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IMPACT OF DIETARY PROTEIN AND AQUA-MAX PLUS® ON GROWTH, FEED EFFICIENCY, AND PHYSIOLOGICAL RESPONSES OF MONO-SEX NILE TILAPIA (*Oreochromis niloticus*, L. 1758)

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SUMMARY

This experiment was designed to estimate the effect of different levels of dietary protein with the addition of Aqua-Max Plus® (as a feed additive) on growth performance, feed utilization, the chemical composition of the whole fish body, and physiological responses of mono-sex Nile tilapia, *Oreochromis niloticus*. Six hundred of mono-sex Nile tilapia fingerlings (at average initial bodyweight 52.64 ± 5.2 g) were distributed randomly to five treatments. Experimental diets contain different levels of dietary crude protein 21, 23, 25, 27% (referred to No. T_1, T_2, T_3, and T_4, respectively) and that were compared to the control diet contains 25% without adding Aqua-Max Plus® (T_0). Results revealed that the increasing dietary protein in diets leads to an improvement in growth performance and hematological parameters as well as total protein, albumin, and globulin concentrations in blood serum. Fish fed on a diet containing 21% protein (T_1) recorded the lowest values of growth performance, feed efficiency, and hematological parameters. The addition of Aqua-Max Plus® to diets containing 23% (T_2) and 25% (T_3) crude protein enhanced growth performance and feed efficiency parameters compared to the control diet. Protein content was significantly improved, whereas fat, ash and EC contents were reduced by increasing the dietary protein. The addition of Aqua-Max Plus® to the experimental diets had no significant effect on serum biochemical parameters. Finally, the addition of Aqua-Max Plus® in the Nile tilapia diets improve the nutritional values of low protein diets, and it is a way to reduce the cost of fish feed.

***Keywords**: Nile tilapia, protein, growth, feed efficiency, physiological responses and Aqua-Max Plus®.*

INTRODUCTION

In the latest years, animal protein becomes a key problem in numerous countries like Egypt. Therefore, there are attempts to reduce the gap between protein production and animal protein demand and consumption, especially fish protein. In Egypt, aquaculture grows quickly to meet the demand for fish protein (as an animal protein source). Currently, the total fish production in Egypt increased from 724,300 tons in 2000 to 1,762,174 tons in 2016 (GAFRD, 2018). Egypt produces about 1,048,276 tons of tilapia fish, which represents 80% of total fish production in 2016 (GAFRD, 2018). This increase is a result of the intensification of production from the existing fish farms and the expansion of areas under cultivation (El-Ebiary, 2002). However, feed costs are considered the highest recurrent cost in aquaculture, being ranged from 60 to 70% of the total production cost. Fish requires high quality feeds with high protein content, where the protein was considered the most expensive nutrient in fish diets (Ai and Xie, 2005). Therefore, any reduction in feed costs through either diet development, improvement husbandry or other direct or indirect means is therefore crucial to the development and well-being of the industry (De Silva and Anderson, 1995).

Recently, there has been a concerted effort to improve the nutritive value of feedstuffs and unconventional feedstuffs by the use of several feed supplements such as dietary exogenous enzymes, probiotics, yeast, amino acids, antioxidants, betaine, carnitine, colorants, vitamins, hormones, aromatic compounds, plant extracts, yucca products, and some organic acids (Finley *et al.*, 2005). Numerous studies displayed the benefits of one or more of these supplements include improved growth-promoting factors and feed value, enzymatic contribution to digestion, and increase immune response (Lara-Flores and Olvera-Novoa, 2013; Liu *et al.*, 2014; Mehrim *et al.*, 2017; Abdel-Mohsen *et al*., 2018; Ali *et al*., 2018).

Aqua-Max plus® is a commercial product containing many components as exogenous enzymes, probiotics, zinc, and sodium butyrate. In our previous study, Farrag *et al.* (2019) recommended the beneficial addition of 2 g Aqua-Max Plus® / kg diet as a growth promoter agent for Nile tilapia. Therefore, the main objective of this study was to evaluate the effects of graded levels of dietary protein (21, 23, 25, and 27 %) with the addition of a new commercial probiotic Aqua-Max plus® at level 2 g/kg diet, regarding their growth performance, feed efficiency, the chemical composition of the fish body, blood hematological and biochemical parameters of Nile tilapia.

MATERIALS AND METHODS

The experimental management:

Six hundred of mono-sex Nile tilapia fingerlings at average initial bodyweight 52.64 ± 5.2 g were adapted for a week on experimental conditions. The trial was carried out in concrete ponds at the private farm of Yousef Assal, Al-Gamaliah, Dakahlia Governorate, Egypt. After the adaptation period, fish were distributed randomly to five treatments. Each concrete pond divided into two equal replicates (3 m^3) by a separator network where the water quality was similar within the treatment. Fish were stocked at a rate of 20 fish / m^3, (120 fish per treatment). The light period was controlled by a timer to provide a 14 h light : 10 h dark as a daily photoperiod. Approximately 20 % of the water volume of each concrete pond was changed two days per week by siphoning and replaced by new freshwater from Al-Salam Canal in Al-Gamaliah, Dakahlia Governorate, Egypt.

The experimental diet and feeding:

Aqua-Max Plus® is a commercial product of a mixture of growth promoters such as enzymes, organic acids, probiotics, and zinc, as described in Table (1). In this study, Aqua-Max Plus® was added to the experimental diets at level 2 g/kg diet according to the previous study by Farrag *et al.* (2019).

