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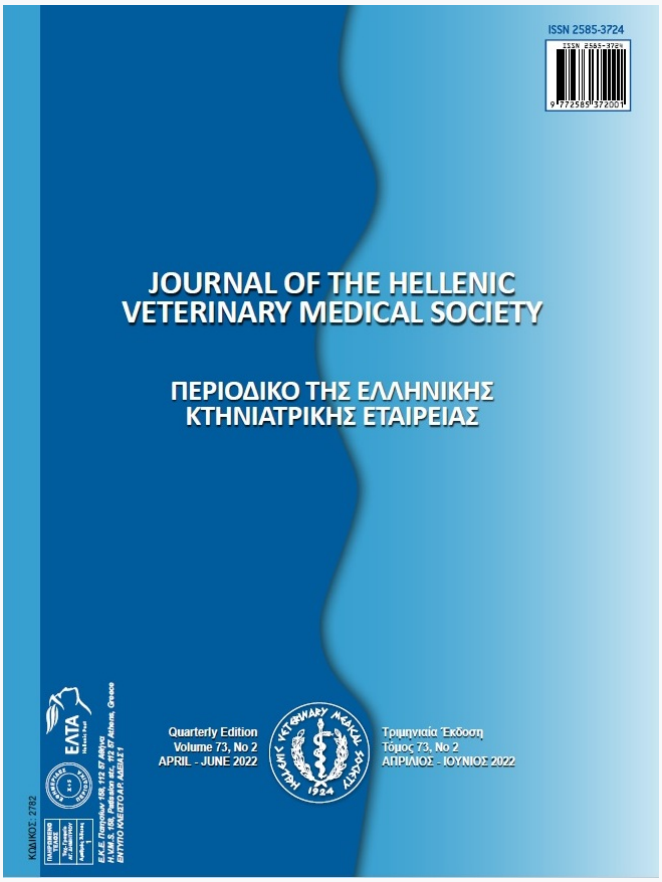

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Effectiveness of Allzyme SSF and Polizyme® multimix feed additives on Growth Performance, Feed Utilization and Immunological Parameters of Nile Tilapia (*Oreochromis niloticus*) Fingerlings

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ABSTRACT: This study was carried out to analyze the potential benefits of some feed additives (Allzyme SSF® at levels, 1.0, 2.0 and 3.0 kg/ton) and (Polizyme® multimix FM at levels, 1.0, 2.0 and 3.0 kg/ton) in Nile Tilapia (*Oreochromis niloticus*) diet on growth performance, feed efficiency, body chemical composition, blood parameters and economical efficiency under Egyptian conditions. Three hundred and fifteen fingerlings of Nile Tilapia *(O. niloticus)* with average initial weight (8.5 ± 0.5 g), were randomly distributed into 7 treated groups allotted into 21 glass aquaria (three replicates of 15 fish / each treatment). Each aquarium measured 60× 35× 40 cm^3 (84 Lt.). The fish were fed at 3% fish biomass throughout the experiment which went on for 12 weeks. The results revealed significant enhancements in growth and all feed utilization parameters in the prebiotic supplemented groups. The diets containing Allzyme SSF® (0.1%) and Polizyme® multimix (0.1%) showed the highest growth and protein utilization parameters values. Experimental fish carcass composition was generally influenced by the different dietary treatments. The hematological, biochemical and immunological parameters of the experimented groups indicated critical increase in Allzyme SSF® and Polizyme® multimix treated groups. The obtained outcomes cleared that Allzyme SSF® and Polizyme® multimix at levels of 0.1% could be used in Nile tilapia diets without negative impacts on growth, feed utilization, blood and immunological parameters. Subsequently, Allzyme SSF® and Polizyme® multimix could be added to commercial diets to improve tilapia fingerlings production and immune response.

***Keywords*:** Allzyme SSF®**;** growth, immunity; *Oreochromis niloticus*; Polizyme® multimix

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INTRODUCTION

Fish constitutes a significant source of protein for many people throughout the world and fish consumption has expanded in significance among health-conscious people because it provides healthy and low cholesterol sources of protein and other nutrients (Agusa *et al.,* 2005 and Burger & Gechfeld, 2005). Tilapia production is of expanding importance in aquaculture globally and is the second after carp in production volume estimates (FAO, 2012). Among the several species of tilapia cultured commercially, Nile tilapia, *Oreochromis niloticus* is the most abundant and important species. Worldwide aquaculture production of tilapia has expanded from around 200 000 metric tons in 1990 (FAO, 2012) to about 4.2 million metric tons in 2012 and forecasted to reach a total production of 5.0 million metric tons in 2015 (FAO, 2016).

