# Appraisal of a high protein distillers dried grain (DDG) in diets for European sea bass, *Dicentrarchus labrax* fingerlings on growth performance, hematological status and related gut histology

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# Appraisal of a high protein distillers dried grain (DDG) in diets for European sea bass, *Dicentrarchus labrax* fingerlings on growth performance, hematological status and related gut histology

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**Abstract**

High protein distillers dried grains (HP-DDG) is a co-product of ethanol production that uses pre-fractionation technology. An 8 week growth trial was conducted to investigate the effect of partial replacement of soybean meal (SBM) by three levels of HP-DDG (30, 40 and 50%) on growth performance, physiological parameters and histological changes of the intestine of European seabass, *Dicentrarchus labrax*. The results indicated that an increased dietary level of HP-DDG of more than 30% significantly increases growth performance and improved the FCR of seabass. In addition, replacement of SBM by HP-DDG enhanced feed intake efficiency and the health status of fish. Hematology and serum biochemistry (hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), packed cell volume (PCV %) and humeral immune parameters including total protein, albumin, globulin, cholesterol, lysozyme activity and total antioxidant capacity significantly increased with increase HP-DDG inclusion levels. The findings of this study indicated that HPDDG is a good complementary protein source for inclusion aquaculture diets and levels above 30% as a replacement of SBM did not compromise growth performance and physiological parameters of seabass whilst enhancing some important indices of health status in this species

**Keywords**: *Dicentrarchus labrax,* high protein distillers dried grains, physiological parameters, growth performance, feed utilization, histology, hematological indices

## Introduction

Aquaculture production is expanding to fill the increasing demand of fish for human consumption globally. Farmed production of fish, particularly carnivorous fish, is still based on high quality feed with many limitations on supply and cost. Nevertheless the use of these commodities in aqua-feeds has been decreasing due to a higher demand of fisheries products, world limited availability, market supply fluctuations and raising prices, which have stimulated research on the use of more sustainable alternative feedstuffs for generating more sustainable diets to meet various specifications (El-Haroun et al., 2007; 2009; El-Husseiny et al., 2018). Due to the expected increase in human population, the world will require an additional 23 million tons of aquatic food by 2030 to maintain current per capita fish consumption. This must come from aquaculture, as fisheries production has stabilized over the last decades (FAO, 2016). As fish consumption is expected to continue to increase, it is important to develop cost effective protein sources to reduce the feed cost and support the rapid expansion of fish industry (Qiu and Davis, 2017; Hassan et al., 2018a).

Feed formulation is a central operation in fish production, confirming that feed ingredients are economically used for maximum growth. The search for least-cost feed formulation must remain a high priority to ensure the improvement of sustainable and profitable aquaculture industries. The objective of commercial feed formulation software is to achieve nutrient balance in diets and also meet the nutritional requirements of animals at least-cost. Therefore, the success of commercial aquaculture production by reducing the cost of prepared diet, without reducing fish performance, leads to a positive impact on the profitability of commercial fish production (Md Mostafizur et al. 2015). Recently (Hassaan et al., 2017 and Hassan et al., 2018a; Kumar et al., 2018) observed that the use of plant feedstuffs (such as wheat gluten, sunflower meal, soybean meal or soy protein concentrate) imposes some concerns due to the “food-feed competition”, rising prices, and carbon footprint involved in their production and importation. Thus, there is an

increasing need to seek alternatives, particularly underutilized commodities, such as by products obtained from food, fermentation and pharmaceutical industries, rather than being highly dependent of imported plant feedstuffs, such as soybean meals, for aquafeeds formulation (Matos et al. 2016). Within these alternative plant feedstuffs, distillers' dried grains with solubles (DDGS), which are by-products from cereal fermentation and subsequent distillation for ethanol production (Goda et al. 2011). 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 majority of the dry-grind ethanol plants produces a DDGS by-product containing 26–34% protein, depending on the grain source, and has reduced anti-nutritional factors compared 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, and 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 aquafeeds. Currently we are witnessing a concerted effort by nutritionists and feed formulators to reduce aqua-feeds costs by replacing expensive protein sources for others less costly. The objective of the present study was to investigate the effect of partial replacement of dietary soybean meal (SBM) by various levels of high protein distiller's dried grains (HP-DDG) on growth performance, physiological and health status parameters of European seabass, *Dicentrarchus labrax* fingerlings.

## Materials and methods

**Fish and experimental facilities**

One hundred and twenty European seabass, *Dicentrarchus labrax* fingerlings with an average initial body weight of 7.5 ± 0.5 g/fish obtained from a private commercial fish farm "El-Shref farm, Wady Marriott, Alexandria" Egypt were used for the assessment trial. After acclimation for two weeks in indoor circular fiberglass tanks (1 cubic meter), a controlled feeding experiment with control diet containing 45% crude protein and partial replacement of soybean meal (SBM) by three levels of HP-DDG was conducted (Table 1). Then, fish were randomly distributed into twelve glass aquaria measuring (70x40x30cm each) representing four treatments (each in triplicate) at a stocking density of 10 fish per aquaria. Daily water exchange rate was 50% of pond volume was salinity water (37 ppt) and rearing conditions (18 ± 1.0°C), pH (7.0 ± 0.50), and a photoperiod regime (12:12 h light: dark).

**Experimental design and diets**

Four isonitrogenous (~45% crude protein) and isolipidic (~13% crude lipid) experimental diets were formulated (Table 1). HP-DDG was produced in the USA (Mirasco Inc. Address: 900 Circle 75 Pkwy SE, Suite 1660, Atlanta, GA 30339, United States) and supplied by a local distributer in Egypt . The control contained no HPDDG. The other experimental diets were formulated to contain HP-DDG replacement with soybean meal at levels of 30, 40 and 50 %. Fish were fed two times a day (7:00 and 12:00 h) to apparent satiation for eight weeks duration of the trial, (six days a week). The experimental diets were processed by blending the dry ingredients into a homogeneous mixture, and then passing the mixed feed through a laboratory pellet mill (a California Pellet Mill, San Francisco, CA, USA). The chemical compositions of the experimental diets are presented in Table 1, the resulting moist pellets were dried at 40 °C for two days. The diets were stored in plastic bags in a refrigerator (-4°C) until use. The ingredients and proximate compositions of the experimental diets are shown in Table (1).