Table (1): Formulation of the experimental Aqua-Max Plus® fed to fish according to the manufacturer's configuration.

Ingredient	g per 2 kg	Effective Material	Amount
Hostazym® X	250.00	Xylenes enzyme (IU)	1500000
Hostazym p10000	50.00	Phytase enzyme (IU)	500000
Gustor Aqua BP70	500.00	Sodium butyrate $NaC_4H_5O_6$ (g)	350
Biomet zinc Aqua	75.00	Zinc (g)	1650
ECOBIOL Aqua	1000	*Bacillus amyloliguefaciens*	1×10^{12}
$CaCO_3$ was supplemented up to 2 kg			

Table (2): Details of the experimental treatments.

Treatment	Details
T_0	Diet 25% CP + 0 g Aqua-Max Plus® / kg diet (as a control)
T_1	Diet 21% CP + 2 g Aqua-Max Plus® / kg diet
T_2	Diet 23% CP + 2 g Aqua-Max Plus® / kg diet
T_3	Diet 25% CP + 2 g Aqua-Max Plus® / kg diet
T_4	Diet 27% CP + 2 g Aqua-Max Plus® / kg diet

The experimental design was shown in Table (2). The ingredients of different experimental diets were homogeneously mixed, Aqua-Max Plus® was added to experimental diets (T_1, T_2, T_3, and T_4), while it was not added to the control diet (T_0), pressed by manufacturing machine (pellets size 1 mm).

The ingredients of experimental diets and proximate chemical analysis were presented in Table (3). The experimental diets were introduced manually twice daily at 8.00 a.m. and 15.00 p.m. at 4 to 2% of the fish biomass in each replicate. Fish were weighed every two weeks to adjust their feed quantity according to the actual body weight changes.

Table (3): Formulation (g / kg) and the chemical analysis of experimental diets.

Ingredient	Dietary protein (%)			
	21	23	25	27
Fishmeal	100	100	110	120
Corn gluten	35	50	50	50
Soybean meal	60	70	90	120
DDGS[1]	80	100	120	130
Broken lentils	70	100	100	100
Wheat bran	130	120	110	100
Rice bran	100	90	80	80
Yellow corn	355	300	270	230
Oil	50	50	50	50
$CaPO_4$	5	5	5	5
Salt	7.50	7.50	7.50	7.50
Vit & min[2]	7.50	7.50	7.50	7.50
Total	1000	1000	1000	1000
Nutrient composition (% on dry matter basis)				
Dry matter (DM %)	84.67	85.32	86.29	86.21
Crude protein (CP)	21.77	23.73	25.70	27.56
Ether extract (EE)	9.77	9.28	9.09	7.96
Ash	7.53	6.47	7.41	6.74
Crude fiber	4.5	4.5	4.5	4.75
Total carbohydrates (NFE)	60.94	60.52	57.80	57.73
Gross energy (GE) (Kcal/100g DM)[3]	483.94	488.67	486.80	487.43
Protein/energy (P/E) ratio[4]	44.98	48.59	52.80	56.56

[1] *DDGS: Dried distillers grains with soluble*

[2] *Vitamins & minerals premix each 3 kg contains: Vitamin A, 10000000 IU; Vitamin D_3, 2500000 IU; Vitamin E, 20000 IU; Vitamin K_3, 3000 IU; Vitamin B_1, 200 mg; Vitamin B_2, 5000 mg; Vitamin B_6, 2000 mg; Vitamin B_{12}, 15 mg; Biotin, 60 mg; Folic acid, 1000 mg; Nicotinic acid, 30 g; Pantothenic acid, 10 g; Mn 80 g; Cu 88g; Zn 70g; Fe 100 g; Co 0.4 g; I 1 g and Se 0.3g. Calcium carbonate was supplemented up to 3 kg*

[3] *GE (Kcal/100 g DM) = CP x 5.64 + EE x 9.44 + Carbohydrates x 4.11 calculated according to* ***(Macdonald et al., 1973)****.*

[4] *P/E ratio (mg protein/Kcal gross energy) = CP/GE x 1000.*

Water quality parameters:

Water temperature, dissolved oxygen, total ammonia, total solids, and pH values were measured twice a week. The dissolved oxygen (DO, mg/l) was determined by HANNA HI 9146-04–Romania. Total ammonia (TA) concentration of the water was tested by direct Nesslerization methods using CHEMETS® test kits (CHEMETRICS, INC, USA) according to APHA (1992) every two weeks. Total solids (TS) (g/l, ‰), and pH-value were measured by Consort C860 – Belgium. Water temperature, pH, DO, TA, TS were 25.17±2.77 °C, 8.24±0.20, 7.56±0.31 mg/l, 0.01±0.001 mg/l, and 0.94±0.35 g/l, respectively. All tested water quality was appropriate for rearing Nile tilapia (*O. niloticus*).

Growth performance and feed efficiency measurements:

At the end of the experiment, fish body weight in each treatment was measured to calculate the growth performance parameters such as total weight gain (TWG, g), average weight gain (ADG, g/fish/day), relative growth rate (RGR, %), specific growth rate (SGR, %/day). Also, feed efficiency parameters like feed intake (FI, g), feed conversion ratio (FCR), feed efficiency (FE, %), protein efficiency ratio (PER), and protein productive value (PPV, %) were calculated. The weight and length of the fish body were individually measured in each treatment to calculate the condition factor (K, %) according to the following equation; K = (fish weight (g) × 100) / total length^3 (cm).

