Effect of Using Probiotics (ZADO®) on the Productive Performance of Nile Tilapia Fish (*Oreochromis niloticus*)

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Abstract

The research was performed at the Department of Animal Production's fish laboratory, National Research Center, Dokki, Egypt. The research was conducted to assess the impact of commercial powder probiotic (ZADO®) on growth efficiency of Nile tilapia mono-sex fish (*O. niloticus*), which were fed on basal experimental diet (the control group) and other 4 experimental diet, which was the basic diet augmented with 0.25, 0.5, 0.75 and 1% of ZADO® for T1, T2, T3, T4 and T5, respectively. The Five experimental treatments were performed in three replicates each, the experimental aquaria were part of closed recirculating system, where environmental parameters were kept constant throughout the experimental period. Fifteen aquaria 60 × 40 × 30 cm, width, depth, and height, respectively each was stocked with 15 fish. The mean individual initial body weight (4.04 g/fish) was recorded at the start of the experiment. All fish in each aquarium throughout the entire experimental period were weighed every two weeks. The experimental fish were fed 32 % crude protein-based diet for (98 days). The daily feed allowances were calculated as 5% of fish body weight and were divided into 3 portions fed at 8 am, 12 pm and 4 pm. The Results of the experiment indicated that T2 had the largest significant (P<0.05) final body weight (FBW g/fish), average weight gain (AWG, g/fish), specific growth rate (SGR % day), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) among all the experimental groups. In addition, no substantial variations (p > 0.05) were found in protein productive value (PPV). Although significant effects (p <0.05) have been reported in fish body crude protein (CP) and fat (EE) in T2. By increasing ZADO levels in experimental diets higher than 0)25% no significant effect were observed.

Keywords: Tilapia, probiotics, ZADO®, growth performance and feed utilization, carcass composition

1 Introduction

Tilapia (*O. niloticus*) is a freshwater fish species commonly grown worldwide. There are many benefits to this fish, such as fast growth, dense flesh, easy to reproduce and simple to culture (Molina et al 2009). Any of these benefits make possibility to culture tilapia in high intensive culture systems. The intensive culture of tilapia, on the other hand, faces a range of issues, involving relatively high feed prices not followed by the product
sales price. Thus, there was a need for alternative ways to improve growth by enhancing the digestibility of tilapia and feed use efficiency.

Through the use of chemical essences and veterinary drugs, in particular antibiotics, deterrence and control of fish diseases has been accomplished in previous decades, although these approaches were later discovered to be responsible for creating major public health risks by encouraging the selection, proliferation and persistence of strains resistant to bacteria (FAO, 2016).

In order to hydrolyze feed nutrients, probiotics could raise the activity of endogenous enzymes and the role of normal flora in fish’s digestive tract. Olvera et al (2001) concluded that, when fish were cultured under stress, probiotics have a positive impact on fish output by reducing dietary protein, contributing to enhanced growth and feeding quality. The positive benefits of probiotics, such as improving feed use, intestinal microflora modulation, enhancing immune responses, and pathogen antagonism.

The probiotics of live microbes have to be effective in mitigating the impacts of stress, contributing to increased output. Aquaculture has emerged to be among the most viable and successful sources to support human food and nutritional security, with growing demand for animal protein. Due to disease outbreaks, slow growth rates and low fish survival in most of the intensive aquaculture farms faced significant hindrances. Fish vaccinations and probiotics were used in aquaculture practices to alleviate these problems. In addition to reducing the usage of harmful antimicrobial compounds, especially antibiotics, the use of probiotics in aquaculture has an eco-friendly and sustainable way, enhanced the appetite and/or bio-growth efficacy of cultured species. The specific objective of this research is to define the impact of ZADO® as a commercial probiotic at various levels on Nile tilapia fish growth and feed parameters.

2 Materials and Methods

Total of five treatments with triplicates (Table 1) were carried out at the Department of Animal Production’s fish laboratory, National Research Center, Dokki, Egypt. This study was performed to assess the impact of commercial probiotic (ZADO®) on growth efficiency, feed usage and body composition of mono-sex Nile tilapia (O. niloticus) fingerlings.

