytokine that regulates the inflammatory response and prevents immunopathology in response to inflammation, but also participates in potentiation of the humoral immune response via enhancement of IgM production in Nile-tilapia, as explained by Wu *et al.*[45], which explains the correlated up-regulation of IgM gene expression along with IL-10 in the afore-mentioned groups. The upregulation of IgM expression values may be attributed to the stimulatory effects of IL-10 on B lymphocytes and their differentiation to plasma cells with subsequent stimulation of antibody production [46], or the improvement of B cell survival rates [47, 48]. Our results

were consistent with those of Zhang *et al*. [32], and Abd-Elaziz *et al*. [49], who revealed that IgM levels were significantly increased in fermented and non-fermented *M. oleifera*-treated groups, particularly in groups supplemented with 5% moringa leaf powder.

Conclusion and recommendation

In conclusion, this study confirmed that the immune-stimulatory activity of *M. oleifera* is strictly dependent on the formulation and concentration of the herbal plant in the diet, as the multi-strain microbially fermented *M. oleifera* (FMO) showed a potent immunostimulatory impact on Nile tilapia in terms of phagocytic capacity, NO production, and up-regulation of immune-related cytokines and IgM genes, with the greatest effects at MFO10%. These results suggest that fermented *M. oleifera* can be utilized as a feed additive for aquaculture to enhance the immune responses of fish.

Conflict of interest

None of the authors has any conflict to declare.

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الملخص العربى

إستخدام المورينجا المتخمرة لتنشيط القدرة البلعمية للخلايا المناعية وإنتاج اوكسيد النيتريك و رفع مستوي التعبير الجيني للستوكينات والأجسام المناعية المضادة في أسماك البلطي النيلي

أسماء أحمد محمد أحمد نصار ، أحلام عبد العزيز ، ساره يوسف عبد الجليل ، أشرف محمود عبد الوهاب عمر و جمال عبد المنعم محمد المولد

قسم الميكروبيولوجي – كلية الطب البيطرى – جامعة الزقازيق44511- مصر

تم استخدام المنتجات العشبية في العلائق الغذائية للبحريات و من هذا المنطلق تم تصميم هذه الدراسة لإستبيان الأثر التحفيزي المناعي لإستخدام خليط أوراق و جذور المورينجا بتركيزين 5% او 10% في صوره خام أو بعد تخميرها كإضافات علائق غذائية في أسماك البلطي النيلي ولتحقيق هذا الهدف تم تقسيم 180 زريعة سمك بلطي نيلي عشوائيا إلي خمسة مجموعات تحتوي كل منها علي عدد 36 سمكه وبعد ذلك تم تغذية الأسماك في كل مجموعة علي واحة من خمسة علائق مختلفة علي النحو التالي: عليقه أساسية (ضابط) عليقه محتوية علي مورينجا خام بتركيز 5% أو مورينجا خام بتركيز 10% أو مورينجا متخمره بتركيز 5% أو 10% و ذلك لفتره 30 يوم تم تجميع عينات دم لتقييم قدرة البلعمة للخلايا المناعية علي إبتلاع الميكروبات و سيرم لقياس منسوب إنتاج أوكسيد النيتريك وعينات من الطحال وذلك لتقييم التغير في التعبير الجيني للسيتوكينات المناعية و الأجسام المضادة ولقد أظهرت النتائج أن الأسماك التي تم تغذيتها علي خليط أوراق و جذور المورينجا الخام بتركيز 10% قد أدي إلي تراجع معنوي ($P < 0.05$)في قدره الخلايا علي الإلتهام وإنتاج أوكسيد النيتريك و كذلك القدرة علي إفراز السيتوكينات المناعية والأجسام المناعية المضادة علي النقيض أظهرت نتائج الأسماك التى تم تغذيتها علي وجبات مدعمه بأوراق و جذور المورينجا المتخمرة بأي تركيز مستخدم علي إرتفاع معنوي في المعايير المناعية تحت الدراسة و بناءا عليه يوصي ا باستخدام المورينجا المتخمرة كمنشط مناعي في اسماك البلطي النيلي.