Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(3): 149 – 166 (2022)
www.ejabf.journals.ekb.eg

Effect of phytase supplementation on growth performance, body mineral composition, and effluent phosphorus content of the seabream (*Sparus aurata*)

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ARTICLE INFO

Article History:
Received: April 18, 2022
Accepted: April 29, 2022
Online: May 22, 2022

Keywords:
phytase;
phosphorus;
growth;
effluent;
sea bream

ABSTRACT

This study evaluated the effects of the dietary phytase on growth, feed utilization, body chemical composition, body minerals composition, and effluent phosphorus (P) content in sea bream (Sparus aurata). Treatment 1 (the control group) contained mineral premix (MP) and inorganic phosphorus (IP) (mono-calcium phosphate) at concentrations of 6 g and 5 g kg–1 diet, respectively, without the addition of phytase. The other four treatments contained one level of dietary phytase supplementation at 0.2 g kg–1 (5000 FTU kg), in the presence or absence of MP and IP, separately or in combination, at concentrations of 6 g and 5 g kg–1 diet, respectively. Sea bream (S. aurata) (0.32±0.01 g fish–1) (initial mean weight ± SD) fed the experimental diets for 60 days. Growth and feed utilization were significantly enhanced by dietary phytase-supplemented diets. Final weight, weight gain, average daily gain, specific growth rate, feed intake, and protein efficiency ratio were higher in the phytase-containing treatments than in the control diet, regardless of the presence or absence of mono-calcium phosphate or the mineral mixture. Following the same pattern, the feed conversion ratio was better in the phytase-containing treatments than in the control diet. Body crude protein, ash, and mineral composition were significantly increased by the dietary inclusion of phytase regardless of the presence or absence of IP or MM. Furthermore, the addition of phytase into S. aurata diets reduced the phosphorus concentration in their effluents. Thus, this study emphasizes the important role of the dietary phytase in S. aurata in enhancing the growth performance, enriching the body's nutritional composition, and reducing the environmental impacts of fish farming.

INTRODUCTION

The gilthead sea bream (Sparus aurata), one of the most important maricultured species in the Mediterranean Sea, produced 258.754 tons in 2019 (FAO, 2022). Due to their proper market price, high survival rate and low food chain feeding habits, gilthead seabream are an excellent choice for aquaculture in the Mediterranean. They are now cultured at very high densities, ranging from 15 to 45 kg m–3 (FAO, 2022). In parallel, there is a

necessity to reduce the possible coastal environmental impact of sea bream aquaculture, such as discharges of organic matter, phosphorus, and nitrogen, which can cause eutrophication (FAO, 2022).

On the other hand, fishmeal (FM) has been used as the main source of protein in fish feed. The importance of fishmeal is due to its high content of essential nutrients such as amino acids, fats, vitamins, and growth factors, as well as its high digestibility and low level of carbohydrates (Zhou et al., 2004; Morales et al., 2014). However, the scarcity and high cost of FM has led to an inevitable need to use low-cost plant-derived protein sources such as cottonseed meal, rice bran, sunflower meal, and soybean meal (Guimarães et al., 2008; Aanyu, et al., 2014; Morales et al., 2014; Koch et al., 2016). On the other hand, one of the main problems hindering the use of plant proteins in fish feeds is their contents of anti-nutritional factors. These anti-nutritional factors include saponins, tannins, glucosinolates, non-starch soluble sugars, gossypol, and phytate that are not degraded or inactivated by product manufacturing processes or during diet extrusion methods (Hardy, 2010; Roy et al., 2014). Phytate (myo-inositol-1,2,3,4,5,6-hexakisphosphate), the major storage form of phosphorous (P), may reach 80% of the total phosphorous content in plants and is not bioavailable for monogastric or agastric aquatic animals (NRC, 1993). For example, in soybean meal, it contains about 14 g kg–1 of phytic acid, which makes 60-80% of phosphorous in the form of phytates (Raboy 1997; Deak and Johnson, 2007). Besides its effect on phosphorous availability, phytic acid also chelates with many cations such as Na, Ca, K, Mg, Zn, Mn, Fe, and Cu (Leiner 1994). Consequently, fish excrete phytate-P into the surrounding water, which may cause pollution and algal growth (Liebert and Portz, 2005). In addition, the absorption and bioavailability of essential minerals in fish such as calcium, magnesium, zinc, and iron are reduced by the formation of insoluble chelated complexes with phytate (Papatryphon et al.,1999). Phytates can also bind to proteins and vitamins and form insoluble complexes, resulting in decreased activity, efficiency and availability of them (Liu et al.,1998; Sugiura et al., 2001). Phytate-protein complexes are less hydrolyzed by proteolytic enzymes (Ravindran et al., 1995). Moreover, some digestive enzymes such as pepsin, amylopsin, and amylase can be inhibited by phytate (Ravindran et al., 1995). furthermore, phytate may also interfere with the digestion of lipid and starch (Cosgrove, 1966). In fact, the effect of phytates on growth, feed conversion efficiency, and mineral retention in commonly cultured fish species such as rainbow trout (Oncorhynchus mykiss), striped bass (Morone saxatilis), and Nile tilapia (Oreochromis niloticus) has been reported (Papatryphon et al., 1999; Francis et al., 2001; Liebert and Portz, 2005; Morales et al., 2014).