Fifteen aquaria 60 × 40 × 30 cm, width, depth, and height, respectively each was stocked with 15 fish with average individual initial body weight (4.04 g). 32% crude protein based diet was fed to the experimental fish. The experimental fish lasted from 30th November 2017 to 8th March 2018 (98 days). As an adaptation period, the fish were fed on a control diet for fifteen days before the beginning of the experiment.

2.1 The rearing system

In this system, the water drained from the rearing aquaria were piped into mechanical and biological filtration system and then returned to the fish aquaria. Mechanical filtration and biological filtration carried out by screens and submersible media bags, respectively. The system was provided with a thermostatic water heater (2.5KW) to control temperature at desired levels. Rearing aquaria were continuously aerated by air stones that were connected to air lines of a small air blower (0.12 Hp).

Table 1. Experimental group design

| Treatments | Diets                  |
|------------|------------------------|
| T1         | Basal diet with no supplement (control) |
| T2         | Basal diet supplemented with 0.25% ZADO® |
| T3         | Basal diet supplemented with 0.5% ZADO® |
| T4         | Basal diet supplemented with 0.75% ZADO® |
| T5         | Basal diet supplemented with 1% ZADO® |
Effect of Using Probiotics (ZADO®) on the Productive Performance of Nile Tilapia Fish (Oreochromis niloticus)

2.2 Water quality assessments

Criteria for water quality have been tested every day at 8:00 am to determine water temperature and dissolved oxygen by oxy-meter (Lutron model Do – 5509). Other water parameters (pH, ammonia (NH₃), nitrate-nitrogen (NO₃-N) and nitrite-nitrogen (NO₂-N) were carried out in the Limnology and Plankton Laboratories in the Department of Limnology of Central Laboratory for Agricultural Climate as per the American Public Health Association’s standard methods (APHA, 1992) Boyd (1990). Values of pH were measured in wastewater samples using a combined electrode connected to a pH meter (Coming Co. pH meter model 345). Ammonia-Nitrogen (NH₄⁺), (NH₃), nitrate (NO₃) were calculated using the methods mentioned by Sauter and Stoub (1990). The water quality parameters were given in (Table 2). Due to the continuous water exchange during the whole experimental period, all parameters of water quality herein are in the acceptable range for the rearing of the Nile Tilapia as mentioned by Abdel-Hakim et al (2002).

Table 2. Averaged water quality determinations during the experimental period

| Parameter             | Readings   |
|-----------------------|------------|
| Average Temperature (°C) | 25 ± 3°C   |
| Oxygen (mg / L)       | 6.5 ± 0.5  |
| PH                    | 7.4 ± 0.4  |
| Ammonia (mg / L)      | 0.05 ± 0.01|
| NO₂-N (mg / L)        | 0.03 ± 0.01|
| NO₃-N (mg / L₀)       | 0.5 ± 0.02 |

2.3 Probiotics

A commercial probiotic (ZADO®) containing Ruminococcus flavefaciens (28*10⁴ CFU) was mixed thoroughly with the experimental diets as 0, 0.25, 0.5, 0.75, 1% for T1, T2, T3, T4 and T5, respectively.

2.4 Experimental diets

Five isonitrogenous (32% CP) and isocaloric (4600 kcal GE/ kg) diets were formulated. The experimental diets were displayed in Table 3. The diet ingredients were bought from the local market, where grains were well ground and then were mixed thoroughly with other ingredients according to the formula. Warm water was added to each diet to make dough. Pellets were made by using meat mincer machine with 2 mm die. Diets are left to a dry in dry oven at 60°C overnight. Experimental diets were chemically analyzed to measure dry matter (DM), crude protein (CP), ether extracts (EE), crude fiber (CF) and ash based on the approach defined by AOAC (2012).

2.5 Fish sampling and nutritional criteria

Tilapia fish (O. niloticus, L) fry were obtained from AL-Delineate city, at Alexandria Governorate. A total of 225 fish fry with an average initial body weight of (4 g± 0.04) were distributed randomly in 15 aquaria. For the whole experimental period, fish were weighed every two weeks in each aquarium. The following parameters were calculated depending on fish body weight data: weight gain, average daily gain, specific growth rate and nutritional parameters were calculated according to Cho and Kaushik (1985) as following:

Initial weight (IBW) g/ fish
Final weight (FBW) g/ fish
Average weight gain (AWG) (g/fish) = average final weight –average initial weight
Average daily gain (ADG)

