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Efficacy of dietary exogenous enzyme supplementation on growth performance, antioxidant activity, and digestive enzymes of common carp (*Cyprinus carpio*) fry

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Abstract Fishmeal (FM) is the main source of protein in fish diets, but its supply is stable. So, available plant protein sources could be used instead of FM in aquafeeds. These plants-source proteins may negatively affect feed intake and/or absorption of nutrients due to bad digestion. Therefore, the efficacy of a dietary exogenous enzymes mixture (Hostazyme X; HX) on growth performance, antioxidant capacity, and digestive enzymes in common carp, *Cyprinus carpio*, was assessed. Dietary HX was included in practical diets at levels of 0.0 (control), 0.1, 0.5, 1.0, and 2.0 g/kg diet. Fish (0.84 ± 0.003 g) were fed on the tested diets up to apparent satiation twice daily in triplicate groups for 8 weeks. The fish performance was significantly improved ($P < 0.05$) by increasing dietary HX up to 1.0 g/kg diet after which fish growth and feed intake were almost the same. Also, intestinal amylase, lipase, and protease significantly increased ($P < 0.05$) due to HX supplementation leading to increased feed intake and overall growth performance. Likewise, the antioxidant activity of common carp was stimulated by HX supplementation in a dose-related manner, where the activities of superoxide dismutase, catalase, and glutathione peroxidase were significantly higher ($P < 0.05$), while malondialdehyde value was significantly lower ($P < 0.05$) in HX-fed fish groups than those fed the control diet. In conclusion, dietary HX could be used as a feed supplement to confer better growth performance and health of common carp fry with an optimal level of 1.0 g/kg diet.

Keywords Common carp · Exogenous enzymes · Growth performance · Digestive enzymes · Antioxidant activity

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Introduction

Fish farming represents one of the fastest-growing food-producing sectors worldwide. The expansion in the aquaculture industry is accompanied by a growing need for protein sources for aqua-feeds production. The most important ingredient in fish diets is fishmeal (FM), which is mainly obtained from a wild fish catch (Gill 2007). As the catch from natural fisheries has stabilized, the supply of FM is stable; meanwhile, its demand increases, thereby causing higher prices (Tacon and Metian 2009; Tacon et al. 2011). In recent years, one illustrious area of research in aquaculture has focused on the replacement of fishmeal with plant-based protein ingredients to support the extending of the worldwide aquaculture industry and to assure its sustainability (Gatlin et al. 2007; NRC 2011). Even so, the mainstream of plant-based feedstuffs has a wide variety of anti-nutritional agents such as non-starch polysaccharides and protease inhibitors, which may decline nutrient consumption, additionally, to decline fish performance, and overall health (Francis et al. 2001; NRC 2011). Hence, fish nutritionists have been working hard to

improve the nutritional value of fish diets by the dietary supplementation of exogenous enzymes to diets.

Presently, supplementing the fish diets with exogenous enzymes may improve the digestibility of diets containing high plant protein sources. The implementation of digestive enzymes in aquatic diets has been rising to improve the overall quality of diets containing these economical plant protein sources. However, exoenzyme supplementation is effective to eradicate the anti-nutritional aspects and improve the consumption of dietary energy and amino acids, ensuing fish performance promotion (Farhangi and Carter 2007; Lin et al. 2007; Soltan 2009). The digestibility of all nutrients, however inclusive carbohydrates, protein, and minerals, appear to be influenced by exoenzymes (Felix and Selvaraj 2004). The growth performance of cultured fish might be boosted by the adding of exogenous enzymes to diets; thus, boosting the digestibility of nutrients via raising activities of intestinal digestive enzymes (Drew et al. 2005; Farhangi and Carter 2007; Lin et al. 2007).

Common carp, *Cyprinus carpio* L., is one of the most widely spread freshwater fish species all over the world, representing 71.9% of freshwater production (Dawood and Koshio 2016), and its global production increased gradually from 2.41 million tons in 2000 to 4.08 million tons in 2013 (FAO 2014). With the expansion of the farmed fish and the stability of FM production, aqua-feeds should contain a minimal amount of FM. To develop sustainable aqua-feeds, exogenous enzymes should be added to advance nutrient digestibility. But, the properties of exogenous enzymes can be varied and are reliant on numerous agents, for instance, the age of the animal and the quality and type of diet (Bedford and Schulze 1998; Acamovic 2001). Consequently, the present study was established to evaluate the influence of the inclusion of multiple digestive enzymes (xylanase, amylase, cellulose, α-galactosidase, β-glucanase, pectinase, lipase, and protease) to a soybean-based diet on the secretions of intestinal digestive enzymes, growth performance, and antioxidant capacity of common carp (*Cyprinus carpio*) fry.