Phytase (myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate phosphohydrolase), a phosphatase enzyme, catalyzes the hydrolysis of phytate into inositol and inorganic P (Debnath et al. 2005; Lemos and Tacon, 2017). Therefore, exogenous phytase has been included in fish diets to degrade phytate and increase the nutrients digestibility (Cao et al.

2007). Phytase can increase the mineral bioavailability of elements such as phosphorus, calcium, iron, manganese, magnesium, and zinc (Cheng and Hardy, 2003; Cao et al., 2007; Roy et al., 2014; Lemos and Tacon, 2017). Morales et al. (2014) assessed the effect of dietary phytase on the available P, soluble protein, total amino acids, and the activity of the main digestive proteases in the stomach, proximal intestine and distal intestine contents of gilthead sea bream. They found that phytase reduced the amount of soluble P–phytate in the stomach and increased overall gastric protease activity by 60% (Morales et al., 2014). Other studies have been carried out on the role of supplemental phytase in improving the nutrient utilization and growth of different cultured fish species such as, red sea bream (Biswas et al., 2019), rainbow trout (Oncorhynchus mykiss) (Cain and Garling, 1995; Forster et al., 1999), channel catfish (Ictalurus punctatus) (Li and Robinson 1997), and Nile tilapia (Oreochromis niloticus) (Liebert and Portz, 2005; Tudkaew et al., 2008).

In fact, rare studies have been performed to study the effect of dietary phytase on gilthead seabream (sparus aurata) in terms of the body mineral composition or phosphorus content in the effluent of S. aurata. Thus, the aim of the present study was the evaluation of the dietary phytase as a feed additive on the growth performance, feed utilization, mineral composition, and phosphorus levels in the effluent of gilthead sea bream (S. aurata) larvae.

MATERIALS AND METHODS

2.1. Experimental diets and feeding regimes

The composition and proximate analysis of the diets are shown in Table 1. Five isonitrogenous (474 g kg^{-1} diet) and isocaloric (507 kcal 100 g^{-1}) diets were prepared. Diet 1 has been formulated as a control diet that did not contain phytase but contained the minerals premix (MP) and inorganic phosphorus (mono-calcium phosphate) (IP) at concentrations of 6 and 5 g kg^{-1} diet, respectively. Diet 2 contained phytase and MP. Diet 3 contained phytase without adding a MP or IP. Diet 4 contained phytase, MP and IP. Diet 5 contained phytase and IP. Phytase was added at fixed level 0.2 g kg^{-1} (5000 FTU kg) (FTU is the quantity of enzyme that produces one micromole of inorganic P per minute from 0.015 mol L^{-1} sodium phytate at 37°C and pH 5.5). The microbial phytase was the commercial product Ronozyme P with 5,000 FTU/g provided by DSM Nutritional Products, Bangkok, Thailand. In a Wiley mill, the materials were first ground to a small particle size (about 250 m) (Labx Company). Prior to adding water to get moisture content of 40%, all dry ingredients in the diets were properly mixed. Diets were processed through a mincer with a die into spaghetti-like strands of the right diameter, sun dried, and stored in airtight containers.

Table 1. The composition and proximate analysis of the experimental diets fed to gilthead sea bream (*Sparus aurata*)

Ingredients (g kg^{-1})	**Diets (g kg^{-1} dry-weight basis)**				
	1	**2**	**3**	**4**	**5**
Fish meal[1]	300	300	300	300	300
Shrimp meal	50	50	50	50	50
Soybean meal[2]	342	342	342	342	342
Corn gluten meal	155	155	155	155	155
Wheat middlings	30	30	30	30	30
Corn starch	10.3	15.1	21.1	10.1	16.1
Fish oil	50	50	50	50	50
Soybean oil	25	25	25	25	25
Citric acid	20	20	20	20	20
Mono-calcium phosphate	5	0	0	5	5
Mineral premix[3]	6	6	0	6	0
Vitamin premix[4]	6	6	6	6	6
Ascorbic acid	0.2	0.2	0.2	0.2	0.2
Antioxidant	0.5	0.5	0.5	0.5	0.5
Phytase[5]	0	0.2	0.2	0.2	0.2
Chemical composition (g kg^{-1}, DM)					
Dry matter	954.0	936.3	946.2	938.3	933.6
Crude protein	474.7	469.4	464.0	478.0	486.1
Ether extract	153.8	159.9	164.2	165.8	169.9

Ash	97.9	87.1	81.9	92.8	105.9
Total carbohydrate	273.6	283.6	289.9	263.4	238.1
Total phosphorus	15.41	10.61	10.61	15.41	15.41
Calcium	18.75	17.049	17.039	18.75	18.74
Gross energy (kcal $100g^{-1}$ DM)	500.71	507.59	511.19	509.70	507.75

[1] Fish meal (Peru steam-treated), crude protein 675 g kg^{-1} dry matter, crude lipid 92 g kg^{-1} dry matter;

[2] Soybean meal (solvent extracted), crude protein 480 g kg^{-1} dry matter.