Nile tilapia is an economically important cultured species in several areas of the world (El-Saidy & Gaber, 2005 and El-Husseny *et al.,* 2007). Egypt made an impressive increase in aquaculture tilapia production, from 24916 mt in 1990 to 487000 mt in 2005m accounting for 55% of Egyptian total fish production (879000, mt year-1). (FAO 2004 and GAFRD, 2006). *Oreochromis niloticus* is considered as the most common and popular fish in Egypt, and has proved to be vital, contributing from 30 to 40 % of the total production of the Lake Nasser in Egypt (Latif & Rashed, 1983). However, Khouraiba (1997) reported that tilapia are constantly gaining importance in aquaculture, especially in the tropics and subtropics. In Egypt, tilapia constitutes approximately 45 % of inland water fishery production.

In the recent years, application of dietary supplements such as vitamins, probiotic, prebiotic and herbal plants as growth promoters and immuno-stimulants have increased in beluga sturgeon; (Falahatkar *et al.* 2006; Akrami *et al.* 2009; Hoseinifar *et al.* 2011a, b; c and Binaii *et al.* 2014).

Prebiotic are non-digestible food ingredients that beneficially influence the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the gut there by improving the host intestinal balance (Ghaedi *et al.,* 2015). Despite the potential benefits to health and performance as noted in various terrestrial animals, the use of prebiotics in the farming of fish and shellfish has been less investigated. The studies of prebiotics in fish and shellfish have investigated the following parameters: effect on growth, feed conversion, gut microbiota, cell damage/morphology, resistance against pathogenic bacteria and innate immune parameters such as alternative complement activity (ACH50), lysozyme activity, natural haemagglutination activity, respiratory burst, superoxide dismutase activity and phagocytic activity (Ringo *et al*., 2010).

Digestive enzymes are aiding in the sustenance assimilation process by changing the non-edible ingredients into more absorpable supplements (Yigit & Keser, 2016). Utilizing of digestive enzymes is additionally prescribed to encourage the absorption procedure of complex ingredients in plant proteins, for example, starch, cellulose and protein into simple substances (Goda *et al*., 2012). Amylases, lipases, cellulases, xylanases, phytases and proteases are various types of exogenous digestive enzymes which have been connected effectively in animal feeding and aquafeed (Feord, 1996 and Forster *et al.,* 1999). Since enzyme preparations have become profitable tools for financially controlling digestive processes in animals (Johnson *et al.,* 1993), there are also impressive interests in utilizing as supplements in fish feeds (Goda *et al*., 2012 and Yigit & Keser, 2016).

An approach to increase the nutritive value of a fish feed is the supplementation of the feed by different additives that permit an upgrade of the bio-beneficial performances of the species utilized. Adding enzymes allows an increase of the feed efficiency in the reared species that subsequently determines an enhancement of the productive indices. The utilization of enzymes as fish feed additives was tried first by utilizing enzyme extracts of animal origin by Dabrovski & Glogowski (1977) and Dabrovska *et al.,* 1979 that revealed positive results on common carp.

One of the most utilized enzymes is Allzyme SSF®, an enzyme product with seven enzyme activities (amylase, cellulase, phytase, xylanase, betaglucanase, pectinase and protease). Other utilized enzyme is Polizyme® Multmix Fm an enzyme product withseven enzyme activities (xylanase, glucanase, Cellulase, Alpha-amylase, Pectinase, Beta-mannanase and Phytase). The use of the enzyme complex Allzyme SSF® before pelleting can increase the carbohydrates, phosphates and nitrogen digestibility (Filler *et al.,* 2007). The beneficial outcome of the Allzyme SSF® enzyme complex was also reported by Filler *et al.* (2010) and Moura *et al.* (2012) in a research carried out on Nile tilapia (*Oreochromis niloticus*).

The current study was directed to investigate the potential benefits of Allzyme SSF®, and Polizyme® Multmix Fm dietary supplementation on growth performance, feed efficiency, body chemical composition, hematological parameters, immunological response and economical efficiency of Nile Tilapia (*O. niloticus*) fingerlings under Egyptian conditions.

MATERIALS & METHODS

This study was conducted in fish Aquaculture Research Unit in Kafrelsheikh Governorate.

Ethical Approval

All handlings of fish were directed according to the guidelines for animal care and use for scientific purposes built up by the Ethics Committee of the Faculty of Agriculture, Kafrelsheikh University, Egypt (Approval Date: 18-03-2018).