**Fish growth performance and survival**

126 The mean final body weight (FBW) in experimental treatment was determined by dividing
127 the total fish weight in each aquarium by the number of fish. Weight gain (WG), specific growth
128 rate (SGR), feed conversion ratio (FCR), economical conversion rate (ECR), and survival (%)
129 were calculated using the following equations, according to Tiews (1980):
130 WG = final body weight (g) - initial body weight (g).
131 SGR = 100× [(ln final body weight (g) - ln initial body weight (g))/ duration of feeding (day)]
132 FCR = feed intake (g)/weight gain (g).
133 ECR = cost of diet ($ $kg^{-1}$) x Feed Conversion Ratio (FCR)
134 Survival (%) = 100 × (initial number of the fish/final number of fish).
135 **Hematological parameters**
136 The total red and white blood cell counts (RBC; $10^6$ $mm^{-3}$ and WBC; $10^3$ $mm^{-3}$,
137 respectively) were obtained by using a standard Neubauerhemocytometer chamber using Shaw's
138 solution as diluting fluid (Stoskopf, 1993). Hemoglobin (Hb; g $dL^{-1}$) was determined
139 colorimetrically using commercial kits (Diamond, Egypt) according to the cyan- methemoglobin
140 procedure (Drabkin, 1945). Packed cell volume (PCV %) was determined after centrifugation at
141 10,000 x g for 5 min (Stoskopf, 1993).
142 **Biochemical and immune parameters**
143 The total protein (g $dL^{-1}$) was determined in plasma samples of fish from the different
144 experimental groups by the Biuret method according to Doumas et al. (1981). Albumin (g $dL^{-1}$)
145 was determined by the bromocresol green method (Reinhold, 1953) and globulin (g $dL^{-1}$) was
146 calculated as the difference between total protein and albumin. Cholesterol using a commercial kit
147 (Pasteur, Lab, France, Egypt) (Yousefi et al. 2011). Lysozyme activity (U $mg^{-1}$ protein) in serum
148 was determined according to the method of Ellis (1990) based on the lysis of the lysozyme
149 sensitive gram-positive bacterium Micrococcus lysodiekticus (Sigma, St. Louis, MO). Lysozyme
150 acts upon susceptible bacteria by combining with and breaking down a mucopolysaccharide. This

mucopolysaccharidehas has been shown to be situated in the bacterial cell wall *M. lysodeikticus*, is normally highly sensitive to lysozyme3 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. lysodiekticus suspension (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). Briefly, 0.2 ml of 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 (%).

**Histological examination**

Randomly, four individual specimens from each replicate of *D. labrax* were chosen and dissected for tissue removal. The intestine were removed, thoroughly washed with a physiological saline (0.9% Nacl) solution and fixed in Bouin's fluid. The material was dehydrated, cleared and finally embedded in paraffin wax. Serial sections were cut to the thickness of *5-6µm*. The sections were stained with haematoxylin counterstained with eosin and mounted in DPX (Yano, 1988). The sections were examined with an Olympus light microscope and photographed with digital camera as required. The histological examination was carried out according to Culling (1983).

**Economical evaluation**

The economic value of the diets was determined according to the following equations (Abdel Rahman et al. 2010 and Salama et al. 2010).

Feed cost per kg fresh fish (LE) = Cost/ kg diet ($) × consumed feed to produce 1 kg fish.

Relative feed cost/kg fresh fish = Values of feed cost/kg fresh fish / the minimum value of the same parameter.

Feed cost/ 1 kg gain ($) = Feed intake per kg gain (FCR) × cost/kg diet ($).

Economic conversion rate (ECR) = Cost of diet ($ $kg^{-1}$) x Feed conversion ratio (FCR).

**Statistical analysis:**

Differences in the obtained results were tested using one-way ANOVA with post hoc Duncan's new multiple range test (Duncan (1955) and considered significant at $P<0.05$.".The data of the experiments were statistically analyzed using GLM (general linear model) procedure according to Statistical Analysis System (SAS 2004).

**Results**

None of the major essential amino acids were limiting, methionine meeting the requirements for seabass and lysine, arginine, histidine and threonine in excess of levels in the control diet. Leucine was noted to be particularly higher for the HP-DDGS diets. Phosphorous (P) that is normally limiting and lower in soybean meal was appreciably higher in the HP-DDGS diets (3.25-3.46%) compared to 1.43% in the control diet. This would likely have been more available than P contained in soybean meal bound as phytate. HP-DDG could be a good alternative for available P and these needs to be confirmed in a future study for digestibility.

**Growth performance**

The results showed that fish fed various levels of HP-DDG in diets, showed improved growth performance compared to the control diet (Table 2), suggesting that the addition of HP-DDG to the diet enhanced the growth performance of seabass. The highest values of FW, WG and SGR were recorded for fish fed 50% HP-DDG, while the lowest values were observed for fish fed

the control diet. Moreover, feed intake and FCR exhibited were significantly enhanced with the increasing HP-DDG levels compared to fish fed the control diet. No mortality was observed in the dietary treatments

**Hematological parameters**

The white blood cells ($10^3$), red blood cells ($10^6$m), Hemoglobin (g $dI^{-1}$) and pack cell volume (%) values differed significantly ($P<0.05$) between the control fish and fish fed various levels HP-DDG diets (Table 3). The WBCs, RBCs, Hb and PCV were tended to increase with increasing dietary HP-DDG levels (Table 3).

**Biochemical and immune parameters**

Total protein, albumin, globulin and cholesterol were significantly elevated in fish fed diets containing various levels of HP-DDG diet compared to the control fish (Table 4) and the highest value recorded in HP-DDG dose (50%) diet compared to all treatments. The same trend was observed for the Lysozyme and total antioxidant capacity (TAC) (Table 4).

**4.1.5. Histology of intestinal tract**

Histology of the intestine of sea bass fed control diet and different levels of HP-DDG are displayed in figures (1–4).The histological changes in fish intestines were assessed with light microscopy. Visual assessment revealed that the fish fed the control diet (high soybean (no HP-DDGS) had smaller intestinal mucosal folds compared to other diets with lower soybean meal at the expense of DDGS (Figure 1). There was evidence of hyperplasia and corrosion of the mucosal folds for seabass intestine fed the control diet. (Figure 2). The superior mucosal fold length and width, improved gut integrity and uniformity recorded of fish fed up to 50% HP-DDG compared with fish fed control diet (Figure 4).

