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Use of high protein distiller's dried grains (HPDDG) with enzyme phytase as a cost effective ingredient in the diet of fingerlings European sea bass, *Dicentrarchus labrax*

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ABSTRACT

The present study was conducted to evaluate the effect of various dietary levels of high protein distiller's dried grains (HPDDG) supplemented with enzyme phytase on growth performance, feed utilization and haematological indices of European sea bass *Dicentrarchus labrax* fingerlings. A total of 150 *D. labrax* fingerlings with an average body weight of 7.5 ± 0.5g were divided in the five experimental treatments (in triplicates each). The experiment was conducted for 56 days. Fish were fed to apparent the satiation six days a week. Five isonitrogenous 45% CP experimental diets were formulated. The control (C) diet had no high protein distiller's dried grains (HPDDG) and phytase enzyme added. Diet 2 (C^+) was formulated as control diet and supplemented with phytase at a level of 0.5 g/kg. Diets 3-5 each contained HPDDG at levels of 20, 30, and 40 %, respectively with soybean meal and supplemented each with phytase at a level of 0.5 g/kg. Over the 56 days feeding period, all fish fed HPDDG-supplemented with phytase diets resulted in higher growth than the control diet, suggesting that the using of HPDDG-supplemented with phytase enhanced the growth performance of sea bass fingerlings. The same trend was recorded for the best FCR and PER. The hematology and serum biochemistry (hemoglobin (Hb), red blood cells (RBCs) count, white blood cells (WBCs) and hematocrit (Hct %) and immune parameters (total protein, albumin, globulin, cholestorl, lysozyme activity and total antioxidant capacity)

of *D. labrax* fingerlings significantly ($P \geq 0.05$) increased with increasing dietary HPDDG with phytase. Result indicates that HPDDG with phytase is a good alternative protein source for compromising growth performance and physiological parameters of European sea bass, *Dicentrarchus labrax* Fingerlings.

Keywords: *Dicentrarchuslabrax*, high protein distillers dried grains,phytase, physiological parameters, growth.

Introduction

Aquaculture is one the fastest growing agriculture industries in the world. Globally, in 2016, aquaculture was responsible for the production of 171 million tons of fish products, most of which was for human consumption **(FAO, 2018).** With the world's population projected to reach 9.7 billion people by 2050 and global capture fisheries unstable and steadily declining, the spotlight turns to aquaculture production to contribute significantly to global food security and adequate global nutrition and human health **(NASS, 2016).**

More than 70% of the total global aquaculture production is dependent upon the supply of external feed inputs. For the aquaculture sector to maintain its current growth rate, the supply of nutrient and feed inputs will have to grow at a similar rate, while aquatic ingredients production remains static and other sectors compete for same feed resources.

Distillers' dried grains with soluble (DDGS), which are the by-product of cereal distillation for ethanol production. Except for the starch fraction, which is consumed during fermentation, DGGS's nutrient content is almost 3 times more concentrated than the original grain, thus containing higher protein, lipid and fiber levels (**Liu, 2011).** The protein content in DDGS ranges from 25 to 45%, depending on the grain source, and has reduced anti-nutritional factors comparatively to most plant protein sources. Studies on DDGS incorporation in aqua-feeds studies were mainly done in omnivorous fish species, such as channel catfish, *Ictalurus punctatus*; (**Li *et al.*, 2011a),** Nile tilapia, *Oreochromis niloticus* and hybrid tilapia, *Oreochromis niloticus* × *Oreochromis aureus* **(Welker *et al.*, 2014a).** So far, studies performed on the potential use of DDGS in carnivorous species are limited to a few studies with rainbow trout, *Oncorhynchus mykiss* **(Overland *et al*., 2013; Welker *et al*., 2014b),** olive flounder, *Paralichthy solivaceus* **(Rahman *et al.*, 2015;**

Bae *et al.*, 2015), and meager, *Argyrosomus regius* **(Magalhães *et al.*, 2016).**

Currently, the use of various enzymes in aquatic feed has been on the rise to improve the overall quality of diets containing these economical protein sources. Use exogenous enzymes to feeds could improve the growth performance by means of enhancing nutrient digestibility **(Farhangi and Carter, 2007),** increasing activities of digestive enzymes **(Lin *et al.*, 2007),** and improving the histological structure **(Mathlouthi *et al.*, 2002)** and the health of intestine (reviewed by **Castillo and Gatlin, 2015**). They include phytase **(Liu *et al.* 2013),** protease **(Dalsgaard *et al.* 2012),** and xylanase **(Nie *et al*. 2009).** In addition, exogenous enzymes have been used extensively throughout the world as additives in animal diets. The use of these exogenous enzymes has been shown to affect the digestibility of nutrients, including protein, carbohydrates and minerals **(Forster *et al.* 1999).** However, supplementation enzymes help to eliminate the effects of anti-nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved fish performance **(Soltan 2009).** The effects of enzyme supplementation on the growth and survival of several cultured fish species have been demonstrated by several researchers; rainbow trout, *Oncorhynchus mykiss* **(Dalsgaard *et al.*, 2014),** tilapia,*Oreochromis niloticus* × *O. aureus* **(Li *et al.*, 2015),** common carp, *Cyprinus carpio* **(Leng *et al.*, 2008),** Atlantic salmon, *Salmo salar* L. **(Carter *et al.*, 1994),** and white shrimp, *Litopenaeus vannamei* **(Li *et al.*, 2015)** but, there are few published reports on the effect of adding enzymes to diets for sea bass on feed utilization, growth performance and mineral utilization. Phytic acid, a known anti-nutrient, in the diets of non-ruminants can significantly affect growth and overall health of animals, through the formation of complexes with proteins and divalent cations **(Dersjant-Li *et al.* 2015).** Phytic acid is prevalent in plant matter, and especially abundant in ingredients such as cottonseed meal, wheat middlings, and soybean meal. The commercial poultry and swine industries have reported the use of phytases to aid in the destruction of phytic acid and increase bioavailability of minerals, protein, and the absorption of phosphorus **(Selle and Ravindran, 2007).**