Chemical analysis of the experimental diets and fish carcass:

At the start and the end of the experiment, fish samples (*n* = 3 from each replicate) were taken and were kept frozen until chemical analysis. The chemical analysis of diet and fish samples were carried out according to the AOAC (2004).

Blood sampling and analytical methods:

At the end of the experiment, blood samples (*n* = 5 for each treatment) were randomly taken and received in plastic tubes. Blood samples were collected and transferred by centrifugation for 15 minutes at 3500 rpm to obtain blood serum. Serum samples were kept in the deep freezer until the biochemical examination. Serum samples were used for the definition of creatinine (Tietz, 1986), triglycerides (McGowan *et al.*, 1983), total protein (Tietz, 1990) and albumin (Wotton and Freeman, 1982), as well as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities by marketable test kits (Humalyzer 3000 manufactured by Human, Germany. Globulin level was calculated by subtracting albumin from total protein.

The other blood samples were used to test the blood hematology as hemoglobin (Hb), total erythrocytes (RBCs), total leukocytes (WBCS) (Natt and Herrick, 1952) and hematocrit (Hct) (Decie and Lewis, 2006). The hematological indices such as mean cell volume (MCV), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) were calculated according to (Dacie and Lewis, 1995).

Statistical analysis:

All data were statistically analyzed using one-way analysis of variance (ANOVA) procedure according to SAS (2006), with one-way analysis. All ratios and percentages were arcsine-transformed prior to statistical analyses. All mean were statistically compared for the significance ($P \leq 0.05$) using multiple range test (Duncan, 1955), evaluated by using the following model:

$$Yij = \mu + Ti + eij$$

Where; Yij = the data of growth performance, feed efficiency, body chemical composition and blood hematological and biochemical parameters; μ = the overall mean; Ti = the fixed effect of treatments and eij= is the random error.

RESULTS AND DISCUSSION

Growth performance parameters:

Growth performance parameters of Nile tilapia fed on different dietary protein levels were obtained in Table (4). The raising dietary protein levels in diets improved in growth performance parameters. Fish fed on a diet containing 21% protein (T_1) recorded the lowest values of growth performance parameters ($P \leq 0.05$). The addition of Aqua-Max Plus® to diets containing 23% (T_2) and 25% (T_3) crude protein enhanced FW, TWG, ADG, and SGR compared to the control treatment (T_0; contained 25% without Aqua-Max Plus®).

The increase of dietary protein levels from 23% to 27% improved all growth performance parameters. Moreover, the addition of Aqua-Max Plus® results in a positive effect on fish growth, especially the diet contains 23% crude protein, which gave the same results as those of fish fed 25% crude protein (the control group). The beneficial effects of Aqua-Max Plus® maybe containing considered growth promoters such as sodium butyrate, zinc, and probiotics. Several studies showed the positive effect of one or more of these substances on growth performance such as probiotic (Eid and Mohamed, 2008; Lara-Flores and Olvera-Novoa, 2013; Mehrim *et al.*, 2017), sodium butyrate (Liu *et al.*, 2014; Abdel-Mohsen *et al*., 2018; Ali *et al*., 2018). In addition, probiotics had a positive effect on growth caused by the best use of carbohydrates, protein, and energy (Irianto and Austin, 2002), which improved growth performance and reduced feed cost (Eid and Mohamed, 2008). Likewise, sodium butyrate can improve feed digestibility, especially for proteins and amino acids (Hoseinifar *et al.*, 2017), increasing the availability of several essential amino acids and nucleotide derivatives (Robles *et al.*, 2013), which reflected to enhance fish growth and survival.

Table (4): Effect of dietary protein (%) with added Aqua-Max Plus® on growth performance parameters of Nile tilapia.

Treatment	FW (g)	TWG (g)	ADG (g/fish/day)	RGR (%)	SGR (%/day)	K (%)
T_0	176.5^{c} ±0.17	123.8^{c} ±0.17	0.842^{c} ±0.00	236.4^{c} ±0.20	1.034^{c} ±0.00	1.819^{c} ±0.04
T_1	167.5^{e} ±0.15	114.8^{e} ±0.15	0.781^{e} ±0.00	246.9^{b} ±0.12	0.989^{e} ±0.00	1.807^{c} ±0.03
T_2	175.9^{c} ±0.03	123.2^{c} ±0.00	0.838^{c} ±0.00	254.8^{a} ±0.00	1.031^{c} ±0.00	1.884^{b} ±0.02
T_3	190.2^{b} ±0.03	137.5^{b} ±0.03	0.936^{b} ±0.00	254.9^{a} ±0.23	1.098^{b} ±0.00	1.957^{ab} ±0.04
T_4	196.7^{a} ±0.15	144.0^{a} ±0.12	0.980^{a} ±0.00	255.3^{a} ±0.38	1.126^{a} ±0.00	2.020^{a} ±0.05

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). FW: Final weight; TWG: Total weight gain; ADG: Average daily gain; RGR: Relative growth rate; SGR: Specific growth rate; K: condition factor.

