

## Article

# Exogenous $\beta$ -Propeller Phytase and Prebiotic Mannan Oligosaccharide (MOS) Supplementation of Formulated Diets Applied to Juvenile Nile Tilapia, *Oreochromis niloticus*: Impact on Growth Performance and Nutrient Digestibility

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**Abstract:** The growth of the aquafeed sector is highly dependent upon the availability of fish feed with a balanced nutritional composition. The use of prebiotics and probiotics can be an effective solution to increase the bioavailability of feed components. In this study, we evaluated the effect of dietary supplementation with  $\beta$ -propeller phytase (0, 600, 1200, 1800 and 2400 U/kg) from *Bacillus* and mannan oligosaccharide (MOS) (0, 2, 4 and 8 g/kg) on growth performance and nutrient digestibility of Nile tilapia over 45 days. The findings showed that adding phytase significantly ( $p < 0.05$ ) increases the growth performance and nutrient digestibility; the 1200 and 1800 U/kg PHY levels showed the maximum weight gain (WG), specific growth rate (SGR) and protein efficiency ratio (PER) and best feed conversion ratio (FCR). Furthermore, phytase increases carcass mineral composition (phosphate and calcium). At the end of the experiment, there were no significant differences among all feeding groups in survival rates (above 90%). Regarding MOS inclusion, insignificant differences were seen in WG, SGR and SR. However, significant effects of MOS were observed on FCR, feed intake (FI) and PER when supplemented at 4 and 8 g/kg of feed. Taken together, our results suggest that supplementation of Nile tilapia feed with adequate amounts of  $\beta$ -propeller phytase from *Bacillus* and MOS increases growth performance and nutrient digestibility.

**Keywords:** Nile tilapia; feed additives; phytase; mannan oligosaccharide; growth performance; digestibility

**Key Contribution:** This study presents the effects of exogenous  $\beta$ -propeller phytase and prebiotic mannan oligosaccharide (MOS) supplemented in diets on the growth performance and nutrient digestibility of Nile Tilapia, *Oreochromis niloticus*, and the findings may benefit the application of these two additives.

## 1. Introduction

Aquaculture represents one of the fastest-increasing industries in terms of food production, producing more than half of the seafood consumed worldwide [1]. However, this expansion is entirely dependent upon the development of aquafeed products [2]. Over

the past few decades, there has been a tendency toward substituting readily available plant-based ingredients for fish meal, which is largely dependent on fisheries as the main protein ingredient, to meet the growing demand for feeds while ensuring the sustainability of aquaculture expansion [3–5]. Now, more than ever, feed formulations incorporate components of a plant-based origin. However, currently and commonly utilized ingredients of plant-based origin including soybean, rapeseed and sunflower meals contain a variety of antinutritional elements that reduce fish performance [5,6]. Antinutritional factors such as fibers, protease inhibitors, and phytic acid have been found to have a negative impact on nutrient digestibility and mineral absorption, decreasing the effectiveness of nutrient utilization and fish growth [6–9]. Hence, it is critical to reduce the antinutritional effects of plant-based substances to effectively use them in fish diets.

One of these antinutrients is phytate, which can be found in plant-based ingredients that are frequently utilized as feed components in aquafeeds. This biomolecule represents a form of phosphorus (P) storage, and it is not bioavailable or hardly assimilated by most fish species because of their lack of sufficient intestinal phytase enzymes [8,10,11]. Moreover, phytate is a relatively heat-stable molecule that binds to vitamins, minerals and proteins, decreasing their absorption and use [12–14]. In fact, the growth performance of fish is hardly impacted by the detrimental antinutritional effects of phytate [15]. So, fish that are fed diets based on plant ingredients need to be supplemented with inorganic P to meet their P nutritional requirement as this mineral is involved in diverse biological processes [16]. However, the addition of non-renewable mineral P is a costly affair and contributes to environmental pollution. Therefore, the breakdown of phytate–P complexes contained in diets based on plant byproducts is required because of the above-mentioned negative effects. Phytase can lower the dependency and the need for inorganic P supplementation in aquaculture, which is disadvantageous for both animals and the environment [17–19]. In numerous fish species, the use of phytase as a feed supplement has been shown to promote digestion and growth [10–12,15,16].