In accordance with the following formula, daily gain was calculated:

\[ \text{ADG} = \frac{(w_{t2}-w_{t1})}{t} \]

Where:
\[ w_{t1} = \text{initial fish weight in grams.} \]
\[ w_{t2} = \text{following fish weight in grams.} \]
\[ t = \text{period in days.} \]
Table 3. Experimental diets formulation (% dry matter bases)

| Ingredient                        | T1  | T2  | T3  | T4  | T5  |
|-----------------------------------|-----|-----|-----|-----|-----|
| Soybean meal (44% CP)             | 34% | 34% | 34% | 34% | 34% |
| Rice bran                         | 18% | 18% | 18% | 18% | 18% |
| Yellow corn                       | 22% | 21.75% | 21.5% | 21.25% | 21% |
| Fish meal (65% CP)                | 20% | 20% | 20% | 20% | 20% |
| Plant oil                         | 2%  | 2%  | 2%  | 2%  | 2%  |
| Fish oil                          | 1%  | 1%  | 1%  | 1%  | 1%  |
| Lime stone                        | 1%  | 1%  | 1%  | 1%  | 1%  |
| Salt                              | 1%  | 1%  | 1%  | 1%  | 1%  |
| Vitamin & minerals premix a       | 1%  | 1%  | 1%  | 1%  | 1%  |
| ZADO® b                           | 0%  | 0.25% | 0.5%  | 0.75% | 1%  |

Chemical composition % (DM)

| Moisture (%)                      | 10.1 | 10.7 | 10.6 | 10.1 | 10.3 |
| Crud protein (%)                  | 32.01 | 32.20 | 32.04 | 32.50 | 32.71 |
| Ether extract (%)                 | 5.6  | 5.81 | 5.27 | 5.52 | 5.12 |
| Crude fiber (%)                   | 6.46 | 6.45 | 6.44 | 6.44 | 6.43 |
| Ash (%)                           | 6.90 | 7.1  | 6.88 | 6.59 | 6.8  |
| NFE (%)                           | 48.99 | 48.44 | 49.37 | 48.95 | 48.94 |
| GE (kcal/100g)                    | 462  | 464  | 459  | 463  | 460  |

1- One kg premix contained:

Vitamins: 48x10^5 I. U (A), 6x10^5 I. U. (D_3), 144 mg (E), 400 mg (B_1), 1600 mg (B_2), 4x10^3 mg (Pantothenic acid), 4 mg (B_12), 4x10^2 mg (Niacin), 2x10^5 mg (Choline chloride), and 400 mg (folic acid).

Minerals: 12x10^3 mg Iron, 16x10^3 mg Manganese, 12x10^2 mg Copper, 120 mg Iodine, 80 mg Cobalt, 40 mg Selenium, and 16x10^3 mg Zinc.

2- NFE = Nitrogen free extract (100 – [CP + Ash + CF + EE]).

3- GE = Gross Energy measured as the protein, lipids and carbohydrates 5.64, 9.44 and 4.11 Kcal / gram respectively. (NRC, 2011).

Specific growth rate (SGR %/day)

SGR = (Ln wt2 - Ln wt1) x 100/t.
Where:
Ln = (log 10x)^3.03
wt1 = initial fish weight in grams.
wt2 = final fish weight in grams

Feed conversion ratio (FCR) has been calculated the following equation:

FCR = Total feed consumption (g)/Final body weight (g) - initial body weight (g)

Feed efficiency ratio (FER) has been measured using the following equation:

FER = final body weight (g) - initial body weight (g)/Total feed consumption (g)

Protein efficiency ratio (FER) has been measured using the following equation:

PER = Final body weight (g) – initial body weight (g)/protein intake (g)

Protein productive value (PPV %) is the percentage ratio of protein consumed in fish body:

PPV (%) = 100 X Final body protein (g/fish) / Initial body protein (g/fish)

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Effect of Using Probiotics (ZADO®) on the Productive Performance of Nile Tilapia Fish (Oreochromis niloticus)

Survival rate:

\[(Z/X) \times 100\]

Where:

- \(Z\) is the surviving number of fish
- \(X\) is the total number of fish

Statistical analysis

Using the general linear models (GLM) method, data were subjected to analysis of variance (ANOVA), the software used was SPSS (Version 16.0) (SPSS, 1997). For comparing control and treatment groups, Duncan multiple range tests (1955).