**Economic efficiency**

The cost of feed per unit of fish gain of fish fed diets contain HP-DDG was significantly lower than the feed used as the control (Table 5). The data also indicate that seabass fingerlings

fed diet containing up to 50% of HP-DDG exhibited significantly lower feed intake, best FCR and ECR values. The diet 50% HP-DDG had the lowest total cost and relative feed cost/kg gain, while fish fed the control diet recorded the highest in total feed cost/kg fish gain.

**Discussion**

Maize co-products from dry-grind bio-ethanol production, such HP-DDG, are attractive ingredients for use in aquaculture feeds due to their energetic content, protein, highly digestible phosphorus and lower amounts of anti-nutritional factors (ANFs). There ingredients are low-priced and may reduce diet cost compared to conventional plant protein ingredients. They are also cost effective compared to conventional plant protein ingredients such a soybean meal. In the present study, HP-DDG were evaluated in diets for seabass fingerlings by incrementally replacing dietary soybean meal protein, while the fish meal levels were kept constant to avoid confounding effects of differences in fish meal levels.

The present results suggest that HP-DDG can be utilized in seabass diets by as much as 50% inclusion by replacing soybean meal without affecting growth performance and physiological parameters. The same positive effect of DDGS on growth performance has been reported in other fish species such as rainbow trout, *Oncorhynchus mykiss* (Øverland et al.2013), Pacific white shrimp, *Litopenaeus vannamei* (Achupallas et al., 2016). The positive contribution of using HP-DDG to improve growth performance and feed utilization could be attributed to the residual yeast, *Saccharomyces cerevisiae* present in DDG during the fermentation step of DDG products in aquaculture feeds (Øverland et al., 2013). The yeast protein is known to be an excellent source of essential amino acids with only methionine being sometimes limiting for certain fish species. Omar et al (2012) reported very good results for yeast Protein Concentrate (YPC) for carp and noted the high BV (Biological Value) of the protein in these products from bioethanol production from cereals. Furthermore, yeast cells are also potent sources of nucleic acids, mannan oligosaccharides (MOS), and β-glucans that can be used as immune-stimulants and growth promoters in fish diets

(Øverland et al. 2013; Hassaan et al., 2018b, 2019). Also, It has been reported that live *Saccharomyces cerevisiae* yeast can settle in the intestinal mucosa of rainbow trout (Øverland et al. 2013) which may have some effect on the fish larval development (i.e. by quickening the maturation of the digestive system, and having prebiotic and probiotic effects). In the present study, feeding HP-DDG improved FCR compared to the control diet (Table 3). These results could be attributed to the yeast fermentation processing and activation of endogenous microbial enzymes which are capable of degrading inhibitors and ANFs during the manufacture of DDGS co-products which enhance the appetite of the fish and consequently improving their feed intake and feed efficiency (Lim et al., 2007). The results are consistent with (Abdel-Tawwab et al., 2008) who found an improvement in the growth performance of Nile tilapia juveniles fed diets supplemented with commercial live yeast (*S. cerevisiae*).

Seabass fed various levels of high protein distillers dried grains (HP-DDG) indicated an increasing of specific hematological parameters such as (RBCs, WBCs, Hb and PCV) with the incremental rise of HP-DDG (Table 4). Fish fed diets containing 50% HP-DDG had significantly higher RBCs, WBCs, Hb and PCV than the group fed the control diet. These results agree with these of Ahmad et al. (2014) who found significant elevation in Hb, RBCs, and WBCs in all fish groups fed on β–glucan diets in comparison to fish fed the control diet. Similarly Lim et al. (2007) observed significant differences among hematological parameters (RBCs, WBCs, Hb, and PCV) of Nile tilapia fed diet containing 10, 20 and 40% of DDGS. It is unknown whether the increase in Hb ,WBCs and RBCs values observed in our study and noticed by Welker et al. (2007) was related to the effect of β-glucan or the presence of additional nutrients, especially vitamins present in DDGS diets (Hassaan et al. 2018b). Furthermore, these increases could be attributed to the presence of *Saccharomyces cerevisiae* during the process of ethanol production that yields DDGS.

The results indicated that fish fed 50% HP-DDG recorded the highest total serum protein, albumin, globulin and cholesterol contents (Table 5). The present results are consistent with Md

Mostafizur et al., (2015) who found that the haematological parameters of juvenile flounder, *Paralichthys olivaceus* total protein, albumin, globulin and cholesterol contents were similarly affected by fish fed different dietary levels of distillers dried grain (DDG). The improvement of haematological parameters of fish fed DDG may be due to the anabolic effect of β-glucan supplementation, which has specific receptors on the phagocytic cells, (heterophiles and monocytes) and β-glucan binds to receptor molecules on the surface of circulating and tissue phagocytes.

Fish fed diets contained 50% HP-DDG recorded the highest Lysozyme and TAC activity (Table 5), these results are in agreement with Staykov et al. (2005) who found that supplementation of mannan oligosaccharide (MOS) in rainbow trout diets improved growth rate, anti-oxidant activities and immune function, these results could be attributed to the role of HP-DDG as an anti-microbial activities due to its yeast compounds. Furthermore, these results suggest that the HP-DDG supplementation could increase the non-specific immune system (humeral immunity) of seabass resulting increase in fish resistance. Recently it was also reported that fermented plant products have positive effect on non-specific immune response, antioxidant activities and diseases resistance of fish (Pham et al., 2007; Hassan et al., 2018). In addition, an overall improvement of mucosal fold length and width observed in seabass fed diet contained 50% HP-DDG (Figure 4) was observed. This improvement in the villi structure may enhancement the nutrient absorption due to increased absorption surface. This may be related to the effect of β-glucan or the presence of additional nutrients, especially vitamins present in DDGS-containing diets because yeasts present in DDGS are a rich source of B-complex vitamins. In addition our findings also show that DDG inclusion could enhance the intestinal morphology in terms of deeper and more regular mucosal folds and goblet cell secretion of mucins. The present results are consistent with the observations of Panagiotidou et al. (2009) who found that seabass fed different levels of yeast extract showed improving of liver morphology. Also, Jarmolowicz et al. (2012) reported that

changes in intestinal morphology of juvenile pikeperch, *Sander lucioperca* (L.) fed brewer's yeast extract result in greater cell absorption activity and better digestion of nutrients in the intestine, which usually leads to improved growth performance and feed utilization.