In the aquafeed sector, the idea of functional feed additives, like probiotics and prebiotics, has particularly received attention. The improvement of growth performance, high nutrient protein digestibility, high digestive enzyme activities and immunostimulation are a few advantages of these supplements [20,21]. Prebiotics, indigestible feed components that promote the activity and proliferation of helpful bacteria in the gastrointestinal system of the host, can be used as a natural preventive supplement in place of chemotherapy, and among the most popular prebiotics utilized in aquaculture are mannan oligosaccharide (MOS) and  $\beta$ -glucan. The incorporation of MOS in various fish species diets was conducive to improved growth performance and nutrient digestibility efficiency [22,23], enhancement of the immune system [21,24,25], amelioration of antioxidative parameters [26] and resistance ability against disease and farming stressors [21,27]. The key factors affecting MOS effectiveness are the fish species, its size, the period of feeding, the supplementation amount and the circumstances of farming [28].

Nile tilapia ranks third among all cultured fish species, with a total volume of production of 8.3% [1]. The omnivorous dietary habits of tilapia, as well as the possibility of gastrointestinal tract fermentation, promote the growth of tilapia farming. In Tunisia, the optimization of tilapia farming has been successfully conducted and developed in geothermal water resources. For the sustainable development of this species, efforts were focused on the optimization of nutritional requirements, selection of proper diets and formulation of efficient dietary supplements [25,29–32]. Despite the beneficial effects of dietary supplementation with phytase and prebiotics in Nile tilapia, there is still scarce knowledge about their effects on growth performance and nutrient digestibility. Therefore, the present study was designed to investigate the impacts of different concentrations of phytase and MOS on growth parameters, nutrient digestibility, and feed conversion efficiency of Nile tilapia subjected to intensive rearing conditions.

## 2. Materials and Methods

This research work using Nile tilapia as an animal model was conducted in conformity with the recommendations of the local ethics committee (IACUC) and approved by the Pasteur Institute of Tunis, Tunisia, under registration number IRB00005445, FWA00010074.

### 2.1. Preparation of Diets

The  $\beta$ -propeller phytase from the *Bacillus* genus (PHY US 417) used during this study was provided by the Laboratory of Microbial Biotechnology, Enzymatic and Biomolecules at the Biotechnology Center of Sfax—Tunisia. The origin of this bacterial enzyme is *Bacillus subtilis* US417 [33]. To prepare experimental diets containing prebiotics, different levels of commercial MOS were incorporated into a powdered basal diet.

Based on the proximate composition of the different ingredients and the nutritional requirements of Nile tilapia [34], isonitrogenous and isolipidic experimental diets were formulated according to NRC [35] and Furuya et al. [36]. The ingredients used in the feeding trial and their rate of incorporation in the experimental regimes are shown in Table 1. Fish meal (Fm) and soybean meal (SBM) represent the source of proteins.

**Table 1.** Ingredients and proximate composition of the experimental diets (% dry matter).

	Experimental Diets							
	Control	PHY1	PHY2	PHY3	PHY4	MOS2	MOS4	MOS8
Phytase (U/kg)	0	600	1200	1800	2400	0	0	0
MOS (g/kg)	0	0	0	0	0	2	4	8
Ingredients (%)								
Fish meal	20	20	20	20	20	20	20	20
Soybean meal	55	55	55	55	55	55	55	55
Maize meal	18	18	18	18	18	18	18	18
Soybean oil	5	5	5	5	5	5	5	5
CMV <sup>a</sup>	2	2	2	2	2	2	2	2
Chromic oxide <sup>b</sup>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate analysis (% Dry matter)								
Moisture	9.56	8.22	8.15	8.20	8.08	8.13	8.21	8.18
Crude protein	34.88	35.04	33.94	33.95	33.24	33.97	33.81	33.85
Crude lipids	9.44	9.78	9.33	9.60	8.40	9.25	9.33	9.45
Ash	11.36	11.96	12.08	11.41	11.82	12.01	11.94	11.83
NFE <sup>c</sup>	34.75	35.00	36.49	36.83	37.47	37.13	36.95	36.73
Gross energy (KJ/g)	17.58	17.79	17.62	17.79	17.28	17.38	17.48	17.81

<sup>a</sup> Vitamin premix and mineral premix were described by Azaza et al. [7,32]. <sup>b</sup> Cr<sub>2</sub>O<sub>3</sub>; inert marker, used only for digestibility trial. <sup>c</sup> N-free extract calculated by difference = 100 – (crude protein + crude lipid + crude fiber + ash).