[3] Minerals premix (mg kg^{-1} diet): $FeSO_4.7H_2O$: 348.5 mg; $CuSO_4.5H_2O$: 6 mg; $ZnSO_4.7H_2O$: 108.2 mg; $MnSO_4.H_2O$: 20.5 mg; KI: 14.5 mg; $NaSeO_3$: 12.5 mg; $CaCO_3$: 4490 mg.

[4] Vitamin premix (mg kg1 diet): retinol palmitate, 60 mg; cholecalciferol, 10 mg; DL-a-tocopherol acetate, 100 mg; menadione, 40 mg; thiamine-HCl, 25 mg; riboflavin, 25 mg; D-calcium pantothenate, 80 mg; pyridoxine-HCl, 20 mg; meso-inositol, 1000 mg; D-biotin, 40 mg; folic acid, 7.5 mg; para-aminobenzoic acid, 25 mg; niacin, 100 mg; cyanocobalamin, 0.05 mg.

5 Phytase: RONOZYME P 5000

2.2 | Experimental fish and facilities

The study was conducted at the National Institute of Oceanography and Fisheries, Al-Max research station in Alexandria, E gypt. Larvae of gilthead sea bream (*Sparus aurata*) were procured from a commercial hatchery in the governorate of Al-Ismailia. Fish were randomly placed in 12 hapas (1 m^3) set in concrete ponds with a total volume of 12 m^3 (each pond contains 3 hapas representing the replicates of each treatment). In ponds (a flow-through system), the water depth was 50 cm. Each hapa was filled with 20 sea bream, each with an initial body weight of 0.32±0.01 g (mean±SD). Every 15 days, fish from each hapa were weighed. Using marine filtered water, half of the rearing water was replaced every two days. An air compressor was used to provide constant aeration. The water temperature was 25°C±1°C. Fluorescent bulbs were used to keep a 12:12 light:dark cycle in the culture unit. Dissolved oxygen, pH, and total ammonia (NH4) were all regularly measured as water quality indicators. Throughout the study, the average values for these parameters were 6.1 mg L^{-1}, 7.8 ppm, and 0.29 ppm, respectively. As an adaptation phase to the experimental diets, the experimental fish were fed the control diet for one week. After that, fish in each hapa were weighed and their initial weights were recorded. Then, fish were fed twice a day (6 days a week) until they appeared satiated. The test diets were produced to the fish for 60 days. All fish in each hapa were bulk weighed at the end of the feeding trial.

2.3 | Analyses

2.3.1 | Growth performance measurements

All fish in each hapa were bulk weighed at the end of the 60-day feeding trial. The proportion of the final number of sea bream divided by the initial number of sea bream was used to determine survival. The following formulas were used to determine growth performance and feed utilization:

$$\text{survival rate} = \frac{\text{initial number of fish}}{\text{final number of fish}},$$

$$\text{feed intake} = \text{amount of consumed feed},$$

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

$$\text{specific growth rate (SGR) (\%/ day)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{trial duration}} \times 100,$$

$$\text{average daily gain (ADG) (g/day)} = \frac{\text{final weight(g)} - \text{initialweight (g)}}{\text{trial duration}},$$

$$\text{feed conversion ratio (FCR)} = \frac{\text{total dry feed intake (g)}}{\text{total fish weight gain (g)}}, \text{ and}$$

$$\text{protein efficiency ratio} = \frac{\text{WG (g)}}{\text{total dry protein intake (g)}}$$

where wf and wi are fish final weight and initial weight, respectively.

2.3.2 | Chemical analysis of diets and whole fish bodies

A standard methodology was used to determine the proximate analyses of the prepared diets and fish carcasses (AOAC, 2009). The carcasses of nine randomly picked fish from each treatment (3 fish/replicate) were collected at the end of the experiment for analysis. The crude protein content was determined using an Automatic Kjeldahl System (UDK 139, VELP Scientifica). The crude lipid content was determined using the Soxhlet extraction method. The ash content was determined using a furnace muffler (600°C for 2 hr). The moisture content was determined using a dry oven (105°C for 24 hr).

2.3.3. Whole body mineral composition

The carcasses of nine randomly picked fish from each treatment (3 fish/replicate) were collected at the end of the experiment for analysis. Each fish sample was dried in an electric oven at 70–80°C until it reached a stable weight. About 2 g from each sample was weighed. All samples were digested using concentrated nitric acid. The whole-body minerals have been estimated using atomic absorption spectrophotometer according to

AOAC (2009). Levels of phosphorus in the whole fish body and diets were determined using UV/ VIS spectrophotometer at absorbance of 720 nm according to AOAC (2009).

