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Effects of soy protein base diet supplemented with lysine and methionine on digestive enzymes activity and hematological parameters in silvery-black porgy (*Sparidentex hasta*) juveniles

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Abstract

The effect of dietary partial replacement of fish meal (FM) with soybean protein (SP) alone or in combination with lysine (Lys) and methionine (Met) supplementation were tested in a 60-day feeding trial for silvery-black porgy (*Sparidentex hasta*) juveniles. Seven isoproteic (*ca.* 50% crude protein) and isoenergetic (*ca.* 22.4 MJ kg^{-1}) diets were formulated in which 45% (SP45), 60% (SP60) and 75% (SP75) of FM protein were replaced by SP and the control diet (FM) was prepared with FM as the major source of protein. In $SP45^+$, $SP60^+$ and $SP75^+$ diets, 45 to 75% of FM was replaced by SP with supplementing blends of Lys and Met (98% of purity). The activities of the trypsin, lipase and α-amylase were higher in fish fed SP diets with crystalline amino acids supplementation than in the other groups ($p<0.05$). Fish fed SP75 and $SP75^+$ diets had the lowest red blood cell count and hematocrit level ($p<0.05$). The results of the current study indicated that anti-nutritional factors in a soy-protein based diet rather than lysine and methionine deficiencies may have adverse effects on digestive enzymes activities and health condition in silvery-black porgy juveniles.

Keywords: Soy protein, Lysine, Methionine, Digestive enzymes activity, Hematology, *Sparidentex hasta*

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Introduction

The diversification of the aquaculture industry, which is based on social, economic and ecological considerations, is the main tool for the sustainability of this fast-growing industry. In this regard, silvery-black porgy (*Sparidentex hasta*, Valenciennes, 1830) is considered as a potential candidate for aquaculture diversification in the Persian Gulf and Oman Sea regions (Pavlidis and Mylonas, 2011). Thus, this species has received considerable attention from the scientific community in order to develop its intensive culture and improve diet formulation. In this sense, the dietary protein requirements for this sparid species are estimated to be *ca.* 48%, fish meal (FM) being the major source of protein in the diet (Hossain *et al.,* 2014). Due to the high cost of fish meal and other considerations like uncertainty of fish meal availability in the future, there are interests in the partial or total replacement of this ingredient with less expensive plant protein meals without adverse effects on growth and health of aquaculture species (Oliva-Teles *et al*., 2015). Previous studies also showed that approximately 20% to 60% FM could be replaced by SP when crystalline AA is supplemented in diets for marine carnivorous fish species without significant effects on growth and feed efficiency (Oliva-Teles *et al*., 2015). Moreover, Yaghoubi *et al.* (2016) reported that the silvery-black porgy juvenile has low tolerance to dietary fish meal substitution with soy products (SP) (16.5–27.3%). Soybean meal is the most available and economical plant protein source with relatively high digestible protein and a good amino acid profile (Gatlin *et al*., 2007; Oliva-Teles *et al*., 2015). Several studies have also shown that the replacement of FM with SP in marine carnivorous fish species have adverse effects on digestive enzyme activities (Tibaldi *et al.,* 2006; Lin and Luo, 2011; Li *et al.,* 2014) and health condition (Gatlin *et al.,* 2007; Ye *et al.,* 2011) as a consequence of the presence of anti-nutritional factors (ANFs) in this alternative protein source. Anti-nutritional factors such as protease inhibitors, lectins, phytic acid, phytoestrogens, antivitamins and allergens as well as indigestible carbohydrates may have negative impacts on digestive enzyme activities which in turn may have adverse effects on feed utilization, growth performance and health status (Francis *et al.,* 2001; Gatlin *et al.,* 2007; NRC, 2011). Moreover, methionine (Met) followed by lysine (Lys) are the main limiting essential AA (EAA) in SP, which might lead to a nutritional imbalance in the diet (Gatlin *et al.,* 2007). In this context supplementation with CAA was shown an efficient strategy for replacing FM with PP in different fish species (Kaushik *et al.,* 1995; Mambrini *et al.,* 1999; Gaylord and Barrows, 2009). In regard to sparid species, it has been reported that replacement of dietary FM with SP up to 40% and 60% did not have any adverse effect on growth performance and health status of gilthead sea bream (*Sparus aurata*) and sharpsnout sea bream (*Diplodus*

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puntazzo) when crystalline EAA were supplemented in diets (Venou *et al.,* 2006; Hernández *et al.,* 2007).

There have been many recent advances in the field of fish nutrition of farmed fish raised on plant feed, including improvements of dietary manipulations, feed supplementation with additives and processing technologies of raw vegetable material to enhance growth and feed efficiency (Klinger and Naylor, 2012). Consequently, FM replacement with a blend of plant protein (PP) sources in fish feeds is presently a major trend in aquaculture (Gatlin *et al*., 2007; Naylor *et al*., 2009). Thus, the aim of the present study was to assess the effect of using SP with Lys and Met supplementation to replace FM in practical diets on digestive enzymes activities and health status in silvery-black porgy.

