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IMPACT OF DIETARY PROTEIN AND HYDROYEAST AQUACULTURE® LEVELS ON GROWTH, FEED EFFICIENCY, PHYSIOLOGICAL, AND HISTOMETRIC RESPONSES OF *Oreochromis niloticus* FINGERLINGS

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SUMMARY

Tilapia is one of the most important farmed fish in the world. The main objective of this study was to evaluate the effects of supplementation the graded levels (0, 5, 10, and 15 g / kg diet) of a new commercial probiotic Hydroyeast Aquaculture® to low crude protein (CP) levels (20 and 25%) in diets of male monosex *Oreochromis niloticus* fingerlings for 14 weeks, regarding their growth performance, feed utilization, carcass composition, hematological, and histometeric parameters of the intestine. Fish with an average initial body weight (7.5 ± 0.001 g) were distributed into eight treatments (three replicates per treatment). Fish in each treatment were stoked at 5 fish / aquaria (90×40×35 cm).The results revealed that the high levels of CP (25%) and probiotic (15 g / kg diet)had positive significant ($P \leq 0.05$) effects on fish growth performance parameters, feed utilization, fish carcass composition (DM, CP, and EC), hematological constituents (hemoglobin, red blood cells, packed cell volume, and blood platelets) and intestine histometric parameters compared with other treatments, but the interaction between them was not significantly affected on all the above parameters. Therefore, it could be concluded that the high level of probiotic 15 g Hydroyeast Aquaculture® / kg diet is useful with low dietary CP level (25%) in feeding fish at early age stage for enhancing the production performance, physiological responses and histometeric parameters of the intestine of male monosex *O. niloticus* fingerlings, thus this level of probiotic may lead to increase the economic efficiency at large scale in the fish farms.

Keywords: *Nile tilapia, probiotic, growth performance, fish physiology, histometric*.

INTRODUCTION

Tilapias are the most successfully cultured fish in the world because of their fast growing and high efficiency to utilize the natural and artificial supplemented feeds. Tilapias have become increasingly popular for farming as they are able to reproduce rapidly, easily bred in captivity, tolerate to a wide range of environmental conditions, and highly resistant to diseases (Rana, 1997).Tilapias are second only to carps as the most widely farmed freshwater fish in the world (FAO, 2010).

Nutrition is the most important factor of the culture process; it is often represent the major operating cost of aquaculture. Under intensive culture system, fish totally depend on complete balanced diets during their life stages. It is advisable for aquaculturist to know the optimum quality and quantity of feeds introduced to fish to avoid poor growth, health and reproduction. Fish cannot grow well without feeds and they should not be underfed. Conversely, overfeeding also should be avoided because feeds are expensive and excess feeding can also result in poor growth and water quality (Landau, 1992). Optimal feeding regimes may result in reduced feed costs by minimizing expenditure of metabolic rate of fish. Studies on feed stimulants can provide information on physiology of the animals concerned and may also detect additives, which can be incorporated into aquaculture feeds. Attractive feed may be looted and consumed quickly, thus reducing losses by leaching of essential water-soluble components. An addition of chemo-attractants to palletized feeds may increase ingestion rates and improve growth, survival and food conversion (El- Sayed *et al*., 2005).

Nowadays, a number of preparations of probiotics are commercially available and have been introduced to fish, shrimp and molluscan farming as feed additives, or are incorporated in pond water (Wang *et al*., 2005). Probiotics are defined as live microorganisms including many yeast and bacteria, which when administered in adequate amounts could enhance the growth and health of the host (Irianto

and Austin, 2002).The benefits of such supplements include improved feed value, enzymatic contribution to digestion, and inhibition of pathogenic microorganisms, antimutagenic and anti-carcinogenic activity, and increased immune response. Moreover, probiotic supplementation may provide vitamins, short chain fatty acids and/or digestive enzymes, and therefore may also contribute to host nutrition (John *et al*., 2006). The research into the use of probiotics for aquaculture is increasing with the demand for environment-friendly sustainable aquaculture (Vine *et al*., 2006). Many studies have pointed out that probiotics in fish diet improved growth and feed efficiency (Ghazalah *et al*., 2010 and Merrifield *et al*., 2010a), physiological responses (Zhu *et al*., 2012 and Abdelhamid *et al*., 2013a), as well as the fish intestine functions (Pirarat *et al*., 2011 and Nakandakare *et al*., 2013).

On the other hand, feed cost about over 50% of the variable costs in most aquaculture operations, with protein being the most expensive dietary source (El-Sayed, 1999), therefore applying the best feeding strategy can have a significant impact on optimizing profit, which is the primary goal of commercial aquaculture. Also, if more fish are able to survive until they are of marketable size, the subsequent cost of production would be reduced drastically. Consequently, the main objective of this study was to evaluate the effects of graded levels (0, 5, 10, and 15 g / kg diet) of a new commercial probiotic Hydroyeast Aquaculture® to the low dietary crude protein levels (20 and 25%) of male monosex Nile tilapia, *Oreochromis niloticus* fingerlings regarding their growth performance, feed utilization, carcass composition, hematological, and intestine histometric parameters for a period of 14 weeks rearing in glass aquarium.