Materials and methods

Diet preparation and fish culture

Hostazyme X (HX) containing xylanase, amylase, cellulose, α-galactosidase, β-glucanase, pectinase, lipase, and protease was purchased from NOREL-Misr Co., Giza, Egypt and added to ingredients of each diet (40.35% crude protein) at levels of 0.0 (control), 0.1, 0.5, 1.0, or 2.0 g/kg diet (Table 1). However, HX of every diet was perched in 100 ml, and then blended with the other components for 40 min to make a paste of each diet. The pastes were independently passed across a mill and pelleted (1 mm diameter) in a paste extruder, then, the diets were dried in an oven at 55 °C for 24 h and after that stored in plastic bags at − 2 °C for further use.

Fry of common carp, *C. carpio* L., were brought from the fish hatchery, Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were reserved in an indoor rectangle fiberglass tank for 14 days for acclimation to the lab conditions. Fish (0.84 ± 0.03 g) were randomly distributed at a density rate of 25 fish for 100-L aquarium in triplicates. Every aquarium was provided with compressed air through air-stone using an air pump. The diets were provided to fish up to apparent satiation twice a day at 9:00 and 14:00 h for 8 weeks. Fish waste with a three-quarter of the aquarium's water was siphoned every day and replaced by clean and aerated water from a storage tank. Fish death was recorded daily and dead fish were taken away.

Analysis of water quality parameters

Water quality was determined biweekly after collecting samples from each aquarium. With an oxygen-meter (970 portable DO-meter, Jenway, London, UK), water temperature and dissolved oxygen have been measured in site for each aquarium. The unionized ammonia concentration was determined by using the HACH comparison apparatus following the method reported by Boyd and Tucker (1998). The pH was determined using a pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). Ranges of water temperature in all treatments were 28.3–29.7 °C, dissolved oxygen level was 5.4–5.7 mg/L, unionized ammonia level was 0.04–0.06 mg/L, and pH was 7.7–7.8. In all treatments, the values of water quality parameters are within the acceptable ranges for the fish growth (Boyd and Tucker 1998).

Growth and feed utilization parameters

At the end of the experiment, fish were gathered from each aquarium, counted, and group-weighed.

Parameters of growth performance and feed utilization were calculated as follows:

$$\text{Weight gain (g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{Weight gain\%} = 100\,[\text{final weight (g)} - \text{initial weight (g)}] / \text{initial weight (g)}$$

Specific growth rate (SGR; % g/day) = 100 (Ln W_2 − Ln W_1) / T, where W_1 and W_2 are the initial and final weight, respectively, and T is the experimental period

$$\text{Feed intake} = \text{the summation of the diet offered throughout the experiment}$$

$$\text{Feed conversion ratio (FCR)} = \text{feed intake (g)} / \text{weight gain (g)}$$

$$\text{Fish survival (\%)} = 100\,(\text{fish number at the start} / \text{fish number at the end})$$

Proximate chemical analysis

The experimental diets and fish from every treatment were analyzed matching to AOAC (1995) standard methods for moisture, crude protein, total lipids, and total ash. Moisture composition was measured by drying samples in an oven at 85 °C until constant weight and calculating weight loss. Nitrogen composition was estimated by using a micro Kjeldahl device, and crude protein was approximated by multiplying the nitrogen content value by 6.25. Total lipid content was approximated by ether extraction, and ash was estimated by burning the samples in a muffle furnace at 550 °C for 6 h. Gross energy was estimated according to NRC (2011).

Intestinal digestive enzyme activities assay

At the end of the feeding trial, fish were starved for 24 h and five fish from each aquarium were sampled randomly for estimating activities of an intestinal amylase, lipase, and protease activities. Fish were dissected instantly and the whole intestines were evacuated and blotted dry with filter paper. Then, the intestine specimen was washed, weighed, and homogenized in ice-cold 0.85% NaCl solution, with volumes nine times the weight of the intestine, using a manual glass homogenizer on ice. Homogenates were then centrifuged (4500×*g* for 10 min at 4 °C), and supernatants were conveyed into clean test tubes and the enzyme activities were analyzed within 12 h.