The present study was undertaken to determine the effect of various dietary levels of high protein distiller's dried grains (HPDDG) supplemented with enzyme phytase on growth performance, feed

utilization and haematological indices of European sea bass *Dicentrarchus labrax* fingerlings.

Material and Methods

Fish and experimental facilities

Total 150 fingerlings of European sea bass, *Dicentrarchus labrax* with an average initial body weight of 7.5 ± 0.5g/fish was obtained from a private commercial fish farm (El- Shref farm, Wady Marriott, Alexandria). During fish acclimation for one week in indoors circular fiberglass tanks (1 cubic meter), fish was fed experiment diet contained 45% crude protein. Then, fish were randomly distributed into 15 glass aquaria measuring (70x40x30cm each) (five treatments, each in triplicate) at a stocking density of 10 fish per aquaria. Daily water exchange rate was 50% underground salinity water (37 ppt). Water temperature, dissolved oxygen, pH, and ammonia were monitored during the trial, to maintain water quality at optimum range for *D. labrax*. Water temperature was maintain at18 ± 1.0°C, dissolved oxygen (DO) at 5.7 mg/L and pH at 7.0 ± 0.50. Fish was held under natural light (12:12 h light:dark schedule).

Experimental design and diets

Five isonitrogenous (45% CP) and isolipidic (11% EE) experimental diets were formulated (Table 1). The control (C) diet had no high protein distiller's dried grains (HPDDG) and phytase enzyme added. Diet 2 (C^+) was formulated as control diet and supplemented with phytase at a level of 0.5 g/kg. Diets 3-5 each contained HPDDG at levels of 20, 30, and 40 %, respectively and supplemented each with phytase at a level of 0.5 g/kg. The daily ration was divided into two equal amounts and offered two times a day (09.00 and 13.00 h). The fish were fed one of the five experimental diets for 56 days, six days a week.

Experimental diets were prepared by mixing the dry ingredients of each diet were thoroughly mixed and 200 ml of water was added per kg diet thereafter, the mixture (ingredients and water) was blender to make a paste of each diet. pelleting of each diet was carried out by passing the blended mixture through laboratory pellet matching was a 1mm diameter matrix, the resulting wet pellet were dried at room temperature for two days. The diets were stored in plastic bags in refrigerator (-2°C) until use.

Table (1): Shows the feed ingredients (%) and chemical composition (%) of the diet.

Ingredients	Experimental Diets				
	Control (C)	C^+	$D_{20\%}{}^+$	$D_{30\%}{}^+$	$D_{40\%}{}^+$
Fish meal (68 % CP)	300.00	300.00	300.00	300.00	300.00
Soy bean meal (47% CP)	375.00	375.00	262.50	225.00	187.50
Corn gluten (60% CP)	90.00	89.5	89.50	89.50	89.50
Rice bran (12% CP)	65.00	65.00	50.00	50.00	50.00
Wheat medling (13% CP)	70.00	70.00	83.80	84.80	85.80
HDDGS (47% CP)	0.00	0.00	112.50	150.00	187.50
Soy bean oil	40.00	40.00	41.00	40.00	40.00
Fish oil	48.8	48.8	49.00	49.00	48.00
Di-calcium phosphate	8.00	8.00	8.00	8.00	8.00
Premix [1]	2.00	2.00	2.00	2.00	2.00
Vit. C	0.20	0.20	0.20	0.20	0.20
Antytocsec	1.00	1.00	1.00	1.00	1.00
Phytase	0.00	0.50	0.50	0.50	0.50
Chemical composition (%, dry matter basis)					
Dry matter (DM)	93.80	93.80	93.77	93.80	93.60
Crude protein (CP)	44.10	44.10	44.40	44.97	45.20
Ether extract (EE)	12.56	12.56	11.00	10.48	9.96
Nitrogen free extract (NFE)[2]	29.09	29.09	30.50	31.15	30.74
Crude fiber (CF)	3.25	3.25	4.30	3.40	3.10
Ash	11.00	11.00	9.80	10.00	11.00
Gross energy (GE; Mj/kg DM)[3]	20.93	20.93	20.81	20.70	20.42

[1] Vitamin and mineral mixture (supplements per kg of the mixed feed): vitamin A, 4,500 IU; vitamin D3, 4,500 IU; vitamin E, 400 mg; vitamin B1, 30 mg; vitamin B2, 40 mg; vitamin B6, 40 mg; vitamin B12, 0.08 mg; vitamin K3, 15 mg; ascorbic acid, 750 mg; nicotinic acid, 300 mg; Ca-pantothenate, 100 mg; folic acid, 10 mg; biotin, 3 mg; inositol, 500 mg; p-amino benzoic acid, 200 mg; Ca, 2.1 g; Fe, 250 mg; Mn, 40 mg; Zn, 60 mg; I, 4 mg; Cu, 12 mg; Se, 0.3 mg; Co, 2 mg.[2]NFE: calculated using the following equation: NFE = 100 (crude protein + ether extract + crude fiber + ash).[3]Gross energy (GE) contents of diets were calculated according to gross caloric values of Brett (1973) using the values of 23.6, 39.5, and 17.2 kJg^{-1} for crude protein, crude fat, and total carbohydrate, respectively.

