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EFFECTS OF DIETARY LIPASE SUPPLEMENTATION ON DIGESTIVE ENZYME ACTIVITY AND GROWTH METRICS OF JUVENILE GILTHEAD SEABREAM (*Sparus aurata* L.)

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Abstract

This study investigated the effects of exogenous lipase supplementation on the growth performance, digestive enzyme activity and body chemical composition of *Sparus aurata* L. A total of 840 *Sparus aurata* (7.72 ± 0.05 g) were fed eight diets for 60 days. Diets were coded Group 1: Control 1 (G1–14% Lipid); Group 2: Control 2 (G2–22% Lipid); Group 3: 14% Lipid + 0.5 g lipase (G3–0.5 g/kg); Group 4: 14% lipid + 1.0 g lipase (G4–1 g/kg); Group 5: 14% Lipid + 1.5 g lipase (G5–1.5 g/kg); Group 6: 22% lipid + 0.5 g lipase (G6–0.5 g/kg); Group 7: 22% Lipid + 1.0 g lipase (G7—1 g/kg) and Group 8: 22% Lipid + 1.5 g lipase (G8–1.5 g/kg). There were 8 dietary treatments that included a protein and lipid diet containing (14% and 22%), crude protein (50%) and supplemented with graded levels of exogenous lipase supplementation activity at 0.5, 1, 1.5 and 2 g/kg diet. As a result of the study, G3 group was better in the final weight (64.42 ± 1.06 g), the specific growth rate (3.55 ± 0.01) and the feed conversion rate (1.20 ± 0.18) compared to other groups ($P < 0.05$). Protease, trypsin and alkaline phosphatase activity support growth and their activity increased during the trial. As regards amylase, lipase and aminopeptidase, a decrease in their activity was detected during the research.

Key words: lipase, digestive activity, enzyme, feed additives

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Introduction. Aquaculture is one of sectors that provide nutritional requirement [1]. Fish are valuable source of protein and play an important role in human nutrition. The total rise of world fish production has rised up to 179 Mt (million tonnes), from which 96.4 Mt derives from capture fisheries and the rest 82.1 Mt derives from aquaculture industry during 2018. Intensive aquaculture system relies on the governance of water quality and diets [2]. However, experts predict limited supply of high-quality fish feed [3]. Commercial fish feeds include in high amount fish meal as the major protein source. But today, fish meal is usually avoided due to its scarcity and high cost. Therefore, aquaculture nutrition has been trying to ameliorate the nutritional value of fish feed by enzyme supplementation, to find suitable alternatives to fish meal. Enzyme addition to feeds is important nutritional progress in the aquaculture sector in the last few years. Besides, supplementation with enzymes may help to remove the effects of antinutritional factors and ameliorate the use of dietary energy and amino acids, resulting in better performance of fish. Dietary lipids play an important role in absorption of fat-soluble vitamins in fish diets. Also, lipids are an energy source and the source of essential fatty acids (EFAs), which is necessary for fish ordinary growth but cannot be synthesized by themselves [3]. Lipase which plays a very important role in the way living organisms function, is an enzyme that catalyses the formation or cleavage (hydrolysis) of fats (lipids). Lipase has a significant number of uses physiologically as role in the digestion, transport, and processing of dietary lipids in most organisms [4]. Gilthead seabream (*Sparus aurata* L.) is one of the most significant aquaculture species with maximum production. Thus, the aim of the present study was to evaluate the effects of dietary lipase supplementation on growth and digestive enzyme activity of gilthead seabream (*Sparus aurata*).