Activities of digestive enzymes were estimated using the diagnostic reagent kits matching to the manufacturer's instructions (Cusabio Biotech Co. Ltd., Wuhan, Hubei, China). The activity of amylase was estimated following the methods submitted by Bernfeld (1955). The activity of amylase was examined on the fundamental of maltose that freed per mg protein per hour using soluble starch (15 mg/mL) as a substrate in 0.1 M phosphate buffer (pH 6.5) with 0.05 M NaCl at 37 °C. Lipase activity was estimated matching to the method explained by Shihabi and Bishop (1971). The reaction was fundamental on lipase's capability to hydrolyze triglyceride in the stabilized emulsion of olive oil, which resulted in a decrease in optical density when estimated at 420 nm with adjusting the optical density of Tris buffer to zero. One unit of lipase activity was defined as 1 μmol substrate consumed per min per g protein in intestinal supernatants at 37 °C. The activity of protease was quantified using the azocasein hydrolysis assay matching the method of Ross et al. (2000). In short, aliquots of 100 ml of intestine supernatants (already diluted 1/10 in 100 mM ammonium bicarbonate buffer) were incubated with 125 ml of 100 mM ammonium bicarbonate buffer containing 2% azocasein (Sigma) for 24 h at 30 °C. The reaction was stopped by adding 10% trichloroacetic acid (TCA). Then, the mixture was centrifuged at 6000×*g* for 10 min and the supernatants were transmitted to a 96-well plate in triplicate having 100 ml 1 N NaOH and measured at 450 nm using a plate reader. Serum was substituted by trypsin (5 mg/ml, Sigma), as a positive control (100% of protease activity), or by the buffer, as negative controls (0% activity).

Antioxidant activity assays

At the end of the feeding trial, five fish from every aquarium were dissected and liver tissues of these fish were homogenized. The antioxidant enzymes were estimated using the diagnostic reagent kits matching to the manufacturer's instructions (MyBioSource Inc., San Diego, California, USA). Malondialdehyde (MDA) level was measured at 532 nm by the thiobarbituric acid method discussed by Ohkawa et al. (1979). Superoxide dismutase (SOD), catalase (CAT), and glutathione

Table 1 Ingredients and proximate composition (on dry matter basis) of a practical diet supplemented with different taurine levels

Ingredients	Hostazym X levels (g/kg diets)				
	HX 0.0 (control)	HX 0.1	HX 0.5	HX 1.0	HX 2.0
Fishmeal[a]	100	100	100	100	100
Soybean meal[b]	700	700	700	700	700
Wheat bran	110	110	110	110	110
Sun flower oil	20	20	20	20	20
Fish oil	20	20	20	20	20
Vitamins premix[c]	15	15	15	15	15
Minerals premix[d]	15	15	15	15	15
Dicalcium phosphate	10	10	10	10	10
Starch	10	9.9	9.5	9	8
Hestazime x	0	0.1	0.5	1.0	2.0
Total	1000	1000	1000	1000	1000
Proximate analysis (%)					
Dry matter	90.23	90.21	90.21	90.23	90.22
Crude protein	40.35	40.34	40.36	40.35	40.36
Ether extract	6.29	6.28	6.29	6.27	6.28
Crude fiber	5.73	5.7	5.71	5.73	5.72
Ash	7.27	7.25	7.27	7.26	7.25
NFE[e]	40.36	40.43	40.37	40.39	40.39
Gross energy (kcal/g)[f]	476.848	476.86	476.86	476.78	476.89

[a] Danish fishmeal: 72.0% protein, TripleNine Fish Protein, DK- 6700 Esbjerg, Denmark

[b] Egyptian soybean flour 45.0% protein, National Oil Co., Giza, Egypt

[c] Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; α-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU

[d] Mineral premix (per kg of premix): CaHPO4·2H2O, 727.2 g; MgCO3·7H2O, 127.5 g; KCl 50.0 g; NaCl, 60.0 g; FeC6H5O7·3H2O, 25.0 g; ZnCO3, 5.5 g; MnCl2·4H2O, 2.5 g; CuCl2, 0.785 g; CoCl3·6H2O, 0.477 g; CaIO3·6H2O, 0.295 g; CrCl3·6H2O, 0.128 g; AlCl3·6H2O, 0.54 g; Na2SeO3, 0.3 g

[e] Nitrogen-free extract = 100 − (protein % + lipid % + total ash % + crude fiber %)

[f] Gross energy was calculated according to NRC (1993) as 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively

peroxidase (GPx) activities were estimated by methods matching to McCord and Fridovich (1969); Aebi, (1984), and Paglia and Valentine (1967), respectively.