Materials and methods

Fish maintenance

The growth trial was conducted at the Mariculture Research Station of the South Iran Aquaculture Research Center (SIARC), Sarbandar, Iran. Silvery-black porgy juveniles were randomly distributed into 21 cylindrical polyethylene tanks (250 L), and each tank was stocked with 15 fish (mean body weight=16.7±0.1 g). Fish were acclimated for 2 weeks before beginning the trial. Tanks were supplied with filtered running seawater (1 L min^{-1}); salinity ranged between 47 and 49 ‰ (48.0±0.5 ‰) and temperature between 22 and 29 °C (25.9±1.3 °C) during the experimental period. Average values for dissolved oxygen and pH were 6.8±0.4 mg L^{-1} and 7.7±0.2, respectively. The photoperiod was left under natural conditions (30°32′N, 49°20′E; 12 L: 12 D).

Experimental diets and feeding

In the current study seven grossly isoproteic (*ca.* 50%) and isoenergetic (*ca.* 22.4 MJ/kg) experimental diets were prepared in which the control diet (FM) contained FM as the major protein source (Table 1). In diets SP45, SP60 and SP75, 45, 60 and 75% of FM was replaced by SP (soybean meal and isolated soy protein, not in a constant proportion for compensating reduced protein levels in the diets) without CAA supplementation, whereas in diets SP45^{+}, SP60^{+} and SP75^{+}, 45, 60 and 75% FM was replaced by SP with supplementing blends of Lys and Met. All ingredients were thoroughly mixed for 30 min, distilled water was added to form a soft dough, and then wet-extruded (meat grinder) to obtain pellets of the desired size (3 mm). Pellets were dried in a convection oven at 25 °C and stored in re-sealable plastic bags at −20 °C until use. During the experimental phase, diets were tested in triplicate; fish were fed by hand to visual satiety three times per day (0900, 1300 and 1700h) for 60 days.

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Table 1: Ingredient, proximate composition and amino acids profile of the experimental diets.

	Diets						
Dietary ingredients (g kg^{-1} dry diet) [a]	**FM**	**SP45**	**SP45^{+}**	**SP60**	**SP60^{+}**	**SP75**	**SP75^{+}**
Fish meal[b]	620	340	340	250	250	155	155
Casein[c]	70	70	64	70	63	70	61
Gelatin	40	40	40	40	40	40	40
Isolated soy protein[e]	0	150	150	180	180	210	210
Soybean meal[f]	0	150	150	240	240	340	340
Corn starch[d]	160	40	40	10	10	0	0
Wheat middling's[d]	20	80	80	70	70	40	40
Fish oil[b]	60	100	100	110	110	115	115
Mineral premix[g]	10	10	10	10	10	10	10
Vitamin premix[h]	15	15	15	15	15	15	15
DL-Methionine	0	0	2.0	0	2.5	0	3.0
L-Lysine-HCl	0	0	4.0	0	4.5	0	6.0
Cr_2O_3[c]	5	5	5	5	5	5	5
***Proximate composition* (%)**							
Dry matter	94.5	92.9	92.7	92.7	92.2	92.6	91.3
Crude protein	50.2	50.8	50.2	51.3	50.3	50.6	50.1
Crude lipid	19.6	18.6	18.4	18.4	18.2	17.6	17.5
NFE[i]	14.9	14.2	15	14.2	14.8	16.2	15.2
Ash	9.3	8.1	8.5	7.5	8.3	6.5	7.9
Gross energy (kJ g^{-1}) [j]	22.2	22	21.8	22.0	21.7	22.0	21.5

[a]Composition of ingredients as % Dry-weight basis [fish meal (63.5% crude protein, 17.7% crude lipid); casein (71.4% crude protein, 4.1%crude lipid); gelatin (85% crude protein, crude lipid, 4.2); soybean meal (41% crude protein, 4.2% crude lipid); isolated soy protein (73.3% crude protein, 2.8% crude lipid); corn starch (2.4% crude protein, 3.7% crude lipid); Wheat middling's (12% crude protein, 9.5% crude lipid)].
[b] Parskilka Mazandaran, Iran (*Clupeonella* sp.).
[c] Sumchun pure chemical, South Korea.
[d] Beyza feed mill, Shiraz, Iran.
[e] Wachsen Industry Co., Ltd. Qingdao, China.
[f] Behpak indastrial company, Behshahr, Mazandaran, Iran.
[g]Vitamin premix U kg^{-1} of premix: vitamin A, 5000000 IU; vitamin D_3, 500000 IU; vitamin E, 3000 mg; vitamin K_3, 1500 mg; vitamin B_1, 6000 mg; vitamin B_2, 24000 mg; vitamin B_5, 52000 mg; vitamin B_6, 18000 mg; vitamin B_{12}, 60000 mg; Folic acid, 3000 mg; nicotinamide 180000 mg; antioxidant, 500mg, career up to1 kg, Damloran pharmaceutical company, Broujerd, Iran.
[h] Mineral premix U kg^{-1} of premix: copper, 3000 mg; zinc, 15000 mg; manganese, 20000 mg; Iron, 10000 mg; potassium iodate, 300 mg, career up to 1 kg, Microvit®,Razak laboratories, Tehran, Iran.
[i] Nitrogen-free extract =100-(protein+lipid+ash+fiber+moisture).
[j] Calculated on gross energy values of 23.6 kj g^{-1} proteins, 39.5 kj g^{-1} fat and 17.2 kj g^{-1} carbohydrates (NRC, 2011).