The previously established method was used to prepare trial diets. To prepare the experimental diets, all ingredients were ground (Ultra Centrifugal Mill ZM 200 Retsch GmbH, Haan, Germany) and then sieved (0.25 mm). After that, the dry powder of ingredients for every diet was perfectly mixed with dissolved vitamin–mineral premix and vegetable oil by using a food mixer (CAM A30, Retsch GmbH, Haan, Germany). Each supplemented diet was mixed progressively with distilled water in the amount of 40 g/100 g of diet to achieve a homogeneous dough. Phytase was later added to the test diets as defined by Cao et al. [37]. The enzyme was incorporated at levels of 0, 600, 1200, 1800 and 2400 UF/kg (diets PHY-0, PHY-1, PHY-2, PHY-3 and PHY-4 respectively) according to previous studies [36,38]. For the MOS trial, we incorporated the prebiotic in the diet at 0, 2, 4 and 8 g/Kg of diet (diets MOS0, MOS2, MOS4 and MOS8 respectively).

The suitable dough was then passed along to a pellet maker (model: amb TC22SL, Oni2, Bruxelles, Belgium) to produce identical pellets of about 2.5 mm in diameter. After extrusion, the pellets were air dried, stored in a freezer at  $-20$  °C to avoid oxidation and

bagged in marked plastic bags until further use. At the fish farm and before feeding, the dried diets were fragmented, and feed particle sizes were adjusted according to fish size as stated in the model developed by Azaza et al. [39]. During the experimental period, the supplementation of the feeding regimes with prebiotics and phytase was produced as required every two weeks.

## 2.2. Fish and Experimental Conditions

The two independent growth experiments were conducted at the Tunisian Research Station of the National Institute of Marine Sciences and Technologies (INSTM). The experimental diets were tested on *Oreochromis niloticus* juveniles, with an initial average weight of 2 g. The fish were weighed individually and randomly distributed in 27 cylindrical fiberglass tanks of 120 L useful volume, 30 fish per tank, thus forming nine treatments in triplicate (five phytase treatments and four MOS treatments), each corresponding to a dietary treatment. The fish were maintained in the tanks 10 days before the start of the experiment to acclimatize them to the new conditions. As previously mentioned by Azaza et al. [32], each tank was a component of an open recirculation system. The tanks were supplied with geothermal water at  $28 \pm 1$  °C and at a flow rate of 4 to 6 L/min, ensuring an oxygen level above 80% saturation.

The fish were manually fed to satiety with the experimental diets, four meals daily (8 a.m., 11 a.m., 2 p.m. and 5 p.m.), for 45 days. When the first feed refusal was noticed, the animal was considered to be satiated. Every 15 days, the fish were weighed, and the tanks were rotated to avoid the tank effect.

Every morning and before the first feeding, we siphoned the bottom of the tanks to ensure a certain level of cleanliness and hygiene in the experimental tanks. During the experiment, the quantity of ingested food was recorded daily to calculate the total feed intake (FI). The physicochemical parameters (pH, O<sub>2</sub>, T) were monitored daily with a modular multi-parameter measurement system (WTW, MIQ-C184, (Niles, IL, USA); accuracy of 0.1 °C and 0.1 mg O<sub>2</sub>/L).

## 2.3. Digestibility Trial and Chemical Analyses

The diets used in the digestibility assessment were processed to contain a nutritional composition similar to that given to fish throughout the development experiment. The apparent digestibility coefficients (ADCs) were assessed using an indirect technique in a separate trial of the experimental diets containing 40 to 50 fish/tank. Chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) was included in all trial diets in the role of an inert digestibility indicator (0.5% of dry weight). During a ten-day adaptation period, fish were fed the corresponding experimental diets prior to any fecal specimens being harvested. This ensured fish adaptation to diets supplemented with chromic oxide. After this period, 10 fish from every tank were manipulated and killed with a high dose (200 mg/L) of tricaine methane sulfonate. The dissection procedure presented by Fernandez et al. [40] was used to collect and prepare fish feces for chemical analysis as previously described in [7,32]. In brief, after 8 h of fish feeding, feces samples were collected from the posterior region of the intestine and then centrifuged for 15 min at 4000 rpm. The product was lyophilized, thinly ground with an ultrafine grinder and stored in a freezer at  $-20$  °C until further analysis.