Commercial phytase enzyme product was used for supplementation of the experimental diets at levels of 0.5g/kg diet. One gram phytase contains the enzymatic activity of 5000 Phytase Units (FTU), phytase was dissolved into 100 mL water at 37 °C **(Yoo *et al.*, 2005).** The solution was added to the experimental diets and incubated for 24 hours at room temperature according to the method of **Danwitz *et al.*, (2016).** Pellets were stored in the refrigerator at - 4 °C (Ambasankar *et al.*, 2009).

Growth Indices

The mean final body weight (FBW) in experimental treatment was determined by dividing the total fish weight in each aquarium by the number of fish. Body weight gain (BWG), feed conversion ratio (FCR), protein efficiency ratio (PER) and specific growth rate (SGR) were calculated using the following equations, according to **Cho (1990) and Castell and Tiews (1980):**

BWG = final body weight (g) - initial body weight (g).
FCR = feed intake (g)/weight gain (g).
SGR (%/day) = 100× [(ln final body weight (g) - ln initial body weight (g))/ duration of feeding (day)].
PER = weight gain (g)/protein intake (g)

Blood Sampling

Blood samples were collected at the end of the experiment. Each of the experimental treatment was sampled once, with five fish/ net enclosure for hematological indices analysis and five fish/ net enclosure bled for plasma content analysis. The fish were anesthetized with t-amyl alcohol and the blood samples were taken by puncturing the caudal vessels. The collected blood was divided into two tubes, one containing heparin as anticoagulant agent for haematological assessment and the other was anticoagulant free for biochemical estimation. The haematological parameters are expressed in international units (SI).

The total red and white blood cell counts (RBC; 10^6 mm^{-3} and WBC; 10^3 mm-3, respectively) were obtained by using a standard Neubauer-hemocytometer chamber using Shaw's solution as diluting fluid **(Stoskopf, 1993).**Hemoglobin (Hb; g ⁄ dL^{-1}) was determined colorimetrically using commercial kits (Diamond, Egypt) according to cyanmethemoglobin procedure **(Drabkin, 1945 and Stoskopf, 1993).**

The total protein (g dL^{-1}) was determined in plasma samples of fish from the different experimental groups by the Biuret method according to

(Doumas *et al.* 1981) .Albumin (g dL^{-1}) was determined by the bromocresol green method (Reinhold ,1953) and globulin (g dL^{-1}) was calculated as the difference between total protein and albumin. Cholesterol using a commercial kit (Pasteur, Lab, France, Egypt) **(Yousefi *et al.* 2011).**

Lysozyme activity (U mg^{-1} protein) in serum was determined according to the method of **Kim and Austin, (2006)** and **Ellis, (1990)** based on the lysis of the lysozyme sensitive gram-positive bacterium Micrococcus lysodiekticus (Sigma, St. Louis, MO). Lysozyme acts upon susceptible bacteria by combining with and breaking down a mucopolysaccharide. This mucopolysaccharidehas been shown to be situated in the bacterial cell wall. M. lysodeikticus, one of the gram positive bacteria, is normally highly sensitive to lysozyme.3 dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 25 μg mL^{-1} (in 0.1 M phosphate-citrate buffer, pH 6) (Sigma, USA) were used as the standard. Prepared standard solutions were placed along with the undiluted serum sample (25 μL) in the wells of a 96-well plate in triplicate, 175 μL of M. lysodiekticussuspension (750 μg mL-1) was prepared in the same buffer

Total antioxidant capacity (TAC) level was estimated spectrophotometrically at 532 nm following the method with Tween 80 oxidation **(Galaktionova *et al.* 1998 and Prieto *et al.* 1999).** Briefly, 0.2 ml of tissue homogenate was added to 2 ml of 1% Tween 80. Instead of the sample, the blank assay included 0.1 ml of distilled water. The mixture was incubated for 48 hours at 37 ºC. After cooling, 1 ml of 40% TCA was added. The mixture was centrifuged at 3,000 g for 10 min. After centrifugation, 2 ml of supernatant and 2 ml of 0.25% TBA reagent were mixed in. The mixture was heated in a boiling water bath at 100 ºC for 15 minutes. The absorbance of the solution obtained was measured at 532 nm and was compared with the blank. The TAC level was expressed in %.

Statistical analysis:

One-way ANOVA and **Duncan's, (1955)** multiple range tests were calculated effects with a probability of $p<0.05$ were considered significant. The data of the experiments were statistically analyzed using GLM (general linear model) procedure according to Statistical Analysis System (**SAS 2004**). However, data are presented untransformed to

facilitate comparisons. The relationship between hematological indices was tested using simple correlation analysis.

