ENHANCING GROWTH PERFORMANCE AND FEED UTILIZATION USING PREBIOTICS IN COMMERCIAL DIETS OF NILE TILAPIA (*Oreochromis niloticus*) FINGERLINGS

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SUMMARY

An experiment was undertaken to study the impact of commercial β-glucan on growth performance, feed utilization and immunomodulatory effects of Nile tilapia fingerlings. Four isocaloric and isonitrogenous diets were formulatedwith three levels of β-glucan (0, 0.6, 1.2 and 1.8%) and fed to the fish for 84 days. The tested diets were applied in 12 fiberglass tanks (80 x 60 x 60 cm) each was supplied arbitrarily with 20 Nile tilapia fingerlings with anmoderate starting body weight of 10 ± 0.20g. Generally, We found that adding β-glucan to the diet improve fish growth performance, feed conversion ratio, protein efficiency ratio and Apparent protein digestibility were enhanced for tilapia fingerlings compared to fish fed on the control diet. In terms of blood estimations no critical contrasts were identified in plasma albumin, total protein and total globulins of fish nourished on the tested diets. Conclusively, the results revealed that It is possible to include concentrations of 0.6 % of β-glucans in the diets for Nile tilapia, with detectable improvement on growth performance, feed utilization and survival.

Keywords: *Feed additives, tilapia, growth performance, feed utilization and blood measurements.*

INTRODUCTION

The addition of beneficial components to diets plays an significant part in manipulating gut microflora which responsible for maintaining good health of the individual and the health of these microbes directly influenced by the intake of prebiotics (Joshi, *etal*.,2018).Functional ingredients, such as probiotics, prebiotics and immunostimulants, are reported to improve growth, feed efficiency, stress tolerance, diseases resistance and health status in fish and thus are nowadays increasingly being used in the aquafeed industry (Oliva Teles, 2012). Prebiotics are non-digestible food stuff that invigorate the development or activity of useful gut commensal bacteria in host thus improves host health (Guerreiro *et al*., 2016).Prebiotics can be developed by using various cereal grains such as corn, wheat, rice, barley, and oats economically. Neeraj *et al*.(2018) showed that fructo-oligosaccharide and Fructose can be produced using immobilized biocatalysts and bioreactors based on inulinase. One important strategy is to develop the innate immune system using immunostimulants, such as β-glucans, as feed supplements (Dalmo and Bogwald 2008).

β-glucans an important immunostimulant among which yeast Beta-glucan has been widely used in aquaculture sector. They are found to be highly beneficial in reducing the mortality due to opportunistic pathogens in juvenile fish (Raa, 2000). They consider a part of a gather of physiologically active compounds generally called "biological response modifiers." And preserved carbohydrates shaping basic components of cell walls of few plants, fungi, yeast, seaweed and bacteria (Vaclav *et al* ., 2013) Consequently, purpose of this study discuss the optimal dose of (β-glucan)in fish diets to enhancement feed utilization, growth performance and body composition of Nile tilapia (*O. niloticus*) fingerlings.

MATERIALS AND METHODS

Experimental fish:

Nile tilapia was delivered from a private hatchery El-Kantara, Ismailia Governorate. The fish weighing about (10±0.2g) on average were carefully acclimatized to laboratory environment and conditions for a couple of weeks before being randomly separated into four equal experimental groups (each set had three identical tanks, 20 fish each). One group was served as control and the other three groups represented the tested feed additive. The experimental fish were periodically weighted every 14 days to calculate the amount of food per each group, which was 3% of the total biomass for the total period of the experiment. During the experimental period Nile tilapia were fed by hand over two meals (8:30, & 17:00) for six days a week.