Sample collection

For the assessment of digestive enzyme activities, 3 fish per tank (n=9 fish per diet treatment) were randomly sampled, anaesthetized with an overdose of anesthetic (2-phenoxyethanol at 0.5 ml L^{-1}; Merck, Schuchardt, Germany), and immediately eviscerated on an ice surface (0–4 ºC). The alimentary tract was dissected, adherent adipose and connective tissues carefully removed and the intestine divided into pyloric caeca, anterior, mid and posterior intestine; and then the dissected

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sections were placed in individually marked plastic tubes and stored at −80 °C until their analysis. Four fish per tank were anesthetized with the same anesthetic and blood was collected from the caudal vein (n=12 fish per diet treatment) with heparinized syringes and pooled together. Blood samples were aliquoted into three parts of 1 mL each. An aliquot of blood was used for hematological parameters and the other aliquots centrifuged (4000 g, 10 min, 4 ºC) and plasma separated and stored at -80 °C until their analysis.

Determination of pancreatic digestive enzymes

Samples were homogenized in 30 volumes (w/v) of ice-cold Manitol (50 mM), Tris-HCl buffer (2 mM) pH 7.0, at a maximum speed for 30s (IKA, Ultra-turrax®, USA), then 100 µL of 0.1M $CaCl_2$ was added to the homogenate and finally the homogenate was centrifuged at 10,000 *g* for 10 min at 4 °C and the supernatants collected and stored in aliquots at −80 °C for further enzyme quantification (Gisbert *et al.,* 2009). Trypsin (E.C. 3.4.21.4) activity was measured with N-α-benzoyl-dlarginine-p-nitroanilide (BAPNA) as substrate. BAPNA (1 mM in 50 mM Tris–HCl, pH 8.2, 20 mM $CaCl_2$) was incubated with the enzyme extract at 37 °C and absorbance was recorded at 410 nm (Erlanger *et al.,* 1961). The activity of α-amylase (E.C. 3.2.1.1) was determined at 25 °C (absorbance: 540 nm) using 1% starch (Sigma-Aldrich) (diluted in a buffer at pH 6.9, 0.02 M Na_2HPO_4 and 0.006 M NaCl) as substrate by the 3, 5-dinitrosalicylic acid method (Worthington, 1991) and absorbance was recorded at 540 nm. Non-specific lipase activity (E.C. 3.1.1) was determined at 25 °C (absorbance: 405 nm) using 0.4 mM 4-nitrophenyl-myristate (Sigma-Aldrich) as substrate and absorbance was recorded at 405 nm (Gawlicka *et al.,* 2000). The protein concentration of the enzyme extracts was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as a protein standard.

Hematological and plasma biochemical analyses

Hematocrit (Hct; %), hemoglobin (Hb; g dL^{-1}) concentration and the number of blood red cells (RBC) were assessed according to methods described by Blaxhall and Daisley, (1973). Blood indices including the mean cell hemoglobin (MCH), the mean cell volume (MCV) and the mean cell hemoglobin concentration (MCHC) were calculated according to the following formulae (Lewis *et al.,* 2001).

Mean cell volume (MCV)=Hct(%)/ RBC($\times 10^6$ µl)×10

Mean cell hemoglobin (MCH)=Hb (g/dL)/RBC($\times 10^6$ µL)×10

Mean cell hemoglobin concentration (MCHC)=(g/dL)=Hb(g/dL)/Hct(%)

Plasma biochemical parameters were analyzed by means of an auto-analyzer (Technicon RA-1000, Technicon Instruments, NY, USA) using commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were conducted for total protein (TP), albumin (ALB), total cholesterol

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(CHOL), triglyceride (TRIG), high-density lipoprotein (HDL) and alkaline phosphatase (ALP).

Statistical analysis

Data were analyzed using the SPSS ver.15.0 (Chicago, Illinois, USA). All the data are presented as mean±standard error of the mean. Arcsine transformations were conducted on all percentage data to achieve homogeneity of variance before statistical analysis. The effects of FM substitutions and CAA supplementation and their interactions on different factors were analyzed using a two-way ANOVA. In case of significant interaction between main factors simple effects were evaluated by one way ANOVA. Duncan procedure was used for multiple comparisons when statistical differences were found among groups using one way ANOVA. The level of significance was set at $p<0.05$ for all tests.

Results

Pancreatic digestive enzymes activities

The specific activity of digestive enzymes in the pyloric caeca, anterior, mid and posterior intestine sections in silvery-black porgy are presented in Table 2. The activities of pancreatic enzymes (trypsin, lipase and α-amylase) decreased in all parts of intestine with increasing dietary SP level ($p<0.05$). On the other hand, trypsin and α-amylase activities were higher in those fish fed supplemented CAA diets than fish fed the corresponding non-supplemented diets ($p<0.05$). Trypsin and α-amylase activity were affected by FM substitution and CAA supplementation in all parts of intestine and pyloric caeca, but lipase activity was only under the effect of FM substitution and there were no differences of lipase activity in the posterior intestine ($p<0.05$).