2.3.4. Phosphorus content in effluent

The phosphorus concentration in each pond effluent was monitored daily as soluble reactive phosphorus (PO_4^{-3}) for the duration of the experiment using Hanna Aquaculture Multi-parameter Photometer, HI83303.

2.3.5. Statistical analysis

For statistical analysis, SPSS 20.0 software (SPSS Inc.) for Windows was utilized. To investigate the significant difference ($p \leq 0.05$) in the measurable parameters and biochemical compositions of fish fed different diets, a one-way analysis of variance (ANOVA) was used, followed by a Duncan's post hoc test (for equal variance).

RESULTS

3.1. Growth performance and Feed utilization

The effect of the experimental diets on growth performance and feed utilization of *s. aurata* is illustrated in Table 2. Final weight (FW), weight gain (WG), average daily gain (ADG), specific growth rate (SGR), feed intake, and protein efficiency ratio (PER) were higher in the phytase-containing treatments than in the control group, regardless of the presence or absence of mono-calcium phosphate or the mineral mixture. Following the same pattern, feed conversion ratio was better in the phytase-containing treatments than in the control group. There was no significant difference in survival rate among the different treatments.

Table 2. Effect of the experimental diets on growth performance and feed utilization of *s. aurata*

Treatment	IW	FW	WG	ADG	SGR	Survival	Feed intake	FCR	PER
1	0.32±0.023^a	3.26±0.08^b	2.94±0.06^b	0.039±0.001^b	3.09±0.05^b	83.33±2.89^a	7.40±0.10^b	2.52±0.43^a	0.83±0.11^b
2	0.34±0.01^a	4.52±0.10^a	4.19±0.9^a	0.056±0.001^a	3.48±0.046^a	86.67±2.89^a	8.19±0.32^a	1.96±0.09^b	1.09±0.53^a
3	0.31±0.15^a	4.64±0.10^a	4.33±0.12^a	0.058±0.001^a	3.61±0.084^a	86.67±2.89^a	8.67±0.21^a	2.00±0.56^b	1.08±0.30^a
4	0.32±0.10^a	4.66±0.34^a	4.34±0.35^a	0.058±0.005^a	3.57±0.121^a	83.33±2.89^a	8.27±0.32^a	1.92±0.21^b	1.10±0.13^a
5	0.32±0.11^a	4.67±0.17^a	4.35±0.17^a	0.058±0.002^a	3.57±0.086^a	85.00±5,00^a	8.57±0.42^a	1.97±0.07^b	1.04±0.038^a

Note: Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± *SD*.

3.2. Body chemical composition

The effect of the experimental diets on the body chemical composition is shown in Table 3. The highest values of crude protein and ash contents were obtained in the phytase-containing treatments followed by the control group ($p < 0.05$). There was no significant difference in crude lipid and dry matter among the different treatments.

Table 3. Effect of the experimental diets on the body chemical composition (%) of *S. aurata*

Treatment	Dry matter	Protein	Lipid	Ash
1	25.56 ± 1.22^{a}	54.62 ± 0.28^{b}	25.59 ± 1.16^{a}	18.50 ± 0.35^{b}
2	24.98 ± 0.74^{a}	56.30 ± 0.67^{a}	24.89 ± 0.62^{a}	20.07 ± 0.71^{a}
3	24.24 ± 0.99^{a}	56.69 ± 0.53^{a}	24.47 ± 0.70^{a}	20.12 ± 0.33^{a}
4	25.83 ± 0.67^{a}	57.35 ± 0.58^{a}	25.33 ± 0.63^{a}	19.68 ± 0.87^{a}
5	24.43 ± 0.67^{a}	56.89 ± 1.09^{a}	24.96 ± 1.38^{a}	19.77 ± 0.49^{a}

Note: Means in the same column bearing different superscripts differ significantly at 0.05 levels. Values are means ± *SD*.

3.3. Whole- body mineral composition

Figures 1 (A-D) and 2 (A-E) show the effect of the experimental diets on the macro- and micro-minerals composition of gilthead sea bream, respectively. Phosphorus showed the highest value in groups 4 and 5, followed by groups 2 and 3, while the lowest result was obtained in group 1 (the control group) (Figure 1A). In comparison to the control group, phytase-treated groups had greater calcium and magnesium results (Figures 1B and 1D, respectively). The potassium results showed no significant differences among the fish groups (Figure 1C). Results of iron were higher in phytase-supplemented groups than the control group (Figure 2A). The phytase-supplemented groups also had higher zinc, manganese and copper levels than the control group (Figure 2B, C, and D, respectively). Following the same pattern, cobalt gave highest results in groups 4 and 5 followed by groups 2 and 3, while the lowest value obtained in group 1 (Figure 2E).