Experimental unit:

The current study had taken place in the Fish Research Center, Faculty of Agriculture, Suez Canal University.12 separated fiber glass tanks (80 x 60 x 60 cm) were prepared for stocking the experimental fish, provided with stored fresh water.In the present study,the photoperiodic conditions was 12h light/ 12h dark environment and physico-chemical parameters of water were measured daily, physico-chemical parameters of water were measured daily and controlled at constant values, the physico-chemical parameters such as temperature(27°C ± 0.2), dissolved oxygen was(above 5.8 mg/l.), pH(7.3 ±0.5) ammonia(1.2 -1.23mg /L) , of water were estimated by following the methods suggested in APHA (1999).

Experimental diets and body composition:

Four isocaloric (393.8 kcal / 100g) and isonitrogenous (25% crude protein) diets were developed out of commercial feed stuff (Table1). Whereas the basal diet was served with no added β-glucan, while prebiotic β-glucan (Sigma-Aldrich Chemie GmbH, Germany) was supplied by diet 1,2 and 3 at 0.6, 1.2, and 1.8 %, respectively. The commercial ingredients were weighting carefully and mixed with additives which were added with corn. Finally, oil and water were added to the components and became appropriate for pushing through CBM granule machine with a diameter of 2mm. The pellets were dried and frozen until experiment begins. The tested diets and fish (Five fish at the start and the end) were completely chemically analyzed except fish crude fiber (CF %) according to the procedures described by A.O.A.C. (2019),composition and chemical analysis of the experimental diets are shown in table(1).

Experimental methodology:

Growth performance parameters:

The growth achievement parameters were calculated with the following equations:
Average weight gain (AWG): -
AWG = Average final weight (g) – Average initial weight (g)
Average daily gain (ADG): -
ADG = [Average final weight (g) – Average initial weight (g)] / time by days
Specific growth rate (SGR %/day): -
(SGR % / day) = 100 [Ln Wt1 – Ln Wt 0 / t]
Where: Ln: normal log ,Wt 0: initial weight (g)., Wt 1: final weight (g), T: time by days.

Feed and protein utilization parameters:

Feed along with protein utilization variables can be calculated with the following equations: -
Feed conversion ratio (FCR): -
FCR = Total feed consumption/ weight gain.
Feed efficiency (FE):-
FE = weight gain/ Total feed consumption
Protein efficiency ratio (PER): -
PER = body weight gain (g)/ protein intake (g).
Survival (%):- SR=Ni x 100/N0
Where: Nt = survived fish number in tank at the end of the experiment.
N0 = survived fish number in tank at beginning of experiment.

Blood measurements:

At the end of the feeding trial, 20 fish were captured and anesthetized for blood sampling from caudal vein, five fish of each aquarium were weighted and prepared to collect the blood samples from caudal vein by heparinized syringes. It was then centrifuged at 3000 rpm for complete 5 minutes to grant a separation of plasma which was subjected to determine albumin (Doumas, *et al.*, 1977) and total protein (Armstrong and Carr, 1964).

Table (1): Proximate analysis and composition of the experimental diets (% on DM).

Item	Experimental diet			
	Control	1	2	3
Feed Ingredient:				
Fish meal (60% CP)	110	110	110	110
Corn gluten (60% CP)	110	110	110	110
Soybean meal (44% CP)	220	220	220	220
Wheat bran	200	200	200	200
Yellow corn	315.5	314.9	314.3	313.7
Soy & fish oil	40	40	40	40
Vitamin & Mineral Mix[1]	3	3	3	3
Di Calcium phosphate	1	1	1	1
(β-glucan)[2]	-	0.6	1.2	1.8
Cr_2O_3[3]	0.5	0.5	0.5	0.5
TOTAL	1000	1000	1000	1000
Chemical composition:				
Dry matter	90.6	90.6	90.4	90.4
Crude protein	25.0	25.4	25.6	25.4
Ether extract	6.7	6.7	6.6	6.5
Crude fiber	6.6	5.9	6.1	6.2
Ash	7.0	7.1	6.8	6.8
N.F.E[4]	54.7	54.9	54.9	55.1
Gross energy Kcal/ 100g[5]	390.7	393.8	393.19	391.95