Diet preparation

A basal diet was formulated from commercial ingredients including fish meal, soybean meal, yellow corn, wheat bran, vitamins, minerals mix and fish oil. The dry ingredients were grounded using a feed processor into minimal size particles. Seven diets were formulated from the basal diets by adding the prebiotics at different concentrations. Contents and chemical composition of each diet were exhibited in Table (1).

The ingredients were weighed and mixed by a mixture blender for 20 minutes. A steady Allzyme® SSF and Polizyme® multimix levels was added for all diets except the control diets. After homogenous mixing, every hundred gram diet was gradually added to the blend as indicated by (Shimeino *et al.,* 1993**).** The diets were cooked on water exaporator for 20 minutes. The diets were pelleted through pelleting machine and the pellets were dried at room temperature for 24 h before used. The pellets were assembled and saved in plastic bags and stored in a refrigerator at 4^0C through the experimental period to dodge nutrients deterioration.

The utilized feed additive, Allzyme® SSF and Polizyme® multimix FM were commercial natural enhancers blend; (Allzyme® SSF; Alltech, Inc., Nich-

Table (1): Composition and Chemical analysis of the experimental diets offered for each group

Ingredients	Diet1 control	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7
Fish meal (72% cp)	10	9.9	9.80	9.71	9.9	9.80	9.71
Yellow corn	22	21.78	21.57	21.36	21.78	21.57	21.36
Soybean meal (45%cp)	42	41.58	41.18	40.78	41.58	41.18	40.78
Wheat bran	20	19.80	19.61	19.42	19.80	19.61	19.42
Fish oil	5	4.96	4.90	4.85	4.96	4.90	4.85
Vit & Min*	1	0.99	0.98	0.97	0.99	0.98	0.97
Allzyme	-	0.99	1.96	2.91	-	-	-
Polizyme	-	-	-	-	0.99	1.96	2.91
Chemical composition							
Dry matter	92	92	92.30	91.60	92.30	92	92.20
Crude protein	29.32	29.47	29.26	29.03	29.50	29.27	29.32
Ether extract	6.27	6.38	6.34	6.30	6.32	6.35	6.38
CF	4.08	4.16	4.30	4.50	4.10	4.20	4.11
Ash	6.43	6.23	6.45	6.43	6.65	6.47	6.50
NFE	53.90	53.76	53.65	53.74	53.43	53.71	53.69
Calculated energy value							
GE (Kcal / 100g)[1]	444.36	445.66	444.10	442.47	443.92	444.06	444.54
DE (Kcal / 100g) [2]	310.83	312.29	310.66	309.33	310.09	310.92	311.4
P / E mg / kcal [3]	65.98	66.12	65.88	65.60	66.45	65.91	65.95

*vitamins and minerals premix (product of victoir) each 3kg contain : 10.000.000 IU vit. A ; 20.00.000 IU vit D3 ; 10.000 mg vit E ; 1000 mg vit K3 ;1000 mg vit B1 ; 1000 mg vit B2 ; 1000 mg vit B6 ;10 mg vit B12 ; 15.000 mg pantothenic ; 10.000 mg Nicotenic ; 2500 mg Folic acid ; 50 mg Biotein ; 100 gm VITC (coated) ; 40 gm Manganese ; 30 gm Zinc ; 20 gm Iron ; 2 gm Copper ; 1 gm Iodine ; 0.1 gm Selenium ; 0.1 gm Cobalt .

[1] (Gross energy) (Kcal/100g), based on 5.6 Kcal/g protein, 9.44 Kcal/g lipid, 4.1 Kcal/g carbohydrate (**NRC, 1993**).

[2] (Digestible energy) (Kcak/100g), based on 5.0 Kcal/g protein 9.0 Kcal/g lipid, 2.0 Kcal/g carbohydrate.

[3] (P/E) (protein to energy ratio)= mg crude protein/Kcal of gross energy.

olasville, KY, USA) and (Polizyme® multimix FM ; Polen ltd. Sti, Turkey).

Experimental design

The experiment was performed using 315 Nile Tilapia (*O. niloticus*) fingerlings (weighing on average 8.5 ± 0.5 g). They were gathered from a private fish farm in Al. Reyad, tolompate 7, Kafr El-Sheikh Governorate. All collected fish were kept in a fiberglass tank, for three weeks for accommodation; where fish were fed a commercial diet (containing 30% dietary protein level (CP)). The tanks are flow-through water irrigation system. After the acclimatization period, the fingerlings were randomly divided into 7 groups of 45 fingerlings / each group allotted into three replicates of 15 fingerlings / each replicate). Fingerlings were put in glass aquaria of 60×35×40 cm^3 cm contained 70 L of water, (15 fish/ aquarium) and were equipped with effective aeration system. The seven Groups; group 1 (control group) were fed a commercial diet, groups 2, 3 and 4 were fed diets supplied with 1, 2, 3 kg/ ton) of Allzyme® SSF While, groups 5, 6 and 7 were fed diets supplemented with 1, 2, 3 kg/ ton of Polizyme® multimix FM, respectively.