Table 2: Digestive enzyme activities (U mg^{-1} protein) in pyloric caeca and different parts of intestine of *Sparidentex hasta* juvenile fed different experimental diets at the end of growth trial (means±SE, n=3). A different superscript in the same row denotes statistically significant differences analyzed by one way ANOVA ($p<0.05$).

	Diets							Two way ANOVA (P value)*		
	FM	SP45	SP45$^+$	SP60	SP60$^+$	SP75	SP75$^+$	FM Substit	CAA Supplement	Interactions
Pyloric caeca										
Trypsin	2.8 ± 0.2 ^{a}	2.1 ± 0.2^b	3.2 ± 0.0 ^{a}	1.5 ± 0.1^{bc}	2.4 ± 0.0	0.4 ± 0.0^d	1.2 ± 0.2 ^{c}	<0.001	<0.001	0.734
Lipase	0.6 ±	1.0 ± 0.2^{ab}	1.2 ± 0.2 ^{a}	0.9 ±	1.2 ± 0.2	0.3 ± 0.1^d	0.5 ± 0.1 ^{cd}	<0.001	0.127	0.704
α-Amylase	0.1 ± 0.0^b	0.1 ± 0.0^b	0.1 ± 0.0 ^{b}	0.1 ± 0.0 ^{b}	0.2 ± 0.0	0.03 ± 0.0^b	0.1 ± 0.0 ^{b}	<0.001	0.009	0.114
Anterior										
Trypsin	3.0 ± 0.1^b	2.2 ± 0.1^c	3.3 ± 0.1 ^{ab}	1.6 ± 0.1 ^{d}	2.5 ± 0.1	0.5 ± 0.0 ^{e}	1.3 ± 0.1 ^{d}	<0.001	<0.001	<0.001
Lipase	0.7 ± 0.3^{abc}	1.2 ± 0.3 ^{ab}	1.4 ± 0.2 ^{a}	1.1 ± 0.3	1.4 ± 0.2	0.3 ± 0.2^c	0.5 ± 0.2 ^{bc}	<0.001	0.155	0.780
α-Amylase	0.3 ± 0.0 ^{ab}	0.2 ± 0.0 ^{b}	0.3 ± 0.0 ^{ab}	0.2 ± 0.0^b	0.4 ± 0.0	0.1 ± 0.0 ^{c}	0.1 ± 0.0 ^{c}	<0.001	<0.001	0.012
Mid intestine										
Trypsin	2.0 ± 0.0^b	1.8 ± 0.1 ^{c}	2.3 ± 0.1 ^{ab}	1.1 ± 0.1^d	2.5 ± 0.0	0.2 ± 0.1^e	0.8 ± 0.1 ^{d}	<0.001	<0.001	<0.001
Lipase	0.9 ± 0.3	1.6 ± 0.4^a	1.8 ± 0.2 ^{a}	1.4 ± 0.5	1.8 ± 0.0	0.4 ± 0.2^c	0.6 ± 0.2 ^{bc}	<0.001	0.091	0.771
α-Amylase	0.6 ± 0.2 ^{b}	0.4 ± 0.1^d	0.7 ± 0.0 ^{a}	0.5 ± 0.2^c	0.7 ± 0.0	0.2 ± 0.0^e	0.2 ± 0.0 ^{e}	<0.001	<0.001	<0.001
Posterior										
Trypsin	1.3 ± 0.0^b	1.0 ± 0.0^c	1.5 ± 0.0 ^{ab}	0.7 ± 0.1^d	1.6 ± 0.0	0.2 ± 0.1 ^{f}	0.6 ± 0.1 ^{d}	<0.001	<0.001	0.005
Lipase	0.3 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.08	0.435	0.995
α-Amylase	0.7 ± 0.0^a	0.5 ± 0.0^c	0.8 ± 0.0 ^{a}	0.6 ± 0.0^b	0.9 ± 0.0	0.1 ± 0.0^d	0.2 ± 0.0 ^{d}	<0.001	<0.001	<0.001

*The significance of the two main effects (FM substitution and AA supplementation) and interaction was analyzed using two-way ANOVA.

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Hematological and plasma biochemical parameters

Hematological and plasma biochemical parameters were significantly affected by replacing dietary FM with SP (Tables 3 and 4). Fish fed the SP75 and SP75$^+$ diets had the lowest RBCs and Hct levels (p<0.05). Hemoglobin was not affected by dietary treatments but MCV, MCH and MCHC increased in SP75 and SP75$^+$ relative to the other treatments (p<0.05). These differences were mostly due to FM substitution (p<0.05). Plasma TP and ALB levels were significantly affected by FM replacement and its interaction with CAA supplementation. In this sense, plasma TP and ALB were highest in fish fed with SP60 and SP75$^+$ diets, respectively (p<0.05). Plasma total cholesterol and HDL were not affected by dietary treatments (p>0.05). Plasma TRIG was mainly affected by CAA supplementation and its interaction with FM replacement, and fish fed with SP75 diet had higher plasma TRIG concentrations (677.0±5.5) than other groups (p>0.05). Plasma ALP levels were significantly affected by FM substitution (p=0.019) and were relatively higher in fish fed SP75 and SP75$^+$ diets than other groups.