1- Each KgVitamin & Mineral mixture premix contained Vitamin A, 4.8 million IU, D_3, 0.8 million IU; E, 4 g; K, 0.8 g; B_1, 0.4 g; Riboflavin, 1.6 g; B_6, 0.6 g, B_{12}, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg , Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

2-- Control diet was served with no feed additives, on the other hand, the other diets were supplied with the applied glucan (Sigma-Aldrich Chemie GmbH, Germany) was an extract from the cell walls of the yeast Saccharomyces cerevisiae and provided as dried Powder

3- Cr_2O_3: Chromic Oxide

4- Nitrogen free extract, calculated by (100-(CP+EE+CF+ ash)) `

5- Gross energy. Based on 5.65 Kcal/g protein, 9.45 Kcal/g fat and 4.11carbohydrate Kcal/g (NRC, 2011)

Digestibility study:

Feces and diets collected seriously were measured through the final 14 days of the experimental time and siphoning any uneaten feed carefully out of each tank approach about 30 minutes after the final feeding. Feces were collected separately by siphoning method from each replicate tank in the morning .It filtered, oven dried at 60°C and kept in airtight containers to estimate the chemical analysis and Apparent protein digestibility in accordance to (Furukawa and Tuskahara, 1966) method.

Statistical analysis:

The results were analysed using (SAS 2000).A one-way ANOVA (analysis of variance) was used to compare the data of growth performance, feed utilisation and body composition. Multiple comparisons were analysed with Duncan’s test (Duncan, 1955) to clear up differences between treatment means at 5% significant level.

RESULTS AND DISCUSSION

Growth performance:

As illustratedin (Tables 2), at the end of the experiment, groups of fish fed the added prebiotic (β-glucan) diets significantly ($P<0.05$) increased final body weight, weight gain, and SGR rather than the group of fish fed on control diet.The differences among the groups for averages of BW at the experimental start were insignificant ($P\geq0.05$) indicating the homogeneity of the experimental groups.At the end of the experiment, the group of fish fed on control diet was the most reduced final body weight (72g), while the final body weight of Nile tilapia fingerlings were (89, 87 and 78 g) for group of fish fed on diets 1 , 2 and 3 ,respectively. This indicated that the group of fish fed on diet 1 and 2 had significantly higher ($P<0.05$) final body weight than the other tested groups of fish fed diet 3 and control diet. SGR followed the same trend of weight gain and final body weight, analysis of variance of SGR proved that The fish groups fed on diets 1, 2, had significantly higher ($P<0.05$) SGR than group fed diet 3 the control diet. The values were 2.21, 2.58, 2.59 and 2.45%/d , respectively for fish groups fed on diets control, 0.6, 1.2 and 1.8 % (β-glucan) additive, respectively. Some authors suggested that somatic growth is enhanced due to the production of glucanase that decomposes β-glucans to generate energy (Lopez *et al*., 2003), while others by the improvement of the intestinal immune response and indirectly promoting growth (Dalmo,. and Bogwald,.2008).These results met with the results of (Misra *et al*., 2006, Fabiana *et al.,* 2017 , Hoseinifar, *et al.,* 2017 and Mohamed *et al.,* 2018). Also, (Guerreiro, *et al.,* 2016) found that Prebiotic compounds like fructo -oligosaccharides, mannan-oligosaccharides, inulin or β- glucan are considered as the most effective prebiotics in aquaculture. While, Mohammad *et al*., (2015) Janine *et al*., (2017) found that the inclusion of β-glucan on the diet had no significant effect on final weight and weight gain.