Feed efficiency parameters:

Feed efficiency parameters of Nile tilapia were presented in Table (5). Increasing dietary protein levels enhanced FI, FCR, and FE in treatments (T_2, T_3, and T_4). Fish fed on a diet containing 21% protein (T_1) recorded the lowest values of feed efficiency parameters ($P \leq 0.05$). Fish fed on diets containing 23% (T_2) gave the same values as those for the control group (T_0), without adding Aqua-Max Plus®. T_2 recorded the significant highest values of PER and PPV compared to other treatments ($P \leq 0.05$). Results showed that feed efficiency parameters such as FI, FCR, and FE gave the best values ($P \leq 0.05$) in fish fed on the diet containing 25% with Aqua-Max Plus® (T_3) compared to the control treatment (contain 25% CP only).

Table (5): Effect of dietary protein (%) with added Aqua-Max Plus® on feed efficiency parameters of Nile tilapia.

Treatment	FI (g)	FCR	FE (%)	PER	PPV (%)
T_0	265.5^{c} ±0.12	2.143^{b} ±0.00	46.65^{c} ±0.04	1.815^{d} ±0.00	23.06^{d} ±0.02
T_1	256.2^{e} ±0.33	2.230^{a} ±0.00	44.82^{d} ±0.01	1.587^{e} ±0.00	20.22^{e} ±0.30
T_2	264.0^{c} ±0.71	2.140^{b} ±0.01	46.68^{c} ±0.12	1.969^{a} ±0.01	23.94^{a} ±0.08
T_3	273.3^{b} ±0.58	1.987^{c} ±0.00	50.32^{b} ±0.10	1.958^{b} ±0.00	24.51^{c} ±0.05
T_4	279.3^{a} ±0.36	1.940^{d} ±0.00	51.56^{a} ±0.02	1.874^{c} ±0.00	26.42^{b} ±0.01

Mean in the same column having different small letters are significantly different ($P \leq 0.05$). FI: feed intake; FCR: feed conversion ratio; FE: feed efficiency; PER: protein efficiency ratio; PPV: protein productive value.

The addition of exogenous enzymes in fish feed can be an alternative to increase the utilization of nutrients coming from plant ingredients. Exogenous enzymes increase the digestibility, reduces a load of pollutants from waste, and decrease production cost. Several scientific papers exhibited the benefits of using enzymes in fish diets for aquatic animals (Ai *et al.*, 2007; Filer and Shafer, 2011; Jiang *et al*., 2014). The results reported herein exhibited improvement utilization of dietary nutrients, especially dietary protein by adding Aqua-Max Plus®, where it raised the nutritional value of the 23% CP diet to an equal value as 25% CP diet. The same trend was observed in fish fed on the 25% diet by adding Aqua-Max Plus®/kg. The improvement in the nutritional value of low protein diets may be due to the combined effect of exogenous enzymes (phytase and xylanase), probiotic, and sodium butyrate that improve the nutritional value of low protein diets and appeared in increased PER and PPV. Xylanase supplementation improves conjugated bile acid function in intestinal contents and increases villus size of the small intestine wall (Bar *et al*., 2012), improving the activities of trypsin, chymotrypsin, lipase, and amylase in the hepatopancreas and intestine (Jiang *et al*., 2014), which enhanced diet nutritional value. This affirmative effect of active substances containing in Aqua-Max Plus® was observed in many studies by Jiang *et al.* (2014) and Ai *et al.* (2007) using exogenous enzymes; Liu *et al.* (2014) on sodium butyrate, and Ahmed and Sadek (2015) on probiotic.

Chemical composition of the fish body:

Dietary protein levels and Aqua-Max Plus® in the diet had a great effect on DM, ash, protein, fat, and EC contents in the fish body of the Nile tilapia (Table 6). Increasing the dietary protein levels, was significantly increased protein content, while fat, ash and EC contents were significantly reduced ($P \leq 0.05$). DM and Fat contents of T_2 were the significant highest values compared with other treatments ($P \leq 0.05$).

Table (6): Effect of dietary protein (%) with added Aqua-Max Plus® on chemical composition of the fish body of Nile tilapia.

Treatment	DM (%)	On dry matter basis			
		Ash (%)	Fat (%)	Protein (%)	EC (Kcal/100g)
T_0	21.67^{b} ±0.13	23.45^{a} ±0.54	12.12^{cd} ±0.17	62.74^{b} ±0.35	475.2^{d} ±3.23
T_1	23.62^{a} ±0.11	21.85^{b} ±0.09	13.70^{b} ±0.15	62.87^{b} ±0.32	490.4^{b} ±0.58
T_2	23.95^{a} ±0.73	20.65^{c} ±0.01	14.64^{a} ±0.09	62.60^{b} ±0.38	500.0^{a} ±0.99
T_3	21.08^{b} ±0.16	21.99^{b} ±0.55	12.61^{c} ±0.19	63.54^{ab} ±0.28	485.0^{bc} ±3.15
T_4	22.51^{ab} ±1.07	21.89^{b} ±0.29	11.89^{d} ±0.26	64.52^{a} ±0.58	483.1^{c} ±0.78

Mean in the same column having different small letters are significantly different ($P \leq 0.05$); DM= Dry matter; EC= Energy content.