Table 3: Hematological parameters of *Sparidentex hasta* juvenile fed different experimental diets at the end of growth trial (means±SE, n= 3). A different superscript in the same row denotes statistically significant differences analyzed by one way ANOVA (p<0.05).

	Diets							Two way ANOVA ($p$value)*		
Hematological parameters	FM	SP45	SP45$^+$	SP60	SP60$^+$	SP75	SP75$^+$	FM Substitution	CAA Supplementation	Interactions
RBC ($\times10^6$ µl)	2.1 ± 0.1 ^{ab}	1.7 ± 0.0 ^{ab}	1.4 ± 0.2 ^{abc}	2.0 ± 0.1^{ab}	2.2 ± 0.4 ^{a}	0.8 ± 0.0^{c}	0.8 ± 0.0 ^{c}	<0.001	0.815	0.319
Hb (g dl^{-1})	4.2 ± 0.3	4.0 ± 0.2	4.3 ± 0.1	4.1 ± 0.3	4.1 ± 0.1	4.1 ± 0.1	3.9 ± 0.3	0.782	0.965	0.673
Hct (%)	30.0 ± 1.1^{a}	25.9 ± 1. 2 ^{ab}	27.0 ± 1.5 ^{ab}	23.6 ± 0.7 ^{b}	27.7 ± 1.2 ^{ab}	17.7 ± 1.0^{c}	18.4 ± 0.6 ^{c}	0.001	0.131	0.445
MCV (mm^3)	142.4 ± 9.5^{cd}	150.5 ± 5.0^{cd}	212.5 ± 36.9 ^{bc}	119.3 ± 7.5^{d}	133.6 ± 23.5 ^{cd}	238.9 ± 7.8 ^{a}	230.0 ± 16.3 ^{a}	0.001	0.219	0.121
MCH (pg cell^{-1})	20.1 ± 1.9 ^{c}	23.7 ± 1.7^{c}	34.1 ± 7.6 ^{bc}	20.8 ± 1.5 ^{c}	19.6 ± 3.4 ^{c}	54.0 ± 2.0 ^{a}	48.8 ± 5.4 ^{a}	<0.001	0.699	0.09
MCHC (g L^{-1})	141.5 ± 8.8 ^{b}	157.3 ± 10.2 ^{b}	158.6 ± 9.7 ^{b}	173.9 ± 9.4 ^{ab}	147.1 ± 4.5 ^{b}	233.8 ± 24.3 ^{a}	212.0 ± 8.6 ^{a}	<0.001	0.146	0.499

*The significance of the two main effects (FM substitution and AA supplementation) and interaction was analyzed using two-way ANOVA.

Table 4: Plasma biochemical parameters of *Sparidentex hasta* juvenile fed different experimental diets at the end of growth trial (means ± SE, n= 3). A different superscript in the same row denotes statistically significant differences analyzed by one way ANOVA (p<0.05).

	Diets							Two way ANOVA (P value)*		
Parameters	FM	SP45	SP45$^+$	SP60	SP60$^+$	SP75	SP75$^+$	FM Substitution	CAA Supplementation	Interactions
TP (g dL^{-1})	3.5 ± 0.1 ^{ab}	3.1 ± 0.4^{ab}	2.5 ± 0.3 ^{b}	5.0 ± 0.7 ^{a}	3.4 ± 0.5^{ab}	3.5 ± 0.5^{ab}	4.8 ± 0.7^{ab}	0.028	0.471	0.041
ALB (g dL^{-1})	0.3 ± 0.0^{b}	0.3 ± 0.1 ^{b}	0.2 ± 0.0^{b}	0.4 ± 0.1 ^{ab}	0.3 ± 0.1^{b}	0.4 ± 0.1 ^{ab}	0.7 ± 0.1 ^{a}	<0.001	0.366	0.002
CHOL (mg dL^{-1})	245.5 ± 25.1	211.3 ± 30.3	166.8 ± 23.4	294.2 ± 40.9	234.2 ± 33.2	273.5 ± 40.1	277.7 ± 45.4	0.054	0.261	0.641
TRIG (mg dL^{-1})	136.5 ± 6.5^{b}	486.2 ± 7.2 ^{ab}	230.8 ± 4.1 ^{b}	434.7 ± 4.8 ^{ab}	480.7 ± 2.9 ^{ab}	677.0 ± 5.5^{a}	254.7 ± 3.9 ^{b}	0.054	<0.001	0.001
HDL (mg dL^{-1})	127.5 ± 18.0	75.5 ± 11.0	73.3 ± 10.9	70.3 ± 12.4	81.2 ± 12.4	74.3 ± 10.8	140.7 ± 37.6	0.167	0.121	0.183
ALP (U L^{-1})	567 ± 40.4^{ab}	362 ± 122.2 ^{b}	635.7 ± 148.1^{ab}	606.5 ± 97.1 ^{ab}	458 ± 86.6 ^{ab}	710 ± 117.5 ^{ab}	973.3 ± 156 ^{a}	0.019	0.192	0.151

*The significance of the two main effects (FM substitution and AA supplementation) and interaction was analyzed using two-way ANOVA.