3.4. Phosphorus content in the effluent

The effect of the experimental diets on phosphorus concentration in the effluent of gilthead sea bream is illustrated in Figure 3. The inclusion of phytase in the diets had a positive effect on the phosphorus levels in *S. aurata* effluent, with lower concentrations in the phytase-supplemented groups compared to the control group. However, group 5 did not differ significantly from the control group.

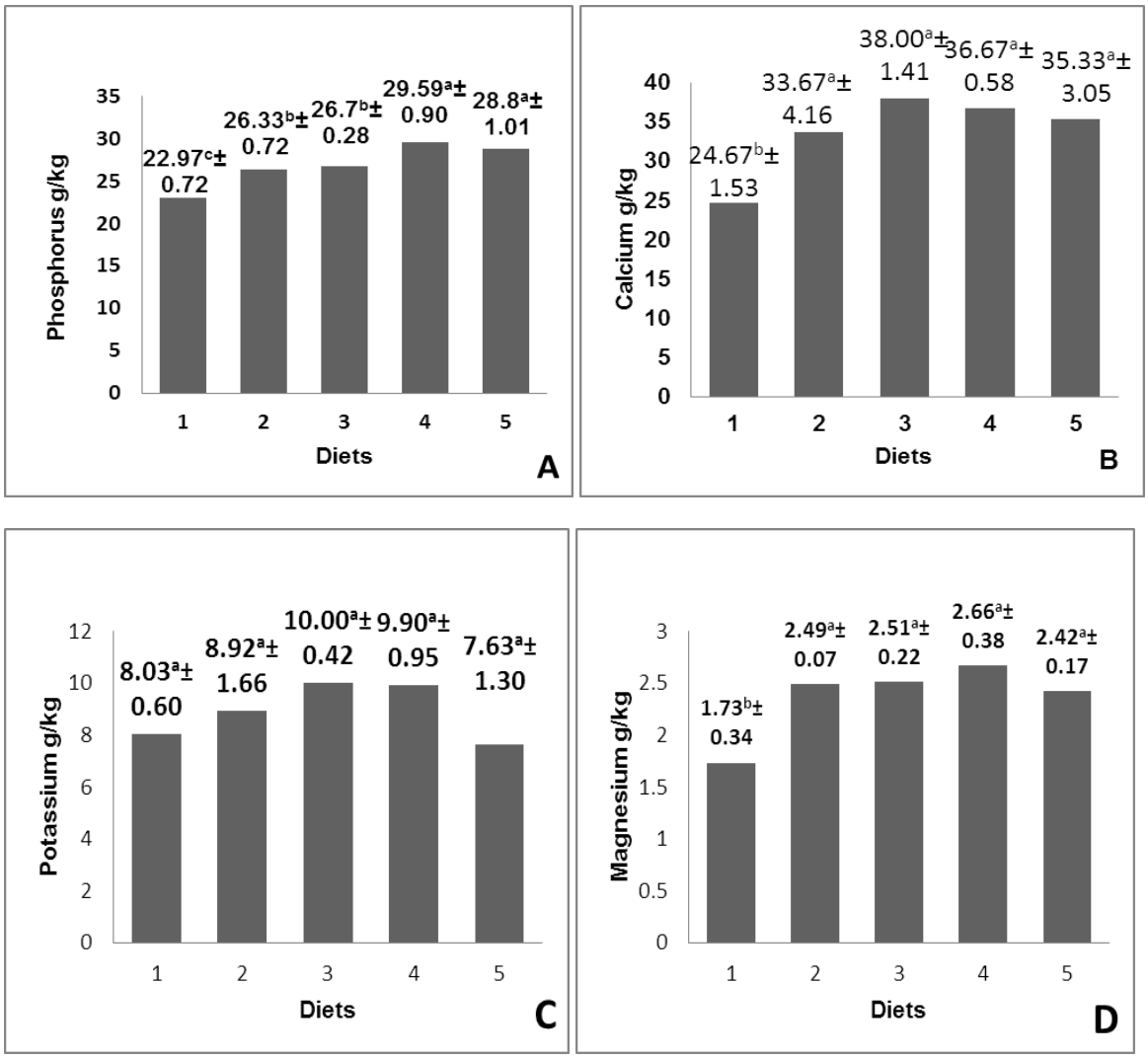

Figure 1. Effect of the experimental diets on macro-minerals concentrations (g kg^{-1}) in the body of gilthead sea bream; A) phosphorus, B) calcium, C) potassium and D) magnesium. Values in the same column with different superscripts are significantly different ($P < 0.05$). The letters (a, b, c, and d) represent the differences among different treatments. All values are mean of three independent biological replicates (n = 3). Means ± standard deviation.