MATERIALS AND METHODS

The experimental management:

This study was conducted in Fish Laboratory Research, Faculty of Agriculture, Mansoura University, Al-Dakahlia Governorate, Egypt. Monosex male *O. niloticus* fingerlings, with an average initial body weight of 7.5 ± 0.001gwere purchased from a private fish farm in Kafr El-Shekh Governorate, Egypt. Fish were stocked into a rearing tank for two weeks as an adaptation period, and fed a basal experimental diet during this period.

A total of 120 *O. niloticus* fingerlings were distributed into eight treatments (three replicates in each), as shown in Table 1. Fish in each treatment were stoked at five fish / aquaria. Each glass aquaria (90 × 40 × 35 cm) was supplied with an air stone connected with electric compressor for water aeration. Light was controlled by a timer to provide a 14h light: 10h dark as a daily photoperiod. The replacement of the aquaria water was done partially every day to remove the wastes then re-new the tap water (Chlorine-free).Water quality parameters were measured including temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter), in all aquaria day by day. Mean values of water temperature ranged between 25.5 and 27.6 ˚C, pH values 6.60 – 7.80 and dissolved oxygen 5.00 – 6.70 mg/ L. All tested water quality criteria in the present study were suitable for rearing *O. niloticus* fingerlings as cited by Mehrim (2009). The tested probiotic, Hydroyeast Aquaculture® formula was comprised of oligosaccharides (50,000 ppm); enzymes (amylase 3.7×10^6, protease 5×10^5, cellulose 2×10^5, pectinase 1×10^5, xylanase 1×10^4, phytase 3×10^3 units / kg); live yeast (5×10^{12} colony forming units (CFU) / kg); and probiotics bacteria (*Lactobacillus acidophilus, Bifedobacterium longhum, B. thermophylu*, and *Streptococcus faecium* 22.5×10^8 CFU / kg for each). It was produced by Agranco corp., Gables, USA.

Table (1): Details of the experimental treatments.

Treat.	Details
T_1	Basal diet (20% CP) + 0 g Hydroyeast Aquaculture®/ kg diet (as a control)
T_2	Basal diet (20% CP) + 5 g Hydroyeast Aquaculture®/ kg diet
T_3	Basal diet (20% CP) +10 g Hydroyeast Aquaculture®/ kg diet
T_4	Basal diet (20% CP) +15 g Hydroyeast Aquaculture®/ kg diet
T_5	Basal diet (25% CP) + 0 g Hydroyeast Aquaculture®/ kg diet (as a control)
T_6	Basal diet (25% CP) + 5 g Hydroyeast Aquaculture®/ kg diet
T_7	Basal diet (25% CP) +10 g Hydroyeast Aquaculture®/ kg diet
T_8	Basal diet (25% CP) +15 g Hydroyeast Aquaculture®/ kg diet

CP: Crude protein.

The experimental basal diet (BD), used in this study contained 20% or 25% crude protein. Ingredients of BD were bought from the local market and proximate chemical analysis was carried out according to AOAC (2004), as shown in Table 2. The ingredients homogeneously mixed and the tested probiotic at levels of 0, 5, 10, and 15 g / kg BD was added. The experimental diets were introduced manually twice daily at 9.00 a.m. and 15.00 p.m. at 5% of the fish biomass in each aquaria. The fish were weighed every two weeks by a digital scale (accurate to ± 0.01 g) to adjust their feed quantity according to the actual body weight changes, the fish biomass present in each aquarium.

Fish sampling and performance parameters:

At the start and at the end of the experiment, fish samples were collected and kept frozen (– 20 °C) till the proximate analysis of the whole fish body according to AOAC (2004). Their energy content were calculated according to NRC (1993). Fish growth and feed efficiencey parameters such as average total weight gain (TWG, g), specific growth rate (SGR, %/day), survival rate (SR, %), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV, %) and energy utilization (EU, %) were calculated according to Bagenal (1978).

Hematological parameters:

At the end of the experiment, blood samples were collected from the fish caudal peduncle of the different treatments. Blood samples from three fish in each aquaria were randomly taken, which were received in plastic tubes. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) using commercial colorimetric kits (Diamond Diagnostic, Egypt). Total red blood cells count (RBCs × 10^6/mm^3), platelets (× 10^3/mm^3) and total count of white blood cells (WBCs × 10^3/mm^3) were counted according to (Dacie and Lewis, 1995) onan Ao Bright – Line Hämocytometer model (Neubauer improved, Precicolor HBG, Germany), as well as the packed cell volume (PCV %) was measured according to Stoskopf (1993).

Table (2): Ingredients and proximate chemical analysis of the experimental basal diets.

Ingredient (g / kg)	Diet 1 (20% CP)	Diet 2 (25% CP)
Fish meal (60% CP)	100	140
Soybean meal (44% CP)	200	280
Yellow corn	300	260
Wheat bran	300	220
Sunflower oil	30	30
Molasses	50	50
Vitamins & minerals[1]	20	20
Composition (% DM)		
Dry matter (DM)	85.24	86.03
Crude protein (CP)	21.80	24.63
Ether extract (EE)	8.01	5.73
Ash	7.28	6.14
Crude fiber (CF)	6.76	8.09
Nitrogen free extract (NFE)	56.15	55.41
Total carbohydrates	62.91	63.50
Gross energy (GE)[2] (kcal/100 g DM)	457.12	453.98
Protein/energy (P/E) ratio (mg CP/kcal GE)	47.68	54.24
Metabolizable energy (ME)[3] (kcal/100g)	382.53	378.85