Results and Discussion

The use plant feedstuff such as (wheat gluten, soybean meal, soy protein concentrate) imposes some concerns due to the "food-feed competition", rising prices, and carbon footprint involved in their production and importation **(Bonaldo *et al.*, 2015).** Thus, there is an increasing need to look for alternatives, particularly underutilized commodities, such as by-products obtained from food, fermentation and pharmaceutical industries, which is highly dependent of imported plant feedstuffs, as soybean meals, for aqua-feeds formulation **(Matos *et al.* 2016).** Within these alternative plant feedstuffs, distillers' dried grains with solubles (DDGS) can consider, which are the by-product of cereal distillation for ethanol production. Except for the starch fraction, which is consumed during fermentation, DGGS's nutrient content is almost 3 times more concentrated than the original grain, thus containing higher protein, lipid and fiber levels **(Liu, 2011).** Recently, the majority of the dry-grind ethanol plants produced a DDGS by-product containing 26–34% protein, depending on the grain source, and has reduced anti-nutritional factors comparatively to most plant protein sources. **(Rosentrater and Muthukumarappan 2006).** However, many ethanol plants are implementing a modified dry milling process called fractionation to increase ethanol yields. In this new process, whole corn is milled, then sorted into separate fractions: corn germ, bran, and the endosperm (which is used for ethanol fermentation). The two main co-products of the modified process are corn germ and high-protein distiller's dried grains (HP-DDG). This HP-DDG product has a protein level of 43–49% and lower levels of fat and phosphorus than that in traditional DDGS because it does not contain the solubles component that would normally be added back to the distiller's dried grains **(Tidwell *et al.* 2017).** The higher protein content of HP-DDG could make them even more attractive for inclusion in fish diets because protein is generally the most expensive nutrient component in aqua-feeds.

Table (2): Growth performance of European sea bass, *Dicentrarchus labrax* fingerlings after 56 days of feeding various levels of high protein distiller's dried grains (HPDDG) with enzyme phytase.

	Experimental diets				
	Control	C^+	$D_{20\%}{}^+$	$D_{30\%}{}^+$	$D_{40\%}{}^+$
IBW (g $fish^{-1}$)	7.55±0.05	7.5±0.05	7.50± 0.06	7.60± 0.01	7.60± 0.03
FBW (g $fish^{-1}$)	$13.95±0.65^d$	$15.50±0.28^c$	$17.60± 0.21^b$	$17.80± 0.15^b$	$18.92±0.21^a$
BWG (g $fish^{-1}$)	$6.55±0.65^c$	$7.86±0.26^c$	$9.80±0.15^b$	$9.95±0.15^b$	$10.73±0.20^a$
SGR (% day^{-1})	$1.25±0.15^c$	$1.25±0.11^c$	$1.37±0.01^b$	$1.45±0.02^{ab}$	$1.58±0.02^a$

Values are mean ±SD of triplicate analyses. Means in the same row bearing different superscript differ significantly *($P \leq 0.05$)*. C: control, D: HPDDG, high protein distiller's dried grains; +: phytase, IBW, initial body weight; FBW, Final body weight; BWG, body weight gain and SGR specific growth rate.

Table(3): Nutrient utilization of European sea bass, *Dicentrarchus labrax* fingerlings after 56 days of feeding various levels of high protein distiller's dried grains (HPDDG) with enzyme phytase.

	Experimental diets				
	Control	C^+	$D_{20\%}{}^+$	$D_{30\%}{}^+$	$D_{40\%}{}^+$
FCR	$1.70±0.07^c$	$1.60±0.04^c$	$1.40±0.05^b$	$1.43±0.03^b$	$1.20±0.03^a$
FI (g $fish^{-1}$)	10.02±0.1	12.13±0.49	13.95±0.40	13.25±0.17	13.10±0.06
PER	$1.48±0.21^c$	$1.47±0.30^c$	$1.58±0.4^b$	$1.67±0.13^b$	$1.81±0.15^a$

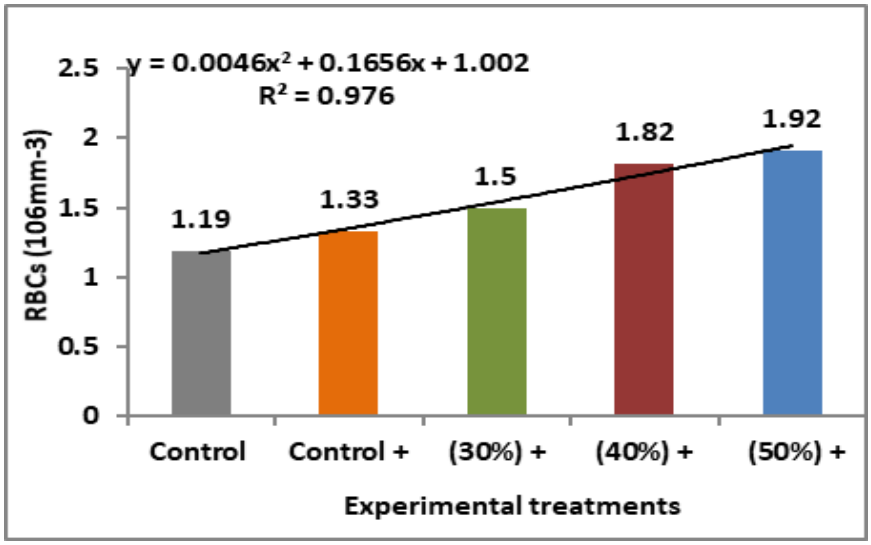
Values are mean ±SD of triplicate analyses. Means in the same row bearing different superscript differ significantly *($P \leq 0.05$)*. C: control, D: HPDDG, high protein distiller's dried grains; +: phytase, FCR: feed conversion ratio, FI: Feed intake and PER: Protein efficiency ratio.

