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Enzyme Supplementation to Soybean Based Diet in Gilthead Sea Bream *(Sparus Aurata)*: Effects on Growth Parameters and Nitrogen and Phosphorus Excretion

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Summary

The aim of the present study was to examine the effects of the diets based on soybean meal supplemented with exogenous enzymes on growth performance, feed utilization, apparent digestibility and waste output of nitrogen and phosphorus in gilthead sea bream *(Sparus aurata)* culture. Diets consisted of 40% fish meal (FM) and 25% dehulled hexane extracted soybean meal (SBM) in commercial feed (TM) and 25% fish meal (FM) and 40% dehulled hexane extracted soybean meal (SBM) in control group (CO), and diet supplemented with enzyme 1 (PRT= Protease; 2 g/kg $^{-1}$), diet supplemented with enzyme 2 (MIX; cellulose, xylanase, endo-β-1,3:1,4-glucanase; 2 g/kg $^{-1}$) and diet supplemented with enzyme 3 (PHY: Pyhtase; 2 g/kg $^{-1}$). A stocking design at 55 fish each tank, gilthead sea bream (initial weights 89.50±1.5 were randomly total of 825 fish allocated into 15 tanks and fed five experimental diets. While weight gain (125.00–133.50) and specific growth rate (0.42–0.50) were statistically similar (P>0.05), feed efficiency ratio (2.52-2.90) and condition factor (1.85-2.08) significantly different among groups (P<0.05) at the end of the experiment. Addition of PRT and PHY into control significantly improved specific growth rate, feed efficiency ratio and condition factor. In this study, showed the highest nitrogen apparent digestibility coefficient (83.20±1.98) in group of PRT whilst the poorest value obtained (69.72±0.01) group of CO. The best (58.57±0.49) and the lowest (42.85±1.98) ADC for phosphorus were obtained from PHT and CO, respectively (p<0.05). In conclusion, this study has shown that dietary different enzyme supplementation can have a positive effect on growth, feed utilization and digestibility in gilthead sea bream. This positive effect has been not only alleviate the negative effect of replacing dietary FM with SBM but also provide about better performance compared to fish fed a higher FM containing diet.

Keywords: *Gilthead sea bream (Sparus aurata), Enzymes, Growth performance, Nitrogen, Phosphorus*

Soya Küspesi Temeline Dayalı Çipura *(Sparus aurata)* Yemlerine Enzim İlavesi: Azot-Fosfor Atılımı ve Büyüme Özellikleri Üzerine Etkileri

Özet

Bu çalışmada, soya küspesi (%40) kullanılarak yetiştiriciliği yapılan çipura *(Sparus auratus)* balık yemlerine bazı ticari enzimlerin ilave edilmesinin büyüme performansı, yemden yararlanma, sindirilebilirlik ve azot-fosfor atılımının etkilerini araştırma amaçlanmıştır. Denemede %40 balık unu + %25 soya küspesi ticari çipura yemi (CF) ve %25 balık unu + %40 soya küspesi (enzim ilavesiz) kontrol grubu (CO) ile enzim 1 ilaveli (PRT: Proteaz; 2 g/kg $^{-1}$), enzim 2 (MIX; cellulose, xylanase, endo-β-1,3:1,4-glucanase; 2 g/kg $^{-1}$), enzim 3 (PHY: Fitaz; 2 g/kg $^{-1}$) içeren toplam 5 grup yem kullanılmıştır. Denemede 2 x 0.5 x 0.23 m. ebadında, tanklar kullanılmış ve her tanka 55 adet (başlangıç ağırlığı ort. 89.50±1.5 g) çipura balıkları 5 grup halinde üç tekerrürlü rastgele olarak yerleştirilmiş ve deneme 12 hafta sürmüştür. Deneme sonunda gruplar arasında ağırlık artışı (125.00–133.50 g) ve spesifik büyüme oranı (0.42–0.50) istatistiki olarak farklılık göstermezken (p>0.05), özellikle proteaz (PRT) ve fitaz grubu (PHY) kontrol grubuna (CO) göre yem değerlendirme (2.52–2.90) ve kondüsyon faktörü (1.85–2.08) açısından farklılık elde edilmiştir (P<0.05). Bu çalışmada en yüksek azot sindirilebilirliği proteaz (PRT) grubunda (83.20±1.98), en düşük kontrol (CO) grubunda (69.72±0.01) elde edilmiştir. Fosfor sindirilebilirliği ise en iyi fitaz (PHY) grubunda (58.57±0.49), en düşük ise kontrol (CO) grubunda (42.85±1.98) gözlenmiştir (P<0.05). Sonuç olarak bu çalışmada farklı enzim uygulamalarının büyüme, yemden yararlanma ve sindirilebilirlik üzerine pozitif etkisi gözlenmiştir. Bu pozitif etki sadece balık unu yerine soya küspesi kullanımı açısından değil, aynı zamanda performans bakımından da olumlu sonuçlar ortaya çıkarmıştır.