Material and method. The experiment was carried out at the Marine Research Station of Fisheries Faculty, University of Çukurova, Yumurtalık, Turkey. Gilthead sea bream (*Sparus aurata*) juveniles were supplied by Akuvatur Hatchery, Adana, Turkey. Triplicate groups of fish were stocked into 24 fiberglass tanks (water volume 500 L) with 35 fish per tank. Also, in every 15 days interval fish weight measurements were taken; 5 fish were taken from each tank for enzyme analysis. In this experiment 840 individuals (7.75 ± 0.05 g Initial Weight, IW) were randomly distributed into eight groups (24 tanks) and coded Group 1: Control 1 (G1 – 14% Lipid); Group 2: Control 2 (G2 – 22% Lipid); Group 3: 14% Lipid + 0.5 g lipase (G3 – 0.5 g/kg); Group 4: 14% lipid + 1.0 g lipase (G4 – 1 g/kg); Group 5: 14% Lipid +1.5 g lipase (G5 – 1.5 g/kg); Group 6: 22% lipid + 0.5 g lipase (G6 – 0.5 g/kg); Group 7: 22% Lipid + 1.0 g lipase (G7 – 1 g/kg) and Group 8: 22% Lipid + 1.5 g lipase (G8 – 1.5 g/kg). The fish were fed (Table 1) three times each day at 8:00, 12:00 and 16:00 for 60 days. Experiment period fish were kept at the natural photoperiod, temperature was $22.01 \pm 2.35\,^{\circ}$C, dissolved oxygen 7.52 ± 0.08 mg/L. Ten fish taken at the starting of the experiment and

five fish from each tank at the end were stored at −20 °C until chemical analysis. Growth performance and feed utilization were calculated according to the following formulae:

Specific Growth Rate (SGR, %day^{-1}) = ((ln Final Weight – ln Initial Weight)/ days) ×100 [5].

Weight gain (WA, %) = ((Final – Initial Weight)/(Initial Weight)) ×100 [6].

Feed Conversion Rate (FCR) = Amount of feed consumed (g)/Weight again (g) [7].

T a b l e 1

Content of the feeds used

	GROUPS							
Raw materials (g/kg)	1	2	3	4	5	6	7	8
Fish meal[1]	470	470	470	470	470	470	470	470
Soybean meal	220	220	220	220	220	220	220	220
Blood meal	40	40	40	40	40	40	40	40
Fish oil[1]	75	145	75	75	75	145	145	145
Dextrin[2]	80	10	80	80	80	10	10	10
Vitamin mix[3]	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Mineral mix[4]	3	3	3	3	3	3	3	3
Bonkalit	110	110	110	110	110	110	110	110
Lipase enzyme (g/kg)[5]	0	0	0.5	1	1.5	0.5	1	1.5
TOTAL	1000	1000	1000	1000	1000	1000	1000	1000
	Calculated Chemical Composition (% dry matter)							
Moisture	8.83	8.71	8.83	8.83	8.83	8.71	8.71	8.71
Crude Protein	50.32	50.13	50.32	50.32	50.32	50.13	50.13	50.13
Crude Lipid	14.42	22.02	14.42	14.42	14.42	22.02	22.02	22.02
Crude Ash	9.85	9.84	9.85	9.85	9.85	9.84	9.84	9.84
NFE[6]	23.96	16.56	23.96	23.96	23.96	16.56	16.56	16.56
Gross Energy (GE)[7] MJ/kg)	21.74	23.43	21.74	21.74	21.74	23.43	23.43	23.43
P:E[8]	23.15	21.39	23.15	23.15	23.15	21.39	21.39	21.39

[1]Fish meal ve Fish oil: Sibal A. Ş.
[2]Dextrin: Sunar Mısır Ent. Tes. San. ve Tic. A. Ş.
[3]Vitamin mix: kg/feed 4 000 000 IU vit. A, 480 000 IU vit. D3, 40 000 mg vit. E, 2400 mg vit. K3, 4 000 mg vit. B1, 6 000 mg vit. B2, 40 000 mg niasin, 10 000 mg Calcium D – pantothenate, 4 000 mg vit. B6, 10 mg vit. B12, 100 mg D-biotin, 1 200 mg folic acid, 40 000 mg vit. C, 60 000 mg inositol.
[4]Mineral mix.: kg/feed 23 750 mg Mn, 75 000 mg Zn, 2 000 mg Co, 2750 mg I, 100 mg Se, 200 000 mg Mg i.
[5]Lipase enzyme: ABP Mühlenchemie Gıda San ve Tic. A. Ş.
[6]NFE (Nitrogen-Free Extractive): 100 – (Crude Protein + Crude lipid + ash + cellulose).
[7]Gross Energy (GE) (MJ/kg): 23.4 MJ/kg protein, 39.2 MJ/kg lipid ve 17.2 MJ/kg.