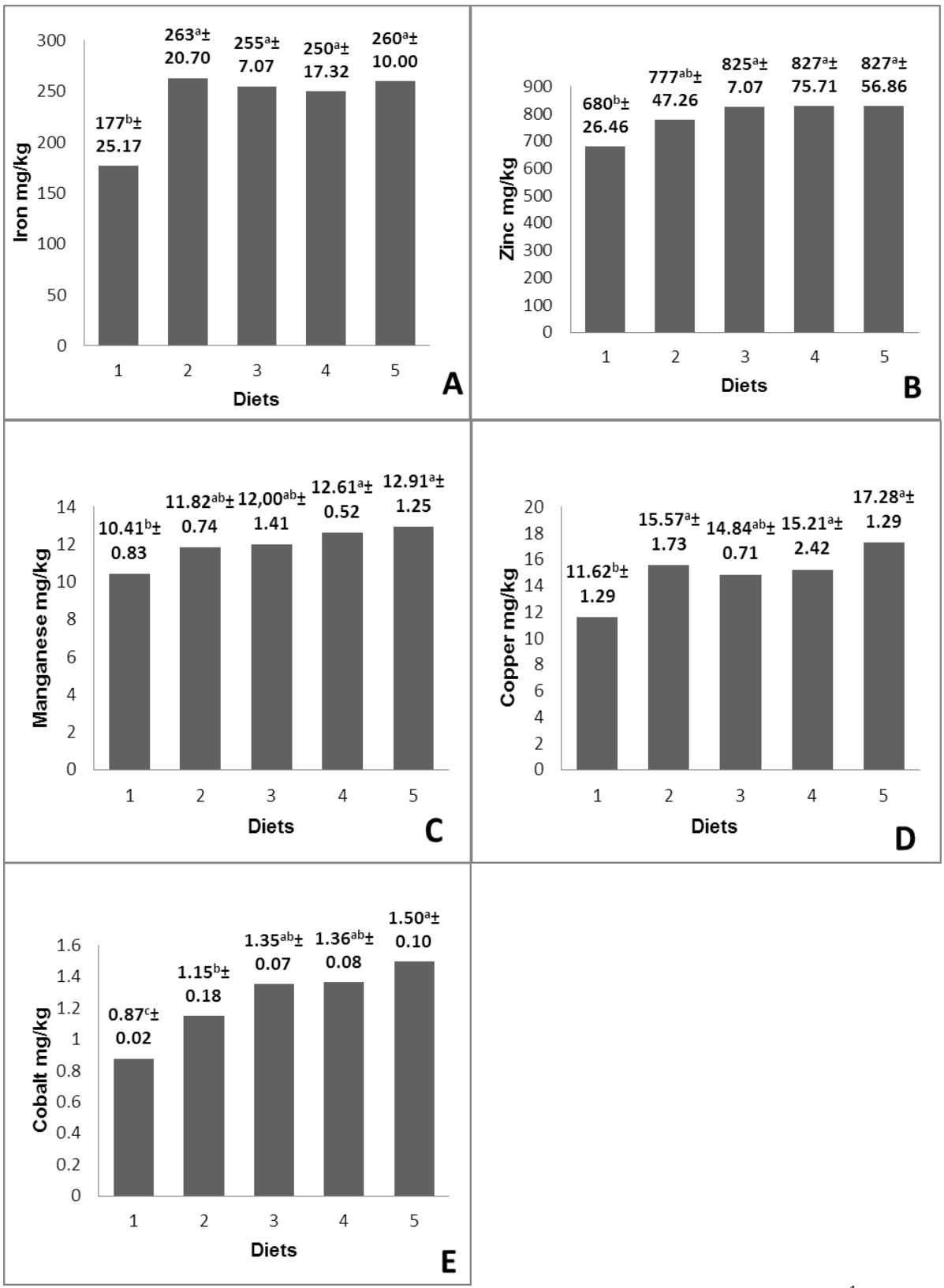

Figure 2. Effect of the experimental diets on micro-minerals concentrations (mg kg^{-1}) in the body of gilthead sea bream; A) iron, B) zinc, C) manganese, D) copper and E) cobalt. Values in the same column with different superscripts are significantly different ($P < 0.05$). The letters (a, b, c, and d) represent the differences among different treatments. All values are mean of three independent biological replicates (n = 3). Means ± standard deviation.