The apparent digestibility (ADC) was determined for dry matter, protein, fat and carbohydrates according to the method described by Furukawa and Tsukahara [41]. The ADCs were calculated using the standard formulas:

$$\text{ADC Dry matter (\%)} = 100 [(1 - (Dy/Fy))]$$

$$\text{ADC}_i = 100 [1 - (Fi/Di) (Dy/Fy)]$$

Fy and Dy represent the proportions of chromic oxide in the feces and diet, respectively, and Fi and Di represent the percentages of nutrients in the feces and diet, respectively. The biochemical composition of the experimental diets and feces samples was determined in

triplicate using the AOAC procedures [42]. Dry matter (DM) was established after 6 h of oven drying at 105 °C. The Kjeldahl apparatus was used to quantify the crude protein content after acid digestion (calculated as %N × 6.25), and crude fat was quantified by ether extraction using the Soxhlet method. Ash content was established by ignition (muffle furnace) of the samples at 550 °C for 6 h. For total carbohydrate (as NFE) calculation, the following formula was used:  $NFE = 100 - (\% \text{ protein} + \% \text{ lipids} + \% \text{ ash} + \% \text{ fiber})$ . Gross energy was assessed using the following conversion factors: protein: 23.6 MJ/kg; fat: 39.5 MJ/kg; carbohydrates: 17.2 MJ/kg [43]. As stated by Morais et al. [44], the amount of digestible energy was calculated using the following conversion factors: 17.2 MJ/kg for carbohydrates, 23.6 MJ/kg for protein and 39.5 MJ/kg for fat (average nutritional digestibility values: for protein, 90%; for fat, 85%; for carbohydrates, 50%).

#### 2.4. Calculations and Statistical Analyses

Different formulas for the growth parameters calculation were used.

- Specific growth rate (SGR) was calculated as follows:  
 $SGR (\% M \text{ day}^{-1}) = 100 (\ln M_f - \ln M_i) / (t_x - t_1)$ , where  $\ln$  is loge and  $M_x$  and  $M_i$  are the mean body masses of fish at times  $t_x$  and  $t_1$ , respectively.
- Feed conversion ratio (FCR) was calculated as follows:  
 $FCR (g/g) = FI / (B_f - B_i + M_d)$ , where  $B_i$  and  $B_f$  are the total body masses (g) of fish at the start and end of the experiment,  $M_d$  is the biomass of fish dying throughout the experiment and  $FI$  (g) is the quantity of distributed feed during the rearing period.
- Protein efficiency ratio (PER) was calculated as follows:  
 $PER = (\text{wet mass gain, g}) / (\text{protein intake, g})$ .

The results were analyzed for normal distribution using the Shapiro–Wilk test and for homogeneity of variance using Barlett’s test [45,46]. Arcsine transformations of percentage data and log transformations for other data were performed to achieve homogeneity of variance. Data that were normal and homogeneous were analyzed by one-way ANOVA using Statistica® version 5.1 software (Statsoft Inc., Tulsa, OK, USA). When significant differences were detected, Duncan’s multiple-range test (DMRT) was used for post hoc analysis. All differences were significant at a probability level of 0.05. Values are expressed as mean ± error standard of means of three replicates (ESM;  $n = 3$ ).

### 3. Results

#### 3.1. Phytase Incorporation

##### 3.1.1. Survival, Feed Intake, Growth Performance and Feed Utilization

The average values of the whole growth performance (WG (g), WG (%), FCR, SGR, FI) and survival rate of *O. niloticus* feeding on PHY test diets are presented in Table 2. The findings showed that adding PHY to the experimental diet significantly ( $p < 0.05$ ) increased the growth parameters compared to the control diet (PHY-0). According to our results, *O. niloticus* that were given a PHY-based diet at levels of 1200 and 1800 U/kg (PHY-2 and PHY-3, respectively) showed maximum weight gain, DWG and SGR. At PHY doses of 1200, 1800 and 2400 U/kg, we found the lowest conversion of feed into flesh compared to other groups ( $p < 0.05$ ). However, the growth indices observed in the fish fed PHY-2, PHY-3 and PHY-4 test diets were not significantly different among the mentioned fish groups. Pertaining to the possible effect on survival, we found that the inclusion of PHY in the diets of Nile tilapia had no impact on the study’s survival rate (Table 2).