Protein Efficiency Ratio (PER) = Weight gain (g)/protein intake (g) [8].

Lipid Efficiency Ratio (LER) = Weight gain (g)/lipid intake(g) [6].

Liver Somatic Index (LSI, %) = (liver weight (g)/fish weight (g)) [5].

Visceral Somatic Index (VSI, %) = (Total internal organ weight (g)/fish weight × 100(g)) [5].

Total Fat (%) (TF, %) = (Perivisceral Fat (%) + Peritoneal Fat (%)) [9].

Thermal Growth Rate (TGR) = ((Final Weight 1/3 – Initial Weight 1/3)/average temperature) ∗ 1000).

Enzyme analysis. Trypsin activity was tested at 25 °C using BAPNA (N-α-benzoyl-dl-arginine p-nitroanilide) as substrate in 50 mM Tris–HCl, 20 mM $CaCl_2$ buffer, pH 8.2 [10]. Amylase activity was measured according to [11]. Lipase activity (U/ml) was defined as the µmol of substrate hydrolyzed per min per ml of enzyme extract [12].

Results. In this study, Gilthead sea bream was fed diets containing different amounts of lipase enzyme for 60 days and at the end of the experiment, the growth parameters of the fish were calculated and presented in Table 2. After 60 days of experiment, the growth performance of fish in the experimental group was improved in different levels. Results show that the final body weight (FW), Specific growth rate (SGR) and Daily growth rate (DGR) in G3 were significantly higher than the control group ($P < 0.05$), while the feed conversion ratio (FCR) reduced significantly in G3 group ($P < 0.05$). The Daily feed intake (DFI) was increased in G3 group. A significant improvement was observed in G3 group compared to the control group ($P < 0.05$), but reduced to the control group level in G6 and G8 groups. Compared to the control, LSI of G3, G4 and G5 groups was significantly higher ($P < 0.05$). VSI of G3, G4 and G7 groups was remarkably decreased compared to the control group ($P < 0.05$). TF of G8 was significantly lower ($P < 0.05$) than the other groups. The best protein efficiency ratio (PER) was found in G3 between all groups. Initial and final carcass composition of the fish fed the experimental diets is presented in Table 3. The final carcass composition showed significant variation of their proximate composition as a result of the diet formulations. Protein, lipid content of G3 groups was significantly higher than other groups ($P < 0.05$). Effects of dietary lipase supplementation and lipid levels on juvenile gilthead seabream and the activity of digestive enzymes are presented in Table 4. Amylase activity was significantly affected by dietary lipase enzyme. When comparing different diets, amylase activity was higher (diet effect $P < 0.05$) in G2 groups than other groups at the end of the 60 days. Fish from G3 group showed significantly better trypsin enzyme activity than fish fed other experimental diets ($P < 0.05$). Minimum trypsin activity was observed in G8 group. The lowest lipase activity was obtained in G1 group compared to other experimental groups ($P < 0.05$). Aminopeptidase activity was also lower in G3 group than in the other groups (Table 4). Maximum Alkaline Phosphatase activity was observed in G3 group ($P < 0.05$).