The positive effect of increasing dietary protein levels and Aqua-Max Plus® on the chemical composition of the fish body was confirmed by their positive effects on fish growth performance (Table 4), and feed intake and nutrient utilization (Table 5). These results agree with those obtained by Khalil *et al.* (2015) who reported that fish fed dietary 25% CP level was significantly higher in DM and CP contents and with reduction of EE and ash contents in their carcass compared to a diet containing 20% CP. These changes in protein and lipid content in the fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rates (Abdel-Tawwab *et al.*, 2006). Similar results were observed by Khattab *et al.* (2004a). The addition of Aqua-Max Plus® to low protein diets (23% CP) results in increased DM and fat contents with non-significant effect in protein content. These changes in the chemical composition of the fish body maybe attributed to Aqua-Max Plus® content on *Bacillus amyloliguefaciens* (as probiotics). Numerous studies have shown that probiotics have an affirmative effect on the chemical composition of the fish body (Eid and Mohamed, 2008; Mehrim, 2009) In contrast, some recent studies indicated that the dietary sodium butyrate supplementation level showed no significant effect on the body contents of moisture, lipid, protein, and ash of grass carp (Liu *et al*., 2014) and Nile tilapia (Ali *et al*., 2018).

Hematological parameters:

Summarized in Table (7) are the effects of dietary protein levels and Aqua-Max Plus® on hematological parameters of Nile tilapia. By growing the levels of dietary protein, RBCs count, Hb, PVC, and WBCs count were significantly augmented, while MCHC was declined ($P \leq 0.05$). T_1 (21% CP + Aqua-Max Plus®) reported the significant lowest values of RBCs count, Hb, PVC, and WBCs, and significant highest values of MCV and MCH compared with other treatments ($P \leq 0.05$). No significant differences among treatments in platelet counts were detected ($P \geq 0.05$).

Monitoring fish health can be done using the hematological and biochemical profile of its blood (De-Pedro *et al*., 2005). Increasing the dietary protein levels caused significant augmentation of hematological parameters such as RBCs count, Hb, PVC, and WBCs count. Furthermore, the addition of Aqua-Max Plus® improved hematological parameters of low dietary protein (23% CP and 25% CP) compared to the high dietary protein (27% CP). This indicates an enhancement of the fish health with increased dietary protein levels and/or adding of Aqua-Max Plus®, which were reflected to improve growth performance and feed efficiency of Nile tilapia. Khattab *et al.* (2004b) reported the same positive effects of testing probiotic on fish health. Reda and Selim (2015) indicated that the use of *B. amyloliquefaciens* as a feed supplement has a beneficial effect on Nile tilapia. Aqua-Max Plus® contains *B. amyloliquefaciens,* sodium butyrate and zinc, which may have a positive effect on hematological parameters in Nile tilapia fish (El-Saidy *et al.,* 2012; Abdel-Mohsen *et al*., 2018).

Table (7): Effect of dietary protein (%) with added Aqua-Max Plus® on blood hematological of Nile tilapia.

Treatment	RBCs ($\times10^6$/mm^3)	Hb (g/dL)	PCV (%)	Platelets ($\times10^3$/mm^3)	Blood indices			WBCs
					MCV (µ3)	MCH (pg)	MCHC (%)	($\times10^3$/mm^3)
T_0	1.678^a ±0.07	8.620^a ±0.11	35.63^b ±3.63	39.50 ±1.91	179.1^b ±4.34	51.67^c ±2.44	26.86^a ±0.84	44.63^a ±0.63
T_1	1.273^c ±0.06	7.533^b ±0.21	34.26^b ±1.13	33.70 ±1.18	258.5^a ±4.92	59.66^a ±3.67	23.11^{ab} ±0.89	36.13^b ±0.18
T_2	1.500^b ±0.02	8.380^a ±0.34	40.05^a ±2.64	39.63 ±1.94	267.5^a ±19.4	58.60^{ab} ±1.05	21.21^b ±1.74	46.13^a ±0.17
T_3	1.730^a ±0.03	8.933^a ±0.22	42.85^a ±2.85	38.25 ±3.65	247.2^a ±12.5	51.63^c ±0.72	21.03^b ±0.94	44.83^a ±0.47
T_4	1.780^a ±0.03	9.030^a ±0.26	42.05^a ±4.21	37.10 ±2.51	259.4^a ±13.2	52.89^{bc} ±0.51	21.97^b ±1.66	44.43^a ±1.70

Mean in the same column having different small letters are significantly different ($P \le 0.05$). RBCs: Red blood cells; Hb: Hemoglobin; PCV: Packed cell volume; Platelets: Blood platelets; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBCs: White blood cells

Serum biochemical parameters:

The effect of dietary protein (%) with added Aqua-Max Plus® on serum biochemical parameters of *O. niloticus* was presented in Table (8). No significant differences among treatments in the activates of ALT and AST and concentration of TCH and TG were detected ($P \ge 0.05$).

Total protein, albumin, and globulin concentrations were significantly augmented with increasing dietary protein ($P \le 0.05$). The addition of Aqua-Max Plus® to the experimental diets showed no significant effect on serum biochemical parameters ($P \ge 0.05$).

