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. T1, T2, T3, and T4, respectively) and that were compared to the control diet contains 25% without adding Aqua-Max Plus® (T0). 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 (T1) recorded the lowest values of growth performance, feed efficiency, and hematological parameters. The addition of Aqua-Max Plus® to diets containing 23% (T2) and 25% (T3) 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 m³) by a separator network where the water quality was similar within the treatment. Fish were stocked at a rate of 20 fish / m³, (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,H,O₆ (g) | 350        |
| Biomet zinc Aqua     | 75.00      | Zinc (g)                 | 1650       |
| ECOBIOL Aqua         | 1000       | Bacillus amyloliquefaciens | 1×10¹²    |

CaCO₃ was supplemented up to 2 kg

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Table (2): Details of the experimental treatments.

| Treatment | Details                       |
|-----------|-------------------------------|
| T₀        | Diet 25% CP + 0 g Aqua-Max Plus® / kg diet (as a control) |
| T₁        | Diet 21% CP + 2 g Aqua-Max Plus® / kg diet |
| T₂        | Diet 23% CP + 2 g Aqua-Max Plus® / kg diet |
| T₃        | Diet 25% CP + 2 g Aqua-Max Plus® / kg diet |
| T₄        | 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 (T₁, T₂, T₃, and T₄), while it was not added to the control diet (T₀), 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          | 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)

| Nutrient            | 21    | 23    | 25    | 27    |
|---------------------|-------|-------|-------|-------|
| 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; 

\[ K = \frac{(\text{fish weight (g)} \times 100)}{\text{total length}^3 (\text{cm})} \]
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 ≤ 0.05) using multiple range test (Duncan, 1955), evaluated by using the following model:

\[ Y_{ij} = \mu + T_i + e_{ij} \]

Where; \( Y_{ij} \) = the data of growth performance, feed efficiency, body chemical composition and blood hematological and biochemical parameters; \( \mu \) = the overall mean; \( T_i \) = the fixed effect of treatments and \( e_{ij} \) = 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 (T1) recorded the lowest values of growth performance parameters \( (P \leq 0.05) \). The addition of Aqua-Max Plus® to diets containing 23% (T2) and 25% (T3) crude protein enhanced FW, TWG, ADG, and SGR compared to the control treatment (T0; 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 (%) |
|-----------|--------|---------|------------------|---------|-------------|-------|
| T₀        | ±17.6  | ±13.8  | ±8.42            | ±26.4   | ±1.034      | ±1.819|
| T₁        | ±0.17  | ±0.08  | ±0.00            | ±0.20   | ±0.00       | ±0.04 |
| T₂        | ±17.5  | ±14.8  | ±7.81            | ±24.9   | ±0.989      | ±1.807|
| T₃        | ±0.15  | ±0.15  | ±0.00            | ±0.12   | ±0.00       | ±0.03 |
| T₄        | ±17.9  | ±12.3  | ±0.83            | ±25.4   | ±1.031      | ±1.884|
| T₅        | ±0.03  | ±0.00  | ±0.00            | ±0.23   | ±0.00       | ±0.02 |
| T₆        | ±190.2 | ±137.5 | ±9.36            | ±25.4   | ±1.098      | ±1.957|
| T₇        | ±0.03  | ±0.03  | ±0.00            | ±0.23   | ±0.00       | ±0.04 |
| T₈        | ±196.7 | ±144.0 | ±9.80            | ±25.3   | ±1.126      | ±2.020|
| T₉        | ±0.15  | ±0.12  | ±0.00            | ±0.38   | ±0.00       | ±0.05 |

*Mean in the same column having different small letters are significantly different (P ≤ 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 (T₂, T₃, and T₄). Fish fed on a diet containing 21% protein (T₀) recorded the lowest values of feed efficiency parameters (P ≤ 0.05). Fish fed on diets containing 23% (T₁) gave the same values as those for the control group (T₀), without adding Aqua-Max Plus®. T₀ recorded the significant highest values of PER and PPV compared to other treatments (P ≤ 0.05). Results showed that feed efficiency parameters such as FI, FCR, and FE gave the best values (P ≤ 0.05) in fish fed on the diet containing 25% with Aqua-Max Plus® (T₄) 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 (%) |
|-----------|--------|-----|--------|-------|---------|
| T₀        | ±265.5 | ±12 | ±143   | ±46.65| ±1.815  | ±23.06 |
| T₁        | ±256.2 | ±0.00| ±230   | ±44.82| ±1.587  | ±20.22 |
| T₂        | ±0.33  | ±0.00| ±0.01  | ±1.08 | ±0.08   | ±0.30 |
| T₃        | ±264.0 | ±0.71| ±140   | ±46.68| ±1.969  | ±23.94 |
| T₄        | ±273.3 | ±0.58| ±1.987 | ±50.32| ±1.958  | ±24.51 |
| T₅        | ±279.3 | ±0.58| ±1.940 | ±51.56| ±1.874  | ±26.42 |
| T₆        | ±0.36  | ±0.00| ±0.02  | ±0.00 | ±0.05   | ±0.01 |