Anahtar sözcükler: *Çipura (Sparus aurata), Eksojen enzimler, Büyüme performansı, Azot, Fosfor*

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INTRODUCTION

In aquaculture, improved feed efficiency is possible with the use of high quality formulated feeds. The use of exogenous enzymes, called natural feed additives as well, has become widespread in higher animals such as pigs and chickens via collaborative studies in biotechnology and animal nutrition [1-3]. Advances in biotechnology have brought new insights into the more efficient utilization, improvement of nutrient digestibility and reduction of antinutritional factors of alternative plant protein sources to fish meal. Soybean meal (SBM) is considered the most convenient alternative protein source in terms of its price, availability and nutritional value. Despite the advantages, the inclusion of it in aqua feeds is limited by the presence of antinutritional factors [4-9]. The inclusion of the enzymes aims to reduce the feed cost by improving the feed efficiency. In addition, it was stated that the use of cheaper feedstuffs could be possible with a level of similar to or sometimes better success than the expensive ones through the supplemental enzymes [3,7]. Protease is the most widely used and constitutes half of the total. This is followed by carbohydrates. Phytase is an enzyme specific to phytate hydrolysis. This enzyme is not present in the digestive tract of many animals including fish [10]. Approximately two-thirds of total phosphorus in plant derived ingredients widely used is present as phytate, which is not efficiently utilized or the bioavailability of which is very limited by fish [11]. As these animals, do not have enough phytase to hydrolyze phytate, diets containing plant ingredients should be added with phytase in order for fish to digest phytate more efficiently [12-14].

Supplemental phytase increased the availability of phosphorus of diets based on plant ingredients and reduced phosphorus level in feces in monogastric animals, including fish [15]. By applying phytase in plant protein-based fish diets, the digestibility of phytate-phosphorus in fish diets will be increased. Total-phosphorus discharged into water will also be reduced when total-phosphorus levels are lowered by changing in diet formulation [16]. Since non-starch polysaccharides present in soybean meal reduces its nutritional value, the addition of the exogenous enzymes to diets including soybean improves availability of nutrients [17].

Nitrogen (N) and phosphorus (P) metabolic wastes produced by fish are at the origin of most dissolved N and P waste produced by intensive aquaculture operations. Dissolved N and P waste impose constraints on productivity of the operations and may lead to environmental degradation [18]. Approximately 80–90% of N metabolic wastes excreted by fish are as ammonia. Urea generally only represents 10 to 15% dissolved N waste outputs [19]. The main factor affecting N metabolic waste outputs are those that influence the catabolism and deposition (retention) of amino acids (protein) by the fish [18].

Phosphorus is generally the first limiting nutrient for plants and algae in the freshwater environment, while it is usually nitrogen in seawater and brackish environments [8]. Metabolic P wastes are excreted mostly as phosphate via the urine. This type of relationship between plasma phosphate and urinary phosphate excretion was recently confirmed with rainbow trout [20].

Dietary phosphorus requirement of rainbow trout is 0.5-0.8% of the diet [11]. However, in commercial feeds phosphorus should be included 1.5 times as much as this amount, because about 70% of total phosphorus in plant derived ingredients is present as phytate and this is not efficiently utilized fish [21] due to the fact that they do not have enough phytase to hydrolyze phytate [11]. Commercial phytase preparations can increase availability phytate phosphorus [22]. Lanari et al.[23] and Sugiura et al.[10,24] found an increase in digestibility of phosphorus and a reduction in phosphorus loses in rainbow trout fed a diet supplemented with phytase. Unlike this, a 25% of reduction in phosphorus discharge was reported in juvenile carp fed a diet containing phytase [24]. In addition, Deguara [25] observed higher digestibility coefficients of phosphorus in sea bream fed diets supplemented with low and high pH active protease+α-gactocidase than fish fed the basal diet.