Hematological and biochemical changes in the blood are important indicators used in monitoring physiological and pathological changes in fish (Satheeskumar *et al*., 2012). Dietary protein level or Aqua-Max Plus® has no effect on the activities of AST and ALT, and TCH and TG. This is inconsistent with the results obtained by Abdel-Tawwab *et al.* (2010) who found that hematological variables were significantly affected by dietary protein level, fish weight, and their interactions except for serum lipids, which was not significantly affected by the interactions. Activities of AST and ALT in serum, liver, and muscles were significantly affected by the dietary protein level and fish weight (Abdel-Tawwab *et al.,* 2010). However, no significant differences were detected in plasma total protein, plasma albumin and plasma total globulins of *O. niloticus* fed on Biogen® and Pronifer® (as probiotics) (Eid and Mohamed, 2008). The same trend was observed in Nile tilapia fed diets containing Biogen® or sodium butyrate, where the activities of serum enzymes alanine aminotransferase and aspartate aminotransferase were not significantly affected by dietary treatments (Ali *et al.,* 2018).

Table (8): Effect of dietary protein (%) with added Aqua-Max Plus® on serum biochemical parameters of Nile tilapia.

Parameter	Treatment				
	T_0	T_1	T_2	T_3	T_4
ALT (IU/L)	36.33 ±1.24	35.1 ±1.72	36.23 ±0.70	35.67 ±0.64	34.03 ±0.88
AST (IU/L)	132.5 ±7.86	150.6 ±5.39	145.6 ±6.76	146.6 ±3.57	149.8 ±9.10
Total Cholesterol (mg/ dL)	149.6 ±4.06	146 ±2.48	153.3 ±7.26	160.7 ±2.32	157.5 ±9.71
Triglyceride (mg/ dL)	163 ±10.62	147.3 ±5.50	153 ±10.93	168 ±6.67	161 ±6.48
HDL (mmol/L)	64.67[a] ±1.43	46.75[b] ±2.78	47.33[b] ±4.17	58.67[a] ±1.55	61.67[a] ±2.25
LDL (mmol/L)	55.38[b] ±0.65	73.87[a] ±1.41	75.40[a] ±4.61	72.18[a] ±2.64	57.84[b] ±5.31
Albumin (g / dL)	3.680[bc] ±0.03	2.910[d] ±0.02	3.460[c] ±0.13	3.900[b] ±0.15	4.213[a] ±0.06
Globulin (g / dL)	1.838[b] ±0.03	1.310[d] ±0.04	1.613[c] ±0.07	1.900[b] ±0.07	2.243[a] ±0.06
Total protein (g / dL)	5.518[b] ±0.02	4.220[d] ±0.04	5.073[c] ±0.14	5.623[b] ±0.12	6.455[a] ±0.10
AL/GL ratio	2.005[ab] ±0.05	2.230[a] ±0.08	2.158[a] ±0.12	2.050[ab] ±0.02	1.883[b] ±0.05

Mean in the same row having different small letters are significantly different ($P \leq 0.05$). ALT: Alanine aminotransferase; AST: serum aspartate aminotransferase; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; Al / Gl ratio = albumin / globulin ratio.

CONCLUSION

Generally, results exhibited the importance of using the Aqua-Max Plus® (as a feed additive) to improve the nutritional values of low protein diets (23% CP), which is similar to 25% CP (without adding Aqua-Max Plus®) in growth performance, feed efficiency, chemical composition, and physiological responses of fish, and it is a way to reduce the cost of fish feed. Hence, further studies are required to evaluate the effects of Aqua-Max Plus® on using different plant protein sources, as well as study the changes in digestive enzymes and microbiology of the intestine.

REFERENCES

Abdel-Mohsen, H.H.; E.A. Wassef; N.M. El-Bermawy; N.E. AbdelMeguid; N.E. Saleh; K.M. Barakat and O.E. Shaltout (2018). Advantageous effects of dietary butyrate on growth, immunity response, intestinal microbiota and histomorphology of European Seabass (*Dicentrarchus labrax*) fry. Egypt. J. Aquat. Biol. & Fish., 22 (4): 93- 110.

Abdel-Tawwab, M.; M.H. Ahmad; Y.A. Khattab and A.M. Shalaby (2010). Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *O. niloticus*. Aquaculture, 298 (3-4): 267-274.

Abdel-Tawwab, M.; H.I. El-Marakby and M.H. Ahmad (2006). Cannibalism in *Oreochromis niloticus* (Linnaeus): effect of stocking density, feed quality and submerged macrophytes. Indian J. Fish., 53: 245-251.

Ahmed, H. A. K. M. and Sadek (2015). Impact of dietary supplementation of sodium butyrate and/or protexin on the growth performance, some blood parameters, and immune response of *Oreochromis niloticus*. Soc. For Anim. Manag., 85-101.

Ai, Q.; K. Mai; W. Zhang; W. Xu; B. Tan; C. Zhang and H. Li (2007). Effects of exogenous enzymes (phytase, non-starch polysaccharide enzyme) in diets on growth, feed utilization, nitrogen and phosphorus excretion of Japanese seabass, *L. japonicus*. Comparative Bioc. and Phys. Part A: Molecular & Integrative Phys., 147 (2): 502-508.

Ai, Q. and X. Xie (2005). Effects of replacement of fish meal by soybean meal and supplementation of methionine in fish meal/soybean meal-based diets on growth performance of the southern catfish *silurus meridionalis*. J. World Aquac. Soc., 36 (4): 498-507.