Statistical analysis

Before statistical analysis, all data were tested for normality of distribution using the Kolmogorov-Smirnov test. The homogeneity of variances was examined among different treatments using Bartlett's test. After that, data were subjected to one-way ANOVA to estimate the effect of HX supplementation. Differences between means were examined at the 5% probability level using the Duncan test. The optimum HX concentration for fish growth was estimated using polynomial regression analysis. All the statistical analyses were done using the SPSS program version 20 (SPSS, Richmond, VA, USA) according to Dytham (2011).

Results

Fish growth performance

In the current study, all growth parameters improved significantly with increasing HX levels ($P < 0.05$; Table 2). This was evident in the final weight of fish as the highest values were observed when fish fed 1–2 g

Table 2 Growth performance of common carp fed diets supplemented with different levels of dietary Hestazime C for 8 weeks

Hestazime x levels (g/kg)	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain%	SGR (%g/day)	Feed intake (g feed/fish)	FCR	Survival (%)
Control	0.84 ± 0.000	7.77 ± 0.18d	6.93 ± 0.18d	824.63 ± 20.99d	3.97 ± 0.04d	10.03 ± 0.09c	1.45 ± 0.03a	98.67 ± 1.33
0.1	0.84 ± 0.003	8.57 ± 0.03c	7.72 ± 0.04c	915.87 ± 7.93c	4.14 ± 0.01c	10.67 ± 0.07b	1.38 ± 0.01b	97.67 ± 1.20
0.5	0.85 ± 0.003	9.07 ± 0.07b	8.22 ± 0.07b	970.87 ± 6.82b	4.23 ± 0.01b	11.03 ± 0.15b	1.34 ± 0.01bc	98.00 ± 1.15
1.0	0.85 ± 0.003	9.80 ± 0.06a	8.95 ± 0.06a	1057.53 ± 8.19a	4.37 ± 0.01a	11.73 ± 0.03a	1.31 ± 0.01c	98.67 ± 1.33
2.0	0.84 ± 0.003	9.57 ± 0.20a	8.72 ± 0.21a	1034.63 ± 28.08a	4.34 ± 0.05a	12.00 ± 0.35a	1.37 ± 0.01b	98.00 ± 1.15

Means having the same letter in the same column are not significantly different at $P < 0.05$

HX/kg diet (9.80 and 9.57 g, respectively), while lowest final weight was observed in the control group (7.77 g). Similarly, weight gain and weight gain % increased significantly ($P < 0.05$; Table 2) and their highest values were obtained with fish fed 1–2 g HX/kg diet (8.95 and 8.72 g, respectively for weight gain and 1057.53 and 1034.63%, respectively for weight gain %); their lowest values were obtained at the control group (6.93 g and 824.63%, respectively). Also, there were a significant improvement in both specific growth rate and feed intake, where they increased significantly from 3.97%g/day and 10.03 g feed/fish, respectively at the control group to 4.37 and 4.34%g/day and 11.73 and 12.00 g feed/fish, respectively for specific growth rate and feed intake when fish fed 1–2 g HX/kg diet, respectively. Moreover, FCR improved significantly by increasing dietary HX concentration and the best value was at level 1.0 g/kg diet (1.31), while the worst value was observed in the control (1.45). Furthermore, HX supplementation did not significantly affect fish survival and its range was 97.67–98.67% ($P > 0.05$; Table 2).

The relation of final fish weight with dietary HX levels (Fig. 1) was best expressed by the second-order polynomial regression equation as follows:

$y = -0.2071X^2 + 1.6529\ X + 6.2$ (R2 = 0.9718.

The regression curve showed that the most suitable HX level for optimum fish growth was 1.0 g/kg diet (Fig. 1). Moreover, fish fed on diets including 2 g HX/kg consumed more diet (12.00 ± 0.35 g feed/fish) than the other treatments; meanwhile, the optimum FCR value was observed at 1.0 g/kg diet (1.31).

Whole-fish body proximate composition

The dietary HX concentration significantly affected the whole-fish body components ($P < 0.05$; Table 3). Dietary HX supplementation compared to the control group increased significantly contents of protein and total lipids, which were 8.49 and 8.47 for crude protein and 3.48 and 3.53 for total lipid when fish fed 1–2 g/kg diet of HX, respectively. Meanwhile, compared to the control group, dietary HX supplementation decreased significantly contents of moisture and total ash content in the whole-fish body ($P < 0.05$; Table 3), so lowest moisture and ash values in treatments of at 1 and 2 g HX/kg diet were 86.30 and 86.33 for moisture and 1.56 and 1.54 for ash, respectively.