In conclusions, the results of the present study indicated that the diet in which up to 50% of the SBM is replaced by HP-DDG yielded improved growth performance and feed utilization for seabass juveniles. Various physiological processes were affected positively in favor of supporting fish health status and nonspecific immune parameters. The potential of enhanced distillers dried grains from the bioethanol and beverage industries appear to be a most useful by-product and could be explored to further reduce the cost of diet manufacture. HP-DDG is presently less costly than standard grades of SBM. In the USA, such products as NexPro™, a next-generation protein ingredient derived from the dry-mill ethanol production process is available for aquafeeds. NexPro™ is a 50 percent protein product containing 25 percent yeast and trials with several fish species have been very positive with significant capacity to replace soybean and fishmeal. From this study, we recommended HP-DDG type products for producing next generation sustainable diets for *D. labrax* fingerlings and suggest more work be undertaken to explore its use in grow-out feeds to harvest size fish where the cost of feed is much higher in relation to production of fish over time. It would also be imperative to also focus on the attributes of HP-DDG in terms of functional properties due to the higher yeast content and bioactive capacity. Applications to other fish species should be thoroughly investigated and cost benefit analysis demonstrated for the substitution of various feed ingredients in complete dietary formulations.

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## References

Abdel Rahman, S.H; Abdel Razek, F.; Goda, A.M. A.-S; Ghobashy, A.-F and Ragaiy A. (2010). Partial substitution of dietary fish meal with soybean meal for speckled shrimp, *Metapenaeus monoceros* (Fabricius, 1798) (Decapoda: Penaeidae) juvenile. Aquaculture Research, 41, 299-306

Abdel-Tawwab, M., Abdel-Rahman, A.M. & Ismael, N,E.M. (2008) Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry

Nile tilapia, *Oreochromis niloticus* (L.) challenged in situ with *Aeromonas hydrophila*. Aquaculture, 280, 185–189.

Achupallas, J.M., Zhou, Y. & Davis, D. (2016) Use of grain distillers dried yeast in practical diets for juvenile Pacific white shrimp, *Litopenaeus vannamei*. Journal. of World Aquaculture Society., 47, 220–229.

Ahmad, M.H., El-Mousallamy, A., Awad, S.M.M. & Abd El-Naby A.S. (2014) Evaluation of Bio-Mos® as a feed additive on growth performance, physiological and immune responses of Nile tilapia, *Oreochromis niloticus* (L). Journal of applied sciences research, 9 (10), 6441-6449.

Culling, C.F. (1983): Handbook of histopathologic and histochemical staining. 3rd Ed., Butterworth London.

Doumas, B.T., Bayso, D.D., Carter, R.J., Peters, T. and Schaffer, R. (1981) Determination of total serum protein. Clinical. Chemistry. 27, 1642 - 1643.

Drabkin, D. (1945) The crystographic and optical properties of the haemoglobin of man in comparison with those of other species. Journal of Biological .Chemistry, 164, 703 - 723.

Duncan´s, D.B. (1955) Multiple ranges and multiple F test. Biometric, 11, 1-42.

El-Haroun, E.R., Azevedo, P.A., & Bureau, D.P. (2009). High dietary incorporation levels of rendered animal protein ingredients on performance of rainbow trout, *Oncohrynchus mykiss* (Walbaum, 1972). Aquaculture, 290, 269-274.

El-Haroun, E.R., & Bureau, D.P. (2007). Comparison of the bioavailability of lysine in blood meals of various origins to that of L-lysine HCL for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 262, 402–409.

El-Husseiny, O.M., Hassan, M. I., El-Haroun, E.R. & Suloma, A. (2018). Utilization of poultry by-product meal supplemented with L-lysine as fish meal replacer in the diet of African

376 catfish, *Clarias gariepinus* (Burchell, 1822). Journal of applied aquaculture. DOI:
377 10.1080/10454438.2017.1412844
378 Ellis, A.E. (1990) Lysozyme Assays. In: Techniques in Fish Immunology, Stolen, J. S., T. C.
379 Fletcher, D.P. Anderson and W.B. van Muiswinkel (Eds.). Vol. 1, SOS Publications, New
380 Jersey, USA, pp. 101-103.
381 FAO (2016) The State of World Fisheries and Aquaculture. Contributing to food security and
382 nutrition for all. Rome. 200 pp.
383 Galaktionova, L.P., Molchanov, A.V., El'chaninova, S.A. & Varshavskii, B.I. (1998) Lipid
384 peroxidation in patients with gastric and duodenal peptic ulcers – Klin. Lab. Diagn., 6,
385 10-14.
386 Goda, A.M.A-S., Wafa, M.A. & Hassaan, S.M. (2011b) Effect of replacing dietary soybean meal
387 with distiller's dried grains with solubles on growth performance of Nile tilapia,
388 *Oreochromis niloticus* monosex in cement ponds. Egyptian Journal of Aquatic Research,
389 2(B), 15-28.
390 Hassaan, M.S., Goda, M.A-S. & Kumar, V. (2017) Evaluation of nutritive value of fermented de-
391 oiled Physic nut, Jatropha curcas seed meal for Nile tilapia *Oreochromis niloticus* fingerlings.
392 Aquaculture nutrition, 23(3), 571-584.
393 Hassaan, M.S., Soltan, M.A., Mohammady, E.Y., Elashry, A.M., El-Haroun, E.R., & Davies, S.J.
394 (2018a) Growth and physiological responses of Nile tilapia, *Oreochromis niloticus* fed dietary
395 fermented sunflower meal inoculated with *Saccharomyces cerevisiae* and *Bacillus*
396 *subtilis*. Aquaculture, 495, 592-601.
397 Hassaan, M.S., Mahmoud, S., Jarmolowicz, S., El-Haroun, E.R., Mohammady, E.Y. and Davies,
398 S.J. (2018b). Effects of dietary baker's yeast extract on the growth, blood indices, and histology
399 of Nile tilapia, *Oreochromis niloticus* L, fingerlings. Aquaculture Nutrition. Volume 24, Issue
400 6, 1709-1717

Hassaan, M.S., El-Sayed, A.I.M., Soltan, M.A., Iraqi, M.M., Goda, A.M., Davies, S.J., El-Haround, E.R., Ramadan, H.A. (2019). Partial dietary fish meal replacement with cotton seed meal and supplementation with exogenous protease alters growth, feed performance, hematological indices and associated gene expression markers (GH, IGF-I) for Nile tilapia, Oreochromis niloticus. Aquaculture, 503: 282–292.