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Discussion

In the present study, the activities of the pancreatic enzymes (trypsin, lipase and α-amylase) decreased in all parts of the intestine with increasing dietary SP level. Increasing dietary PP levels also led to the decrease in digestive enzymes activities in other fish species (Santigosa *et al.,* 2008; Cheng *et al.,* 2010; Deng *et al.,* 2010; Lin and Luo, 2011; Bowyer *et al.,* 2013; Li *et al.,* 2014) maybe due to the presence of trypsin and lipase inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins and allergens (Francis *et al.,* 2001; Gatlin *et al.,* 2007; NRC, 2011). In contrast, Gisbert *et al*. (2016) reported that replacement of dietary FM with blends of PP sources did not have any adverse effects on digestive enzymes activities in flathead grey mullet (*Mugil cephalus*). On the other hand, the presence of ANFs such as lipase and co-lipase inhibitors in SP may have led to a decrease in lipase activity in fish fed with SP-based diets (Mambrini *et al.,* 1999). In addition, oligosaccharides and non-starch polysaccharides in SP are involved in the binding action with bile salts and/or by an obstructing action on digestive enzymes coupled with changes in digesta viscosity and transit rate, which may reduce the digestibility and bioavailability of all nutrients (Francis *et al.,* 2001). Furthermore, higher activities of trypsin and α-amylase in those fish fed supplemented CAA diets were observed in the current study. Similar to our results, it was reported that AA such as Met (Xiao *et al.,* 2011) and Lys (Zhou *et al.,* 2008) had positive effects on digestive enzyme activities in the intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian). This positive influence may be because of the effects of CAA on stimulating pancreatic enzymes secretion. In this context, it has been demonstrated that AA can be absorbed without prior digestion when present in the free form such as CAA or digestive end-products like, L-lys (Rønnestad *et al.,* 2007; Nunes *et al.,* 2014). These forms of AA can act directly on the pancreatic acinar cells to stimulate enzyme secretion through activating neuropeptides such as bombesin and cholecystokinin (Rønnestad *et al.,* 2007; Murashita *et al.,* 2015).

The highest MCV, MCH and MCHC followed by the lowest RBC and Hct levels were observed in fish fed SP75 and SP75+ diets, which were also out of their normal ranges (Mozanzadeh *et al.,* 2015). These anemic signs were mainly induced by FM substitution, whereas the CAA supplementation in those diets did not improve the hematological condition in silvery-black porgy juveniles. In this regard it has been reported that high levels of ANFs in PP such as saponins or phytic acid may also be responsible for the hemolysis of RBCs and induce iron deficiency by chelating di-and tri-valent ions, respectively (Francis *et al.,* 2001), and they may also lead to an anemic condition (Zhou *et al.,* 2005; Lim and Lee, 2008; Lim *et al.,* 2011).

Plasma protein levels are often used as an indicator of nutritional and physiological status in fish (Peres *et al.,* 2014). In the current study, an increase

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in plasma TP or ALB that are observed in fish fed with SP60 and SP75$^+$ diets, respectively may be an adaptation to bind the excess trypsin inhibitors in the plasma as the consequence of high dietary SP levels, as has been described by Xu *et al.,* (2012) in juvenile Amur Sturgeon *Acipenser schrenckii*. In contrast, it has been reported that serum TP decreased with increasing dietary SP levels in red sea bream and Japanese flounder (Takagi *et al.,* 2001; Ye *et al.,* 2011), because of the low quality of SP in comparison with FM protein. It has also been reported that the dietary inclusion of SP lead to an increase in nitrogen losses and high postprandial ammonia excretion as a result of an excess in AA catabolism in European sea bass *Dicentrarchus labrax* (Kaushik *et al.,* 2004) and Asian sea bass *Lates calcalifer* (Tantikitti *et al.,* 2005). In this study, plasma total cholesterol and HDL were neither affected by FM replacement nor CAA supplementation. As HDL is specialized for reverse cholesterol transport from the extrahepatic tissues to the liver (Leger, 1985); thus, no differences in plasma total cholesterol among various treatments led to stable plasma HDL levels. Plasma TRIG was highest in fish fed SP75 diet and was affected by CAA supplementation as well as its interaction with FM replacement. Supplementation of Lys and Met might have increased carnitine biosynthesis, which in turn might have elevated fatty acid β-oxidation resulting in a reduction of plasma TRIG levels in fish fed supplemented CAA diets (Harpaz, 2005). Alkaline phosphatase is one of the most reliable hepatic biomarkers for assessing nutritional and health conditions in silvery-black porgy (Mozanzadeh *et al.,* 2017). In the current study, plasma ALP levels in fish fed SP75 and SP75$^+$ were relatively higher than in fish fed with the other diets and they were significantly affected by FM substitution (p=0.019). This result may be because of liver damage as the consequence of the presence of ANFs or by raising AA metabolism and generating more metabolic wastes, as the consequence of high dietary SP level as it has also been reported in Amur sturgeon (Xu *et al.,* 2012).

In conclusion the results from this study showed that the high levels of SP in diets led to a decrease in digestive enzymes activities and also have adverse effects on health status of silvery-black porgy juveniles as a consequence of increasing ANFs in SP base diets rather than EAA deficiencies.