**Table 2.** Growth performance, feed utilization efficiency and biological parameters of juvenile Nile tilapia fed experimental diets supplemented with phytase. Each value is a mean  $\pm$  SEM derived from three replicates ( $n = 3$  tanks per diet) <sup>a</sup>.

Variable	Experimental Diets (U/kg)				
	PHY-0	PHY-1	PHY-2	PHY-3	PHY-4
	0	600	1200	1800	2400
IBM (g)	2.8 $\pm$ 0.08	2.47 $\pm$ 0.09	2.43 $\pm$ 0.1	2.42 $\pm$ 0.07	2.44 $\pm$ 0.06
FBM (g)	22.79 $\pm$ 1.39 <sup>a</sup>	21.54 $\pm$ 2.32 <sup>a</sup>	26.14 $\pm$ 2.73 <sup>b</sup>	25.92 $\pm$ 1.52 <sup>b</sup>	24.87 $\pm$ 2.02 <sup>b</sup>
SR (%)	96.66 $\pm$ 2.31	97.78 $\pm$ 2.67	96.66 $\pm$ 2.31	95.55 $\pm$ 1.33	91.33 $\pm$ 5.42
DWG (g)	0.45 $\pm$ 0.09 <sup>a</sup>	0.42 $\pm$ 0.06 <sup>a</sup>	0.53 $\pm$ 0.08 <sup>b</sup>	0.52 $\pm$ 0.05 <sup>b</sup>	0.50 $\pm$ 0.1 <sup>b</sup>
SGR (%/day)	5.02 $\pm$ 0.06 <sup>a</sup>	4.81 $\pm$ 0.08 <sup>a</sup>	5.28 $\pm$ 0.07 <sup>b</sup>	5.26 $\pm$ 0.06 <sup>b</sup>	5.15 $\pm$ 0.05 <sup>b</sup>
FCR (g/day)	1.95 $\pm$ 0.05 <sup>a</sup>	1.97 $\pm$ 0.08 <sup>a</sup>	1.67 $\pm$ 0.1 <sup>b</sup>	1.60 $\pm$ 0.09 <sup>b</sup>	1.73 $\pm$ 0.1 <sup>b</sup>
FI (g/day)	26.5 $\pm$ 2.33	25.0 $\pm$ 2.58	26.4 $\pm$ 3.71	25.1 $\pm$ 2.12	25.9 $\pm$ 3.10
PER	1.21 $\pm$ 0.11 <sup>a</sup>	1.20 $\pm$ 0.07 <sup>a</sup>	1.54 $\pm$ 0.08 <sup>b</sup>	1.61 $\pm$ 0.04 <sup>b</sup>	1.56 $\pm$ 0.04 <sup>b</sup>

<sup>a</sup> Values in the same row followed by different superscript letters (a and b) are significantly different (DMRT,  $p < 0.05$ ). IBM, initial body mass; FBM, final body mass; SR, survival rates; DWG, daily weight gain; SGR, specific growth rate; FCR, feed conversion ratio; FI, feed intake; PER, protein efficiency ratio.

### 3.1.2. Nutrient Digestibility

The findings indicated that fish fed phytase-based diets have higher ADCs of nutrients compared to the control diet. With increasing phytase levels, the ADCs of nutrients were improved with the 1200 U/kg level-based diet and reached their maximum with the 2400 U/kg diet (Table 3). The results showed that maximum values were noted for ADCs of protein (88.93%) at 2400 U/kg and ADCs of carbohydrates and energy at 1800 U/kg (77.21% and 84.22%, respectively). Statistically, these average values were higher ( $p < 0.05$ ) than those for non-supplemented and PHY-1-based diets. However, no significant differences ( $p > 0.05$ ) in fat and dry matter digestibility were observed between the groups.

**Table 3.** Apparent nutrient digestibility coefficients (ADCs) of experimental diet components after phytase supplementation.