Jarmolowicz, S., Zakes, Z., Siwiccki, A., Kowalska, A., Hopko, M., Glabski, E. & Demaksa-Zakes, Partyka, k. (2012) Effects of brewer's yeast extract on growth performance and health of juvenile pikeperch *Sander lucioperca* (L.). Aquaculture Nutrition, 18, 457-464.

Kumar, V., Habte-Tsion, H.M., Allen, K., Bowman, B., Thompson, K., El-Haroun, E., Filer, K. & Tidwell, J. (2018) Replacement of fish oil with Schizochytrium meal and its impacts on the growth and lipid metabolism of Pacific white shrimp (*Litopenaeus vannamei*) . Aquaculture Nutrition, 15-758-792.

Li, E., Lim, C., Klesius, P. & Cai, C. (2012) Enhancement effects of dietary wheat distiller's dried grains with solubles on growth, immunity, and resistance to *Edwardsiella ictaluri* challenge of channel catfish, *Ictalurus punctatus*. Journal of the World Aquaculture Society, 43, 814–827.

Lim , C., Garcia , J.C., Yildirim-Aksoy, M., Klesius, P.H., Shoemaker, C.A. & Evans, J.J. (2007) Growth response and resistance to *Streptococcus iniae* of Nile tilapia, *Oreochromis niloticus*, fed diets containing distiller's dried grains with solubles. Journal of the World Aquaculture Society, 38, 231–237.

Liu, K.S. (2011) Chemical composition of distillers grains, a review. Journal of Agriculture Food Chemistry. 59, 1508-1526.

Matos, E., Dias, J., Dinis, M.T. & Silva, T.S. (2016) Sustainability vs. Quality in gilthead seabream *(Sparus aurata L.*) farming: are trade-offs inevitable? Reviews in Aquaculture 10, 1–22

425 Md Mostafizur, Rahman. & Jin Choi., Sang-Min Lee. (2015) Influences of dietary distillers dried
426 grain level on growth performance, body composition and biochemical parameters of
427 juvenile olive flounder (*Paralichthys olivaceus*). Aquaculture Research, 46, 39–48.
428 NRC, National Research council (1993) Nutrient requirements of fish. National Academy
429 Press, Washington D. C.USA.
430 Omar, Samad S., Daniel L. Merrifield Holger Kühlwein Peter E.V.Williams, Simon J. Davies
431 (2012) Biofuel derived yeast protein concentrate (YPC) as a novel feed
432 ingredient in carp diets. Aquaculture, 330, 54-62
433 Øverland, M., Krogdahl, Å., Shurson, G. & Skrede, A., Denstadli, V. (2013) Evaluation of
434 distiller's dried grains with solubles (DDGS) and high protein distiller's dried grains
435 (HPDDG) in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 416, 201–208.
436 Panagiotidou, M., Nengas I., Henry M., Rigos G., Charalambous C., Sweetman J.1. (2009). Effect
437 of different dietary levels of yeast extract (nupro® on growth, feed utilisation and immune
438 system of sea bass (Dicentrarchus labrax). 9th Symposium on Oceanography & Fisheries,
439 Proceedings, Volume II, 1080-1084
440 Pham M.A., Lee, K.J., Lim, S.J. & Park, K.H. (2007) Evaluation of cottonseed and soybean meal
441 as partial replacement for fishmeal in diets for juvenile Japanese olive flounder
442 (*Paralichthys olivaceus*). Fisheries Science, 73, 760–769.
443 Qiu, X . and Davis, D. (2017) Evaluation of flash dried yeast as a nutritional supplement in plant-
444 based practical diets for Pacific white shrimp *Litopenaeus vannamei*. Aquaculture.
445 Nutrition. 23, 1244-1253.
446 Reinhold, R.R, (1953) Determination of serum albumin. Clinical Chemistry, 21, 1370 - 1372.
447 Rosentrater, K.A. & Muthukumarappan, K. (2006) Corn ethanol coproducts: generation,
448 properties, and future prospects. International Sugar Journal, 108, 648–657.

449 Salama, F.A, Ttonsy, H.D, Labib, E.M. and Mahmoud, S.H. Zaki, M.A. (2010) Nutrtional studies
450 on partial and total replacement of soybean meal by distillers dried grain with soluble
451 (DDG'S) in diet for Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Nutrition
452 and Feeds, 13 (1), 165-176.
453 SAS, (2004) Statistical Analysis System User's Guide (version 9). SAS Institute, Inc., Cary, North
454 Carolina, USA.
455 Staykov , Y., Denev , S . & Spring, P. (2005) The effects of mannan oligosaccharide (Bio-Mos)
456 on the growth rate and immune function of rainbow trout (*Salmo gairdneriirideus G.)*
457 growth in net cages. In: Howell B, Flos R, eds. Lessons from the past to optimize the
458 future Bangkok, Thailand: European Aquaculture Society, 427,432-435.
459 Stoskopf, M.K. (1993) Fish Medicine W.B. Saunders Comp. Philadelphia.
460 Tidwell, J.H., Coyle, S.D. & Bright1, L.A., Pires, L. (2017) Effects of Varying Levels of High-
461 Protein Distiller's Grains on Growth Performance of Channel Catfish, *Ictalurus*
462 *punctatus*, and Post-Extrusion Feed Pellet Characteristics. Journal of the World
463 Aquaculture Society, 48, 426-434.
464 Welker, T.L., Lim, C., Yildirim-Aksoy, M., Shelby, R. & Klesius, P.H. (2007) Immune response
465 and resistance to stress and *Edwardsiella ictaluri* challenge to channel catfish, *Ictalurus*
466 *punctatus*, fed diets containing commercial whole-cell yeast or yeast *subcomponents*.
467 Journal of the World Aquaculture Society, 38, 24–35.
468 Wu, Y.V., Rosati, R.R. & Brown, P.B. (1997) Use of corn-derived ethanol coproducts and
469 synthetic lysine and tryptophan for growth of tilapia (*Oreochromis niloticus*) fry. Journal
470 of Agriculture Food Chemistry, 45, 2174–2177.
471 Yano, I. (1988) Oocyte development in the kuruma prawn, *Penaeus japonicus*. Mar. Biol., 99,
472 547–553.