[1]Each 3 kg premix contains : Vit. A, 12000,000 IU; Vit. D_3, 3000,000 IU; Vit. E, 10,000 mg; Vit. K_3, 3000 mg; Vit. B_1 200 mg; Vit. B_2, 5000 mg; Vit. B_6, 3000 mg; Vit. B_{12}, 15 mg; Biotin, 50 mg; Folic acid 1000 mg; Nicotinic acid 35000 mg; Pantothenic acid 10,000 mg; Mn 80g; Cu 8.8g; Zn 70 g; Fe 35 g; I 1g; Co 0.15g and Se 0.3g.
[2]GE (kcal/100 g DM) = CP x 5.64 + EE x 9.44 + Total carbohydrates x 4.11 calculated according to NRC (1993).
[3]ME (kcal/100g DM) = Metabolizable energy was calculated by using factors 3.49, 8.10 and 4.50 kcal/g for total carbohydrates, EE and CP, respectively according to Pantha (1982).

Intestine histometric examination:

At the end of the experiment, three fish in each aquaria were sacrificed, and fish intestine were taken for histometric examination. Samples were fixed in 10% neutralized formalin solution followed by washing with tap water, then dehydrated using different grades of alcohol (70, 85, 96 and 99%). Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were sectioned to six microns and

stained with hematoxyline and eosin (H & E stains) for preparing the histological slides according to Roberts (2001) and then subjected to histometric examination according to Radu-Rusu *et al*. (2009).

Statistical analysis:

The obtained data was statistically analyzed using general linear models (GLM) procedure according to SAS (2001) for users guide (version 9.2), with factorial design (2 × 4) evaluated by using the following model:

$$Y_{ijk} = \mu + L_i + M_j + LM_{ij} + e_{ijk}$$

Where: Y_{ijk} is the data of growth performance, feed utilization, carcass composition and blood hematological and intestine histometric examination of mono-sex Nile tilapia, μ is the overall mean, L_i is the fixed effect of the crude protein (CP) levels (20 and 25%) in diets, M_j is the fixed effect of the dietary supplementation of Hydroyeast Aquaculture® levels (0, 5, 10 and 15%), LM_{ij} is the interaction effect between the crude protein levels and dietary supplementation of Hydroyeast Aquaculture® levels and e_{ijk} is the random error. All ratios and percentages data were arcsine-transformed prior to statistical analysis. The differences between mean of treatments were compared using Tukey's post hoc significant test, and differences were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Growth and feed efficiency parameters:

Growth and feed efficiency parameters of *O. niloticus* fingerlings fed different levels of crude protein (CP) and Hydroyeast Aquaculture® are illustrated in Table 3. Results revealed that the 25% CP treatment significantly ($P \leq 0.05$) increased final weight, total weight gain, and SGR, as well as significantly ($P \leq 0.05$) improved FCR compared to 20% CP treatment. In contrast, data of PER showed a significant ($P \leq 0.05$) increase in dietary 20% CP than 25% CP. However, PPV and EU were not affected by dietary CP levels. Also, 15g Hydroyeast Aquaculture®/kg BD was the best treatment, followed by 10 g /kg BD, which gave significantly high growth and feed efficiency parameters ($P \leq 0.05$) than 5 and 0 g /kg diet treatments. However, SR was not affected by CP levels, or probiotic levels. In addition, the effect of interaction between dietary CP levels and probiotic levels on all growth and feed efficiency parameters was not significant. Consequently, the high level of CP (25%) and high levels of tested probiotic (15 or 10 g / kg diet, respectively) showed positive effects on the growth and feed efficiency parameters of *O. niloticus* fingerlings among other dietary treatments.

Functional additive, like probiotics, is a new concept on aquaculture (Li and Gatlin III, 2004), where the addition of microorganisms to diets shows a positive effect on growth caused by the best use of carbohydrates, protein, and energy (Irianto and Austin, 2002). In this respect, many researchers suggested the positive effects of probiotics on tilapia growth and feed efficiency (Eid and Mohamed, 2008 and Mehrim, 2009). Olvera-Novoa *et al*. (2002) reported that *O. mossambicus* fed 25 – 30% (of the dietary crude protein) yeast diets showed the best growth performance, feed conversion, protein efficiency ratio, nitrogen utilization, incidence cost, and profit index. Also, Abdel-Tawwab *et al*. (2008) confirmed that the better feed intake in yeast supplemented diets (1.0–5.0 mg/kg diet) may have been due to increased fish appetite resulting in a higher feed intake and therefore improved growth. Additionally, Mehrim (2009) tested the same probiotic (Biogen®) and reported similar positive effects on growth performance and feed conversion ratio of the Nile tilapia fish. Moreover, Khalil *et al*. (2012) reported that Hydroyeast Aquaculture® probiotic is useful at levels of 15 g /kg diet and 10 g /kg diet for enhancing production performance of adult males and females *O. niloticus*, respectively. Recently, Abdelhamid *et al*. (2013 b) suggested the same positive effects on growth and feed efficiency of *O. niloticus* fed some dietary biological additives. Conversely, He *et al*. (2009) found that supplementation of dietary DVAQUA® showed no effects on growth performance, and survival rate of the hybrid tilapia (*O. niloticus* ♀ × *O. aureus* ♂). The reasons for the differences between fish species have not been elucidated, but might be due to the differences in aquaculture and physiological conditions, and the type of basal ingredients in diets.