Table 2

Growth and feed evaluation parameters of the groups at the end of the experiment (60 days) ($n = 3$, mean ± standard deviation)

Growth Parameters	14% Lipid				22% Lipid			
	Control (G1)	%14—0.5 (G3)	%14–1 (G4)	%14–1.5 (G5)	Control (G2)	%22—0.5 (G6)	%22–1 (G7)	%22–1.5 (G8)
IW (gr)	7.72±0.11^{a}	7.67±0.06^{a}	7.70±0.11^{a}	7.76±0.08^{a}	7.74±0.05^{a}	7.75±0.01^{a}	7.65±0.05^{a}	7.71±0.04^{a}
FW (gr)	61.83±1.15^{b}	64.42±1.06^{a}	56.61±1.06^{c}	55.36±1.33^{c}	57.18±0.14^{c}	57.38±0.79^{c}	53.08±1.59^{d}	50.75±1.20^{e}
SGR (% Day^{-1})	3.47±0.05^{b}	3.55±0.01^{a}	3.32±0.04^{c}	3.27±0.06^{c}	3.33±0.01^{c}	3.34±0.02^{c}	3.23±0.04^{cd}	3.14±0.04^{d}
DGR (%Day^{-1})	0.90±0.02^{b}	0.95±0.02^{a}	0.82±0.02^{c}	0.79±0.02^{c}	0.82±0.00^{c}	0.83±0.01^{c}	0.76±0.02^{c}	0.72±0.00^{d}
TGR	1.50±0.02^{a}	1.54±0.01^{a}	1.41±0.02^{b}	1.39±0.03^{b}	1.42±0.00^{b}	1.42±0.01^{b}	1.35±0.03^{bc}	1.31±0.02^{bc}
DFI (%Day^{-1})	1.72±0.03^{a}	1.86±0.02^{a}	1.80±0.07^{a}	1.78±0.13^{a}	1.82±0.04^{a}	1.65±0.09^{ab}	1.73±0.08^{a}	1.55±0.08^{ab}
FCR(%Day^{-1})	1.58±0.07^{ab}	1.20±0.18^{b}	1.47±0.09^{ab}	1.41±0.08^{ab}	1.43±0.05^{ab}	1.54±0.13^{ab}	1.42±0.07^{ab}	1.68±0.04^{a}
PER (%)	1.20±0.02^{a}	1.26±0.02^{a}	1.08±0.02^{b}	1.05±0.03^{b}	1.09±0.00^{b}	1.10±0.01^{b}	1.00±0.34^{bc}	0.95±0.02^{bc}
LER (%)	3.86±0.08^{ab}	4.05±0.07^{a}	3.49±0.07^{a}	3.40±0.10^{a}	2.24±0.00^{b}	2.25±0.03^{b}	2.06±0.07^{b}	1.95±0.05^{b}
LSI	2.12±0.50^{a}	2.13±0.42^{a}	1.93±0.52^{a}	2.02±0.42^{a}	1.67±0.49^{a}	2.17±0.29^{a}	2.10±0.50^{a}	1.78±0.40^{a}
VSI	3.81±0.58^{a}	3.57±0.97^{a}	3.52±0.75^{a}	3.77±0.48^{a}	3.63±0.79^{a}	3.67±0.60^{a}	3.59±0.93^{a}	3.82±0.65^{a}
TF	1.48±0.58^{a}	1.21±0.83^{a}	1.36±0.61^{a}	1.11±0.64^{a}	1.01±0.41^{a}	1.15±0.58^{a}	1.47±1.21^{a}	0.92±0.60^{a}

Two–way ANOVA	FW (gr)	SGR (%Day^{-1})	DGR (%Day^{-1})	TGR	DFI (%Day^{-1})	FCR (%Day^{-1})	PER (%)	LER (%)	LSI	VSI	TF
Lipid	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	0.003	0.068	$P < 0.001$	$P < 0.001$	0.026*	0.536	0.131
Enzyme	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	0.077	0.161	$P < 0.001$	$P < 0.001$	0.002*	0.221	0.023*
Lipid∗Enzyme (Interaction)	0.084	0.118	0.074	0.106	0.004*	0.004*	0.074	$P < 0.001$	0.988	0.793	0.234

* Two way ANOVA was made according to $P < 0.01$ significance level;
* Symbol represents difference according to $P < 0.05$ significance level

Table 3

Body nutritional composition of the groups at the end of the research (60 days, $n = 3$, mean ± standard deviation)