Feed utilization:

Table (2) illustrate the results of feed utilization. The use of β-glucans has been shown to improvefeed utilization, the results showed that (FCR) was (1.75, 1.44, 1.46 and 1.61) for group of fish fed diets control, diet 1, diet 2 and diet 3 , respectively. These significant differences in FCR and %weight gain between the control and treated groups may be due to poor efficiency of feed resulting in lower weight gain. The lower weight gain and FCR is supported by the findings of Dalmo and Bogwald (2008). These effects proved that addition of β-glucans in fish diets enhance the feed utilization; these identical outcomes have been pronounced for the use of β-glucans. Composition of microbes in the gut is straight forwardly influenced within the same diet Fabiana *et al.,* (2017).In practical aspect, this indicates that the use of prebiotics can reduce the amount of feed necessary for fish growth, which can highly result in remarkable reductions of fish production cost. Also, the results of PER values for groups fed on diets 1 and 2 were higher ($P<0.05$) comparing with the different groups. The PER was listed to be 2.28, 2.73, 2.67 and 2.44 for control, diet 1, diet 2, and diet 3, respectively.The PER values appeared that the diets supplemented with prebiotics significantly ($P<0.05$) altogether moved forward protein utilization in commercial diets of tilapia. Also, the results of feed efficiency were enhanced with adding the commercial feed additives (prebiotics) and it was the same as FCR and PER which was listed to be 0.69, 0.68 for diets 1, 2 and 0.62 for diet 3. This results are matching withdietary prebiotics and prebiotics can improve the growth performance, feed utilization, body indices (Talpur *et al.,* 2014 and Hoseinifar *et al.,* 2017).

Blood measurements:

No significant elevation in plasma albumin, plasma total protein, and plasma total globulins of fish fed the supplemented prebiotic diets and diet without adding it (control) This results in agreement with Gurreiro *et al*.,(2016) while, it was in contrast with a number of studies (Mohamed *et al*., 2018; Misra *et al*. 2006; Sych *et al.,* 2013). Also, Kühlwein *et al,* (2013) found that the blood monocyte fraction was significantly higher in mirror carp *(Cyprinus carpio* L.) fed the 1% and 2% β glucan diets and no significant changes were observed in the other blood parameters assessed.

Table (2): Growth Performance and feed utilization of *O. niloticus* fingerlings fed on experimental diets.

Parameter	Experimental diet			
	Control	1	2	3
Initial avg. wt. (g)	10.0	10.2	9.8	10.0
final avg. wt. (g)	72.0±2.1[c]	89±1.8[a]	87±1.9[a]	78±2.3[b]
Weight gain (g)	62±1.8[c]	78.8±2.1[a]	77.2±1.6[a]	68±1.9[b]
SGR%/d[3]	2.21±0.04[c]	2.58±0.06[a]	2.59±0.07[a]	2.45±0.07[b]
FCR	1.75±0.07[c]	1. 44±0.04[a]	1.46±0.03[a]	1.61±0.06[b]
PER	2.28±0.04[d]	2.73±0.07[a]	2.67±0.01[b]	2.44±0.02[c]
FE	0.57±0.03[c]	0.69±0.04[a]	0.68±0.07[a]	0.62±0.04[b]
Feed intake (g)	108.5	113.5	112.7	109.5
APD (%)	74.3±0.71	79.5 ±0.61	78.5 ±0.45	77.3 ±0.71
PTP (g/dl)	4.98±0.10	5.20 ±0.140	5.15 ±0.12	5.20 ±0.12
PA (g/dl)	2.06±0.12	2.14 ±0.12	2.17 ± 0.10	2.16 ±0.12
PTG (g/dl)	2.92±0.14	3.06 ±0.10	2.98 ±0.10	3.04 ±0.10
Survival rate (%)	97±0.03[b]	100±0.03[a]	100±0.03[a]	100±0.03[a]

Value in the same row with a common superscript are not significantly different (P<0.05).
APD = Apparent protein digestibility. PTP = Plasma total protein. PA = Plasma albumin, PTG = Plasma total globulins

Digestibility study:

Table (2) elevated that the apparent protein digestibility were significantly improved (P<0.05) with adding β-glucan in tilapia diets. The better digestibility gained with the addition of prebiotics improved diet and protein digestibility, which may in turn described the better growth and feed efficiency observed with the supplemented diets. These results came in conformity with Ringo *et al*., (2010**),** this results indicated that addition of β-glucan had no negative impact on the activity of the major enzymes in stomach and intestine.