Probiotics help in feed conversion efficiency and live weight gain(Saenz de Rodriguez *et al*., 2009).Additionally, all the probiotic-supplemented diets resulted in growth to be higher than that of the control diets, suggesting that the addition of probiotics mitigated the effects of the stress factors. This resulted in better *O. niloticus* performance, with better growth parameters in the diets supplemented with the yeast (Lara-Flores *et al*., 2010).Accordingly, to the positive results of the tested probiotic on growth

and feed efficiency parameters in the present study (Table 3) and those obtained by other attempts; probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet, and by breakdown of indigestible components (Irianto and Austin, 2002), increased nutrient digestibility (Burr *et al*., 2005). Better digestibility obtained with the supplemented diets suggests that the addition of probiotics improved diet and protein digestibility, which may in turn explain the better growth and feed efficiency seen with the supplemented diets (Wachéet *al*., 2006). In addition, enzymes in probiotic lead to improve the growth and feed utilization, as well as they lead to digestive enzyme activation (Saxena, 2008).

Table (3): Effect of dietary protein (%), probiotic (Hydroyeast Aquaculture®, g / kg diet) levels and their interaction on growth performance and feed efficiency of *Oreochromis niloticus* fingerlings.

Treat.	Final weight (g)	Total weight gain (g)	SGR (% / d)	SR (%)	FCR	PER	PPV (%)	EU (%)
Crude protein level, % (CP)								
20	36.40^{B}	29.38^{B}	1.67^{B}	88.33	2.08^{A}	2.32^{A}	30.84	14.22
25	45.22^{A}	38.10^{A}	1.88^{A}	96.66	1.83^{B}	2.06^{B}	29.81	15.07
±SE	0.479	0.471	0.011	4.249	0.062	0.067	1.086	0.529
P-value	0.0001	0.0001	0.0001	0.184	0.014	0.014	0.510	0.273
Probiotic level, g / kg diet (P)								
0	36.43^{D}	29.48^{D}	1.68^{D}	86.66	2.15^{A}	1.97^{B}	23.95^{B}	11.12^{C}
5	39.65^{C}	32.50^{C}	1.74^{C}	96.66	1.95^{AB}	2.18^{AB}	30.34^{A}	13.80^{B}
10	42.25^{B}	35.20^{B}	1.81^{B}	96.66	1.81^{B}	2.36^{A}	34.35^{A}	16.73^{A}
15	44.91^{A}	37.80^{A}	1.87^{A}	90.00	1.91^{AB}	2.25^{AB}	32.67^{A}	16.93^{A}
±SE	0.678	0.666	0.015	6.00	0.088	0.096	1.536	0.748
P-value	0.0001	0.0001	0.0001	0.570	0.021	0.041	0.031	0.0001
Interaction (CP*P)								
(T_1) 20/0	32.43	25.49	1.57	86.66	2.23	2.14	23.76	10.90
(T_2) 20/5	35.82	28.67	1.64	93.33	2.09	2.28	30.60	13.11
(T_3) 20/10	36.96	30.04	1.70	93.33	1.86	2.58	37.78	17.13
(T_4) 20/15	40.39	33.34	1.78	80.00	2.14	2.28	31.24	15.74
(T_5) 25/0	40.43	33.47	1.79	86.66	2.08	1.80	24.14	11.34
(T_6) 25/5	43.48	36.33	1.84	100.00	1.81	2.07	30.08	14.49
(T_7) 25/10	47.55	40.37	1.92	100.00	1.76	2.14	30.92	16.33
(T_8) 25/15	49.44	42.26	1.97	100.00	1.68	2.22	34.10	18.13
±SE	0.958	0.943	0.022	8.498	0.125	0.135	2.173	1.058
P-value	0.445	0.508	0.877	0.696	0.507	0.528	0.185	0.499

Mean in the same column in each category having different capital letters are significantly different ($P \leq 0.05$).

SGR: Specific growth rate; SR: Survival rate; FCR: Feed conversion ratio; PER: Protein efficiency ratio; PPV: Protein productive value; EU: Energy utilization.

Fish Carcass composition:

Proximate chemical analysis of the whole body of *O. niloticus* fingerlings at the start or at the end of the experiment is summarized in Table 4. Fish fed dietary 25% CP showed significant ($P \leq 0.05$) increase of DM and CP contents of fish carcass compared with diet containing 20% CP. In contrast, data of EE and ash showed significant ($P \leq 0.05$) increase with 20 than 25% CP. However, no significant ($P \geq 0.05$) differences were found in EC between both dietary CP levels. Fish fed 15 g Hydroyeast Aquaculture®/kg diet showed significant ($P \leq 0.05$) increase in DM, EE, and EC of fish carcass among other levels of probiotic. However, the diet containing 5 g Hydroyeast Aquaculture®/kg diet significantly ($P \leq 0.05$) increased CP content in fish carcass compared with other probiotic levels and the control group. Also, the control group (free diet from the tested probiotic) showed increase in ash content of fish carcass than levels of probiotic treatments. In addition, the interaction effect between dietary CP and Hydroyeast Aquaculture® levels was not significant on all fish carcass composition parameters. Generally, the high level of CP (25%) and high levels of tested probiotic (15 g Hydroyeast Aquaculture® / kg diet) had positive effects on fish carcass composition (DM, CP, and EC) than other dietary treatments. These

positive effects of the high level of CP (25%) and high level of tested probiotic (15 g/ kg diet) on fish carcass confirmed by their positive effects on fish growth, and feed utilization (Table 3).