Ali, T.E.S.; A.M. El-Sayed; M.A.R. Eissa and H.M. Hanafi (2018). Effects of dietary Biogen and sodium butyrate on hematological parameters, immune response, and histological characteristics of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquacult. Int., 26 (1): 139-150.

AOAC (2004). Official Methods of Analysis of the AOAC. 18th ed. W. Horwitz (Ed.). Association of Official Analytical Chemist Official.

APHA (1992) American Public Health Association. Standard methods for the examination of water and waste water, 18th ed., Washington, DC. pp: 1268.

Bar, N.; S.K. Mukhopadhayay; S. Ganguly; S. Pradhan; N.C. Patra; S. Pal and S. Halder (2012). Study on probiotic effect of xylanase supplementation in broiler feed. Indian J. Anim. Nutr., 29 (1): 100-103.

Dacie, J.V. and S.M. Lewis (1995). Practical haematology. 8th ed. Churchill Livingstone, Edinburgh.

De Pedro, N.; A.I. Guijarro; M.A. López-Patiño; R. Martínez-Álvarez and M.J. Delgado (2005). Daily and seasonal variations in hematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. Aquac. Res., 36 (12): 1185-1196.

De Silva, S.S. and T.A. Anderson (1995). Fish nutrition in aquaculture Ed., Champ man and Hall, 2-6 Bouday Raw, London SEI8 FIN, UK.

Decie, S.I.V. and S.M. Lewis (2006). Practical hematology. 10th Edn., Churchill Livingstone, London. ISBN: 13: 978- 443, PP: 736.

Duncan, D.B. (1955). Multiple ranges and multiple F-tests. Biometrics, 11:1-42.

Eid, A. and K.A. Mohamed (2008). Effect of using probiotic as growth promoter in commercial diets for monosex Nile tilapia (*Oreochromis niloticus*) fingerlings. 8th International Symposium on Tilapia in Aquaculture, Cairo, Egypt, 12-14 Oct., pp: 241-253.

El-Ebiary, E.H. (2002). Modern technology for intensification of fish culture and its environ-mental impact. Report of a workshop held in Ismailia, Suez Canal Uni., 1-2 July 2002.

El-Saidy, D.M.; M.A. El-Kashef; M.A. Wafa and A.M. El-Hais (2012). Effect of zinc supplementation on the growth performance, feed utilization, body composition and hematological parameters of Nile tilapia, *Oreochromis niloticus* (L.). Egypt. J. Nut. and Feeds, 15 (3): 671-679.

Farrag, F.H.; F.F. Khalil; M.M. Refaey; A.A. El-Shebly and A.M. Behery (2019). Effect of different levels of Aqua-Max Plus® on growth performance, feed efficiency, and physiological responses of mono-sex Nile tilapia (*Oreochromis niloticus*, L. 1758). Egypt. J. Aquat. Biol. & Fish., (In press)

Filer, K. and Shafer B. (2011). Evaluation of a natural enzyme complex on performance of Nile tilapia fed diets with animal or plant protein sources. In: Aquaculture America, New Orleans. Anais. New Orleans: World Aquaculture Society, 2011. p.59.

Finley, W., J. Fry, and N. Peacock (2005). Feeding the growth in aquaculture to 2015: the scope for novel 212 GODA nutritional products. Nusa Dua, Bali, Indonesia. World Aquaculture Society, The Annual International Meeting Conference, May 9–13.

GAFRD (2018). General Authority for Fishery Resources Development. Fish Statistics Yearbook 2016. Ministry of Agriculture and Land Reclamation, Egypt.

Hoseinifar, S.H.; Y.Z. Sun and C.M. Caipang (2017). Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view. Aquac. Res., 48 (4): 1380-1391.

Irianto, A. and B. Austin (2002). Probiotics in aquaculture. J. Fish. Dis., 25 (11): 633-642.

Jiang, T.T.; L. Feng; Y. Liu; W.D. Jiang; J. Jiang; S.H. Li and X.Q. Zhou (2014). Effects of exogenous xylanase supplementation in plant protein-enriched diets on growth performance, intestinal enzyme activities and microflora of juvenile Jian carp (*Cyprinus carpio* var. J ian). Aquac. Nutr., 20 (6): 632-645.

Khalil, F.F.; A.I. Mehrim; M.M. Refaey and M.M. Ghanem (2015). Impact of dietary protein and Hydroyeast Aquaculture® levels on growth, feed efficiency, physiological, and histometric responses of *Oreochromis niloticus* fingerlings. Egypt. J. Nut. and Feeds, 18 (1): 151-164.

Khattab, Y.A.; M. Abdel-Tawwab and M.H. Ahmad (2004a). Effect of protein level and stocking density on growth performance, survival rate, feed utilization and body composition of Nile tilapia fry (*Oreochromis niloticus* L.). In Proceedings of the Sixth International Symposium on Tilapia in Aquaculture (pp. 264-276).

Khattab, Y.A.; A.M.E. Shalaby; S.M. Sharaf; H.I. El-Marakby and E.H. Rizkalla (2004b). The physiological changes and growth performance of the Nile tilapia *Oreochromis niloticus* after feeding with Biogen® as growth promoter. Egypt, J. Aquat. Bio. And Fish., 8: 145-158.

Lara-Flores, M. and M.A. Olvera-Novoa (2013). The use of lactic acid bacteria isolated from intestinal tract of Nile tilapia (*Oreochromis niloticus*), as growth promoters in fish fed low protein diets. Lat. Am. J. Aquat. Res., 41(3): 490-497.