Fish were fed the experimental diets for 12 weeks at a rate of 3% of fish biomass on dry matter basis. Diets were applied twice a day (at 8:00 am & 14:00 pm). Fish were weighed at fortnightly intervals along the experimental period and the feed amounts were corrected according to the change in live body weight. Fish excreta and feeding wastes were expelled by siphoning and about half of water in every aquarium was replaced day by day by dechlorinated new water.

Determination of fish growth parameters

The fish were totally weighed (15 fish / each replicate) using an electronic balance**.**

Total weight gain (TWG), average daily gain (ADG), specific growth rate (SGR), survival rate (SR%), feed conversion ratio (FCR), and protein efficiency ratio (PER), were calculated according to the following equations:

Total weight gain (TWG) (g) Final body weight -Initial body weight **(**Annet, 1985**).**

Average Daily Gain (g/fish/day) = TWG (g)/trial period (d)

Specific growth rate (SGR %/day) =[Ln Final body weight- Ln Initial body weight] × 100/trial period (d) (Pouomonge & Mbonglang, 1993**)**

SR= Total number of fish at the end of the experiment × 100/ absolute number of fish at the start of the experiment.

FCR= feed consumption (g)/Live weight gain. (De Silva & Anderson, 1995**).**

PER=Live weight gain (g)/protein intake (g). (De Silva & Anderson, 1995**).**

PPV = 100 × (Retained protein (g) / Protein intake (g).

Determination of diet proximate analysis

Dry matter, crude protein, ether extract, crude fiber and ash contents of the experimental diets and the whole body of fish at the end of the experiment were performed according to AOAC (1990).

Water quality Parameters

Water quality parameters were observed routinely throughout the trial timeframe. Water temperature, dissolved oxygen (DO) and pH, ammonia, nitrite were estimated. Water temperature was measured utilizing thermometer. Dissolved Oxygen level was measured daily at 8 o'clock by using oxygen meter (Model FE 247, EDT Instruments LTD. Dover Kent, UK). The pH was monitored using portable digital pH meter (Martini Instruments Model 201/digital). Ammonia-N was estimated using test kit (Model NI, Cat. No. 22669-00, Hach Co.). Nitrite was measured using test kits (Model NI-14 Cat.No. 14161-00, Hach Co.). Determinations were carried out weekly according to APHA (1989).

Hematological investigations

Toward the end of the experiment, twelve fishes from every group (4 fishes / every replicate) were randomly sampled and weighed. Anti-coagulated blood samples were taken from the caudal vein for blood analysis. Due to the small fish size, blood samples collected from 3-4 fish were pooled according to Urbinate and Carneiro (2006).

Red blood cells count (RBCs×10^6/mm) and white blood cells count (WBCs×10^3/mm) were determined according to the method described by Stoskopf, (1993).

Serum total protein was determined

colorimetrically using commercial kits (TP0100, Sigma - Aldrich,USA). Serum albumin was measured using bromocresol green binding method (Doumas *et al.,* 1971). Serum globulin was calculated by subtracting albumin values from total protein. Albumin/globulin (A/G) ratio was calculated by dividing albumin values by globulin ones. Serum alanine aminotranferase (ATP) and serum aspartate a minotransferase (AST) assays were performed as described by Palti *et al.* (1999).

Determination of internal organs indices

Toward the end of the experiment, four fishes from every treatment were slaughtered and the abdominal cavity was directly opened to evacuate liver, kidney, spleen and gonads, then weighed separately. Liver index (HSI), kidney index (KSI), spleen index (SSI) and gonads index (GSI) were calculated as follows:

Hepato somatic index (HSI%)=100×[liver weight (g) /body weight (g) (Jangaard *et al.,*1967*).*

Kidney somatic index (KSI%)=100×[kidneys weight (g)/body weight (g)] **(**Angelescu *et al.,*1956**).**

Spleen somatic index (SSI%)=100×[spleen weight (g) / body weight (g)] (Jangaard *et al.,* 1967*).*

Gonado somatic index (GSI%)=100×[gonads weight (g)/body weight (g)] (Tseng and Chan, 1982).