Table (4): Effect of dietary protein (%), probiotic (Hydroyeast Aquaculture®, g / kg diet) levels and their interaction on carcass composition of *Oreochromis niloticus* fingerlings.

Treat.	DM (%)	On dry matter basis (%) CP	EE	Ash	EC (kcal / 100 g)
At the start:					
	18.16	57.93	19.47	22.59	510.50
At the end:					
Crude protein level, % (CP)					
20	22.01^{B}	57.81^{B}	24.45^{A}	17.73^{A}	556.91
25	22.59^{A}	61.25^{A}	22.80^{B}	15.94^{B}	560.74
±SE	0.079	0.345	0.391	0.151	2.036
P-value	0.0001	0.0001	0.008	0.0001	0.202
Probiotic level, g / kg diet (P)					
0	19.96^{D}	59.75^{B}	21.66^{C}	18.58^{A}	541.56^{D}
5	21.53^{C}	61.92^{A}	21.67^{C}	16.40^{BC}	553.81^{C}
10	23.46^{B}	59.01^{B}	24.46^{B}	16.52^{B}	563.76^{B}
15	24.26^{A}	57.44^{C}	26.71^{A}	15.84^{C}	576.16^{A}
±SE	0.112	0.488	0.553	0.213	2.880
P-value	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction (CP*P)					
(T_1) 20/0	19.86	55.11	22.91	21.98	527.13
(T_2) 20/5	20.83	61.62	22.35	16.02	558.56
(T_3) 20/10	23.26	59.45	24.49	16.05	566.53
(T_4) 20/15	24.10	55.06	28.06	16.88	575.43
(T_5) 25/0	20.06	64.40	20.42	15.18	56.00
(T_6) 25/5	22.23	62.21	20.99	16.79	549.06
(T_7) 25/10	23.66	58.57	24.43	16.99	561.00
(T_8) 25/15	24.43	59.82	25.37	14.80	576.90
±SE	0.158	0.691	0.782	0.302	4.073
P-value	0.211	0.341	0.326	0.288	0.101

Mean in the same column in each category having different capital letters are significantly different ($P \leq 0.05$).
DM: Dry matter; CP: Crude protein; EE: Ether extract; EC: Energy content.

As the current findings in fish carcass composition was affected by dietary CP and Hydroyeast Aquaculture® levels, the yeast supplementation significantly affected of the whole-fish body composition (Abdel-Tawwab *et al*., 2008). These results suggest that yeast supplementation plays a role in enhancing feed intake with a subsequent enhancement of fish body composition. The proximate chemical analysis of *O. niloticus* whole body including total lipids and total ash were significantly influenced by dietary protein level only; meanwhile yeast supplements significantly affected ash content (Abdel-Tawwab, 2012). On the other hand, changes in protein and lipid content in fish body could be linked with changes in their synthesis, deposition rate in muscle and/or different growth rate (Abdel-Tawwab *et al*., 2006).In this topic, Khattab *et al*. (2004b) reported that crude protein, total lipids and ash were significantly ($P < 0.01$) affected by protein level and increasing stocking density rate of tilapia fish.

The present results are in close agreement with those reported by Khattab *et al*. (2004a), EL-Haroun *et al*. (2006) and Mohamed *et al*. (2007) for tilapia fish. Furthermore, Mehrim (2009) reported positive effects of inclusion of Biogen® at a level of 3g/kg on carcass composition of monosex *O. niloticus* fingerlings. Inversely, Eid and Mohamed (2008) found that no statistical differences were observed in whole body moisture, crude protein, ether extract and ash of monosex *O. niloticus* fingerlings fed diets containing different levels of commercial feed additives (Biogen® and Pronifer®), compared with the control treatment. Also, Khalil *et al*. (2012) studied the effect of Hydroyeast Aquaculture® probiotic in both adult males and females of *O. niloticus* and they suggested that fish carcass composition was took unclear trends between adult males and females within all treatments, which may be due to the differ in sexes, metabolism, physiological responses and sexual behaviors of fish during this stage of life, which affected in biochemical contents in their bodies. Generally, there is a negative relationship between crude protein and crude fat of *O. niloticus*carcass on one hand (El-Ebiary and Zaki, 2003), and a positive

correlation between crude protein and crude ash contents of *O. niloticus*, on the other hand (Abdelhamid *et al*., 2007).

Hematological parameters:

Dietary live yeast may improve growth performance and hematological picture in fish (Kobeisy and Hussein, 1995). In the present study, data of hematological parameters of *O. niloticus* fed different levels of dietary protein and Hydroyeast Aquaculture® probiotic are given in Table (5). Data indicated that dietary 25% CP significant ($P \leq 0.05$) increase of blood platelets than 20% CP. However, there were no

Table (5): Effect of dietary protein (%), probiotic (Hydroyeast Aquaculture®, g / kg diet) levels and their interaction on hematological parameters of *Oreochromis niloticus* fingerlings.