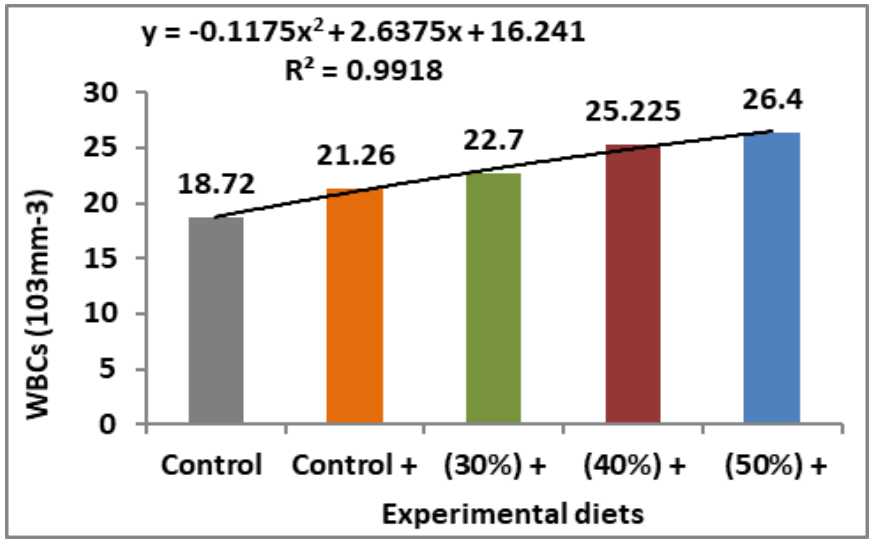
Data in Table 2 shows that all fish fed HPDDG-supplemented with phytase diets resulted in higher growth than the control diet, suggesting that the using of HPDDG-supplemented with phytase enhanced the growth performance of sea bass fingerlings. The final body weight (FBW), body weight gain (BWG) and specific growth rate (SGR) of *D. labrax* increased with increasing dietary HPDDG-supplemented with phytase up to 400g/kg. The same trend was recorded for the best FCR and PER (Table 3). For omnivorous fish species, DDGS may be used to replace fish meal and soybean meal, up to 40% of diet, without lysine supplementation **(Webster *et al.* 1991, 1992, 1993; Lim *et al.* 2009)** and with no negative repercussions on growth performance. Higher dietary replacement levels may be achieved with the adequate restoration of dietary essential amino acid profile, by using amino acid supplements or combination among different protein sources **(Webster *et al.* 1991; Cheng and Hardy 2004). Webster *et al.* (1992)** states that a combination of DDGS with soybean meal (35% DDGS and 49% soybean meal) can be used to totally replace in the diet, with or without lysine supplementation and methionine. In rainbow trout diets, **Cheng and Hardy (2004)** states that DDGS can be used at 22.5% inclusion level or at 75 % with lysine and methionine supplementation. In the same species a replacement of 25 % of fish meal can be achieved with a mixture of corn DDGS and corn gluten meal **(Stone *et al* 2005). Recently, Totok *et al* ., (2017)** reported that adding phytase enzyme supplementation of 1,000 FTU on s feed has highly significant effect to growth of Asian seabass. **Wallace *et al.*, (2016)** reported thatphytase had positive potential impacts on nutrient utilization and growth responses in tilapia. FCR, AWG and SGR improved marginally with inclusions suggesting some level of enzyme counteraction on targeted ANFs ($P > 0.05$).

The addition of enzymes in diets can help to improve the utilization of dietary energy, amino acids and eliminate the effects of anti-nutritional factors (ANFs) resulting in improved performance **(Gitoee *et al.*, 2015),** due to disrupting the cell wall matrix and enhancing the contact between digestive enzyme and cell content, which resulted in improved energy and nutrient digestibility **(Wu *et al.*, 2004). Zhu *et al.*, (2014),** reported that dietary phytase supplementation significantly increased growth performance of seabass. Insignificant effects on growth performance were also reported in Japanese seabass, *Lateolabrax japonicas*, with the supplementation of 500 IU kg^{-1} feed phytase **(Ai *et al.*, 2007)** and in

channel catfish, *Ictalurus punctatus* under similar phytase treatment **(Yan and Reigh 2002).** However, some significantly positive effects of phytase supplementation on growth were observed in rainbow trout **(Vielma *et al.*, 2002).** Therefore, phytase supplementation level is important in the effects on growth performance. The addition of exogenous enzymes to aquafeed has been reported to enhance the digestion of indigestible ingredients in some species of fish such as Altantic salmon **(Carter *et al.* 1994)**, black tiger shrimp *Penaeus monodon* **(Buchanan *et al.* 1997),** and silver perch *Bidyanus bidyanus* **(Stone *et al.* 2003).**