Immunological studies

a- Determination of phagocytic activity (PA) and phagocytic index (PI)

Phagocytic activity was determined according to *Kawahara et al.* (1991).

b- Lysozyme activity in serum

The lysozyme activity was examined by the technique described by Demers and Bayne (1997), in light of the ability of lysozyme to lyses Gram positive lysozyme delicate bacterium; *Micrococcus lysodeikticus*.

Statistical analysis

The obtained data were statistically analyzed utilizing general direct models technique adjusted by SPSS (1997) for users guide, with a restricted ANOVA. Means were statistically compared for the significance ($P < 0.05$) using Duncan's multiple range test (1955).

RESULTS

In the present study, the physiological reactions of *O. niloticus* fingerlings to Allzyme SSF® and Polizyme® multimix FM were investigated through affirmation of fish improvement and hematological parameters. It was observed that there were colossal increment in outright weight gain (TWG) and average daily gain (ADG) in all prebiotics treated groups aside from in T3 group compared with the control group (T1). The most raised characteristics were noticed in the T2 and T5 groups. Notwithstanding, the specific growth rate (SGR) was fundamentally increased in T2 and T5 group only. Survival rate percent (SR%) was 100% in all groups and no mortalities were recorded as shown in table (2: A).

The effects of the two used prebiotics on feed intake, in Table (2: B) showed the highest values inT2 and T5 groups compared with the control group with no significant differences between them concerning feed intake (FI), Protein efficiency ratio (PER), and Protein productive value (PPV%). However, regarding feed conversion ratio (FCR), T6 group indicated the best treatment in correlation to the control group.

Chemical composition of the experimental fish body, average dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash were summarized in table (3:A). There was a critical and basic distinction in the DM in the case of all treated groups except T3 group. While, CP% was fundamentally extended in all groups with the highest levels in T2 and T5 groups with no significant differences among them. On the other hand, ether extract was altogether large reduced in all treated groups contrasted with control one. The most astounding estimation of ash content was recorded in all treated groups and the minimal values were found in T2 and T7 groups.

As summarized in table (3:B), all water physicochemical parameters for all treatments showed that, dissolved oxygen was not least than 5 mg/ litter, total ammonia not more than 0.06 mg/ litter, pH values were between 7.1 and 7.9, nitrite (NO_2) was not more than 0.46 mg/ litter and temperature was about 27ºC.

RBCs, and WBCs count check showed significant increases in all prebiotics supplemented groups in comparison with the control one (Table 4). The highest level of RBCs count was seen in T4 and T7groups with no significant differences among all treatments except in T5 group. However, the highest level of WBCs count was observed in T5 group. The results of serum total proteins indicated high level in T2, T3,

Table (2: A): Effect of Used prebiotics on growth parameters and survival rate of Nile tilapia

Treat	I.W. g/fish	F.W. g/fish	(TWG) g/fish	(ADG) g/fish/day	SGR % / day	(SR) %
T1	8.66 ± 0.00	24.54 ± 0.12^{bcd}	15.03 ± 0.21^{cd}	0.16 ± 0.23^{cd}	1.13 ± 0.00^{bcd}	100 ± 0.00
T2	8.66 ± 0.00	27.81 ± 0.43^{a}	19.15 ± 0.43^{a}	0.21 ± 0.49^{a}	1.30 ± 0.01^{a}	100 ± 0.00
T3	8.66 ± 0.00	22.93 ± 0.50^{d}	14.27 ± 0.50^{d}	0.15 ± 0.05^{d}	1.07 ± 0.02^{d}	100 ± 0.00
T4	8.66 ± 0.00	24.03 ± 0.17^{cd}	15.37 ± 0.17^{cd}	0.17 ± 0.00^{bcd}	1.13 ± 0.00^{bcd}	100 ± 0.00
T5	8.66 ± 0.00	26.00 ± 1.15^{b}	17.34 ± 1.15^{b}	0.19 ± 0.12^{ab}	1.21 ± 0.04^{b}	100 ± 0.00
T6	8.66 ± 0.00	23.73 ± 0.53^{cd}	15.07 ± 0.53^{cd}	0.17 ± 0.01^{bcd}	1.11 ± 0.02^{cd}	100 ± 0.00
T7	8.66 ± 0.00	25.19 ± 0.49^{bc}	16.53 ± 0.49^{bc}	0.18 ± 0.05^{bc}	1.18 ± 0.02^{bc}	100 ± 0.00