*Mean in the same column having different small letters are significantly different (P ≤ 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 ≤ 0.05). DM and Fat contents of T2 were the significant highest values compared with other treatments (P ≤ 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 (%) | Ash (%) | Fat (%) | Protein (%) | EC (Kcal/100g) |
|-----------|--------|---------|---------|-------------|----------------|
| T0        | 21.67b | 23.45a  | 12.11cd | 62.74b      | 475.2d         |
| T1        | ±0.13  | ±0.54   | ±0.17   | ±0.35       | ±3.23          |
| T2        | 23.62a | 21.85b  | 13.70b  | 62.87b      | 490.4b         |
| T3        | ±0.11  | ±0.09   | ±0.15   | ±0.32       | ±0.58          |
| T4        | 23.95a | 20.65c  | 14.64a  | 62.60b      | 500.0c         |
| T5        | ±0.73  | ±0.01   | ±0.09   | ±0.38       | ±0.99          |
| T6        | 21.08b | 21.99b  | 12.61c  | 63.54ab     | 485.0bc        |
| T7        | ±0.16  | ±0.55   | ±0.19   | ±0.28       | ±3.15          |
| T8        | 22.51ab| 21.89b  | 11.89d  | 64.52a      | 483.1c         |
| T9        | ±1.07  | ±0.29   | ±0.26   | ±0.58       | ±0.78          |

Mean in the same column having different small letters are significantly different (P ≤ 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 amyloliquefaciens (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 ≤ 0.05). T1 (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 ≤ 0.05). No significant differences among treatments in platelet counts were detected (P ≥ 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 (×10⁶/mm³) | Hb (g/dL) | PCV (%) | Platelets (×10³/mm³) | MCV (µm³) | MCH (pg) | MCHC (%) | WBCs (×10³/mm³) |
|-----------|-----------------|-----------|---------|---------------------|-----------|---------|----------|----------------|
| T₀        | 1.678ᵃ          | 8.620ᵃ    | 35.63ᵇ  | 39.50               | 179.₁ᵇ    | 51.67ᵇ  | 26.86ᵃ   | 44.63ᵃ        |
|           | ±0.07           | ±0.11     | ±3.63ᵇ  | ±1.91               | ±4.34ᵇ    | ±2.44ᵇ  | ±0.84ᵇ   | ±0.63ᵇ        |
| T₁        | 1.273ᶜ          | 7.533ᵇ    | 34.26ᵇ  | 33.70               | 258.₅ᵃ    | 59.66ᵇ  | 23.11ᵇ   | 36.13ᵇ        |
|           | ±0.06           | ±0.21     | ±1.13ᵇ  | ±1.18               | ±4.92ᵇ    | ±3.67ᵇ  | ±0.89ᵇ   | ±0.18ᵇ        |
| T₂        | 1.500ᵇ          | 8.380ᵃ    | 40.05ᵇ  | 39.63               | 267.₅ᵃ    | 58.60ᵇ  | 21.21ᵇ   | 46.13ᵃ        |
|           | ±0.02           | ±0.34     | ±2.64ᵇ  | ±1.94               | ±1.94ᵇ    | ±1.05ᵇ  | ±1.74ᵇ   | ±0.17ᵇ        |
| T₃        | 1.730ᵃ          | 8.933ᵃ    | 42.85ᵃ  | 38.25               | 247.₂ᵃ    | 51.63ᵇ  | 21.03ᵇ   | 44.83ᵃ        |
|           | ±0.03           | ±0.22     | ±2.85ᵇ  | ±3.65               | ±1.25ᵇ    | ±0.72ᵇ  | ±0.94ᵇ   | ±0.47ᵇ        |
| T₄        | 1.780ᵃ          | 9.030ᵃ    | 42.05ᵃ  | 37.10               | 259.₄ᵇ    | 52.89ᵇ  | 21.97ᵇ   | 44.43ᵃ        |
|           | ±0.03           | ±0.26     | ±4.21ᵇ  | ±2.51               | ±13.2ᵇ    | ±0.51ᵇ  | ±1.66ᵇ   | ±1.70ᵇ        |

Mean in the same column having different small letters are significantly different (P ≤ 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 ≥ 0.05).