ADC (%)	Experimental Diets				
	PHY-0	PHY-600	PHY-1200	PHY-1800	PHY-2400
Dry matter	74.66 $\pm$ 1.27	72.5 $\pm$ 1.43	71.2 $\pm$ 1.15	75.45 $\pm$ 1.04	70.7 $\pm$ 1.89
Protein	83.10 $\pm$ 1.22 <sup>a</sup>	84.17 $\pm$ 1.11 <sup>a</sup>	88.33 $\pm$ 1.65 <sup>b</sup>	87.63 $\pm$ 1.15 <sup>b</sup>	88.93 $\pm$ 1.12 <sup>b</sup>
Fat	88.63 $\pm$ 1.70	86.25 $\pm$ 1.85	88.02 $\pm$ 1.09	84.32 $\pm$ 1.79	83.74 $\pm$ 1.26
Carbohydrates	70.61 $\pm$ 2.55 <sup>a</sup>	72.64 $\pm$ 1.3 <sup>a</sup>	70.66 $\pm$ 1.72 <sup>a</sup>	77.21 $\pm$ 1.0 <sup>b</sup>	76.17 $\pm$ 1.15 <sup>b</sup>
Energy	79.72 $\pm$ 0.07 <sup>a</sup>	77.54 $\pm$ 1.13 <sup>a</sup>	82.59 $\pm$ 1.17 <sup>bc</sup>	84.22 $\pm$ 0.94 <sup>c</sup>	80.38 $\pm$ 1.27 <sup>ab</sup>

Data are expressed as mean  $\pm$  SEM ( $n = 3$ ). Values on the same line and with different superscript letters (a, b and c) are significantly different ( $p < 0.05$ ).

### 3.1.3. Carcass Composition

Our results showed that phytase addition has no effect on carcass ash, fat and carbohydrate composition in fish fed different test diets. Regarding the protein content, we observed a statistically significant ( $p < 0.05$ ) higher content of protein (14%) in fish bodies at 2400 U/kg (Table 4).

**Table 4.** Carcass composition (%) of fish fed mixture-based phytase-added diets after 45-day feeding trial.

Composition (%)	Final Body Composition (Experimental Diets)				
	PHY-0	PHY-600	PHY-1200	PHY-1800	PHY-2400
Moisture	78.54 ± 0.01	82.33 ± 0.06	79.50 ± 0.01	80.48 ± 0.95	78.24 ± 0.02
Protein	12.55 ± 0.15 <sup>a</sup>	12.39 ± 0.14 <sup>a</sup>	12.46 ± 0.19 <sup>a</sup>	12.63 ± 1.23 <sup>a</sup>	14.90 ± 0.73 <sup>b</sup>
Fat	6.89 ± 0.32	6.29 ± 0.19	6.34 ± 0.21	5.71 ± 0.56	5.43 ± 0.29
Ash	2.77 ± 0.56	2.71 ± 0.60	2.36 ± 0.48	2.14 ± 0.86	2.54 ± 0.97
P (g/kg)	1.36 ± 0.07 <sup>a</sup>	1.46 ± 0.05 <sup>a</sup>	1.94 ± 0.06 <sup>b</sup>	1.82 ± 0.03 <sup>b</sup>	1.46 ± 0.09 <sup>a</sup>
Ca (g/kg)	2.5 ± 0.1 <sup>a</sup>	2.8 ± 0.02 <sup>b</sup>	2.55 ± 0.09 <sup>a</sup>	2.8 ± 0.08 <sup>b</sup>	2.95 ± 0.07 <sup>b</sup>

Data are expressed as mean ± SEM (n = 3). Values on the same line and with different superscript letters (a and b) are significantly different ( $p < 0.05$ ).

### 3.2. MOS Incorporation

#### 3.2.1. Growth Performance

Data on growth indices of Nile tilapia fed with experimental diets after MOS treatment are presented in Table 5. MOS did not significantly influence the final body weight, weight gain, specific growth rate and survival rate of Nile tilapia treated with MOS ( $p > 0.05$ ). However, the feed conversion ratio, feed intake and protein efficiency ratio were significantly enhanced by including MOS at 4 and 8 g compared to the control group ( $p < 0.05$ ).

**Table 5.** Growth performance, feed utilization efficiency and biological parameters of juvenile Nile tilapia fed experimental diets supplemented with MOS. Each value is a mean ± SEM derived from three replicates (n = 3 tanks per diet)<sup>a</sup>.