Replacing fish meal with soybean meal or plant protein feedstuffs in fish diet may not only reduce cost of fish diets, but also reduce environmental pollution due to lower discharges of phosphorus.

The study was carried out to examine the effects FM with SBM supplemented with protease, enzyme cocktail and pyhtase enzymes on growth performance (weight gain, specific growth rate), feed conversion ratio, apparent digestibility coefficient and waste output of nitrogen and phosphorus in gilthead seabream culture.

MATERIAL and METHODS

Fish, rearing conditions and experimental design

Gilthead sea bream (initial weight 89.0±2.5 g, mean±SD), used in the experiment were obtained from Institute of Mediterranean Fisheries Research, Production&Training, Beymelek Unit, Antalya. Before the commencement of the study, fish were acclimated to rearing conditions for 2 weeks. The study lasted twelve weeks and during that biometric measurements were taken at the initiation and end of the experiment. Diets were offered at 2% of body weight. Daily amount of feeds were given to fish twice, morning (09:00) and afternoon (16:00). During the experiment, minimum and maximum value of water temperature (16-22°C), salinity (30-32 ppt), dissolved oxygen (7.7-8.2 mgL $^{-1}$), ammonia (0.01-0.10 mgL $^{-1}$), nitrite (0.001-0.0001 mgL $^{-1}$) and pH (7.0-7.5), were obtained respectively. Trial involved five groups of fish each composed of fifty-five fish with similar weight with three replicates per treatment. Fifteen polyester tanks were used in the study. Each tank, having 2x0.5x0.23 m of dimensions and with 2300 liters capacity, received a constant flow of water (45 Lmin $^{-1}$). The total weight of the fish was recorded at the beginning and end of the experiment. After 14 days adaptation period, the fish were fed the experimental diets.

Experimental diets and analysis

Fish in the each of experimental diets was fed to triplicate groups. Five experimental groups were fish fed the commercial feed (TM), containing 40% FM and 25% dehulled hexane extracted SBM and the control group (CO) containing 25% FM and 40% dehulled hexane extracted SBM without supplemental enzyme, control diet supplemented with 2 g/kg $^{-1}$ PRT [20], 2 g/kg $^{-1}$ MIX and 2 g/kg $^{-1}$ PRT. Feeding ratio was determined by considering water temperature and total weight of fish in the tank. Ingredients used were obtained from the local market and ground, mixed at predetermined levels and then, pelleted in Institute of Mediterranean Fisheries Research, Production & Training, Kepez Unit, Antalya. Pelleted diets were stored at +4°C until used. Ingredient compositions and proximate analysis of the diets were given in *Table 1.*

Feed and feces were analyzed for dry matter (DM), crude protein, crude ash and total phosphorus using the vanado-molybdate method according to AOAC [26], crude protein (%Nx 6.25) by the Kjedahl method using a Kjeltech aouto-analyser (Model 1030, Tecator, Höganas, Sweden) and total lipid according to the method of Bligh and Dyer [27].

Digestibility trial

Feces were collected daily (twice a day 10:00- and 17:00 hours) for a week from a sedimentation column fitted to each tank and treated according to the procedure proposed by Cho et al.[28]. The apparent digestibility coefficient (ADC) was determined by an indirect method using chromic oxide as the inert marker. The concentration of chromium in the diet and feces were analyzed for proximate composition and phosphorus by the same methods used for the feed samples [23].

Apparent digestibility coefficients (ADC) were calculated using the following formula for nitrogen (N) and phosphorus (P);

$$ADC = 100 - \{100 \times (\frac{Cr_2O_3 \text{ in diet (\%)}}{Cr_2O_3 \text{ in feces (\%)}} \times \frac{\text{Nutrient in feces (\%)}}{\text{Nutrient in diets (\%)}})\}$$

Statistical analysis

Results were analyzed by one-way analyses of variance (ANOVA) and the treatment means compared by Duncan's [29] multiple range tests. Significance was tested at the $P<0.05$ level. Homogeneity of variance was performed by Levene test and variance was found to be homogenous.