Intestinal digestive enzymes activities

Activities of intestinal amylase, lipase, and protease increased significantly with increasing HX levels reaching their optimum values at the treatment of 1.0 g HX/kg diet (99.00, 251.5, and 220.0 U/g, respectively) ($P > 0.05$; Fig. 2). Meanwhile, their lowest activities were observed with fish fed the control diet (56.5, 80.5, and 105.0 U/g protein for amylase, lipase, and protease activities, respectively).

Antioxidants activities

It is noticed that MDA value decreased significantly; meanwhile, SOD, CAT, and GPx activities were significantly elevated due to increasing HX levels in the tested diets ($P < 0.05$; Fig. 3). Their optimum activities (19.00 nmol/g, 28.00, 24.00, and 25.50 IU/g for MDA, SOD, CAT, and GPx, respectively) were observed with fish fed 1.0 g HX/kg diet ($P > 0.05$; Fig. 3). Fish fed the control diet showed highest MDA value (35.5 nmol/g) together with lowest activities of SOD, CAT, and GPx (11.5, 13.5, and 11.5 IU/g, respectively).

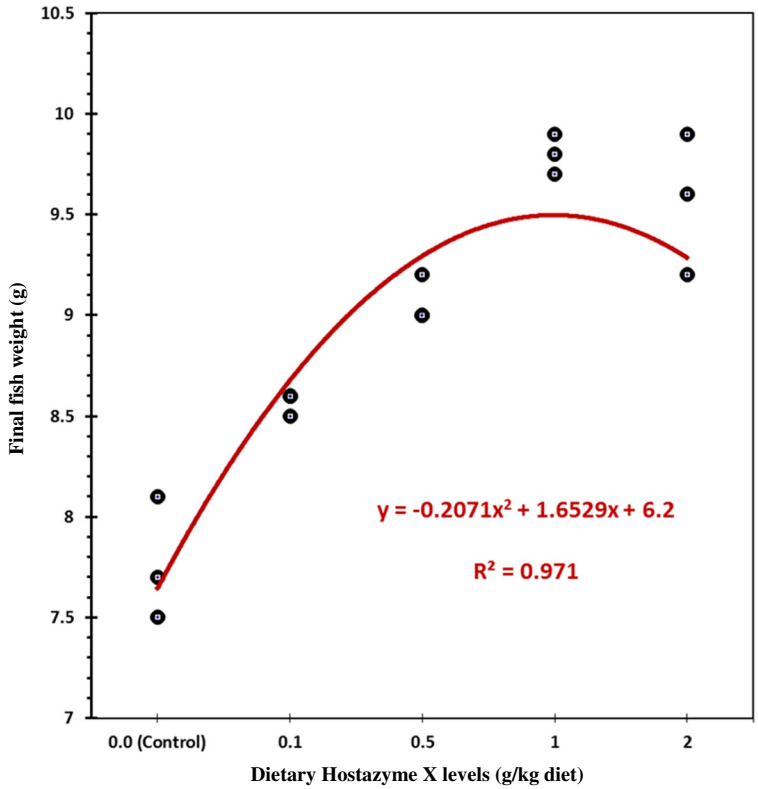

Fig. 1 The relationship between final weight (g) of common carp and different levels of dietary Hostazyme X after feeding for 8 weeks. Dietary Hostazyme X levels (g/kg diet)

Discussion

Fish growth performance

In the present study, fish fed the control diet showed lower growth performance than diets supplemented with exoenzymes, pointing out that the dietary exoenzymes were advantageous for the growth of common carp. Growth response and feed utilization were enhanced with exoenzyme supplementation proposing that the adverse effects of plant ingredients were compensated almost by the addition of the exoenzymes to fish diets because digestive exoenzymes have growth-stimulating effects, and they also are effective to eradicate the anti-nutritional aspects and improve the feed consumption, ensuing fish performance promotion. These results are in endorsement with some previous studies on salmon (Carter et al. 1992, 1994), rainbow trout (Drew et al. 2005), and rabbitfish (Dawood et al. 2019). Bogut et al. (1995) used a multienzyme preparation containing amylase, protease, β-gluconase, β-glucosidase, and cellulase at a rate of 1.5 mg/kg diet on common carp fingerlings, the growth was significantly improved. Adeoye et al. (2016) studied the influence of exogenous enzyme supplementation on Nile tilapia

Table 3 Proximate chemical composition (% on a fresh weight basis) of the whole body of common carp fed diets supplemented with different levels of Hestazime X for 8 weeks