Body nutritional composition	Initial	14% Lipid				22% Lipid			
		Control (G1)	%14—0.5 (G3)	%14–1 (G4)	%14—1.5 (G5)	Control (G2)	%22—0.5 (G6)	%22–1 (G7)	%22—1.5 (G8)
Protein	15.47±0.64	18.13±0.12^{ab}	18.67±0.14^{a}	17.34±0.48^{b}	17.10±0.48^{b}	18.19±0.62^{ab}	17.41±0.72^{b}	17.70±0.30^{ab}	17.94±0.85^{ab}
Lipid	4.75±0.16	13.58±0.13^{ab}	14.06±0.78^{a}	13.04±0.47^{b}	12.24±0.08^{b}	12.75±0.11^{b}	13.95±0.76^{a}	12.55±0.25^{b}	13.21±0.84^{ab}
Dry matter	30.23±0.67	34.69±0.76^{b}	37.08±0.61^{a}	34.29±0.31^{bc}	35.24±0.62^{b}	35.64±0.91^{b}	35.69±0.19^{b}	35.59±0.45^{b}	34.42±0.45^{bc}
Ash	6.08± 0.98	2.44±0.40^{b}	2.32±0.57^{ab}	2.44±0.40^{b}	2.46±0.68^{b}	2.66±0.80^{b}	3.21±0.47^{ab}	3.85±0.66^{a}	2.34±0.38^{b}

Two-way ANOVA	Protein	Lipid	Dry Matter	Ash
Lipid	0.615	0.012*	0.047*	$P < 0.001$
Enzim	0.119	0.122	0.213	0.440
Lipid*Enzim (Interaction)	0.534	0.014*	$P < 0.001$	0.169

* Two way ANOVA was made according to $P < 0.01$ significance level;
* Symbol represents difference according to $P < 0.05$ significance level.

Table 4

Digestive enzyme activity of the groups at the end of the Research (60 days, $n = 3$, mean ± standard deviation)

Enzyme activity (U/ mg protein)	14% Lipid				22% Lipid			
	Control (G1)	%14—0.5 (G3)	%14–1 (G4)	%14–1.5 (G5)	Control (G2)	%22–0.5 (G6)	%22–1 (G7)	%22–1.5 (G8)
Amylase	9.39 ± 0.14^{bc}	7.31 ± 0.55^{c}	7.68 ± 0.94^{c}	11.79 ± 0.45^{b}	13.40 ± 0.60^{a}	8.74 ± 0.78^{c}	10.28 ± 0.59^{b}	11.31 ± 0.69^{b}
Trypsin	1.71 ± 0.03^{c}	1.86 ± 0.04^{a}	1.75 ± 0.03^{b}	1.78 ± 0.04^{ab}	1.83 ± 0.04^{a}	1.76 ± 0.03^{b}	1.79 ± 0.02^{ab}	1.68 ± 0.03^{c}
Lipase	18.99 ± 0.70^{b}	20.74 ± 0.54^{ab}	21.96 ± 0.86^{a}	21.67 ± 0.28^{a}	21.40 ± 0.50^{a}	20.59 ± 0.20^{ab}	21.15 ± 0.69^{a}	20.51 ± 0.55^{ab}
Protease	2.23 ± 0.03^{b}	2.54 ± 0.05^{a}	2.22 ± 0.05^{b}	1.99 ± 0.05^{c}	2.27 ± 0.06^{b}	2.25 ± 0.04^{b}	2.33 ± 0.02^{b}	1.94 ± 0.04^{c}
Aminopeptidase	0.081 ± 0.003^{a}	0.064 ± 0.007^{ab}	0.078 ± 0.001^{a}	0.075 ± 0.001^{a}	0.074 ± 0.009^{a}	0.084 ± 0.004^{a}	0.080 ± 0.002^{a}	0.079 ± 0.007^{a}
Alkaline Phosphatase	0.118 ± 0.002^{c}	0.160 ± 0.09^{a}	0.119 ± 0.004^{c}	0.132 ± 0.001^{b}	0.091 ± 0.003^{d}	0.094 ± 0.004^{d}	0.077 ± 0.010^{e}	0.130 ± 0.002^{b}