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

Hematological and biochemical changes in the blood are important indicators used in monitoring physiological and pathological changes in fish (Sathees Kumar 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 |          |          |          |          |
|----------------------------|-----------|----------|----------|----------|----------|
|                            | T₀        | T₁       | T₂       | T₃       | T₄       |
| ALT (IU/L)                 | 36.33     | 35.1     | 36.23    | 35.67    | 34.03    |
|                            | ±1.24     | ±1.72    | ±0.70    | ±0.64    | ±0.88    |
| AST (IU/L)                 | 132.5     | 150.6    | 145.6    | 146.6    | 149.8    |
|                            | ±7.86     | ±5.39    | ±6.76    | ±3.57    | ±9.10    |
| Total Cholesterol (mg/ dL) | 149.6     | 146      | 153.3    | 160.7    | 157.5    |
|                            | ±4.06     | ±2.48    | ±7.26    | ±2.32    | ±9.71    |
| Triglyceride (mg/ dL)      | 163       | 147.3    | 153      | 168      | 161      |
|                            | ±10.62    | ±5.50    | ±10.93   | ±6.67    | ±4.68    |
| HDL (mmol/L)               | 64.67     | 46.75    | 47.33    | 58.67    | 61.67    |
|                            | a         | b        | b        | a        | a        |
|                            | ±1.43     | ±2.78    | ±4.17    | ±1.55    | ±2.25    |
| LDL (mmol/L)               | 55.38     | 73.87    | 75.40    | 72.18    | 57.84    |
|                            | b         | a        | a        | a        |         |
|                            | ±0.65     | ±1.41    | ±4.61    | ±2.64    | ±5.31    |
| Albumin (g / dL)           | 3.680     | 2.910    | 3.460    | 3.900    | 4.213    |
|                            | bc        | d        | c        | c        |         |
|                            | ±0.03     | ±0.02    | ±0.13    | ±0.15    | ±0.06    |
| Globulin (g / dL)          | 1.838     | 1.310    | 1.613    | 1.900    | 2.243    |
|                            | b         | d        | c        | b        |         |
|                            | ±0.03     | ±0.04    | ±0.07    | ±0.07    | ±0.06    |
| Total protein (g / dL)     | 5.518     | 4.220    | 5.073    | 5.623    | 6.455    |
|                            | b         | d        | c        | b        |         |
|                            | ±0.02     | ±0.04    | ±0.14    | ±0.12    | ±0.10    |
| AL/GL ratio                | 2.005     | 2.230    | 2.158    | 2.050    | 1.883    |
|                            | ab        | ab       | ab       | ab       |         |
|                            | ±0.05     | ±0.08    | ±0.12    | ±0.02    | ±0.05    |

Mean in the same row having different small letters are significantly different (P ≤ 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.

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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 الأسماك، والاستجابات الفسيولوجية لأسماك البلطي النيلي وحيد الجنس. تم توزيع ستة مصادر من أصباغ البلطي النيلي وحيد الجنس (بمتوسط وزن ابتدائي 52.64 ± 5.2 جم) بشكل عشوائي في خمسة علاقات تجريبية. حددت الخلاصة الذاتية الوضعيات المختلفة من البروتين الغذائي (21 و 23 و 25 و 27٪). وتمت مقارنتها مع علقاء التحكم المحتوية على 25٪ في بدون إضافة الأكواماكس بلاس. أظهرت النتائج أن زيادة البروتين الغذائي في للعلائق التجريبية يؤدي إلى تحسن في أداء النمو والصفات الهيماتولوجية. حيث سجلت الأسماك التي تغذت على علقة بها 21٪ من البروتين جودة النمو وكفاءة التغذية، والصفات الهيماتولوجية. أدت إضافة الأكواماكس بلاس إلى العلائق التجريبية التي تحتوي على 23٪ و 25٪ من البروتين خام إلى تعزيز في مقاييس أداء النمو، والكفاءة الغذائية، والصفات الهيماتولوجية مقاورة بعليقة التحكم. أدت زيادة البروتين الغذائي إلى تخفيض في صفاتها، وقليل علوي الدهون والمواد الطاقة في جسم الأسماك. ومع ذلك، فإن إضافة الأكواماكس بلاس إلى علائق التجريبية لم تظهر أي تأثير على الصفات الكيميائية في الجسم. أخيرًا، يمكن أن نستنتج أن إضافة الأكواماكس بلاس في علائق أسماك البلطي النيلي أدت إلى تحسن في الجودة الغذائية لعلاقتنا متخصصة في البروتين. لذلك، يمكن التوصية باستخدام الأكواماكس بلاس في علاقات أسماك البلطي النيلي كوسيلة لتقليل كلفة أعلام الأسماك.