The analysis model was as follows:

\[PY_{ij} = \mu + Ti + E_{ij}\]

- \(\mu\) = the overall average.
- \(Ti\) = the effect of treatment.
- \(E_{ij}\) = the random error

3 Results and Discussion

3.1 Growth efficiency parameters of ZADO® probiotic fed fingerlings of Nile tilapia

Results given in Table 4 showed that fish group fed T2 (0.25% ZADO®) had significant differences (P<0.05) FBW (31.94 g/fish), ADG (0.286 g/fish/day), AWG (28.03 g/fish) and SGR (2.14%/day) than the other experimental groups including the control. By adding higher levels of ZADO® mor than 0.25% the growth performance of tilapia fingerlings did not show any significant improvements (P>0.05). The lowest numerical values were observed by group T3, where 0.5% ZADO® was added. Guimarães et al (2019) reported that tilapia diets containing probiotics showed higher growth rate than the control diets. They described that, the adding of probiotics reduced the impacts of stress factors and improved fish performance than that provided by yeast. Welker and Lim (2011) found that probiotics may stimulate digestion by stimulating digestive enzymes production or other changes in the intestinal environment that may lead to enhanced growth efficiency. Enzymes participating in digestion (carbohydrases, esterase, lipase, phosphatase, peptidase, proteases, and cellulases) are produced by intestinal microbes include some species the widely utilized as probiotics. Nayak (2010) observed that tilapia fish fed diets containing Bacillus subtilis and an unidentified “photosynthetic bacteria” had higher growth performance than the control diet. Honsheng (2010) attributes the increased production of enzymes to improved weight gain and feeding efficiency. Enhanced Nile tilapia growth performance was also recorded by Essa et al (2010) whether fed B. subtilis, Lactobacillus plantarum, a mixture of B. subtilis and L. plantarum, or S. cerevisiae. In the gastrointestinal tract, fish fed the bacterial probiotics, alone or
in mixture, displayed enhanced activity of amylase, protease, and lipase enzymes. Similar results were supported by Khatun and Saha (2017) who found that highest production and survival rate were achieved when fish are fed diets comprising probio-aqua. In the current research probiotic levels were between 0.25, 0.5, 0.75 and 1%, whereby increasing up to 0.25% the growth performance of tilapia fingerlings was increased. No growth improvement was achieved when fish fed with the probiotic level higher than 0.25%. The same results, Soltan et al (2016) observed that when fish fed probiotics in ascending levels (0.1-0.4%) the best growth performance indices (body weight, weight length, weight gain and specific growth rate) were achieved by adding 0.2% probiotic in fish diets. Higher probiotic levels showed reduction in fish growth performance indices. In addition, Abu-Ayyana et al (2017) reported that using ZADO® with low levels improved tilapia fish growth efficiency and feed use parameters rather than higher levels.

The improvement of tilapia average weight gain in the present experiment as shown in (Fig 1) could be attributed to the group of probiotics of anaerobic bacterial found in ZADO® that causes better digested diets and improved fish performance and productivity. The enzymes in the probiotic reach a certain level in gastrointestinal tract and act as a growth enhancer by increasing nutrients digestibility and absorbaility in fish gut. Gatesoupe (1999) in his experiment using B. subtilisone in fish diets found that digestive enzymes as amylase, lipase, protease, can be produced and may led to increase the concentration of intestinal digestive enzymes, which stimulate the synthesis of digestion vitamins. Samuel (2013) concluded that the impact of probiotics on growth efficiency of different fish species might be due to the type of probiotics, fish intestinal microbiota and species of host fish.

The results of survival rate indicated that it was not affected significantly (P>0.05) by ZADO® levels in diets (Fig 2). To the same results, came Guimarães et al (2019), found no significant variations in the survival of tilapia fish when it fed diets included probiotics relative to the control group.