Hematological parameters investigated are presented in Figures (1-5). Mean red blood cell counts (RBCs), mean white blood cell counts (WBCs), hematocrit (Hct), hemoglobin (Hb) (Fig. 1), total plasma protein, ,total plasma globulin (Fig. 2), Cholesterol (Fig. 3), Lysozyme (Fig. 4) and total antioxidant capacity (TAC, %)(Fig. 5) of *D. labrax* fingerlings significantly ($P \geq 0.05$) increased with increasing levels HPDDG with phytase. A highly positive correlation was observed between dietary HPDDG-supplemented with phytase and WBCs (R^2= 0. 976), RBCs (R^2= 0. 991), Hct (R^2= 0. 916), Hb (R^2= 0. 949), Cholesterol (R^2= 0. 962), Lysozyme (R^2= 0. 987) and TAC, % (R^2= 0. 967).

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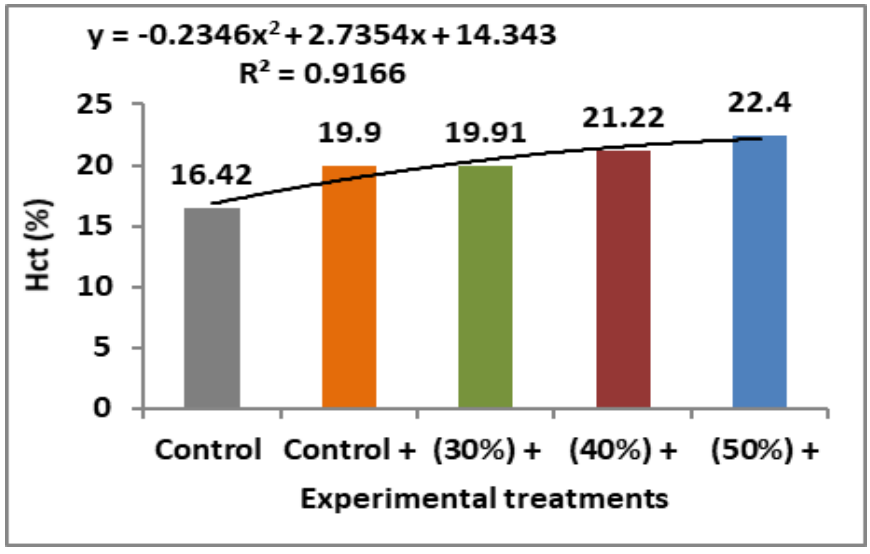
R² = 0.9918
WBCs (103mm-3)
30
25
20
15
10
5
0
18.72
21.26
22.7
25.225
26.4
Control
Control +
(30%) +
(40%) +
(50%) +
Experimental diets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R² = 0.9166
Hct (%)
25
20
15
10
5
0
16.42
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19.91
21.22
22.4
Control
Control +
(30%) +
(40%) +
(50%) +
Experimental treatments

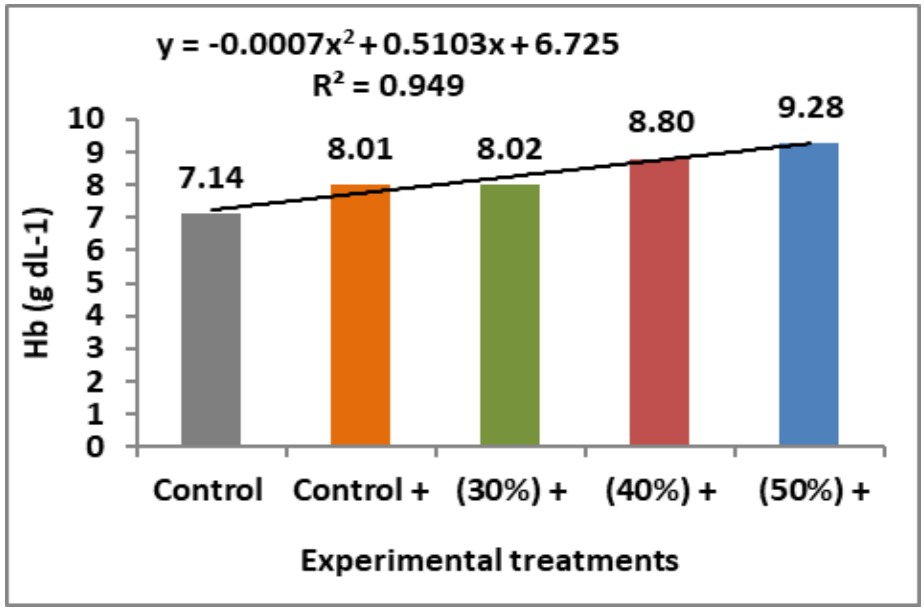

Fig. 1 Illustrated the effect of various dietary levels of high protein distiller's dried grains (HPDDG) with enzyme phytase on blood RBCs, WBCs, Hct and Hb contents of European sea bass, *Dicentrarchus labrax* fingerlings.