Table 1. *Ingredients (g/kg $^{-1}$) and chemical analyses of experimental diets*

Tablo 1. *Deneme rasyonlarının içerikleri (g/kg^{-1}) ve kimyasal analizleri*

INGREDIENTS	DIETS				
	CF [1]	CO [1]	PRT [1]	MIX [1]	PHY [1]
Fish meal	40	25	25	25	25
Soybean meal	20	40	40	40	40
Full fat soybean	5	9.22	9.12	9.12	9.12
Blood meal	5	5	5	5	5
Wheat middlings	18.45	9.23	9.13	9.13	9.13
Menhaden oil	7	7	7	7	7
Vitamin premix [2]	2	2	2	2	2
Mineral premix [3]	1	1	1	1	1
Vitamin C [4]	0.3	0.3	0.3	0.3	0.3
Coline [5]	0.15	0.15	0.15	0.15	0.15
Pellet binder [6]	0.4	0.4	0.4	0.4	0.4
Chromic oxide [7]	0.4	0.4	0.4	0.4	0.4
Antioxsidant [8]	0.3	0.3	0.3	0.3	0.3
Enzyme 1 [9]	-	-	0.2	-	-
Enzyme 2 [10]	-	-	-	0.2	-
Enzyme 3 [11]	-	-	-	-	0.2
TOTAL	100.00	100.00	100.00	100.00	100.00
Nutrient content					
Dry matter, %	91.51	92.33	92.92	91.94	93.54
Crude protein, %	43.81	44.85	44.72	42.81	43.14
Ether extracts, %	13.48	13.36	12.45	13.52	11.89
Crude fiber, %	3.97	3.25	3.25	3.25	3.25
Crude ash, %	7.22	13.74	15.15	11.38	12.29
Metabolizable en. (MJ kg^{-1})	14.48	14.39	14.39	14.39	14.39
Calcium, %	1.6	1.09	1.09	1.09	1.09
Phosphorus, %	1.33	1.45	1.45	1.45	1.45
Lysine, %	3.35	3.17	3.17	3.17	3.17
Methionine+cystine, %	1.55	1.50	1.50	1.50	1.50

[1] **CF: Commercial feed group, no added enzyme;** CO: Control group, no added enzyme, PRT: Protease group: 2%o protease; MIX: Mix group: 2%o enzyme cookteyl; PHT: Phytase group: 2%o phytase, [2] **Vitamin mixture:** Included of per kg; 18.000 IU A, 2.000 IU D_3, 200IU E, 12 mg K, 150 mg C, 30 mg B_2, 20 mg B_1, 0.05 mg B_{12}, 20 mg pyridoxine, 10 mg panthotenic acid, 220 mg niacin, 210 mg inositol, 5 mg folic acid, 0.5 mg biotine & 2.000 mg coline, [3] **Mineral mixture:** Included of per kg: 70 mg zinc, 60 mg mangenese, 60 mg magnesium, 4 mg ferro, 2 mg copper, 1.5 mg iode, 0.5 mg cobalt, 0.05 mg selenium. [4] Vitamin C, Hoffman La-Roche Inc. [5] Coline, Ufuk Kimya İlaç San. ve Tic. Ltd. Sti İstanbul. [6] Lignosülfanat, [7] Cr_2O_4, Merck, [8] Buthylhidroxitoluoen, (powder form), [9] Protease. [10] Cellulose, xylanase, endo-ß-1,3:1,4-glucanase, [11] Pyhtase.

RESULTS

Growth performance

Growth performance data of the treatments were given in *Table 2*. The highest and lowest growth rates were obtained from the group PHY (48.31 and the control group (41.57±2.09) respectively, but there were no significant differences among the treatments (P>0.05). The specific growth rate values ranged from 0.502±0.045 (PHY group) to 0.419±0.015 control group (P>0.05). However, feed conversion ratio values were significantly different among the groups at the end of the study. As seen in *Table 2*, PHY group (2.52±0.07) showed significantly better feed conversation ratio than CO (2.90±0.06) and TM (2.85±0.05) groups (P<0.05) but similar to those PRT (2.70±0.06) and MIX (2.73±0.06) groups. Condition factor (CF) was significantly higher in PRT, PHY and MIX groups than CO and TM groups (P<0.05). There is no mortality during the research periods in experimental groups.