Hestazime X levels (g/kg diet)	Moisture	Crude protein	Total lipids	Total ash
Control	87.30 ± 0.16a	7.28 ± 0.04d	2.87 ± 0.07 c	2.35 ± 0.04a
0.1	87.30 ± 0.20a	7.61 ± 0.11c	3.03 ± 0.05bc	2.03 ± 0.04b
0.5	86.55 ± 0.15b	8.13 ± 0.03b	3.30 ± 0.08ab	1.90 ± 0.01b
1.0	86.30 ± 0.20b	8.49 ± 0.02a	3.48 ± 0.05a	1.56 ± 0.15c
2.0	86.33 ± 0.07b	8.47 ± 0.05a	3.53 ± 0.10a	1.54 ± 0.18c

Means having the same letter in the same column are not significantly different at $P < 0.05$

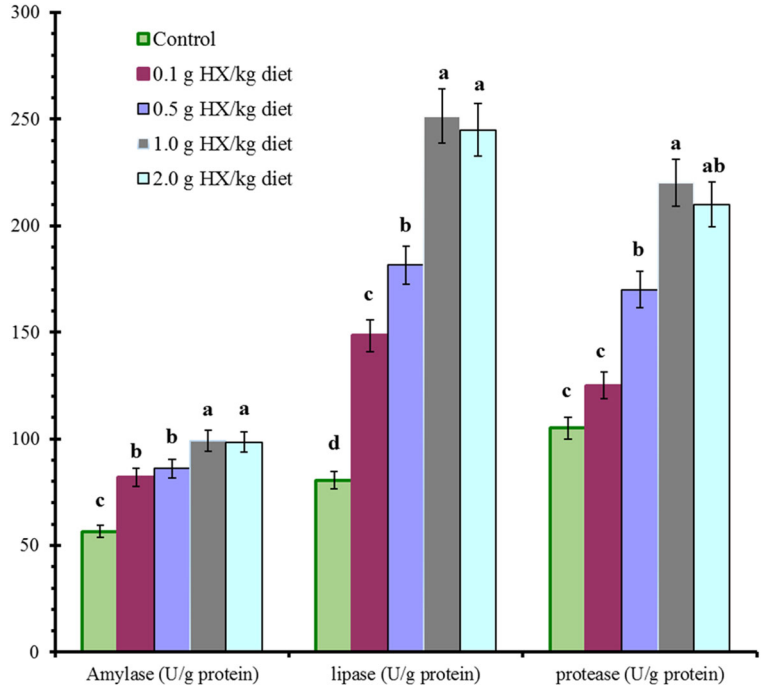

Fig. 2 Activities of intestinal digestive enzyme (U/g protein) of common carp fed diets supplemented with different levels of dietary Hestazime X for 8 weeks. Bars having different letters for the same variable are significantly different at $P < 0.05$

growth, intestinal morphology, and microbiome composition where they found that fish fed diet complemented with multienzymes performed better than those fed the control diet. Moreover, numerous fish researches have been performed with diets enhanced with pancreatic enzymes such as proteases (Carter et al. 1992, 1994; Drew et al. 2005; Lin et al. 2007; Castillo and Gatlin 2015). The authors detected an enhanced growth, feed efficiency,