I.W. = Initial Weight **F.W.** = Final Weight **T.W.G =** Total weight gain **A.D.G =** Average daily gain
S.G.R = Specific growth rate **S.R.% =** Survival Rate

Table (2: B): Feed intake, feed conversion rate and protein efficiency ratio of Nile tilapia in response to prebiotics supplementation

Treat.	FI (g)	FCR	PER	PPV %
T1	39.22 ± 0.08^{bc}	2.58 ± 0.03^{a}	1.30 ± 0.02^{b}	32.15 ± 0.16^{bcd}
T2	42.43 ± 0.26^{a}	2.21 ± 0.03^{b}	1.53 ± 0.02^{a}	36.37 ± 0.86^{a}
T3	36.99 ± 0.73^{c}	2.60 ± 0.14^{a}	1.31 ± 0.07^{b}	29.78 ± 1.05^{cd}
T4	37.34 ± 1.24^{c}	2.42 ± 0.08^{ab}	1.41 ± 0.04^{ab}	33.44 ± 0.04^{abc}
T5	41.33 ± 1.11^{ab}	2.39 ± 0.09^{ab}	1.41 ± 0.05^{ab}	37.23 ± 2.47^{a}
T6	39.46 ± 0.12^{bc}	2.62 ± 0.09^{a}	1.30 ± 0.04^{b}	29.35 ± 0.78^{d}
T7	40.18 ± 0.89^{ab}	2.42± 0.02^{ab}	1.40 ± 0.01^{ab}	35.69 ± 1.07^{ab}

FI: Feed Intake **FCR**: Feed conversion rate **PER**: Protein efficiency ratio **PPV** = Protein productive value

Table (3: A): Composition Analysis of fish body fed graded levels of Allzyme and Polizyme

Treat. No	% on Dry matter basis				
	DM %	CP %	EE %	Ash %	GE (kcal/100g)
T1	24.21±0.13^{b}	58.31±0.09^{e}	2.74±0.01^{a}	19.68±0.08^{d}	484.85±0.46^{a}
T2	24.35±0.04^{b}	63.08±0.03^{ab}	10.27±0.03^{ef}	19.13±0.05^{e}	481.06±0.24^{bc}
T3	24.05±0.17^{bc}	61.19±0.08^{c}	10.43±0.10^{e}	21.19±0.04^{a}	470.61±0.63^{e}
T4	24.29±0.17^{b}	63.03±0.02^{b}	11.06±0.03^{c}	20.51±0.10^{b}	479.51±0.51^{d}
T5	24.26±0.25^{b}	63.23±0.05^{a}	10.76±0.06^{d}	20.16±0.05^{c}	479.66±0.47^{cd}
T6	25.05±0.02^{b}	58.63±0.05^{d}	12.42±0.06^{b}	20.10±0.03^{c}	481.88±0.35^{b}
T7	24.24± 0.03^{b}	63.04± 0.01^{b}	10.19 ±0.03^{c}	19.25±0.03^{e}	480.04±0.32^{cd}

Data shown are Means (±SD) in each row; different superscript letters indicate significant difference (P ≤0.05).
DM= Average dry matter **CP**= crude protein
EE= ether extract **GE**= Gross energy

Table (3: B): Averages of some physico-chemical parameters of Water

Treat No.	Water parameters				
	Temperature C°	PH Value	DO Mg /L	NH_3 Mg/ L	No_2, Mg / L
T1	27.0	7.1	6.4	0.05	0.44
T2	26.5	7.6	5.6	0.04	0.39
T3	26.7	7.3	5.3	0.05	0.46
T4	27.1	7.9	5.7	0.06	0.44
T5	26.4	7.4	5.9	0.04	0.39
T6	27.3	6.7	6.3	0.06	0.40
T7	26.9	7.7	5.7	0.05	0.42

Table (4): Effect of Allzyme and Polizyme on haematological parameters and serum biochemical analysis in *Oreochromis niloticus*