Variable	Experimental Diets			
	MOS0	MOS2	MOS4	MOS8
IBM (g)	2.28 ± 0.01	2.25 ± 0.02	2.25 ± 0.02	2.25 ± 0.02
FBM (g)	20.65 ± 1.19	19.78 ± 0.86	19.62 ± 0.37	20.24 ± 1.28
SR (%)	97.5 ± 1.46	97.5 ± 2.50	94.16 ± 0.83	95.83 ± 2.21
DWG (g/j/ind)	0.40 ± 0.03	0.38 ± 0.02	0.38 ± 0.01	0.39 ± 0.03
SGR (%/day)	4.89 ± 0.12	4.83 ± 0.08	4.81 ± 0.06	4.85 ± 0.14
FCR (g/day)	1.60 ± 0.03 <sup>a</sup>	1.57 ± 0.03 <sup>a</sup>	1.49 ± 0.03 <sup>ab</sup>	1.44 ± 0.04 <sup>b</sup>
FI (g/day)	28.39 ± 1.64 <sup>a</sup>	26.53 ± 0.32 <sup>a</sup>	24.09 ± 0.11 <sup>b</sup>	24.96 ± 0.77 <sup>b</sup>
PER	1.21 ± 0.11 <sup>a</sup>	1.20 ± 0.07 <sup>a</sup>	1.54 ± 0.08 <sup>b</sup>	1.61 ± 0.04 <sup>b</sup>

<sup>a</sup> Values in the same row followed by different superscript letters (a and b) are significantly different (DMRT,  $p < 0.05$ ). IBM, initial body mass; FBM, final body mass; SR, survival rate; SGR, specific growth rate; FCR, feed conversion ratio; FI, feed intake; PER, protein efficiency ratio.

#### 3.2.2. Nutrient Digestibility

The nutrient digestibility coefficients of the experimental diets are presented in Table 6. The dry matter digestibility was higher ( $p < 0.05$ ) in groups of fish fed 4 and 8 mg/kg of MOS. However, the ADCs of protein and fat did not change significantly ( $p > 0.05$ ) among the groups.

**Table 6.** Apparent nutrient digestibility coefficients (ADCs) of experimental diet components.

ADC (%)	Experimental Diets			
	MOS0	MOS2	MOS4	MOS8
Dry matter	71.95 ± 1.14 <sup>a</sup>	73.79 ± 1.38 <sup>a</sup>	78.17 ± 0.87 <sup>b</sup>	81.68 ± 1.56 <sup>b</sup>
Protein	83.80 ± 3.26	85.01 ± 1.96	89.27 ± 1.75	88.86 ± 2.08
Fat	91.12 ± 4.08	92.35 ± 2.18	90.55 ± 3.11	93.86 ± 2.45

Data are expressed as mean ± SEM (n = 3). Values on the same line and with different superscript letters (a and b) are significantly different ( $p < 0.05$ ).

### 3.2.3. Carcass Composition

After the 45 days of the experiment, results of fish carcass composition showed that moisture, protein, fat and ash values were unaffected by supplementation of MOS in diets ( $p > 0.05$ ) compared to those values in fish fed the control diet (Table 7).

**Table 7.** Carcass composition (%) of fish fed mixture-based MOS-added diets after 45-day feeding trial.

Composition (%)	Final Body Composition (Experimental Diets)			
	MOS0	MOS2	MOS4	MOS8
Moisture	73.5 ± 0.4	73.4 ± 0.4	73.4 ± 0.2	73.7 ± 0.5
Protein	15.6 ± 0.3	17.6 ± 3.1	12.5 ± 1.0	10.9 ± 1.4
Fat	5.8 ± 1.3	5.6 ± 0.8	6.8 ± 1.2	6.1 ± 1.2
Ash	3.0 ± 0.2	3.5 ± 0.4	3.7 ± 0.6	4.2 ± 0.5

Data are expressed as mean ± SEM (n = 3).

## 4. Discussion

Fish nutrient digestibility issues may be caused by the presence of phytate in meal-based diets. Additionally, this biological compound can bind to important proteins and minerals found in ingredients of fish species, which particularly reduces the bioavailability of nutrients and amino acids [10,11,47]. In the present study, phytase supplementation significantly improved the SGR, FI and FCR of *O. niloticus* juveniles. The addition of phytase at 1200 and 1800 U/kg improved performance more than the other treatments. A recent study found that supplementation with phytase in the range of 1500 to 2000 U/kg is advised to improve Nile tilapia growth performance and nutrient bioavailability [10]. Similarly, Khaled [48] observed that 1000 U/kg phytase significantly improved the WG, specific growth rate and FCR of Nile tilapia. The optimal levels of PHY supplementation for Nile tilapia juveniles' growth performance are consistent with those discovered in [10,38,49,50] varying between 1200 and 2000 U/kg; nonetheless, they are higher than those established in [36,51,52] ranging from 700 to 1000 U/kg. However, the origin and type of the enzyme, the method of phytase incorporation, the rearing conditions, feed formulation and dietary phytate content could all be factors contributing to the variability in the optimum phytase supplementation level [10,37].