Nitrogen and phosphorus ADC

Dietary and fecal content of nitrogen and phosphorus and digestibility values were given in *Table 3* and *Figure1*. In PRT group, fecal nitrogen was significantly less (P<0.05) compared to MIX, CO and TM groups, but it was similar in PHY group. Faecal phosphorus levels by fish fed PRT and PHY diets were significantly lower than those fed CO and TM diets. However, MIX group showed similar fecal phosphorus to the others, except the PHY group.

In this study, obtained the highest nitrogen apparent digestibility coefficient 83.20% whilst CO presented the poorest (69.72%). The best and lowest ADC for phosphorus was obtained from PHT and CO, respectively (P<0.05).

Table 2. *Growth performance in experimental groups*
Tablo 2. *Deneme gruplarının gelişim performansı*

Item	CF [1] Mean±SE	CO [1] Mean±SE	PRT [1] Mean±SE	MIX [1] Mean±SE	PHY [1] Mean±SE
Initial Weight (g) [2]	88.00±1.0	89.00±1.5	91.00±0.5	91.00±2.5	89.00±2.0
Final Weight (g) [3]	125.00±1.45	126.00±1.15	133.50±2.00	131.00±2.00	132.00±2.50
GR (%) [4]	42.05±2.09	41.57 ±2.09	45.11 ±1.41	43.95±4.38	48.31±4.89
SGR [5]	0.427±0.015	0.419 ±0.015	0.495 ±0.015	0.487±0.035	0.502 ±0.045
FCR [6]	2.85±0.05a	2.90±0.06a	2.70 ±0.06ab	2.73±0.06ab	2.52±0.07b
CF [7]	1.92±0.01c	1.85 ±0.01c	2.10 ±0.01b	1.97±0.02a	2.08±0.02ab

Data (mean±SE) with different letters within a row are significantly different (P<0.05).
[1] COF: Commercial feed group, no added enzyme; CO: Control group, no added enzyme, PRT: Protease group: 2%o protease; MIX: Mix group: 2%o enzyme cocktail; PHT: Phytase group: 2%o phytase
[2] Body weight of initial (WI), [3] Body weight of final (WF)
[4] Growth rate, GR (% increase in weight)[30,31] = ((final body weight – initial body weight)/initial body weight) x 100
[5] Specific growth rate, SGR (%/day) [30,31] = ((Ln final body weight – Ln initial body weight)/days) x 100
[6] Feed conversion ratio, FCR [30,31] = dry feed intake (g) / weight gain (g).
[7] Condition factor [30,31] = (W/L3) x 100

Table 3. *Change of nitrogen and phosphorus in feed and feces in experimental groups*
Tablo 3. *Deneme gruplarında yem ve dışkıda azot-fosfor değişimi*

Item	CF [1] Mean±SE	CO [1] Mean±SE	PRT [1] Mean±SE	MIX [1] Mean±SE	PHY [1] Mean±SE
N- feed, %	6.55±0.04b	6.41±0.03b	6.63±0.01a	6.48±0.06ab	6.59±0.02ab
N- feces, %	5.65±0.06a	5.63±0.05a	4.91±0.13b	5.41±0.03a	5.17±0.08ab
P- feed, %	1.53±0.02a	1.49±0.04a	1.53±0.01a	1.46±0.03a	1.44±0.02a
P- feces. %	0.84±0.02a	0.83±0.02a	0.85±0.02bc	0.74±0.02ab	0.60±0.02c
Dry matter ADC [2]**, %**	82.78±1.19a	81.64±l.06ab	78.04±1.82a	74.89±0.47c	81.79±1.13ab
Protein ADC [2]**, %**	84.56±l.07c	85.03±0.21bc	87.42±0.96b	86.96±0.03bc	87.21±0.86bc
N-ADC2 %	70.45±0.15a	69.72±0.01a	83.20±1.98b	79.52±1.19b	81.25±0.68b
P-ADC2 %	43.15±0.58a	42.85±1.98a	48.77±2.01a	45.69±0.48a	58.57±0.49b