Treat.	Hb (g / dL)	RBCs ($\times 10^6$ / mm^3)	PCV (%)	Platelets ($\times 10^3$ / mm^3)	Blood indices			WBCs ($\times 10^3$ / mm^3)
					MCV (μ^3)	MCH (pg)	MCHC (%)	
Crude protein level, % (CP)								
20	6.52	1.08	16.66	219.62^{B}	143.85	58.00	41.42	181.75
25	7.05	1.24	17.13	321.00^{A}	137.01	56.75	41.62	192.62
±SE	0.307	0.070	1.062	20.987	3.478	3.186	3.113	10.120
P-value	0.261	0.147	0.760	0.009	0.202	0.788	0.964	0.469
Probiotic level, g / kg diet (P)								
0	5.82^{B}	0.82^{B}	14.27^{B}	233.25	146.22	61.97	44.72	176.25
5	7.02^{AB}	1.28^{A}	17.85^{AB}	297.75	137.97	54.97	40.00	185.75
10	6.62^{AB}	1.15^{AB}	15.52^{AB}	290.75	134.85	57.62	42.80	186.75
15	7.67^{A}	1.40^{A}	19.95 ^{A}	259.5	142.67	54.92	38.57	200.00
±SE	0.434	0.100	1.502	29.68	4.919	4.506	4.402	14.312
P-value	0.043	0.018	0.011	0.438	0.424	0.667	0.761	0.714
Interaction (CP*P)								
(T_1) 20/0	4.80	0.35	10.90	168.00	157.90	70.80	49.45	171.00
(T_2) 20/5	7.55	1.43	20.40	247.00	141.20	52.60	37.35	184.00
(T_3) 20/10	6.25	1.12	14.65	239.50	130.80	55.85	42.65	183.00
(T_4) 20/15	7.50	1.42	20.70	224.00	145.50	52.75	36.25	189.00
(T_5) 25/0	6.85	1.29	17.65	298.50	134.55	53.15	40.00	181.50
(T_6) 25/5	6.50	1.13	15.30	348.50	134.75	57.35	42.65	187.50
(T_7) 25/10	7.00	1.18	16.40	342.00	138.90	59.40	42.95	190.50
(T_8) 25/15	7.85	1.37	19.20	295.00	139.85	57.10	40.90	211.00
±SE	0.614	0.141	2.125	41.974	6.957	6.373	6.226	20.241
P-value	0.171	0.113	0.108	0.915	0.241	0.293	0.634	0.970

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

significant ($P \geq 0.05$) effects of both dietary CP levels on other hematological parameters. Fish fed 15g Hydroyeast Aquaculture® / kg diet showed the best group followed by the group of fish fed 5g Hydroyeast Aquaculture® / kg diet, where significant ($P \leq 0.05$) increase of hemoglobin (Hb) content; red blood cells (RBCs) and packed cell volume (PCV) than other groups. Meanwhile, no significant differences were detected on blood platelets, blood indices (MCV; MCH and MCHC) and white blood cells count (WBCs) among all dietary probiotic levels. Also, the interaction effects between dietary CP levels and Hydroyeast Aquaculture® levels did not show any significant differences in all hematological parameters. IN the present study, no significant differences of most hematological parameters as affected by dietary CP or Hydroyeast Aquaculture® levels were recorded. These mean no drastic effects on fish health regarding the tested levels of CP or probiotic. In addition, the high level of Hydroyeast

Aquaculture® (15g / kg diet) had a slight improvement on blood hematological parameters among other levels of the tested probiotic, which confirmed by its positive effects on fish growth, feed efficiency (Table 3), and fish carcass composition (Table 4).

Hematological parameters of fish blood are useful tools that aid in studying the immunopotentiators (Tukmechi *et al*., 2011). The same positive effects of tested probiotic on fish health were reported also by other researchers (Khattab *et al*., 2004a and Marzouk *et al*., 2008). Similarly, Rawling *et al*. (2009) reported that PCV, Hb and RBCs levels were not affected of red tilapia (*O. niloticus*) by dietary inclusion of Sangrovit®. In this perspective, RBCs counts and Hb content total serum protein, albumin / globulin ratio and phagocytic activity in *O. niloticus* fed diets containing probiotic bacteria *Micrococcus* species were higher than that of the control group (Osman *et al*., 2010). Also, *O. niloticus* fed diets containing 1.0–5.0 g yeast/kg exhibited higher RBCs, Hb, and PCV values, whereas glucose, lipid, protein, albumin, and globulin values were increased up to 1.0 g yeast/kg diet after which those parameters decreased (Abdel-Tawwab *et al*., 2008). These results suggest an improvement of fish health when fed a yeast supplement. Also, in a study conducted by Zhu *et al*. (2012) no effects on total number of leukocytes, granulocytes, and lymphocytes were observed, however, the number of monocytes was significantly elevated in fish fed 0.2% dietary yeast polysaccharides. Therefore, the increased number of blood monocytes in *I. punctatus* might indicate an immunomodulatory effect. In addition, Dahiya *et al*. (2012) revealed that probiotic had a positive effect on Hb level which increased approximately to 24% in its value, RBCs count which increased approximately to 10% in its value, WBCs count which decreased approximately to 8% in its value and PCV increased approximately to 20% in its value. This clearly indicated that there was positive response in the values of haematological parameters of Indian magur (*Clarius batrachus* L.). These results agreed with some previous studies (Welker *et al*., 2007 and Hai and Fotedar, 2009).