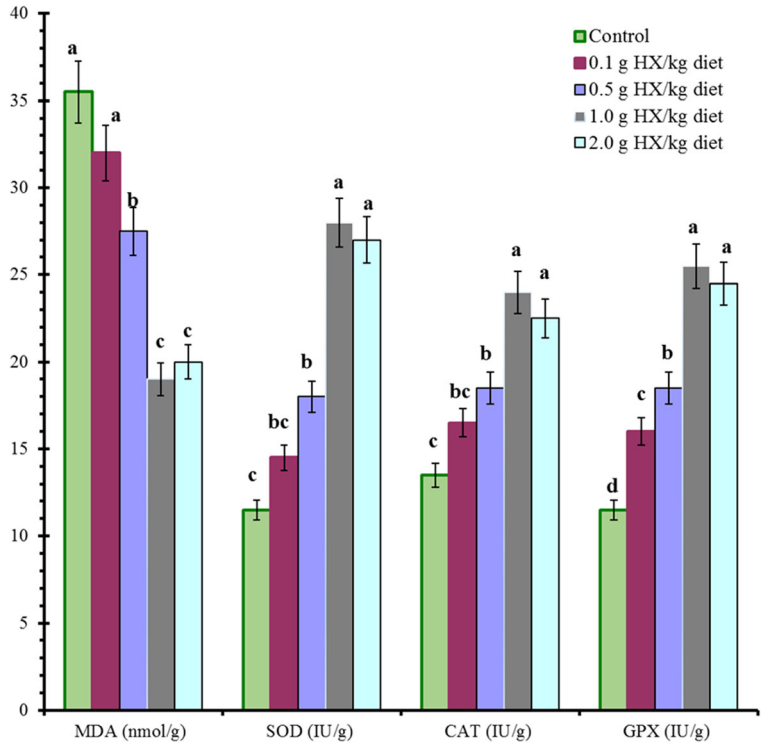

Fig. 3 Changes of hepatic MDA (nmol/g), CAT, GPX and SOD activities (IU/g) of common carp fed diets supplemented with different levels of dietary Hestazime X for 8 weeks. Bars having different letters for the same variable are significantly different at $P < 0.05$

and protein digestibility by supplementation of exogenous enzymes to plant-based protein diets to fish. Besides, Lin et al. (2007) investigated the effects of supplemental exogenous enzymes (protease, β-glucanase, and xylanase) at a dose of 1.0 and 1.5 g/kg on growth performance of juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*), which were fed to feed based on plant ingredients (soybean, rapeseed, and cottonseed meals). These results showed that both proteases besides amylase actions in the intestine; furthermore, the hepatopancreas of juvenile hybrid tilapia significantly improved with rising dietary enzyme rates. These results suggest that exogenous enzyme supplementation promoted the secretion of endogenous enzymes in fish intestines. Hlophe-Ginindza et al. (2016) added a Natuzyme50 (commercial multienzymes containing protease, lipase, α-amylase, cellulase, amyloglucosidase, β-glucanase, pentosonase, hemicellulose, xylanase, pectinase, acid phosphatase, and acid phytase) to a Kikuyu-based diet at a level of 0, 0.25, 0.5, 0.75, and 1.00 g/kg diet for Mozambique tilapia, *O. mossambicus*; they concluded that fish fed diet including Natuzyme50 at a level of 0.50 g/kg diet displayed the best growth performance and boosted the activities of endogenous enzymes enhancing the efficacy of the digestive process. The ideal inclusion level of exoenzymes may differ between fish species and life stages, so tests should be carried out with different dosage levels to find optimum inclusion (Jiang et al. 2014). The summary of results also suggests that the influence of exogenous enzymes may hinge on the amounts and composition of plant feedstuffs in the diet formulation.

Whole-fish body proximate composition

The proximate chemical analysis of whole-fish body inclusive crude protein and total lipids improved significantly due to dietary HX supplementation; meanwhile, ash and moisture content significantly declined. These results propose that HX supplementation boosted protein synthesis and deposition in the fish's body when it was supplemented at optimum levels. The boosted rate of the body protein content may be due to the higher level of digestive enzyme activity and its beneficial effect on digestion and absorption of protein material in the fish gut (Ye et al. 2011; Abdel-Tawwab and Monier 2018). The enhancement in fat digestibility is particularly notable because non-starch polysaccharide is known to increase hydrolysis of bile salts and hence reduce fat consumption (Vahjen et al. 2007). Yildirim and Turan (2010) have reported that in African catfish (*Clarias gariepinus*), body fat increased when exogenous enzyme is used for feeding, but, this increase was not significant. Also, Ghomi et al. (2012) concluded that body fat of sturgeon, *Huso huso*, fingerlings boosted leading to highest fat content (34.53%) when fingerlings fed with exoenzyme-supplemented diets (500 mg/kg diet). Actually, changes in fish body composition such as protein and lipid contents might be linked to changes in their synthesis, deposition rate in muscle, and/or different rates of growth (Fauconneau et al. 1985; Taylor and Onders 2006; Abdel-Tawwab et al. 2006; Abdel-Tawwab and Monier 2018).