Data (mean±SE) with different letters are significantly different (p<0.05).
[1] COF: Commercial feed group, no added enzyme; CO: Control group, no added enzyme, PRT: Protease group: 2%o protease; MIX: Mix group : 2%o enzyme cocktail; PHT: Phytase group : 2%o phytase
[2] ADC; Apparent digestibility coefficient

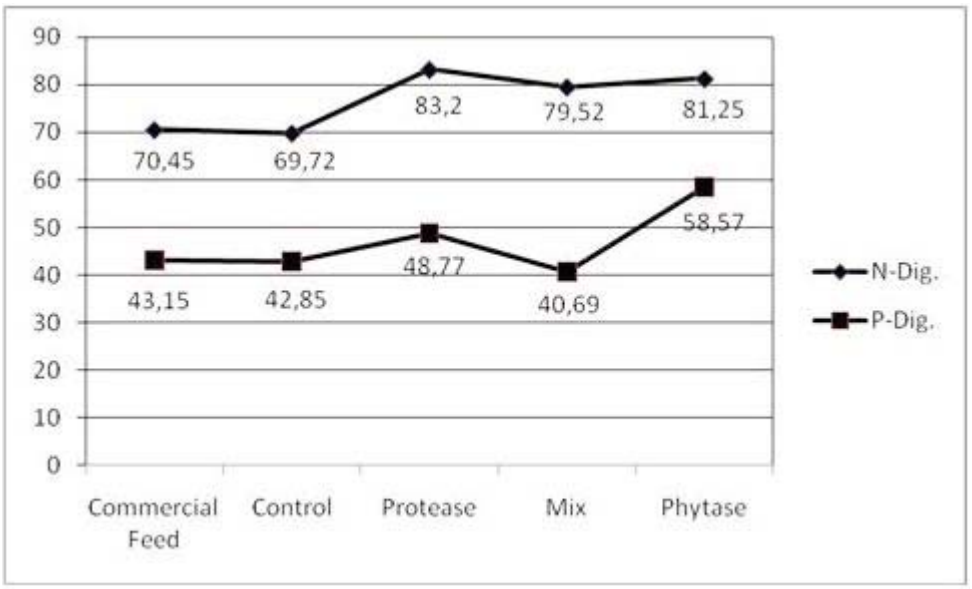

Fig 1. Change of digestibility N and P in experimental diets (%)
Şekil 1. Deneme gruplarında N ve P sindirilebilirliğinin değişimi (%)

DISCUSSION

In this study, a numerically lower weight gain was obtained in fish feed TM diet and CO diet. Supplementing the diets with exogenous PRT and PHY led to 4-6 g of increase in weight gain compared to the CO and TM *(Table 2)*. Deguara [25] reported that inclusion of enzymes (0.1% low and high pH active protease+α-galactocidase) into seabream diet improved weight gain by 18 g and 10 g, respectively, compared to basal diet without exogenous enzyme. The few experiments that have been carried out on the use of supplementary enzymes in juvenile fish diets have generally shown a positive result as compared to fish fed unsupplemented diets. Boguts et al.[32] investigated the effects of enzyme cocktail supplementation; 0.5, 1.0 and 1.5 g levels in carp with 46 g/kg $^{-1}$ initial weigh. The fish fed the 1.5 g/kg Polyzme diet had significantly better feed utilization and growth. Cardenete et al.[33] have been found no difference in growth performance of *Oncorhynchus mykiss* fed control diet (40% protein provided by cottonseed meal) or a commercial diet with enzyme cocktail of 0.4, 1.2 and 3.6 g/kg $^{-1}$. However, the present study did not find any significant effect of feeding 40% SBM diets on weight gain and specific growth rate, which are in agreement with previous studies [34,35].

Specific growth rates (SGR) were 0.42, 0.43, 0.50, 0.49 and 0.50 for CF, CO, PRT, MIX and PHY groups respectively, which were lower than the values reported by Deguara [25] (0.53, 0.77 and 0.71%).

In the current study, the best FCR value (2.52) was obtained from PHY diet, followed by PRT (2.70), MIX (2.73) and CO (2.90). These values showed that the addition of phytase into fish diets resulted in better FCR.

Feeding diets with 40 % SBM in this study resulted in a significantly lower feed efficiency (gain: feed). This means that addition of the enzymes the nutritional improvement; there may have been apparent increase in palatability. Similarly in a different works, feed conversation ratios were 2.82, 2.18 and 2.46 for the control, low and high pH active protease+α-galactocidase added diets, respectively [25].