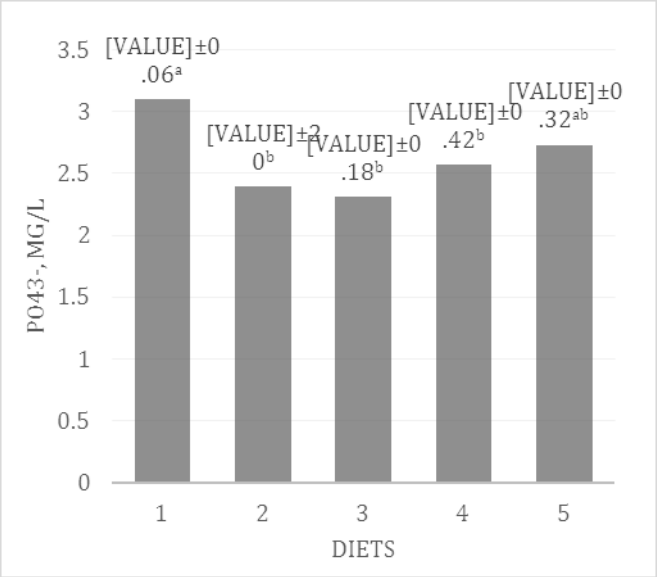

Figure 3. Effect of the experimental diets on phosphorus concentration in the effluent of gilthead sea bream. Values in the same column with different superscripts are significantly different ($P < 0.05$). The letters (a, b, c, and d) represent the differences among different treatments. All values are the mean of three independent biological replicates (n = 3). Means ± standard deviation.

DISCUSSION

The results of this study indicated the positive role of phytase on the growth performance of *S. aurata*. In comparison to the control group, phytase supplemented treatments had significantly higher weight gain and specific growth rate. Furthermore, the presence or absence of IP and MP had no effect on fish growth when the diet was supplemented with phytase, showing that the availability of P in the non-IP or MP diet with phytase enzyme was appropriate for *Sparus aurata*. IP or MP were present in the control diet did not meet the phosphorus requirements of *S. aurata*. In support, Cain and Garling (1995) found that rainbow trout given phytase-treated soybean meal had growth rates and feed conversions that were comparable to or better than those of rainbow trout fed a commercial diet. This could be because soybean meal was pre-treated with phytase enzyme, which boosted the P availability by hydrolysis of phytin P into an inorganic form that could be used by rainbow trout instead of supplemental P in their diet. In addition, in red sea bream, final weight was improved with the phytase supplementation at 1000 and 2000 FTU kg^{-1} diet as compared with fish fed a diet without phytase supplementation (Biswas et al., 2019). Tudkaew et al. (2008) investigated the effect of dietary phytase or inorganic phosphorus supplementation, separately or in combination on sex-reversed red tilapia (*Oreochromis*

niloticus Linn.). They found that phytase or inorganic phosphorus enhanced growth performance to the same extent. In addition, phytase boosted the phosphorus availability, reduced the need for inorganic phosphorus and eliminated the phosphorus waste of tilapia fed diets based on reduced fish meal (Tudkaew et al., 2008).

In parallel, FCR in the present study decreased with the supplementation of phytase, regardless of the presence of IP or MP. A similar result by Cain and Garling (1995) has been revealed where FCR in rainbow trout fed phytase-treated soybean meal was lower than fish fed on a commercial diet without any treatment. The authors attributed this enhanced growth and reduced feed conversion ratio to the lower protein content of the commercial diet compared to the experimental diets.

On the contrary, in other studies, phytase could not enhance the growth performance (Forster et al., 1999; Sajjadi and Carter, 2004; Qiu and Davis, 2017). It is noteworthy that these studies were conducted on different species. Thus, this discrepancy is acceptable because response to addition of phytase enzyme is species-specific (Romano & Kumar, 2018). Additionally, the efficiency of phytase is also affected by other parameters such as pH, phosphorus, Ca, phytase type, and concentration and interactions with other components or non-phytase enzymes (Romano & Kumar, 2018).

Spinelli et al. (1993) proved the negative role of diets containing 0.5% phytic acid on the growth of rainbow trout, which was attributed to the reduced protein availability. Phytates work as chelators, forming protein phytic acid complexes that can impair protein and mineral bioavailability (NRC 1993). Following the same pattern, in the current study, a remarkable effect of phytase on the body crude protein (CP) was observed. All phytase supplemented treatments have higher CP than the control treatment. This could be due to the ability of phytase to improve protein availability by breaking down phytin-protein complexes in the gut and neutralizing the negative effects of phytate on protein (Liebert and Portz, 2005). This improvement in CP in the current study is supported by an improvement in PER as well. Similarly, a considerable increase in protein digestibility was obtained in phytase supplemented diets, which resulted in an improved protein retention efficiency at phytase concentration of 2000 FTU/kg diet compared with the non-supplemented treatment (Biswas et al., 2019). Similar results of the effect of phytase on protein utility was also revealed in rainbow trout (Vielma et al., 2002) and yellow catfish (*Pelteobagrus fulvidraco*) (Zhu et al., 2014). Ash content showed higher results in all phytase-supplemented groups compared to the control group. In support, the inclusion of phytase improved the bone ash and phosphorus concentrations in rainbow trout (*Oncorhynchus mykiss*) (Vielma et al., 1998). Similarly, ash content in scales and vertebrae was significantly increased in Nile tilapia (*Oreochromis niloticus*) feeding on low-phosphorus, plant-based diets supplemented with microbial phytase as a result of improved phosphorus availability in the diet (Liebert and Portz, 2005).

