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Lysine and methionine supplementation in high soy protein content diets for silvery-black porgy (*Sparidentex hasta*) juveniles

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Abstract

A two-month feeding trial was conducted to evaluate the effect of partial replacement of fish meal (FM) by soybean protein (SP) alone or in combination with lysine (Lys) and methionine (Met) supplementation in practical diets for silvery-black porgy juveniles (16.7±0.1 g). 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. Growth performance, feed utilization, and protein and lipid digestibility decreased with increasing dietary SP levels ($p<0.05$). Fish fed SP75 had the highest whole body lipid content, but with the lowest whole body arginine, lysine, histidine, phenylalanine and taurine concentrations ($p<0.05$). Supplementing Lys and Met in SP based diets did not improve growth performance, suggesting that the anti-nutritional factors in soybean protein products rather than Met and Lys deficiency might limit FM substitution with SP in silvery-black porgy juveniles.

Keywords: Fishmeal replacement, Lysine, Methionine, Growth performance, Body composition, *Sparidentex hasta*

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Introduction

Silvery-black porgy (*Sparidentex hasta* Valenciennes, 1830) has been identified as a desirable candidate for aquaculture diversification in the Persian Gulf and Oman Sea regions where it currently supports a small but well established sector of aquaculture (Pavlidis and Mylonas, 2011). Consequently, some investigations have been focused on establishing the nutritional requirements and optimal feed formulations for this warm water marine fish species (Mozanzadeh *et al*., 2015; 2016; 2017 a,b). In this context, the dietary protein requirements for this sparid species are estimated to be *ca.* 48%, being fish meal (FM) as the major source of protein in the diet (Hossain *et al*., 2014). Therefore, optimizing the source and inclusion level of various protein ingredients is critical to developing a cost-effective and high performing feeds for this species. FM becomes one of the most expensive dietary ingredients due to declining fisheries resources and increasing demands because of the progressive growth in global finfish aquaculture (Tacon and Metian, 2008). Thus, finding alternative protein sources such as animal by-products, plant feed stuffs and other novel protein sources is essential to meet increasing global demands of FM for the sustainability of aquaculture industry (Naylor *et al*., 2009). Soybean protein (SP) is the most available and economical plant protein (PP) source with relatively high digestible protein content, a constant composition and good amino acid (AA) profile (NRC, 2011). However, methionine (Met) followed by lysine (Lys) are the main limiting essential amino acids (EAA) in SP, which might lead to a nutritional imbalance in the diet (Gatlin *et al*., 2007). These nutritional imbalances along with the presence of the anti-nutritional factors (ANFs) in SP may lead to a growth reduction, which was associated with a reduction in feed intake in several marine carnivorous fish species (Hernández *et al*., 2007; Kader *et al*., 2012; Silva-Carrillo *et al*., 2012). A common way to improve the levels of EAA in the diet is to supplement the deficient AA with synthetic sources like crystalline amino acids (CAA) (Nunes *et al.*, 2014). Using the nutrient-based formulation is a cost effective approach for adjusting EAA in the diet with minimum changes in macro and micro ingredients (Nunes *et al*., 2014). The dietary Met and Lys requirements for fish range from 0.5 to 1.5 and 1.2 to 3.3% of the diet, respectively (NRC, 2011), and the supplementation of these two EAA in the PP based diets can spare other EAA (Kerr and Easter, 1995). In our previous study, we have found out it is possible to replace 16.5-27.3% of FM by SP in silvery black porgy juvenile; however more than 30% replacement led to decrease the growth performance and feed efficiency because of presence of ANFs and EAA deficiency especially Lys and Met (Yaghoubi *et al*., 2016). As there is no information available on the effects of replacement of FM by SP with CAA in diets for silvery-black porgy, the aim of the present study was to assess the effect of using SP with Lys and Met supplementation to replace FM

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in practical diets for silvery-black porgy.

Materials and methods

Experimental diets

In the current study, seven grossly isoproteic (*ca.* 50%) and isoenergetic (*ca.* 22.4 MJ kg^{-1}) experimental diets were prepared in which the control diet (FM) contained FM as the major protein source (Tables 1 and 2). In diets SP45, SP60 and SP75, 45, 60 and 75% of FM was replaced by SP (soybean meal and isolated SP, 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. Fish oil from *Clupeonella* sp. was used as the main lipid source and chromic oxide (Cr_2O_3) was included in the diets at 0.5% as an internal marker for estimating diets digestibility. All ingredients were thoroughly mixed for 30 min, distilled water was added to form a soft dough, and then wet-extruded 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.