![Fig 1. Average weight gain of the experimental group (g/ fish)](image)

### Table 4. The impact of ZADO® on growth performance of tilapia fingerings Values are the mean ± S.E. of triplicate groups

| Growth parameters | T1 (Control) | T2 | T3 | T4 | T5 |
|-------------------|-------------|----|----|----|----|
| IBW (g/fish)      | 4.04±0.040  | 3.91±0.034 | 4.02±0.023 | 3.8±0.00 | 3.9±0.090 |
| FBW (g/ fish)     | 28.24±0.73  | 31.94±0.70 | 26.49±1.17 | 27.94±1.22 | 29.17±1.51 |
| AWG (g/fish)      | 24.19±0.71  | 28.03±0.69 | 22.46±1.19 | 24.14±1.22 | 25.27±1.49 |
| ADG (g/ fish/day) | 0.247±0.01  | 0.286±0.01 | 0.229±0.12 | 0.246±0.01 | 0.258±0.01 |
| SGR (%/ day)      | 1.98±0.021  | 2.14±0.02 | 1.76±0.047 | 2.04±0.04 | 2.05±0.055 |
| SR (%)            | 94.77±2.22  | 95.55±2.22 | 100.00±0.0 | 97.77±2.22 | 97.77±2.22 |

There are substantially different values of different superscripts in the same raw (P<0.05).

IBW: initial body weight; FBW: final body weight; AWG: average weight gain; ADG: average daily gain; SGR: specific growth rate
Effect of Using Probiotics (ZADO®) on the Productive Performance of Nile Tilapia Fish (Oreochromis niloticus)

3.2 Feeding utilization parameters of Nile tilapia fry fed ZADO®

Data given in Table 5 showed the impact of various ZADO® levels on feed utilization parameters. Slight significant variations ($P<0.05$) were noted between FCR, FER and PER of T2 (0.25% ZADO®) and other treatments. Soltan et al (2016) found that feed intake, (PER), (FCR) were improved by increasing probiotic levels in fish diets. The findings of the current research agree with the findings of Allameh et al (2017); Soltan et al (2016), Abdel-Tawwab and Ahmad (2009) and Abdel-Tawwab et al (2020) they noticed better FCR values when the probiotic-containing diets were fed to Nile tilapia (O. niloticus L).

The noticed growth increase in the first ZADO® level (0.25%) in the current research could be owing to high feed usage and the raised in digestibility of various diet components. El-Haroun et al (2006) also observed a major impact of probiotics on the protein efficiency ratio in Nile tilapia (Oreochromis niloticus L) relative to control (without probiotics). suggest that the best FCR values found in the probiotic-supplemented diet, where probiotics have enhanced feed usage even under stress conditions, and the improved digestibility obtained by supplementing diets indicates that the adding of probiotics has enhanced the diet and digestibility of proteins, that can, in turn, illustrate the improved growth and feed efficiency observed by supplementing diets Lara-Flores et al (2003). Similar findings were reported by Khalil et al (2001), who reviled that the use of feed was the highest in Nile tilapia fed diets supplemented with probiotics, this means that the nutrients have a response as a palatability enhancer with better taste that increase the feed consumption by fish and consequently increase the growth rate. Probiotics improve intestinal flora efficiency, thus enhancing digestion and improving energy utilization, leading to better growth. In addition, some studies have noted that intestinal microorganisms are significant for the health of fish by hindering the formation of pathogenic bacteria in the alimentary tract. The findings of El-Sanhoury and Ahmed (2017), who stated that the use of ZADO® at different levels in broiler feeding up to 0.5 Kg/ton affected positively poultry (FBW). They attributed that to the increase in stomach and intestine in ZADO® groups which consequence led to improve feed utilization.

By increasing ZADO® level in diets higher than 0.25% PPV % was increased. The availability of energy source when ZADO® was added has been increased, where complexed dietary fiber has been converted into monosaccharide (Gado et al 2013), which led to spare protein to be utilized in anabolism rather than used as energy source.