473 Yousefi, M., Abtahi, B. & Kenari, A. (2011) Hematological, serum biochemical parameters, and
474 physiological responses to acute stress of Beluga sturgeon (*Huso huso*, Linnaeus 1785)
475 juveniles fed dietary nucleotide. Comparative Clinical Pathology, 21(5), 1-6.
476

**Table (1):** Formulation, proximate composition (g $kg^{-1}$) of the experimental diets (g/kg).

| Ingredient | Control | 30% HP-DDG | 40% HP-DDG | 50% HP-DDG |
|---|---|---|---|---|
| Fish meal 68 % CP | 300.0 | 300.0 | 300.0 | 300.0 |
| Soy bean meal 47% CP | 375 | 262.5 | 225.0 | 187.5 |
| Corn gluten 60% CP | 90.0 | 90.0 | 90.0 | 90.0 |
| Rice bran 12% CP | 65.0 | 50.0 | 50.0 | 50.0 |
| Wheat midllings13% CP | 70.0 | 83.8 | 84.8 | 85.8 |
| HP-DDG | 0.0 | 112.5 | 150.0 | 187.5 |
| Soy bean oil | 41.0 | 42.0 | 41.0 | 41.0 |
| Fish oil | 48.8 | 49.0 | 49.0 | 48.0 |
| Di-calcium phosphate | 8.0 | 8.0 | 8.0 | 8.0 |
| Vit. and Min. premix[1] | 2.0 | 2.0 | 2.0 | 2.0 |
| Vitamin. C* | 0.2 | 0.2 | 0.2 | 0.2 |
| Total | 1000 | 1000 | 1000 | 1000 |
| Chemical composition (%)[2] | | | | |
| Dry matter (DM) | 938.0 | 937.5 | 931.8 | 937.7 |
| Crude protein (CP) | 448.6 | 448.8 | 449.7 | 446.0 |
| Ether extract (EE) | 125.6 | 131.0 | 129.8 | 128.9 |
| Nitrogen free extract (NFE)[3] | 283.3 | 257.0 | 266.5 | 278.6 |
| Crude fiber (CF) | 32.5 | 43.2 | 34.0 | 32.0 |
| Ash | 110.0 | 120.00 | 120.0 | 114.5 |
| Gross energy (GE; MJ /kg)[4] | 209.8 | 209.3 | 209.1 | 209.6 |

[1]Vitamins and minerals mixture each 1 Kg of mixture contains: 12 m.IU vit. A, 12 mIU vit. $D_3$, 10g vit. E, 2g vit. K, 1g vit. $B_1$, 5g vit. $B_2$, 1.5g vit. $B_6$, 10mg vit. $B_{12}$, 30g niacin, 1000 mg folic acid, 50mg biotin, 10g banathonic acid, 50g zinc, 30g iron, 60g manganese, 10g copper, 100 mg cobalt, 100mg selenium, 1000mg iodine. * Ascorbyl Phosphate

[2] DM –Basis.

[3]NFE: nitrogen-free extract calculated using the following equation: NFE = 100 (crude protein + ether extract + crude fiber + ash).

[4]Gross energy (GE) = Calculated using gross calorific values of 23.63, 39.52 and 17.15 KJ $g^{-1}$ for protein, fat and carbohydrate, respectively according to (NRC, 1993).

Table (2): Phosphorus and essential amino acids content of the experimental diets.

| Ingredient | Control | 30% HP-DDG | 40% HP-DDG | 50% HP-DDG |
|---|---|---|---|---|
| Phosphorus | 0.590 | 1.43 | 3.25 | 3.46 |
| Methionine | 0.444 | 0.47 | 0.49 | 0.49 |
| Cystine | 0.196 | 0.54 | 0.86 | 0.95 |
| Lysine | 0.210 | 1.08 | 1.64 | 1.86 |
| Tryptophan | 0.278 | 1.62 | 2.03 | 2.33 |
| Threonine | 0.296 | 1.25 | 1.76 | 2.05 |
| Iso leucine | 0.321 | 1.39 | 1.68 | 1.89 |
| Histidine | 0.498 | 1.37 | 1.91 | 1.93 |
| Valine | 0.520 | 1.15 | 1.74 | 1.79 |
| Leucine | 0.835 | 1.97 | 4.39 | 4.62 |
| Arg | 0.402 | 0.69 | 1.23 | 1.29 |
| Tyr | 0.283 | 1.72 | 3.28 | 3.47 |
| Phenylalanine | 0.518 | 2.27 | 4.54 | 4.79 |

**Table (3):** Growth performances and feed utilization parameters of European sea bass fed the experimental diets (means ± SE).

| Diets | IBW | FBW (g fish$^{-1}$ ) | WG | FCR | Feed intake (g fish$^{-1}$ ) | SGR |
|---|---|---|---|---|---|---|
| Control | 7.47±0.07 | 14.47±0.64$^{b}$ | 7.00±0.59$^{b}$ | 1.71±0.04$^{a}$ | 11.97±0.89$^{b}$ | 0.87±0.06$^{b}$ |
| 30% HP-DDG | 7.50±0.06 | 17.20±0.58$^{a}$ | 9.70±0.64$^{a}$ | 1.45±0.07$^{ab}$ | 14.17±1.59 $^{a}$ | 1.39±0.11$^{a}$ |
| 40% HP-DDG | 7.50±0.06 | 17.37±0.78$^{a}$ | 9.87±0.81$^{a}$ | 1.46±0.14$^{ab}$ | 13.30±0.10 $^{a}$ | 1.41±0.21$^{a}$ |
| 50% HP-DDG | 7.53±0.03 | 18.03±0.03$^{a}$ | 10.50±0.00$^{a}$ | 1.26±0.06$^{b}$ | 13.20±0.60 $^{a}$ | 1.70±0.00$^{a}$ |

Values are mean ±SD of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).