Intestine histometric measurements:

Histometric characteristics of intestine of experimental fish fed diets containing different levels of protein (20 or 25%) and Hydroyeast Aquaculture®(0, 5, 10 and 15 g / kg diet) were illustrated in Table 6. Fish fed diet containing 25% CP led to significantly ($P \leq 0.05$) increased all intestine histometric parameters compared with 20% CP. However, intensity of villi per mm^2 have not significant ($P \geq 0.05$) differences among treatments. Also, increasing levels of tested probiotic (15 and 10 g / kg diet, respectively) revealed significantly ($P \leq 0.05$) increased musculosa thickness (µm); submucosa thickness (µm); villi length (µm) and absorption area of villous (in 1 mm^2), meanwhile the villi width (µm) and the intensity of villi per mm^2 were significantly ($P \leq 0.05$) decreased compared with the low level of probiotic (5 g / kg diet) or the control group. In addition, the interaction between dietary CP and Hydroyeast Aquaculture® levels did not show any significant differences in all intestine histometric parameters.

These enhancement of the histometric characteristics of intestine by increasing the levels of dietary protein or tested probiotic may be related with protein functions for development the intestine tissue and increase their absorption efficiency, as well as may be related with the role of the tested probiotic components. The tested probiotic inclusion the live yeast or bacteria species, which has stimulating effects for intestine layers, and beneficial to protect the integrity of fish intestinal mucosal layer. In addition, previous studies have regarded the effect of probiotics or immunostimulants in intestinal morphology and a number of them are *in vitro* or *ex vivo* experiments (Merrifield *et al*., 2011 and Zhu *et al*., 2012).

Villous height and crypt depth are a direct representation of the gut function and health. In this respect regarding with the present findings, Uni *et al*. (1995) suggested that an increase in villous height might also indicate a greater absorption area and vice versa. The mucosa enzyme activity per mass of intestine is closely associated with the number of enterocytes per villi. In aquatic animals, intestinal villous height is regarded as a sign of absorption ability. Also, recent studies have demonstrated that ingredients such as mannanoligosaccharides (Dimitroglou *et al*., 2010), *Lactobacillus rhamnosus* (Pirarat *et al*., 2011) and polysaccharide yeast (Zhu *et al*., 2012) are able to increase villus height in different fish species.

A previous study on gilthead Sea bream demonstrated that mannanoligosaccharide administration leads to an increase in villous height length and density, although no final effect was observed in the absorptive surface (Dimitroglou *et al*., 2010). Yet, Merrifield *et al*. (2010b) reported that probiotics (*Pediococcus acidilactici*) may enhance the enterocyte microvilli in rainbow trout (*Oncorhynchus mykiss*). Recently, Gisbert *et al*. (2013) reported that the probiotic-supplemented diet increased the level of leukocyte infiltration in the lamina propria of the intestinal mucosa, the number of goblet cells ($P < 0.010$), and villi height ($P < 0.001$), but did not affect villi width in *O. mykiss* fingerlings. Where,

microvillus height is usually thought to be directly related to the absorptive surface area of the intestine. In addition, Varley (2008) cited that probiotics show real benefits in the synergistic effects with the beneficial bacteria in making inroads into improving gut health. However, to our knowledge, no previous studies have taken into consideration the possible variations in this parameter that are caused by differences in intestinal size .

Table (6): Effect of dietary protein (%), probiotic (Hydroyeast Aquaculture®, g / kg diet) levels and their interaction on intestine histometric measurements of *Oreochromis niloticus* fingerlings

Treat.	MST (µm)	SMT (µm)	Villi			Absorption area of villous (in 1 mm^2)
			Length (µm)	Width (µm)	Intensity (villi / mm^2)	
Crude protein level, % (CP)						
20	27.82 ^{B}	30.90 ^{B}	495.85 ^{B}	128.87 ^{B}	6.12	4.39 ^{B}
25	36.45 ^{A}	36.31 ^{A}	573.55 ^{A}	156.97 ^{A}	6.03	6.15 ^{A}
±SE	1.412	1.446	11.991	3.335	0.074	0.191
P-value	0.0001	0.0025	0.0001	0.0001	0.3614	0.0001
Probiotic level, g / kg diet (P)						
0	27.95 ^{B}	26.63 ^{C}	305.53 ^{D}	172.13 ^{A}	6.46 ^{A}	4.42 ^{C}
5	30.38 ^{B}	30.39 ^{BC}	405.05 ^{C}	150.98 ^{B}	6.50 ^{A}	5.24 ^{B}
10	33.44 ^{AB}	35.76 ^{AB}	664.31 ^{B}	127.02 ^{C}	5.60 ^{B}	5.26 ^{B}
15	36.76 ^{A}	41.64 ^{A}	763.90 ^{A}	121.55 ^{C}	5.73 ^{B}	6.16 ^{A}
±SE	1.996	2.045	16.959	4.717	0.104	0.270
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Interaction (CP*P)						
(T_1) 20/0	25.45	25.50	260.35	158.66	6.46	3.43
(T_2) 20/5	26.98	27.77	339.51	131.82	6.50	3.77
(T_3) 20/10	28.78	31.36	668.60	113.71	5.80	5.12
(T_4) 20/15	30.07	38.97	714.90	111.27	5.73	5.24
(T_5) 25/0	30.45	27.77	350.70	185.60	6.46	5.41
(T_6) 25/5	33.78	33.02	470.59	170.15	6.50	6.72
(T_7) 25/10	38.11	40.16	660.01	140.32	5.41	5.40
(T_8) 25/15	43.45	44.30	812.89	131.82	5.73	7.08
±SE	2.824	2.893	23.983	6.670	0.148	0.382
P-value	0.344	0.511	0.485	0.634	0.388	0.573