Activities of intestinal digestive enzymes

The current results display that the dietary HX supplementation improved the activities of intestinal amylase, lipase, and protease especially when fish fed 1.0–2.0 g/kg diet ($P < 0.05$; Fig. 2). This observation might be because dietary HX includes numerous digestive exoenzymes as carbohydrases, lipase, and protease. It is thinkable that the supplementing exogenous enzymes improved nutrient digestibility by boosting the secretion of endogenous digestive enzymes (Castillo and Gatlin 2015). Also, exogenous digestive enzymes improved the cholecystokinin hormone content in the intestine, which dominates the liberation of digestive enzymes and bile from the pancreas into the intestinal lumen (Jiang et al. 2014). Cholecystokinin excretion is organized by dietary protein (Cahu et al. 2004). These findings of the present study may be consistent with what they have reached by Yildirim and Turan (2010), Ghomi et al. (2012), and Ali Zamini et al. (2014) where they detected a positive impact of various commercial, multienzyme complex (phytase, xylanase, β-glucanase, β-amylase, cellulase, and pectinase) on the growth performance and feed proficiency of African catfish, *C. gariepinus*; great sturgeon, *H. huso*; and Caspian salmon, *Salmo trutta*, respectively. Besides, Hlophe-Ginindza et al. (2016) concluded that fish fed diet including Natuzyme50 at a level of 0.50 g/kg diet boosted the activities of endogenous enzymes enhancing the efficacy of the digestive process. Lin et al. (2007) found that both protease besides amylase actions in the intestine; furthermore, the hepatopancreas of juvenile hybrid tilapia significantly improved with rising dietary enzyme rates. These results suggest that exogenous enzyme supplementation promoted the secretion of endogenous enzymes in fish intestines.

Antioxidants activities

Oxidative stress appears to play a major role in the pathogenesis and progression of many livers and intestinal diseases (Papada et al. 2014; Xiao et al. 2014). The enzymatic antioxidant capacity in fish is a defense system protecting the body tissues from the damage caused by oxidative stress (Ben Ameur et al. 2012). The liver and intestine are involved in different functions and the challenges that they are closely confronted are also different. As the major detoxifying organ in vertebrates, the liver is also central to degrading metabolic products (Lee et al. 2015), and this is constantly challenged by many endogenous and exogenous free radicals. Furthermore, it is reported that antioxidant defenses are more highly developed in the liver than in other organs (Lushchak et al. 2005). Therefore, it is very important to enhance the antioxidant ability of the liver and intestine, thus enhancing the liver and intestine health. Also, fish feeding plays a substantial role in their welfare by preserving their oxidative balance, either by supplying nutrients that enhanced the antioxidant system or avoiding those that would induce an increase of free radical production (Liew et al. 2015; Burgos-Aceves et al. 2016; Hoseinifar et al. 2017). In the present study, it is noticed that MDA value decreased significantly; meanwhile, activities of SOD, CAT, and GPx increased significantly in the liver of fish fed HX-enriched diets than that fed the control diet ($P < 0.05$; Fig. 3). Malondialdehyde (MDA) contents were extensively used as a biomarker for oxidative damage to lipids and proteins respectively (Chen et al. 2009; Xiao et al. 2019). The MDA is a product of lipid peroxidation and directly reflects the level of lipid peroxidation, and a higher level of MDA leads to higher cell toxicity, quicken the breakdown of cells and tissues (Buege and Aust 2004). In the current study, decreasing of the MDA value with increasing dietary HX suggested that dietary HX may depress the lipid peroxidation and protein oxidation in the fish. Furthermore, the lipid peroxidation and protein oxidation can be suppressed by antioxidant enzymes such as SOD, CAT, and GPx activities in fish (Martínez-Álvarez et al. 2005; Xiao et al. 2019). It is known that SOD, CAT, and GPx activities are the main antioxidant enzymes (Mallick and Mohn 2000; Krajcovicova-Kudlackova et al. 2003). Qualitative data related to the evaluation of enzymatic antioxidants in fish such as SOD and CAT show that they are structurally and functionally very similar to those of mammals (Aksnes and Njaa 1981; Wilhelm 1996). The SOD catalyzes the dismutation of the superoxide radical ($O2^{-}$) to hydrogen peroxide, and hydrogen peroxide is then converted to water and oxygen by GPx and CAT. These reactions reduce the extent of oxidative damage in cells (Rzeuski et al. 1998; Saputra et al. 2016). Thus, higher values of hepatic SOD, CAT, and GPx activities in common carp fed HX-enriched diets could be responsible for improving the fish health by scavenging free radicals and improving the antioxidant defense system in the fish. These results indicate that dietary HX supplementation could enhance the resistance of common carp to oxidative stress and would probably confer better fish health. These results are consistent with recently resulting, which dietary nutritional supplements were found to be an efficient method for improving organ antioxidant capacity (Wu et al. 2017)

Conclusion

The present study shows that dietary exoenzymes (Hostazyme X) have a positive effect on the performance and health of common carp fry via elevating their intestinal digestive enzyme activities and improving their antioxidants capacity. However, HX inclusion in common carp diets at a level of 1.0 g/kg diet could improve fish growth, feed utilization, and defense system of fish.

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Data availability Research data are not shared.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of fish were followed by the author.

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