HP-DDG, high protein distiller's dried grains; IBW, initial body weight; FBW, Final body weight; WG, weight gain ; FCR, feed conversion ratio and SGR, specific growth rate.

Table (4): Hematological parameters of European sea bass fed the experimental diets (means ± SE).

| Diets | WBCs ($10^3$) | RBCs ($10^6$) | Hb (g $dL^{-1}$) | PCV (%) |
|---|---|---|---|---|
| Control | $18.73 \pm 0.46^{d}$ | $1.20 \pm 0.02^{c}$ | $7.14 \pm 0.01^{c}$ | $16.43 \pm 0.04^{d}$ |
| 30% HP-DDG | $21.03 \pm 0.14^{c}$ | $1.36 \pm 0.02^{b}$ | $7.68 \pm 0.06^{b}$ | $18.59 \pm 0.07^{c}$ |
| 40% HP-DDG | $23.47 \pm 0.14^{b}$ | $1.51 \pm 0.04^{a}$ | $8.05 \pm 0.14^{a}$ | $19.94 \pm 0.15^{b}$ |
| 50% HP-DDG | $26.97 \pm 0.58^{a}$ | $1.64 \pm 0.05^{a}$ | $8.25 \pm 0.02^{a}$ | $21.56 \pm 0.07^{a}$ |

Values are mean ±SD of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).

HP-DDG, high protein distiller's dried grains; WBCs, white blood cells; RBCs, red blood cells; Hb, Hemoglobin; PCV%, Packed cell volume.

Table (5): Biochemical parameters of European sea bass fed the experimental diets (means ± SE).

| Diets | Total protein (g $dL^{-1}$) | Albumin (g $dL^{-1}$) | Globulin (g $dL^{-1}$) | Cholesterol (mgdI) | Lysozyme (U $mg^{-1}$ protein) | TAC (%) |
|---|---|---|---|---|---|---|
| Control | $3.22 \pm 0.02^{d}$ | $2.11 \pm 0.03^{c}$ | $1.11 \pm 0.00^{c}$ | $125.42 \pm 2.44^{c}$ | $1.51 \pm 0.04^{d}$ | $6.64 \pm 0.04^{c}$ |
| 30% HP-DDG | $3.36 \pm 0.02^{c}$ | $2.16 \pm 0.01^{bc}$ | $1.20 \pm 0.03^{b}$ | $140.12 \pm 2.58^{b}$ | $1.79 \pm 0.09^{c}$ | $7.19 \pm 0.04^{b}$ |
| 40% HP-DDG | $3.51 \pm 0.03^{b}$ | $2.25 \pm 0.02^{ab}$ | $1.26 \pm 0.01^{b}$ | $154.78 \pm 4.79^{a}$ | $2.17 \pm 0.02^{b}$ | $7.42 \pm 0.13^{ab}$ |
| 50% HP-DDG | $3.81 \pm 0.02^{a}$ | $2.29 \pm 0.05^{a}$ | $1.53 \pm 0.02^{a}$ | $161.19 \pm 2.19^{a}$ | $2.79 \pm 0.05^{a}$ | $7.58 \pm 0.11^{a}$ |

Values are mean ±SD of triplicate analyses. Means in the same column bearing different superscript differ significantly ($P \leq 0.05$).

HP-DDG=high protein distiller's dried grains; TAC=Total antioxidant capacity.

Table (6): Economical evaluation of European sea bass fed the experimental diets (means ± SE).

| Diets | Feed cost per kg ($*) | FCR | ECR ($*) | Cost/kg fresh fish($*) | Relative Feed cost/kg |
|---|---|---|---|---|---|
| Control | 0.75 | 1.71 | 1.28 | 0.63 | 122.5 |
| 30% HP-DDG | 0.74 | 1.45 | 1.08 | 0.59 | 114.6 |
| 40% HP-DDG | 0.74 | 1.46 | 1.08 | 0.58 | 113.4 |
| 50% HP-DDG | 0.74 | 1.26 | 0.93 | 0.51 | 100 |

*1$=17.8 L.E. (Egyptian pound).

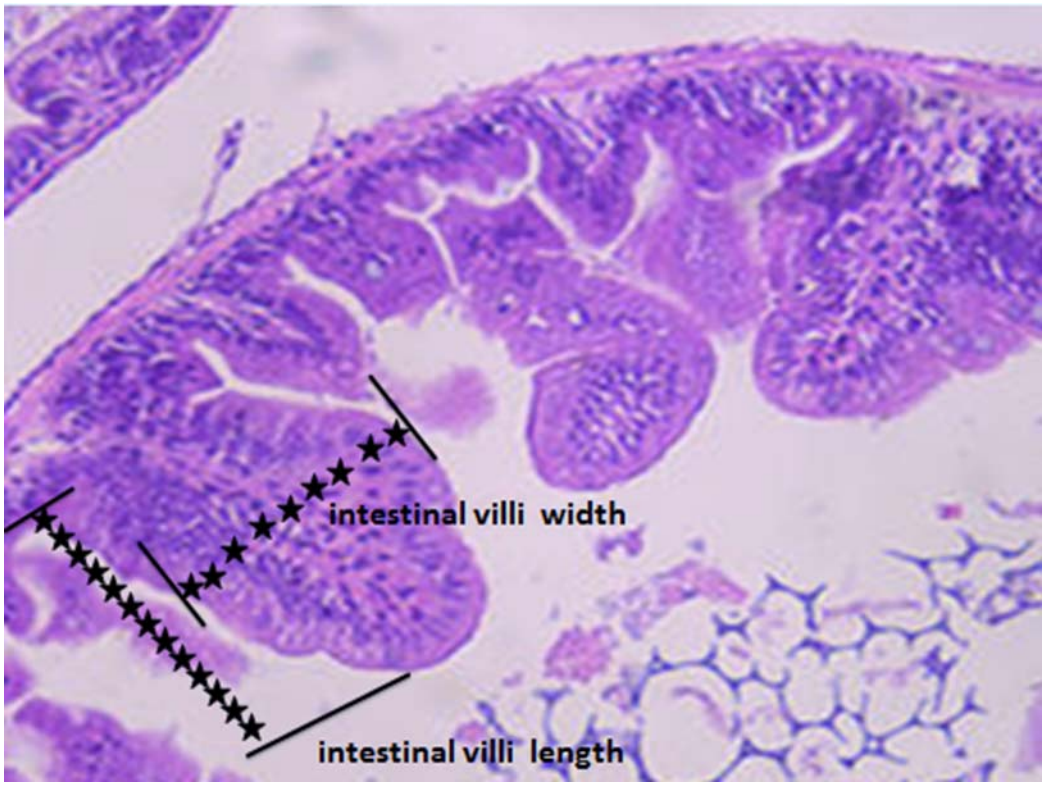


Figure (1): Photomicrograph of sea bass intestine stained with H&E (X400), fed on the control basal diet showing intestinal mucosal folds (villi) length and width but lacking uniformity and some evidence of damage and enteritis in some neighboring mucosal (villi) folds.