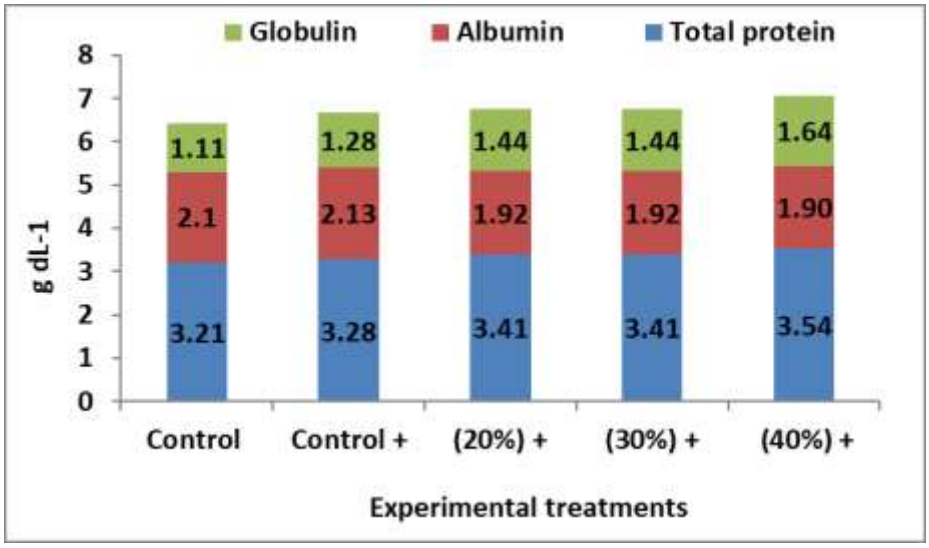

Fig. 2 Illustrated the effect of various dietary levels of high protein distiller's dried grains (HPDDG) with enzyme phytase on total serum protein, albumin and globulin of European sea bass, *Dicentrarchus labrax* fingerlings.

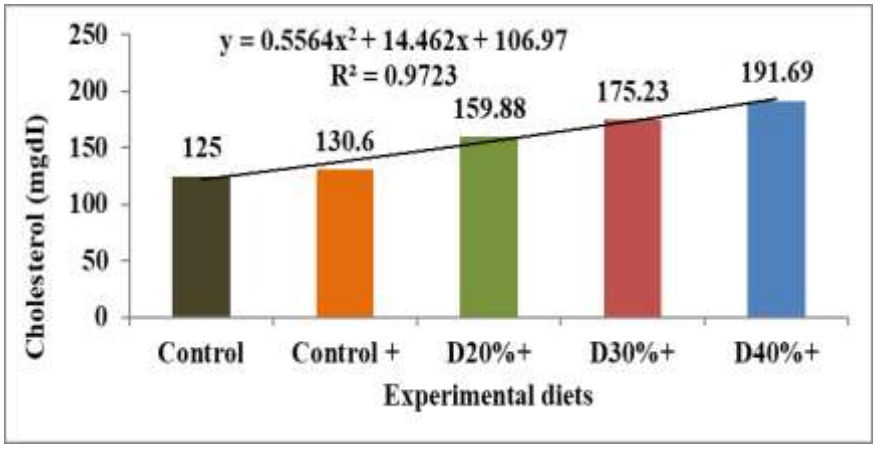

Fig. 3 Illustrated the effect of various dietary levels of high protein distiller's dried grains (HPDDG) with enzyme phytase on blood Cholesterol (mgdI) content of European sea bass, *Dicentrarchus labrax* fingerlings.

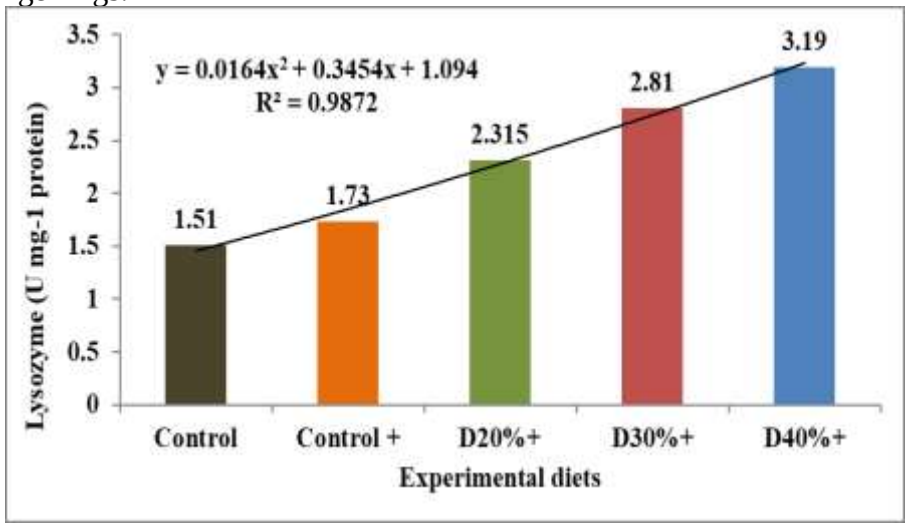

Fig. 4 Illustrated the effect of various dietary levels of high protein distiller's dried grains (HPDDG) with enzyme phytase on blood Lysozyme (U mg-1 protein) content of European sea bass, *Dicentrarchus labrax* fingerlings.