Treat. No.	RBCs (10^6 /mm)	WBCs (10^3 / mm)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	AST (U/L)	ALT (U/L)
T1	2.62±0.22^{b}	28.60±0.41^{d}	4.70±0.00^{a}	2.74±0.05^{ab}	1.96±0.05^{c}	84.05±2.92^{ab}	5.47±0.11^{a}
T2	3.14±0.16^{ab}	35.56±1.47^{bc}	5.27±0.08^{a}	3.05±0.03^{ab}	2.22±0.05^{b}	84.44±3.20^{ab}	4.84±0.4^{bc}
T3	3.17±0.13^{ab}	35.11±2.82^{bc}	5.30±0.18^{a}	3.05±0.15^{ab}	2.25±0.03^{b}	88.56±0.32^{a}	5.39±0.13^{a}
T4	3.74±0.27^{a}	30.88±0.51^{bcd}	5.25±0.26^{a}	3.11±0.17^{a}	2.14±0.09^{bc}	82.32±2.75^{ab}	4.98±0.05^{b}
T5	2.94±0.09^{b}	43.07±2.28^{a}	4.76±1.58^{a}	2.72±0.04^{ab}	2.04±0.04^{bc}	80.32±0.77^{b}	4.60±0.12^{c}
T6	3.70±0.27^{a}	30.36±1.05^{cd}	5.23±0.08^{a}	3.11±0.06^{a}	2.11±0.02^{bc}	80.53±0.33^{b}	4.85±0.05^{bc}
T7	3.72±0.56^{a}	36.86±2.75^{b}	5.14±0.15^{a}	2.58±0.29^{b}	2.56±0.14^{a}	85.55±2.57^{ab}	4.92±0.05^{b}

Data shown are Means (±SD) in each row; different superscript letters indicate significant difference (P ≤0.05).
RBCs = Red Blood Cells **WBCs** = White Blood Cells
ALT = Alanine aminotransferase **AST** = Aspartate aminotransferase

Table (5): Results of Phagocytic activity, phagocytic index and lysozyme activity of Nile tilapia in response to prebiotics supplementation

Treatment	Phagocytic activity	phagocytic index	lysozyme activity
T1	16.52± 1.12^{c}	4.13±0.11^{d}	0.03±0.001^{d}
T2	32.25± 1.53^{ab}	14.36± 0.37^{a}	0.44± 0.003^{a}
T3	23.07± 1.47^{ab}	12.21± 0.39^{ab}	0.24± 0.003^{ab}
T4	20.39± 1.77^{b}	11.31± 0.39^{b}	0.24± 0.003^{ab}
T5	32.05± 1.43^{ab}	13.67± 0.51^{ab}	0.38± 0.003^{ab}
T6	22.87± 2.06^{ab}	9.61± 0.51^{bc}	0.21± 0.014^{b}
T7	19.28± 2.21^{bc}	8.44± 0.64^{c}	0.20± 0.033^{b}

Data shown are Means (±SD) in each row; different superscript letters indicate significant difference (P ≤0.05)

T4 and T6 groups, but there were no significant differences among all treatments. Albumin, showed high level in T2, T3, T4 and T6 however; globulin showed high level in T2, T3 and T7. The T5 group demonstrated a great decrease in AST level. The most elevated amount of ALT was recorded in T3group.

Regarding immunological parameters, phagocytic activity and index and lysozyme activities were altogether extended and expanded in all groups with most extreme measurements in group 2 and group 5, respectively as presented in table (5).

DISCUSSION

Prebiotics, in aquaculture, can be admitted either as feed included substances or as added substances to the water (Moriarty 1998, Taoka *et al.,* 2006). The shape and length of prebiotic and probiotic organization can affect their practicality on fish wellbeing (Welker & Lim, 2011). Recently, exogenous digestive enzymes are broadly utilized everywhere throughout the world as additives in fish feeds to improve the healthy benefit value of diets, especially with the expansion of plant proteins (Kolkovski *et al.,* 1997).

In the present study, there were no significant (p≥ 0.05) differences in the initial body weight among all treatments. While average weight gain (AWG), average daily gain (ADG) and specific growth rate (SGR) of the experimental fish were most higher in fish fed T2 and T5 groups. In general, the fish of T3 recorded the lowest values for AWG, ADG and SGR when compared with the other fish treated groups. The increased growth parameters among prebiotic treated groups may be attributed to improved feed utilization in fish. In other words, the enzymatic treatment with exogenous digestive enzymes (Allzyme and Polizyme) improved the digestion process to be more suitable for increasing anaerobic bacteria and exogenous enzymes activity. Diets containing level of 1 g kg^{-1}Allzyme and Polizyme showed significantly higher SGR than other experimental diets. The results are similar to those reported previously in several fish species including, Channel catfish (Jackson *et al.,* 1996, Debnath *et al.,* 2005), Rainbow trout (Irianto & Austin, 2002; Oz *et al.*, 2018, 2020, 2021), Clarias catfish (Giri*et al.,* 2003), Nile tilapia (El-Haroun *et al.,* 2006; Lin *et al.,* 2007; Ali *et al.,* 2010; Samuel *et al.,* 2017 and Dawood *et al.,* 2019),Sturgeon (Adel *et al*., 2016), Grasscarp (Sara *et al.,* 2012) and Common carp (Singh *et al.,* 2011), respectively; but in contrast to those reported by Genc *et al.,* (2007) and Yigit and Keser (2016).