Minerals are required by all aquatic organisms for important physiological and biochemical functions, as well as for the maintenance of normal life processes. In fish, the importance of macro-minerals (phosphorus, calcium, magnesium, potassium) and micro-minerals (cobalt, copper, iron, manganese, zinc) has been established (NRC, 1993 & 2011). In the current study, the addition of phytase to the gilthead seabream diet has improved the macro- and micro-mineral concentrations of the fish body. This indicated the successful conversion and degradation of the indigestible phytate by the enzyme treatment (Cain & Garling, 1995). In support, treating soybean meal with phytase elevated the inorganic phosphorus level (Cain & Garling, 1995). In another study, both dietary inorganic phosphorus and phytase supplementation significantly increased the concentration of minerals (P, Ca, Mg, and Zn) in the vertebrae of red sea bream (*Pagrus major*) juveniles fed soybean-based diets (Laining et al., 2011). Pacu (*Piaractus mesopotamicus*) fed a phytase-supplemented diet showed higher mineral bioavailability for iron, zinc, and phosphorus retention compared to the control group due to reduced gastrointestinal phytic acid and improved the mineral bioavailability (Cian et al., 2018). Sugiura et al. (2001) studied the effects of microbial phytase at different levels on the utilization of phosphorus, trace minerals, and protein by rainbow trout (*Oncorhynchus mykiss*) fed with soybean meal-based diets. They found that phytase supplementation enhanced the apparent absorption of protein, phosphorus, magnesium, iron, copper, strontium, and zinc in soybean meal-based diets. Similarly, phytase addition in rainbow trout diets increased Mg, Mn, and Zn digestibility, which linked to the higher P digestibility (Cheng & Hardy, 2003). Other studies reported the increased phosphorus availability in freshwater fishes fed phytase such as carp (Sch_efer *et al*., 1995), tilapia (Furuya *et al*., 2001, Phromkunthong *et al*., 2004; Phrom-kunthong and Gabaudan, 2006) and hybrid catfish (Phrom-kunthong *et al*., 2005).

The effluent P levels increased significantly as the fish were fed increasing levels of P. P levels in rainbow trout solid waste can be lowered by feeding diets low in phytin P (Ketola and Harland, 1993). Furthermore, supplementing phytase in a diet high in plant protein has the potential to boost phytin P bioavailability (Cain & Garling, 1995). As a direct consequence, increased availability of phytin P through the dietary enzyme phytase reduces P excretion into the environment (Cain & Garling, 1995). This helps in reducing negative impacts on the aquatic environment such as eutrophication, which can lead to the death of aquatic animals when accompanied by low levels of dissolved oxygen (Correll, 1999; Biswas et al., 2007). This is in agreement with the results of the present study, which showed that the inclusion of phytase in the diets had a positive effect on the phosphorus levels in *S. aurata* effluent, with lower concentrations in the phytase-supplemented groups compared to the control group. Yu et al. (2005) indicated that phytase in soybean-based diets fed by Korean rockfish (*Sebastes schligili*) juveniles helped in reducing the phosphorus contamination in the local environment by converting phytate-P into available phosphorus, improving its bioavailability and reducing the

inorganic phosphorus supplement. This is likely due to the phytase activity, which dephosphorylates phytic acid and phytate-P to increase their availability (Storebakken et al., 1998). Lower dietary levels of phosphorus resulted in a 65-88% reduction in phosphorus in the effluent of juvenile rainbow trout (*Oncorhynchus mykiss*) (Cain & Garling, 1995). When juvenile fish (1.9 g $fish^{-1}$) were fed a phytase-treated diet with no phosphorus supplementation, they released 1.61 g of P kg^{-1} weight gain compared to 4.54 g of P kg^{-1} weight gain when fed a commercial diet, whereas larger fish (16.97g $fish^{-1}$) fed a phytase-treated diet with no supplemental P released only 0.29 g of P kg^{-1} weight gain in the effluent (Cain & Garling, 1995).

Although the phosphorous concentration in the effluent was lower in the 5 fish group compared to the control group, the difference was not statistically significant. In the group 5, MP has been excluded, while it contained phytase and IP. In fact, many factors should be taken into account to understand P absorption, among them the differences due to the addition of specific macro or trace mineral premixes affected the P solubility (Tacon and De Silva, 1983). Therefore, more studies to investigate the interaction between phytase addition and diet minerals content should be performed.

CONCLUSION

In conclusion, phosphorus can be obtained from an IP supply or through phytate hydrolysis in a plant-based diet supplemented with phytase. Because dietary phytate lowers intestinal phosphorus absorption, while also interfering with the availability of other minerals, it appears likely that phytate would be degraded by phytase rather than compensated for with additional IP in the diet. Thus, the current study revealed that phytase surpassed IP in terms of enhancing the growth, improving body nutritional composition, and lowering environmental consequences of gilthead sea bream.

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