Deguara [25] added protease with low and high pH active to the diets and found that low pH active protease showed better growth and protein efficiency ratio, and similar feed intake compared to high pH active protease and the control diet without exogenous enzyme addition.

The nitrogen and phosphorus ADC values were concordant with values reported by Lupatsch and Kissil [36] but were higher than the values reported by Cheng and Hardy [34]. These differences among studies may have stemmed from origin and level differences of the feedstuffs and enzymes used in the experimental diets and different feed preparation techniques and environments.

P found in organic compounds, such as phospholipids and nucleic acids, are apparently highly digestible to fish. P found in phytate (inositol hexaphosphate), also an organic compound, however, is not digestible to fish since fish lack the enzyme necessary to release P (phytase) [35]. Numerous studies have shown that dietary incorporation of microbial phytase improved the ADC of P of fish fed diets containing phytic acid [23]. The use of phytase appropriate only for diets with digestible P contents below the requirement of the fish and containing significant levels of plant ingredients, i.e. in which significant proportion of the P is as phytate-P. There are a number of other factors to consider. Experimental evidences suggest that there is a requirement to maximize growth and maximize P deposition and bone mineralization. P requirement of rainbow trout for maximum growth was 0.37% digestible P (0.19 gMJ $^{-1}$ DE) and 0.53% (0.27 gMJ $^{-1}$ DE) for maximum phosphorus deposition [18,37].

Increasing water temperature results in increasing feed intake, growth and N waste outputs per fish per unit of time. In this study, positive effect obtained might be due to high water temperature between (18–20°C). The activity of this enzyme is affected by environmental temperature and its activity may be limited at low water temperatures, i.e. 5–15°C. Moreover, the enzyme is sensitive to heat and may be destroyed during pelleting and extrusion under standard commercial conditions. This problem may be rather easily circumvented through post-pelleting applications and possibly encapsulation

of the enzyme. Pretreatment of plant ingredients with phytase prior to their incorporation in fish feeds has also been suggested and shown to be highly effective. Low phytate varieties of grains and oilseeds are increasingly available and their use could be helpful to minimize indigestible phytate P in feeds and consequently, fecal P output [10,24,38].

It was found that supplemental phytase increased the nutrient digestibility of diets including plant derived ingredients and growth rate of fish [12,16,25]. Sugiura et al.[24] reported apparent availability of phytate phosphorus to fish increased when diets were added with microbial phytase.

It was measured the levels of nitrogen and phosphorus in diets and feces so as to determine the effect of supplemental enzymes on the availability of nitrogen and so on discharge of it. Fecal nitrogen showed a propensity to reduce in diets with supplemental enzymes. It has been reported that of consumed nitrogen, 30% is retained in the body and 70% excreted through feces, gill and urine; as for phosphorus, the values are 32 and 68%, respectively [39].

The result of this study presented a statistically significant ($P<0.05$) effect of enzymes and interaction between SBM and enzyme supplementation on ADC of dry matter, crude protein and phosphorus *(Table3)*.

Phytase supplementation in diets for gilthead sea bream and other fish species have previously been shown to be effective in releasing nutrients. According to this research, the inclusion rate of phytase plays an important role in releasing phytate-phosphorus in plant protein-based fish diets and that the effectiveness of phytase varies with the plant ingredient source [34]. The uses of phytase, the supplemental levels of trace minerals such as manganese and may be reduced. This can be an advantage in fish production, especially fish growth because most of the feed used in a production cycle is consumed at late growth stage.

Ketola and Harland [40] reported phytate phosphorus in most plant meals was not digestible for fish and the availability of dietary phosphorus could be increased by reducing the level of phytate in the diets. He also reported that the minimum level of phosphorus without phytate might have been 0.5-0.6% of diet, and addition of phytase increased growth rate and digestibility reduced the discharge of phosphorus.

In conclusion, this study has shown that dietary different enzyme supplementation can have a positive effect on growth, feed utilization and digestibility in gilthead sea bream. This positive effect has been not only alleviate the negative effect of replacing dietary FM with SBM but also provide better performance compared to fish fed a higher FM containing diet.

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