In the present study, the two used prebiotics supplementation in Nile tilapia feeds improved feed intake (FI), Protein efficiency ratio (PER) and Protein productive value (PPV) especially in T2 and T5 groups. These findings might be due to the action of enzymes in Allzyme and Polizyme mixture; which might lead to stimulating the digestion of the fibrous components by increasing the rate of fibre digestion and consequently improving the growth rates when fish fed these diets. The improvement of food conversion ratio, the best was recorded in T6 group. This may be attributed to the effect of the used prebiotics in the current study which led to decreased amount of feed necessary for producing one unit of fish leading consequently to production cost reduction. The results were in agreement with many authors (Chakrabarti *et al.,* 1995;Wong *et al*., 1996; Mazloum *et al.,* 2011 and Samuel *et al.,* 2017), where they found that FCR was improved by the addition of some prebiotics in the diet.

The results of chemical composition of the experimental fish body, average dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash, were in a partial agreement to Orban *et al.,* (2007), where they recorded that body composition was firmly influenced by their feed composition. The critical distinction in the DM and CP% among groups may be attributed to the level of feed additives in the diet and/or elevation of dietary Immunogen level. The results were similar to many previous reports (Bock *et al.,* 2007; Genc *et al.,* 2007; Yilmaz *et al*., 2007 and Goda *et al.*, 2012) and in contrast to Lara-Flores *et al.* (2003) and Dawood *et al.* (2019).

In the present study, water quality parameters observed were within the normal ranges required for normal growth of Nile tilapia (Abdelhakim *et al.,* 2002; Khalafalla and Mohsen, 2007 and Ibrahim *et al.,* 2010); consequently any changes in the growth parameters may be attributed to the level of the feed additives incorporated to the diets.

The general health status of the fish can be assessed by estimating the blood parameters which reflect the physiological reaction of fish towards various feeding additives (Dawood *et al.,* 2015).The raised number of WBCs may be explained by the improved resistance as a result of Allzyme and/or Polizyme feed supplementation. The result is similar to those reported by some authors (Ha *et al.,* 2017 and Magouz *et al*., 2019). However, it is opposed to other authors (Welker et *al*., 2007 and Ali *et al*., 2017). The differences in hematological parameters results may be attributed to fish physiological condition, age, environmental conditions, species, prebiotic type and dose and/or dietary everyday practice (Osuigwe *et al.,* 2005). Changes in haematological and serum biochemical parameters might be species-related and depend upon the fuse rates of Allzyme and Polizyme to diet ingredients and/or the raising time span period (Ta'ati *et al.,* 2011). The elevated level of total plasma proteins, albumin and globulin in prebiotic treated groups may be attributed to improved body defense, stronger innate response and fast metabolism of fish as a result of prebiotic supplementation (Andrews *et al.,* 2009, Sahoo & Mukherjee, 2001 and Dawood *et al.,* 2019). The results were in agreement to some authors (Magouz *et al.,* 2019); however, in disagreement with Andrews *et al.* (2009).

This investigation indicated that the immune response of *O. niloticus* fingerlings was generally impacted and extended in all groups with the most extreme level in group 2 and 5. Lysozyme is a champion among the most major safe responses of fish. It is starting from neutrophils and macrophages radiated into blood and mucous fluid to apply bacteriolytic effects (Saurabh & Sahoo, 2008) helping organisms to restrict parasitic, bacterial and viral diseases (Yanu, 1997). The current results exhibited that the components of lysozyme activity were essentially altered due to Allzyme and Polizyme supplementation. The highest serum lysozyme activity was seen in fish supported eating diet with 0.1% from Allzyme and Polizyme. The extended lysozymal and bactericidal activities in the present investigation may be attributed to the immune-stimulatory impacts of dietary Allzyme and Polizyme (Dawood *et al*., 2018).

CONCLUSION

It could be concluded that prebiotics supplementation of Allzyme SSF® and Polizyme® multimix FM are exceedingly valuable in *Oreochromis niloticus* fingerlings diets resulting in an expanded nutrient utilization and improving growth rate, hematological, biochemical parameters, immunological responses and survival rate. From the current outcomes, it is desirable to use Allzyme SSF® and Polizyme® multimix as feed additives at level of 0.1%, with commercial feeds to improve tilapia fingerlings production and immune response.

CONFLICT OF INTEREST

No competing interest to all authors of the current manuscript**.**

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