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	65	70	66
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 (negligible 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).

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Table 2: Amino acids profile of the experimental diets g 100g^{-1} diet.

	Diets						
	FM	**SP45**	**SP45^{+}**	**SP60**	**SP60^{+}**	**SP75**	**SP75^{+}**
Essential amino acid composition							
Arginine	2.9	3.5	3.3	3.6	3.5	3.8	3.6
Histidine	1.1	1.3	1.1	1.3	1.2	1.3	1.2
Isoleucine	2.2	2.4	2.2	2.5	2.3	2.5	2.4
Leucine	3.6	3.9	3.5	4.0	3.7	4.2	3.8
Lysine	3.7	3.7	5.2	3.7	5.2	3.6	5.2
Methionine	1.3	1.0	2.4	1.0	2.3	0.9	2.2
Phenylalanine	2.0	2.3	2.1	2.4	2.2	2.5	2.3
Threonine	1.9	2.0	1.8	2.1	1.9	2.1	1.9
Valine	2.5	2.7	2.4	2.7	2.4	2.7	2.5
Non-essential amino acid composition							
Aspartic acid	4.0	4.4	4.3	4.1	4.5	4.4	4
Glutamic acid	6.5	6.8	6.9	7.1	6.7	6.7	6.6
Serine	1.9	2.5	2.3	2.7	2.4	2.9	2.6
Glycine	3.5	3.2	3.1	3.1	3	30	2.9
Alanine	4.1	3.9	4.1	3.9	4	4.0	4.2
Tyrosine	1.6	1.9	1.6	1.9	1.7	2.0	1.8

Fish maintenance and feeding

The growth trial was conducted at the Mariculture Research Station of the Aquaculture Research Center-South of Iran, Bandar Imam Khomeini . 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 (BW_i) = 16.7±0.1 g, mean± standard error). Fish were acclimated for 2 weeks before beginning of 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 considered under natural conditions (30°32′N, 49°20′E; 12 L: 12 D). Diets were tested in triplicate; fish were fed by hand to visual satiety three times per day (0900, 1300 and 1700h) for 60 days. Uneaten pellets were drained off and counted every day to calculate the total feed intake per tank using a mean dry pellet weight. The digestibility study was carried out during the last two weeks of the trial; tanks were siphoned of any uneaten feed at the night prior to fecal collection and feces were subsequently siphoned and collected with a mesh screen (30 µm) at the following morning, then rinsed with distilled water to remove excess salt before being frozen at −20 ºC until their analysis (Yaghoubi *et al.,* 2016).

Sample collection

At the start of the experiment, six fish were randomly sampled and stored at −18 °C for later determination of initial whole body proximate composition. At the end of the trial, fish were fasted for 24 h before being anaesthetized (2-phenoxyethanol at 0.5 ml L^{-1}; Merck, Schuchardt, Germany) and individually weighed (BW_f). Five specimens from each replicate were sacrificed with an overdose of the same anesthetic to evaluate their hepatosomatic index

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(HSI), viscerosomatic index (VSI) and intraperitoneal fat (IPF) (Yaghoubi *et al.*, 2016).

Fish growth, feed utilization and diet digestibility determination

The weight of fish, liver, intraperitoneal fat and viscera (with liver and intraperitoneal fat) and standard length (SL) were measured to the nearest 0.1g and 1 mm, respectively. The following formulas were used to assess growth performance, feed utilization and body condition parameters:

specific growth rate (%/day, SGR)= [(ln BW_f–ln BW_i)/t]×100, where t is experimental period (60 days); survival (%, S)=(number of fish in each group remaining on day 60/initial number of fish)×100; feed conversion ratio (FCR)=feed intake (g)/weight gain (g); protein efficiency ratio (PER)=weight gain (g)/protein intake (g); hepatosomatic index (%, HSI)=(liver weight (g)/BW_f)×100; viscerosomatic index (VSI, %)=(visceral weight (g)/BW_f); intraperitoneal fat index (IPF; %)=(IPF weight (g)/BW_f)×100; Fulton's condition factor (K)=(BW_f /SL^3)×100.

Apparent digestibility coefficients (ADCs) of proteins and lipids were calculated according to Maynard and Loosli, (1972) and Cho and Slinger, (1979) as follows: ADC of nutrients (%) =100–[100×(Cr_2O_3 in diet/Cr_2O_3 in faeces)]×[(% nutrient in faeces/% nutrient in diet)].