References

Blaxhall, P. and Daisley, K., 1973. Routine haematological methods for use with fish blood. *Journal of Fish Biology,* 5, 771-781.

Bowyer, J.N., Qin, J.G., Smullen, R.P., Adams, L.R., Thomson, M.J. and Stone, D.A., 2013. The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*) feeds at different water temperatures: 2. Soy protein concentrate. *Aquaculture,* 410, 1-10.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein

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utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72, 248-254.

Cheng, Z., Ai, Q., Mai, K., Xu, W., Ma, H., Li, Y. and Zhang, J., 2010. Effects of dietary canola meal on growth performance, digestion and metabolism of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture,* 305, 102-108.

Deng, J., Mai, K., Ai, Q., Zhang, W., Tan, B., Xu, W. and Liufu, Z., 2010. Alternative protein sources in diets for Japanese flounder *Paralichthys olivaceus* (Temminck and Schlegel): II. Effects on nutrient digestibility and digestive enzyme activity. *Aquaculture Research,* 41, 861-870.

Erlanger, B.F., Kokowsky, N. and Cohen, W., 1961. The preparation and properties of two new chromogenic substrates of trypsin. Archives of Biochemistry and Biophysics, 95, 271-278.

Francis, G., Makkar, H.P. and Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture,* 199, 197-227.

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å. and Nelson, R., 2007. Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research,* 38, 551-579.

Gawlicka, A., Parent, B., Horn, M.H., Ross, N., Opstad, I. and Torrissen, O.J., 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (*Hippoglossus hippoglossus*): indication of readiness for first feeding. *Aquaculture,* 184, 303-314.

Gaylord, T.G. and Barrows, F.T., 2009. Multiple amino acid supplementations to reduce dietary protein in plant-based rainbow trout, *Oncorhynchus mykiss*, feeds. *Aquaculture,* 287, 180-184

Gisbert, E., Giménez, G., Fernández, I., Kotzamanis, Y. and Estévez, A., 2009. Development of digestive enzymes in common dentex *Dentex dentex* during early ontogeny. *Aquaculture,* 287, 381-387.

Gisbert, E., Mozanzadeh, M.T., Kotzamanis, Y. and Estévez, A., 2016. Weaning wild flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish meal substitution. *Aquaculture,* 462, 92-100.

Harpaz, S., 2005. L-carnitine and its attributed functions in fish culture and nutrition: A review. *Aquaculture,* 249, 3-21.

Hernández, M., Martínez, F., Jover, M. and García, B.G., 2007. Effects of partial replacement of fish meal by soybean meal in sharpsnout seabream (*Diplodus puntazzo*) diet. *Aquaculture,* 263, 159-167.

Hossain, M., Al-Abdul-Elah, K. and El-Dakour, S., 2014. Evaluation of different commercial feeds for culture of juvenile sobaity (*Sparidentex Hasta* Valenciennes) in Kuwait. *APCBEE Procedia,* 8, 310-316.

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Kaushik, S., Cravedi, J., Lalles, J., Sumpter, J., Fauconneau, B. and Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 133, 257-274.

Kaushik, S., Coves, D., Dutto, G. and Blanc, D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. *Aquaculture,* 230, 391-404.

Klinger, D. and Naylor, R., 2012. Searching for solutions in aquaculture: charting a sustainable course. *Annual Review of Environment and Resources,* 37, 247-276.

Leger, C., 1985. Digestion, absorption and transport of lipids. In: Cowey, C.B., Mackie, A.M. and Bell, G.J., Eds. Nutrition and Feeding in Fish. Academic Press, London, pp. 299–331.

Lewis, S., Bain, B. and Bates, I.D., 2001. Lewis practical haematology. Churchill Livingstone. New York. P: 633**.**

Li, Y., Ai, Q., Mai, K., Xu, W., Deng, J. and Cheng, Z., 2014. Comparison of high-protein soybean meal and commercial soybean meal partly replacing fish meal on the activities of digestive enzymes and aminotransferases in juvenile Japanese seabass, *Lateolabrax japonicus* (Cuvier, 1828). *Aquaculture Research,* 45, 1051-1060.

Lim, S.J. and Lee, K.J., 2008. Supplemental iron and phosphorus increase dietary inclusion of cottonseed and soybean meal in olive flounder (*Paralichthys olivaceus*). *Aquaculture Nutrition,* 14, 423-430.

Lim, S.J., Kim, S.S., Ko, G.Y., Song, J.W., Oh, D.H., Kim, J.D., Kim, J.U. and Lee, K.J., 2011. Fish meal replacement by soybean meal in diets for Tiger puffer, *Takifugu rubripes*. *Aquaculture,* 313, 165-170.

Lin, S. and Luo, L., 2011. Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. *Animal Feed Science and Technology,* 168, 80-87.

Mambrini, M., Roem, A.J., Carvedi, J., Lalles, J. and Kaushik, S., 1999. Effects of replacing fish meal with soy protein concentrate and of DL-methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, *Oncorhynchus mykiss*. *Journal of Animal Science,* 77, 2990-2999.