3.4 Chemical composition of the experimental fish carcass

The results in Table 6 showed the effect of different ZADO® levels on tilapia body composition. There were no significant effects of ZADO® levels on body dry matter, whereas protein content in T2 (57.66) was higher significantly ($P<0.05$) than other treatments. It was found that by adding ZADO® to tilapia diets, crude fat content increased compared to the control group. The highest significant crude fat content (23.33%) was reported by group T2 (0.25% ZADO®). In addition, body ash content was decreased by increasing ZADO® level in diets. The lowest ash content (16.3%) was noticed by group fed T2 and T5. The findings achieved in the current experiment are disagreed with the findings of El-Haroun et al (2006), who proposed that no
Table 5. The effect of probiotics on feed utilization of Nile tilapia

| Feed utilization parameter | T1 Control | T2 | T3 | T4 | T5 |
|---------------------------|-----------|----|----|----|----|
| Feed intake (g/fish)      | 57.02±1.64| 61.2±1.40 | 54.88±1.35 | 55.52±0.50 | 57.90±1.04 |
| FCR                       | 2.31b±0.07 | 2.18±0.11 | 2.45±0.12 | 2.36±0.03 | 2.31b±0.07 |
| FER                       | 0.43±0.012 | 0.46±0.03 | 0.41±0.02 | 0.44±0.01 | 0.44±0.01 |
| PER                       | 1.48±0.04 | 1.59±0.06 | 1.43±0.06 | 1.49±0.02 | 1.49±0.04 |
| PPV                       | 19.62±0.50 | 21.34±1.02 | 22.67±0.36 | 23.52±0.36 | 21.80±0.58 |

Values are the mean ± S.E. of triplicate groups for four treatments. There are substantially different values of different superscripts in the same raw (P<0.05).

Table 6. Nile tilapia fish’s chemical composition (on DM basis)

|          | Initial | T1 (Control) | T2 | T3 | T4 | T5 |
|----------|---------|--------------|----|----|----|----|
| Dry matter (%)     | 23.66±1.45 | 24.10±1.15 | 23.02±1.22 | 28.23±1.52 | 27.31±1.20 | 26.62±0.88 |
| Crude protein (%)  | 52.33±0.88 | 54.66±0.88 | 57.66±1.20 | 54.11±1.15 | 54.21±1.15 | 52.56±1.45 |
| Ether extract (%)  | 17.33±1.45 | 21.66±0.88 | 23.33±0.88 | 19.66±0.88 | 24.11±1.15 | 22.66±1.45 |
| Ash (%)            | 23.66±0.88 | 19.76±1.21 | 16.32±0.88 | 20.32±1.15 | 22.32±1.2 | 16.33±1.45 |

a, b means of the same raw with different superscript are significantly different (p<0.05)

Statistical variations (P>0.05) were found in carcass compositions in dry matter and protein content between various treatments when commercial probiotics were used in Nile tilapia feeds. Lara-Flores et al (2003) and Ghosh et al (2008) observed that the impacts of dietary administration of *B. subtilis* on body composition revealed that probiotics boosted carcass fat content, but no substantial changes in moisture; ash and protein content were observed. Therefore, probiotics do not seem to have a major impact on the body composition of fish and do not significantly influence the synthesis of tissues. Allameh et al (2017) concluded that the administration of probiotics in the Nile tilapia diet (*O. niloticus*) led to an enhancement of the body’s protein and lipid content, but no substantial impact was observed on the moisture and ash content. Dowidar et al (2018) concluded that fish health and innate immunity was significantly enhanced by the dietary administration of any of the probiotics.

4 Conclusions

The results of this experiment concluded that, dietary administering of 0.25% ZADO® may be utilized as a probiotic agent in *O. niloticus* culture hence improved fish growth performance and feed utilization parameters.

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Effect of Using Probiotics (ZADO®) on the Productive Performance of Nile Tilapia Fish (Oreochromis niloticus)

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تأثير استخدام البروبيوتوك على الأداء الإنتاجي لأسماك البلطي

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الموجز

أجريت هذه التجربة في معمل الأسماك في قسم الانتاج الحيواني بالمركز القومي بالدقي - مصر لتقييم تأثير إضافة مسحوق البروبيوتوك التجاري (زادو) على الأداء الإنتاجي لأسماك البلطي النيلي وحيد الجنس والتي غذت على عليقة أساسية مقسمة إلى خمسة علائق تجريبية وهما كالتالي: المعاملة رقم (1) عليقة قياسية (كنترول) بدون إضافة والمعاملة رقم (2) و (3) و (4) و (5) على التوالي وكان لكل معاملة 3 مكررات.

نطلق نتائج تأثير البروبيوتوك على الأداء الإنتاجي. وظائف فتوحت عند استخدام 0.25% زادو تحسن الأداء الإنتاجي ومعدل الاستفادة من الغذاء في أسماك البلطي النيلي.