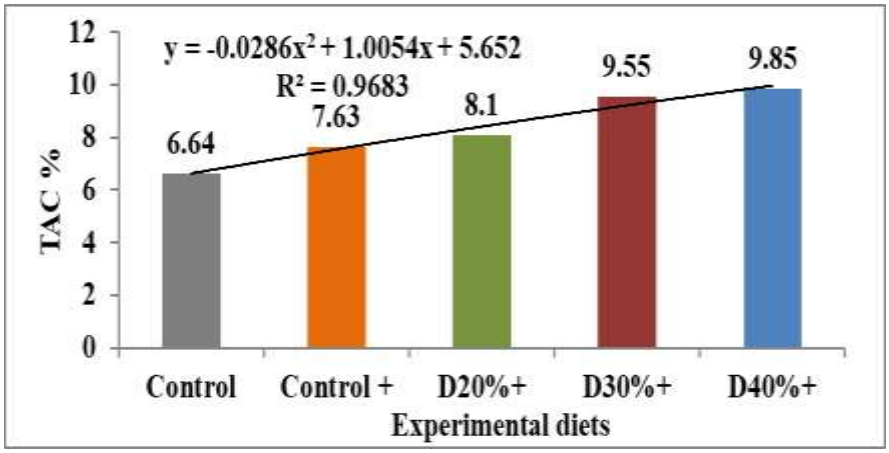

Fig. 5 Illustrated the effect of various dietary levels of high protein distiller's dried grains (HPDDG) with enzyme phytase on total antioxidant capacity (TAC%) of European sea bass, *Dicentrarchus labrax* fingerlings.

Total serum protein is often used as an indicator of physiological condition in fish, as it is one of the most stable components of blood, and so an increase or decrease of total blood proteins, globulins and albumin has clinical relevance in fish. Infection may be followed by marked changes on total blood protein due to impair hepatic synthesis of blood protein, increase catabolism or losses of albumin in urine or synthesis of globulins by the immune system. High plasma albumin and/or globulin has been related to stress, inflammatory and innate immune responses or to feeding immune-stimulants. Also higher levels of plasma non-specific humoral immune parameter, such as lysozyme and complement activity, have been used as indicative of immuno-enhancing properties to certain dietary compounds (**Peres *et al.*, 2015).** The present study observed that highest hematological, biochemical and immune induces values (WBC, RBC, Hb, Hct, total protein, globulin, Cholesterol, Lysozyme activity and TAC) for fish fed diets contain HPDDG compared to control. The increase in hemoglobin concentration could be attributed to the higher oxygen consumption associated with more hemoglobin saturation and dissociation rates **(Yahav *et al.*, 1998).** Commercial catfish diets with high levels of phytase enzymes could significantly improve hematological parameters we observed significantly higher concentration of RBC's, WBCs, Hct% and Hb concentration as a result of phytase super dose fortification to a commercial catfish ration **(Peatman and Beck, 2016).** However, **El-Katcha *et al.* (2014)** found that the enzyme

supplementation had no significant effect on blood serum albumin concentrations when compared with broiler chicken group fed on the same diet without enzyme supplementation.

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إستخدام نواتج تقطير الحبوب العالية البرويتن مع انزيم الفيتاز فى النظام الغذائى لاصبعيات اسماك القاروص الاوربى

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الملخص العربى

أجريت هذة الدراسة بأحدى المفرخات الخاصة بوادى مريوط – بالاسكندرية (مفرخ مدحت الشريف للاسماك البحرية) حيث أجريت التجربة بهدف دراسة تاثير استخدام نواتج تقطير الحبوب عالية البروتين مع انزيم الفيتاز على كفاءة النمو والمعاملات الفسيولوجية في إصبعيات أسماك القاروص البحر الأوروبي.

الهدف من هذة الدراسة هو دراسة تأثير مستويات مختلفة من استخدام نواتج تقطير الحبوب عالية البروتين مع انزيم الفيتاز على كفاءة النمو والمعاملات الفسيولوجية في إصبعيات أسماك القاروص البحر الأوروبي بأستخدام ٣ مستويات من نواتج تقطير الحبوب العالية البروتين (٣٠،٤٠ و٥٠ %) كبديل لفول الصويا فى العلائق مع أضافة انزيم الفيتاز بعدل (٠,٥ جم/كجم علف). بمعدل تخزين ١٠ سمكة بكل حوض وقد استمرت التجربة ٥٦ يوم بوزن أبتدائى (٧,٥ جم) وتمت التغذية حتى الشبع وتقدم العلائق ٣ مرات يوميا.

وقد أظهرت النتائج ما يلى:

تحسن النمو مع زيادة مستويات نواتج تقطير عالية البروتين مع انزيم الفيتاز فى العليقة وكذلك أيضا تحسن المعايير الفسيولوجية للدم .

ومما سبق نستنتج أن أستخدام نواتج تقطير الحبوب عالية لبروتين (HPDDG) بالإضافة إلى أنزيم الفيتاز أظهرت تحسن فى معدلات النمو والمعايير الفسيولوجية للدم لاصبعيات أسماك القاروص الأوربى، حيث ان انزيم الفيتاز حسن بشكل كبير من الخصائص الغذائية للنظام الغذائي.