Mozanzadeh, M.T., Yaghoubi, M., Yavari, V., Agh, N. and Marammazi, J.G., 2015. Reference intervals for haematological and plasma biochemical parameters in sobaity sea bream juveniles (*Sparidentex hasta*, Valenciennes 1830). *Comparative Clinical Pathology,* 24, 1501-1507.

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Mozanzadeh, M., Marammazi, J., Yaghoubi, M., Yavari, V., Agh, N. and Gisbert, E., 2017. Somatic and physiological responses to cyclic fasting and re-feeding periods in sobaity sea bream (*Sparidentex hasta*, Valenciennes 1830). *Aquaculture Nutrition*, 23, 181-191.

Murashita, K., Fukada, H., Takahashi, N., Hosomi, N., Matsunari, H., Furuita, H., Oku, H. and Yamamoto, T., 2015. Effect of feed ingredients on digestive enzyme secretion in fish. *Bulletin of Fisheries Research Agency*, 40, 69-74.

Naylor, R.L., Hardy, R.W., Bureau, D.P., Chiu, A., Elliott, M., Farrell, A.P., Forster, I., Gatlin, D.M., Goldburg, R.J. and Hua, K., 2009. Feeding aquaculture in an era of finite resources. *Proceedings of the National Academy of Sciences,* 106, 15103-15110.

NRC, 2011. Nutrient requirements of fish and shrimp. The National Academies Press, Washington, DC. P: 392.

Nunes, A.J., Sá, M.V., Browdy, C.L. and Vazquez-Anon, M., 2014. Practical supplementation of shrimp and fish feeds with crystalline amino acids. *Aquaculture,* 431, 20-27.

Oliva-Teles, A., Enes, P. and Peres, H., 2015. Replacing fishmeal and fish oil in industrial aquafeeds for carnivorous fish. Feed and Feeding Practices in Aquaculture. pp. 203-233.

Pavlidis, M. and Mylonas, C., 2011. Sparidae: Biology and aquaculture of gilthead sea bream and other species. John Wiley & Sons. P:408.

Peres, H., Santos, S. and Oliva-Teles, A., 2014. Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). *Fish Physiology and Biochemistry,* 40, 1339-1347.

Rønnestad, I., Kamisaka, Y., Conceição, L., Morais, S. and Tonheim, S., 2007. Digestive physiology of marine fish larvae: hormonal control and processing capacity for proteins, peptides and amino acids. *Aquaculture,* 268, 82-97.

Santigosa, E., Sánchez, J., Médale, F., Kaushik, S., Pérez-Sánchez, J. and Gallardo, M., 2008. Modifications of digestive enzymes in trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) in response to dietary fish meal replacement by plant protein sources. *Aquaculture,* 282, 68-74.

Takagi, S., Shimeno, S., Hosokawa, H. and Ukawa, M., 2001. Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream *Pagrus major*. *Fisheries Science,* 67, 1088-1096.

Tantikitti, C., Sangpong, W. and Chiavareesajja, S., 2005. Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (*Lates calcarifer*). *Aquaculture,* 248, 41-50.

Tibaldi, E., Hakim, Y., Uni, Z., Tulli, F., de Francesco, M., Luzzana, U. and Harpaz, S., 2006. Effects of the

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partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). *Aquaculture,* 261, 182-193.

Venou, B., Alexis, M., Fountoulaki, E. and Haralabous, J., 2006. Effects of extrusion and inclusion level of soybean meal on diet digestibility, performance and nutrient utilization of gilthead sea bream (*Sparus aurata*). *Aquaculture,* 261, 343-356.

Worthington, C., 1991. Worthington enzyme manual related biochemical. Freehold, New Jersey, USA. pp, 212-215.

Xiao, W.W., Feng, L., Liu, Y., Jiang, J., Hu, K., Jiang, W.D., Li, S.H. and Zhou, X.Q., 2011. Effects of dietary methionine hydroxy analogue supplement on growth, protein deposition and intestinal enzymes activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Nutrition,* 17, 408-417.

Xu, Q., Wang, C., Zhao, Z. and Luo, L., 2012. Effects of replacement of fish meal by soy protein isolate on the growth, digestive enzyme activity and serum biochemical parameters for juvenile amur sturgeon (*Acipenser schrenckii*). *Asian-Australasian Journal of Animal Sciences,* 25, 1588.

Yaghoubi, M., Mozanzadeh, M.T., Marammazi, J.G., Safari, O. and Gisbert, E., 2016. Dietary replacement of fish meal by soy products (soybean meal and isolated soy protein) in silvery-black porgy juveniles (*Sparidentex hasta*). *Aquaculture,* 468, 50-59.

Ye, J., Liu, X., Wang, Z. and Wang, K., 2011. Effect of partial fish meal replacement by soybean meal on the growth performance and biochemical indices of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquaculture International,* 19, 143-153.

Zhou, Q.C., Mai, K.S., Tan, B.P. and Liu, Y.J., 2005. Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). Aquaculture Nutrition, 11, 175-182.

Zhou, X.Q., Zhao, C.R., Jiang, J., Feng, L. and Liu, Y., 2008. Dietary lysine requirement of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Nutrition,* 14, 381-386.