Two way ANOVA	Amylase	Trypsin	Lipase	Protease	Aminopeptidase	Alkaline Phosphatase
Lipid	$P < 0.001$	$P < 0.001$	0.991	0.044^{*}	0.394	$P < 0.001$
Enzyme	$P < 0.001$	0.143	0.179	$P < 0.001$	0.263	$P < 0.001$
Lipid∗Enzyme	$P < 0.001$	$P < 0.001$	0.032^{*}	$P < 0.001$	0.075	$P < 0.001$

* Two way anova was made according to $P < 0.01$ significance level,
* Symbol represents difference according to $P < 0.05$ significance level

Discussion. Impacts of dietary lipase supplementation was examined on digestive enzyme activity and growth promoting effect in gilthead sea bream. Feeding lipase supplemented diets influenced digestive enzyme activity, growth and FCR in varied manners in *Sparus aurata.* In the present study the addition of lipase to diets including 14% and 22% lipid affects growth and FCR in gilthead seabream (*Sparus aurata*). Specific growth rates (SGR) were between 3.14 and 3.55% on Day-1 which were higher than the values reported by AYHAN et al. [13]. The best FCR value (1.20) was obtained from 0.5 g lipase/kg, 14% lipid. Growth of fish in the G3 group was positively influenced by enzymatic supplementation. In this work, fish fed the control diet showed lower growth performance than those fed diets supplemented with lipase enzymes, pointing out that the dietary lipase enzyme was advantageous for the growth of sea bream. Growth response and feed utilization were enhanced with lipase supplementation to fish diets because digestive exoenzymes have growth-stimulating effects, and they also are effective in eradicating the anti-nutritional aspects and improving the feed consumption, ensuing fish performance promotion. These results are in endorsement with some previous studies on rabbitfish [14] and common carp [15]. Compared with protease and carbohydrase, the data around the effect of exogenous lipase on fish are still limited. The previous study demonstrated that fed the lipase diet in *Sparus aurata* might modify the kinetics of lipid absorption and utilization in teleosts [16]. LIU et al. [17] investigated the effects of exogenous lipase supplementation in young grass carp (*Ctenopharyngodon idella*). The results indicated that optimal exogenous lipase supplementation significantly increased growth in grass carp. In contrast, SAMUELSEN et al. [18] showed that dietary lipase exhibited no effect on growth in rainbow throut. The reason the results are different may be attributed to the use of different fish species, different exogenous enzymes, and the inclusion levels of plant-based feedstuffs [19]. The exogenous lipase also improved the protein sparing effect, as previously reported in some marine fish [17]. In addition, the PER and LER of groups were significantly higher ($P < 0.05$) suggesting that higher utilization of lipids results in a more efficient way of utilizing dietary proteins. The reason for the application of various enzymes in fish feed is to improve the overall quality of diets. The supplementation of exogenous enzymes to feeds could improve the growth performance of cultured fish increasing activity of digestive enzymes. In the presented study fish fed with the G3 group was observed in better growth and development. The increase in growth of body weight reflected the changes in the activity of digestive enzymes (Table 4). The activity of digestive enzymes is considered a significant indicator for fish growth and the level of digestive enzyme depends on the capacity of digestion absorption of nutrients which affects the development and the growth in fish. Liu et al. [17] showed that the activity of digestive enzymes including chymotrypsin, trypsin, amylase, and lipase in the hepatopancreas and intestines of grass carp *C. idella* were significantly developed by adding lipase enzyme. Similar results were reported in

another study [20], where the authors suggested that exogenous lipase promotes the growth of fish through improvement in digestive enzyme activity. The present work reveals the importance of using enzyme in aquaculture to enhance growth and prove that lipase may improve the growth and developement of seabream by increased activity of enzyme. The inclusion level of 0.5 g lipase/kg, 14% lipid was shown to be optimal for use in the diet of gilthead seabream (*Sparus aurata*).

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