Body composition:

Table (3) showed that there were no statistical differences had been discovered in whole body moisture, ether extracts and ash ,Conversely, body crude protein content was higher in the fish fed on diets with β-glucans diets (p<0.05).These results are in close match with the results of (Wafaa *et al*., 2014 and Mohamed *et al.*, 2018)

Table (3): Chemical composition of whole body *O. niloticus* fingerlings fed the experimental diets (as wet basis).

Component (%)	Experimental diets				
	Initial	(Control)	1	2	3
Moisture	76.40±0.21	71.00±0.2	70.98±0.2	71.00±0.2	71.13± 0.2
Crude protein	13.55±0.21	16.15±0.21	16.29±0.21	16.2± 0.21	16.95±0.21
Ether extract	4.5 ±0.21	6.17±0.21	6.07± 0.21	6.09± 0.21	6.01± 0.21
Ash	5.55 ± 0.21	7.68± 0.21	7.66± 0.21	7.64 ± 0.21	7.61± 0.21

Values in the same row with a common superscript are not significantly different (P<0.05).

CONCLUSION

From the acquired results, it can be concluded that added prebiotic (β glucans) with level of (0.6 %) in Nile tilapia diets has a positive growth performance effect and represents the best feed utilization.

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تحسين أداء النمو والاستفاده الغذائيه باستخدام البريبيوتيك في العلائق التجاريه لاصبعيات اسماك البلطي النيلي

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تعتبر البريبيوتيك من الإضافات الغذائية الطبيعية التى تضاف إلى علائق الاسماك بغرض تحسين النمو و زيادة أعداد العشائر البكتيرية النافعة وتقليل المرضية والضارة منها، والتى كذلك لها دور كبير فى تحسين هضم وأمتصاص العناصر الغذائية . لذلك كان الهدف من هذه الدراسة هو تقييم تأثير ثلاثة مستويات مختلفة من البيربيوتيك (بيتا جلوكان) على النمو، والاستقاده الغذائيه فى إصبعيات أسماك البلطي النيلى. تم تطبيق أربع معاملات: ثلاث مستويات من بيتا جلوكان (صفر،0.6 ، 1.2،1.8٪) بالإضافة الى المعاملة الكنترول . تم تطبيق العلائق المختبرة فى 12 حوض من الفيبرجلاس ابعاده (60x60 x80سم) حيث تم التخزين بمعدل 20من أصبعيات أسماك البلطي موزعه عشوائياً بمتوسط وزن 10 ± 0.20 جرام في كل حوض . استمرت التجربة لمدة 84 يوما . وقد كان أفضل أداء للنمو و معامل تحويل الغذائي وكفاءة تمثيل البروتين و معامل هضم البروتين فى أصبعيات أسماك البلطي التى غذيت على العلائق المضاف اليها البريبيوتيك خاصة مستوى 0.6% بالمقارنة بمجموعة الأسماك التى غذيت على عليقة الكنترول. كذلك اظهرت نتائج قياسات الدم عدم وجود اي اختلافات معنويه في البروتين الكلي والالبيومين والجلوبيولين لبلازما الدم للاسماك التي غذيت علي العلائق التجريبيه كما لايوجد ايضا اي تاثير معنوي لها علي مكونات الجسم

التوصية: يستخلص من هذه النتائج أن مستوى (0.6 %) من البريبيوتيك (بيتا جلوكان) يعتبر كافياً للحصول على أفضل أداء للنمو ، والاستفاده الغذائيه لاصبعيات اسماك البلطي النيلي.