Liu, W.; Y. Yang; J. Zhang; D.M. Gatlin; E. Ringø and Z. Zhou (2014). Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (*Cyprinus carpio*) pre-fed with or without oxidized oil. Br. J. Nutr., 112 (1): 15-29.

Macdonald, P.; R.A. Edwards and J.F.D. Greenhalgh (1973). Animal Nutrition, 2nd Ed., Longman, London.

McGowan, M.W.; J.D. Artiss; D.R. Standbergh and B.A. Zak (1983). Peroxidase-coupled method for colorimetric determination of serum triglycerides. Clin. Chem., 29: 538.

Mehrim, A.I. (2009). Effect of dietary supplementation of Biogen® (Commercial probiotic) on mono-Sex Nile tilapia in *Oreochromis niloticus* under different stocking densities. J. Fish. Aquat. Sci, 4(6): 261-273.

Mehrim, A.I.; F.F. Khalil; M.M. Refaey and A.M. Abdalqadir (2017). Impacts of Pro-Grow® probiotic on water quality, growth and feed efficiency, body composition, physiological, immune responses and economic efficiency of adult *Oreochromis niloticus* (Linnaeus, 1758) under stocking density stress. Sci. Int., 5: 113 – 126.

Natt, M.P. and C.A. Herrick (1952). A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poult. Sci., 31: 735–738.

Reda, R.M. and K.M. Selim (2015). Evaluation of *Bacillus amyloliguefaciens* on the growth performance, intestinal morphology, hematology and body composition of Nile tilapia, *Oreochromis niloticus*. Aquacult. Int., 23 (1): 203-217.

Robles, R.; A.B. Lozano; A. Sevilla; L. Márquez; W. Nuez-Ortin and F.J. Moyano (2013). Effect of partially protected butyrate used as feed additive on growth and intestinal metabolism in sea bream (*Sparus aurata*). Fish Physiol. Biochem., 39 (6): 1567-1580.

SAS (2006). SAS statistical guide for personal computer, SAS Institute Inc. Cary, NC.

Satheeshkumar, P.; G. Ananthan; D.S. Kumar and L. Jagadeesan (2012). Hematology and biochemical parameters of different feeding behavior of teleost fishes from *Vellar estuary*, India. Comp. Clin. Path., 21 (6): 1187-1191.

Tietz, N.W. (1986). Textbook of Clinical Chemistry. W.B. Saunders, Philadelphia, 1271.

Tietz, N.W. (1990). Clinical Guide to Laboratory Tests 2nd ed. Philadelphia.

Wotton, I.D. and H. Freeman (1982). Microanalysis in Medical Biochemistry. Churchill, New York, USA.

تأثير البروتين الغذائي والأكواماكس بلاس على النمو وكفاءة التغذية والاستجابات الفسيولوجية لأسماك البلطي النيلي وحيد الجنس

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تهدف هذه الدراسة إلى تقييم تأثير مستويات مختلفة من البروتين الغذائي مع إضافة منتج الأكواماكس بلاس (كإضافة غذائية) على أداء النمو، وكفاءة التغذية، والتركيب الكيميائي لجسم الاسماك، والاستجابات الفسيولوجية للأسماك البلطي النيلي وحيد الجنس. تم توزيع ستمائة من أصبعيات البلطي النيلى وحيد الجنس (بمتوسط وزن ابتدائي 52.64 ± 5.2 جم) بشكل عشوائي في خمسة معاملات تجريبية. احتوت العلائق التجريبية على مستويات مختلفة من البروتين الغذائي الخام 21 و23 و25 و27٪ (والمشار إليها بأرقام معاملات الاولى والثانية والثالثة والرابعة على التوالي) والتي تمت مقارنتها مع عليقة الكنترول والمحتوية على 25 ٪ بدون إضافة الأكواماكس بلاس. أظهرت النتائج أن زيادة البروتين الغذائي في العلائق التجريبية يؤدي إلى تحسن في أداء النمو والصفات الهيماتولوجية وكذلك تركيز كل من البروتين الكلى والألبيومين والجلوبيولين في سيرم الدم. حيث سجلت الأسماك التي تغذت على عليقة بها 21٪ بروتين خام أدنى القيم لأداء النمو وكفاءة التغذية، والصفات الهيماتولوجية. أدت إضافة الأكواماكس بلاس إلى العلائق التجريبية التي تحتوي على 23٪ و25٪ بروتين خام إلى تعزيز في مقاييس أداء النمو والكفاءة الغذائية والصفات الهيماتولوجية مقارنة بعليقه الكنترول. أدت زيادة البروتين الغذائي الى تحسين محتوى البروتين، وتقليل محتوى الدهون والرماد والطاقة في جسم الأسماك. ومع ذلك، فإن إضافة الأكواماكس بلاس إلى العلائق التجريبية لم تظهر أي تأثير على الصفات الكيماوية في الدم. أخيرًا، يمكن أن نستنتج أن إضافة الأكواماكس بلاس في علائق أسماك البلطي النيلي أدت إلى تحسن في القيمة الغذائية للعلائق منخفضة البروتين. لذلك، يمكن التوصية باستخدام الأكواماكس بلاس في علائق أسماك البلطي النيلي كوسيلة لتقليل تكلفة أعلاف الأسماك.