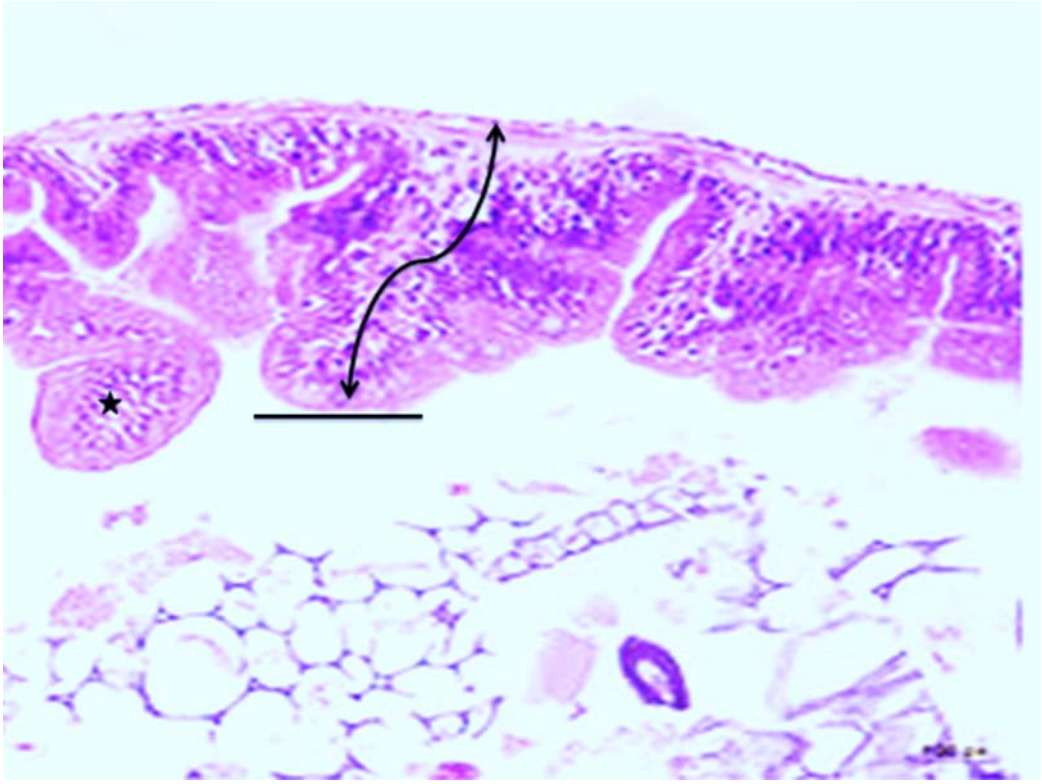

Figure (2): Photomicrograph of sea bass intestine stained with H&E (X400), group (fed on 30% HPDDG) showing moderate improvement in the length of intestinal mucosal folds (villi) with few goblet cells in the lining epithelial.

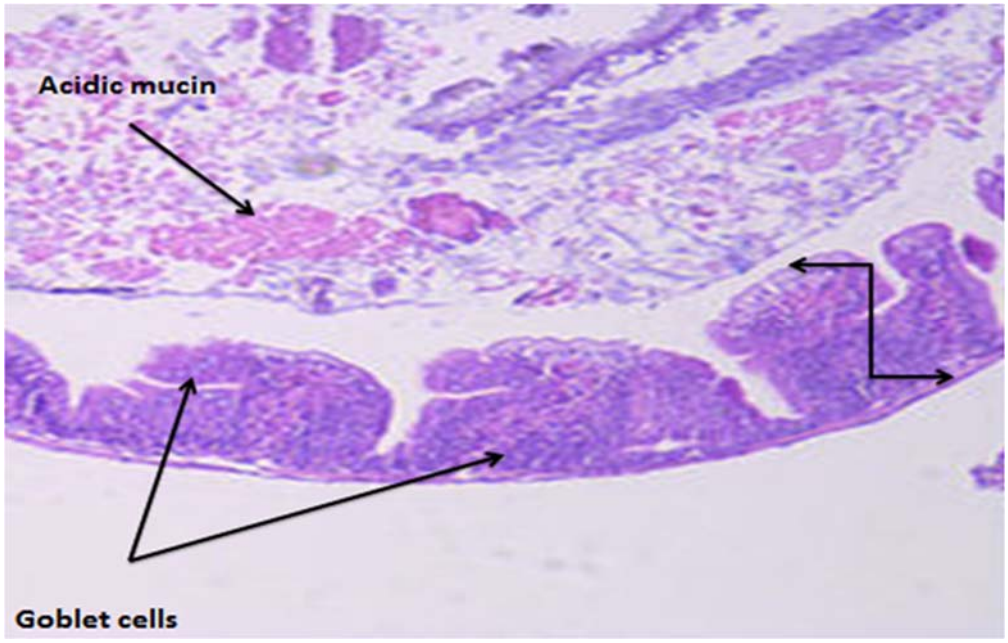


Figure (3): Photomicrograph of sea bass intestine stained with H&E (X400), group (fed on 40% HPDDG) showing improvement in length and width of intestinal mucosal (villi) folds with more regular uniformity as well as active goblet cells and filled with abundant acidic mucin secretion.


Figure (4): Photomicrograph of sea bass intestine stained with H&E (X400), group (feed on 50% HPDDG) showing moderate improvement in length and width of intestinal villi as well as goblet cells and filled with abundant acidic mucin secretion.