In the present study, the ADCs of nutrients were improved with a phytase level of 1200 U/kg diet and reached their maximum at 2400 U/kg diet. The results showed a maximum of protein digestibility at 2400 U/kg. Similarly, Maas et al. [53] noticed that ADCs of protein were enhanced by phytase supplementation up to 1000 U/kg for the same fish species. However, numerous reports reported the advantageous effects of phytase on the ADCs of nutrients [36,38,54]. The release of macronutrients from diets by cleaving the links between phytate–protein and phytate–minerals may be the cause of the increased palatability and conversion rate of diets.

Some studies have shown that poor fish carcass composition could be due to phytate–mineral complexes, which often bind essential nutrients, making them unavailable to fish [37]. Consistent with these studies, in the present research, the carcass composition of fish fed phytase-supplemented diets showed increasing levels of P and Ca, revealing the positive impact of phytase on the release of chelate minerals; that is, the incorporation of PHY increased the mineral content in *O. niloticus* juveniles. Moreover, the group of fish fed a phytase-supplemented diet would benefit from improved nutrient biodigestibility in terms of body composition and bone strength [55]. The addition of phytase may improve the body's ability to retain nutrients via the hydrolysis of the chelated phytate structure, leading to improved fish carcass composition [15,50].

Phosphate is an important cellular component and is involved in all cellular reactions that produce energy. Thus, P is a necessary nutrient for fish to grow, form their skeletons and reproduce [56–58]. On the other hand, P is a harmful contaminant of the aquatic

environment, and excessive P concentrations are the most common cause of eutrophication of aquatic reservoirs [59]. Actually, the addition of microbial phytase to fish diets reduced P excretion and its release into the environment via the open recirculation system.

Indigestible compounds known as prebiotics are created when yeast cell walls and useful carbohydrates are fermented [60,61]. Active prebiotics called mannan oligosaccharides (MOSs) are well known for their capacity to improve gut digestion and immunity, while also being proven to exhibit antibacterial properties [27,62]. According to our results, no differences were observed in the growth performance of fish between the MOS and control groups. However, the feed conversion ratio, feed intake and protein efficiency ratio were improved in the prebiotic groups, particularly in fish fed with diets containing 4 and 8 g/kg (Table 5). In many studies, MOS was found to have a direct beneficial impact on growth behavior and health status, whereas other studies found no discernible effects of employing MOS on fish species [20,63–65]. In this regard, Dimitroglou et al. [66] reported that the mean final weight and specific growth rate (SGR) of fish fed FM or SBM diets remained unaffected by MOS supplementation (2 and 4 g/kg). MOSs are feed additives well known for their capacity to improve intestinal digestion via the enhancement of the activity of intestinal enzymes [27,28,62]. The activation of digestive enzymes by dietary MOS led to higher feed utilization but without a significant effect on growth performance. However, the body proximate composition analysis in the present investigation revealed that MOS supplementation had no effect on any of the evaluated parameters. Similarly, the chemical characteristics of carcass components were not meaningfully impacted by dietary MOS in Thinlip Grey Mullet (*Liza ramada*), while earlier research utilizing 0.4% MOS supplementation in hybrid tilapia [67] and rainbow trout [68] revealed higher body protein levels.

## 5. Conclusions

Based on the obtained results, it can be concluded that PHY levels varying between 1200 and 2400 U/kg proved to be the most efficient in improving the growth performance of *O. niloticus* juveniles. Overall, phytase supplementation positively affected Nile tilapia growth performance and nutrient digestibility and reduced the need for supplementation with mineral phosphate sources. Prebiotic mannan oligosaccharide could improve feed utilization, and 4 g/kg was shown to be the optimum level. However, further investigations are needed to determine the possible impacts of dietary MOS and PHY supplementation on other physiological responses and resistance to disease or stress using molecular tools.

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