Mean in the same columnin each category having different capital letters are significantly different ($P \leq 0.05$).
*MST: Musculosa thickness; SMT: Submucosa thickness; Absorption area of villous (in 1 mm^2) = *Area of one villi × Intensity of villi (villi / mm^2); *Area of one villi = villi length × villi width × 2.*

Inversely with our findings, Cerezuela *et al*. (2012) noted that diets containing *Bacillussubtilis* resulted in a decrease in microvillus height. Interestingly, another study reported a strong decrease in microvillus height provoked by the replacement of fish meal with soybean meal in fish diet (Rombout *et al*., 2011). This suggests that *B. subtilis* had a negative impact on microvilli similar to the one observed in fish fed vegetal diets.Generally, these enhancements of histometric characteristics of fish intestine were distinctly increased by increasing the dietary protein or tested probiotic levels, which confirmed by the significantly increased of the growth and feed efficiency parameters (Table 3). Where, closely related also by the levels or the formula of the tested probiotic, or may be the probiotic properties for stimulating the appetite of fish and consequently increasing the feed intake and nutrients utilization without any adverse effects on the experimental fish (according to the hematological parameters, Table 5).

CONCLUSIONS

From the foregoing results, it could be concluded that the high level of Hydroyeast Aquaculture® probiotic (15 g / kg diet) is the best level with the low dietary CPlevel (25%) in this early stage of life of experimental fish for enhancing the growth and feed efficiency, carcass composition, physiological and intestine histometric responses of monosex male *O. niloticus* fingerlings. Although the cost of supplementing 1500 g Hydroyeast Aquaculture® / tone diet would increase the price by 2.5%, yet this improved the performance in terms of the total growth gain of fish by 128.5% compared to the control group during this experiment. Thus, this probiotic may lead to improve the economic efficiency at large scale in the fish farms without adversely effects on fish health. Also, to confirm these findings, further studies on other dietary probiotics, prebiotics, synbiotics, cheaper ingredients, and protein sources are needed for reducing the feeding costs with better digestibility and fish health.

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تأثير مستويات البروتين الغذائى وهيدروييست أكواكلشر على أداء النمو، الاستفادة الغذائية ، الإستجابات الفسيولوجية والهستوميترية لإصباعيات البلطى النيلى

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قسم الإنتاج الحيواني ــ كلية الزراعة ــ جامعة المنصورة ــ المنصورة ـ مصر

يعد البلطى من أهم الأسماك المستزرعة فى العالم. تهدف هذه الدراسة إلى تقييم تأثيرات إضافة مستويات متدرجة (صفر، 5 ، 10 ، 15 جرام/ كجم علف من البروبيوتيك®Hydroyeast Aquaculture) إلى العلائق ذات مستويات البروتين المنخفض (20 أو 25 % بروتين خام) لإصباعيات البلطى النيلى وحيد الجنس ذكور لمدة 14 أسبوع فيما يتعلق بكفاءة النمو، الأداء والاستفادة الغذائية ومكونات الجسم ، القياسات الهيماتولوجية و الهستوميترية للأمعاء.

وزعت الأسماك ذات متوسط الوزن الإبتدائى (7.5 ± 0.001 جرام) على ثمانية معاملات غذائية (ثلاث مكررات / معاملة). سكنت الأسماك فى الأحواض بمعدل 5 أسماك / حوض (أبعاده 90 × 40 × 35 سم). أوضحت النتائج أن المستوى العالى من البروتين (25% بروتين خام) ، المستوى العالى من البروبيوتيك (15 جرام / كجم علف) أدى إلى تأثيرات إيجابية معنوية على قياسات كفاءة نمو الأسماك، الاستفادة من الغذاء ومكونات جسم السمك (المادة الجافة، البروتين والمحتوى من الطاقة) ، المكونات الهيماتولوجية (الهيموجلوبين ، خلايا الدم الحمراء ، حجم الخلايا المضغوطة ، الصفائح الدموية) ، القياسات الهستوميترية للأمعاء مقارنة بالمعاملات الأخرى، كما أوضحت النتائج عدم وجود تداخل معنوي بين المعاملات لكل القياسات السابقة. لذا يمكن التوصية بأن المستوى العالى من البروبيوتيك 15 جم ®Hydroyeast Aquaculture / كجم علف هو المستوى الأنسب مع المستوى المنخفض من البروتين الغذائى (25%) لهذه المرحلة العمرية للأسماك لتحسين الأداء الإنتاجي ، الإستجابات الفسيولوجية ، القياسات الهستوميترية لأمعاء إصباعيات البلطى النيلى وحيد الجنس ذكور. وبالتالي فإن استخدام البروبيوتيك بهذا المستوى ربما يؤدى إلى زيادة الكفاءة الاقتصادية على نطاق أوسع فى المزارع السمكية.