Biochemical analyses

Proximate analyses of ingredients, diets, whole body (n=3 fish per replicate, n=9 fish per treatment) and feces were determined using standard methods Association of Official Analytical Chemists (AOAC) (2005). Moisture was determined using a moisture analyzer (AMB50, ADAM, UK). Protein was determined by measuring nitrogen using the Kjeldahl method (BÜCHI, Auto-Kjeldahl K-370, Switzerland). To convert total nitrogen to total protein content, as a percentage of dry weight, the factor 6.25 (100/16) was used. Total body lipid was extracted by petroleum benzene using the Soxhlet method (Barnstead/Electrothermal, UK). Fiber content was analyzed with a fiber analyzer (VELP® Scientifica, Italy), while the ash content was determined for each dried sample in a porcelain crucible using a muffle furnace (Finetech, Shin Saeng Scientific, South Korea) at 600 °C for 8 h. Chromic oxide concentrations in the diets and feces was estimated according to the method of Furukawa and Tsukahara, (1966). All analyses were performed in triplicate (methodological replicates). Amino acids (except for tryptophan) were determined after hydrolysis of the samples (diets and whole body). Freeze-dried samples (Freeze dryer, Operon, OPRFDU 7012, Korea) were hydrolyzed in 6N HCl for 24 h at 110 °C in glass vials filled with nitrogen. The o-phthaldialdehyde (OPA) was used as a pre-column derivatization reagent according to Lindroth and Mopper, (1979). Total AA levels were determined by HPLC (Knauer, Germany) using C18 column (Knauer, Germany) at the flow rate of 1 ml min^{-1}

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with fluorescence detector (RF-530, Knauer, Germany).

Statistical analysis

Data were analyzed using the SPSS ver. 15.0 (Chicago, Illinois, USA). All the data were presented as means±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 by the one way ANOVA. The level of significance was set at $p<0.05$ for all tests.

Results

Survival, growth performance and feed utilization

Fish survival was not affected by experimental diets, ranging from 91.1% in $SP75^{+}$ to 100% in FM and SP45 diets ($p>0.05$; Table 3). Growth performance significantly decreased when dietary FM was replaced with SP, whereas BW_f ranged from 33.9±0.6 g (78.6% WG) to 42.7±1.2 g (151.6% WG) in fish fed SP75 and FM diets, respectively ($p<0.05$). Two-way ANOVA results showed that the growth parameters (BW_f, WG and SGR) were affected not only by FM substitution and CAA supplementation but also by their interactions. In the present study, fish fed FM diet had higher FI than the other groups; however, no differences were observed in regards to FM replacement and CAA supplementation and their interactions. FCR and PER increased and decreased respectively in fish fed diets in which over 60% of FM replaced by SP ($p<0.05$). These differences were observed regarding the FM replacement for both FCR and PER and were only affected by CAA supplementation in PER.

ADCs of protein and lipid were affected by experimental diets. Fish fed CAA supplemented diets had lower ADCs of nutrients than other groups. Apparent digestibility coefficients of protein were noticeably affected by FM substitution, CAA supplementation and their interactions, ranging from 96.2 to 98.2% in $SP45^{+}$and FM treatments, respectively ($p<0.05$). The apparent digestibility coefficient of lipid was only affected under the effects of CAA supplementation and ranged from 97.4 to 99.2% in fish fed $SP45^{+}$and FM diets, respectively ($p<0.05$). Fish fed CAA supplemented diets had lower ADCs of nutrients than the other groups. In the present trial, somatic parameters, including the K, HSI, VSI and IPF were not affected by different dietary treatments ($p<0.05$).

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Table 3: Growth, feed utilization and biometric 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).

Growth performance	Diets							Two way ANOVA (p value)*		
	FM	SP45	SP45^{+}	SP60	SP60^{+}	SP75	SP75^{+}	FM substitution	CAA supplementation	Interactions
BW_i (g) [a]	16.7±0.0	16.7± 0.1	16.7±0.1	16.6± 0.1	16.6±0.1	16.6±0.1	16.7± 0.0	-	-	-
BW_f (g) [b]	42.7±1.2^{a}	37.8±0.6^{ab}	38.1±1.8^{ab}	34.3±1.1^{bc}	35.9±1.6^{b}	33.9±0.6^{c}	35.4±1.8^{bc}	0.005	0.011	0.005
SGR (% body weight d^{-1}) [c]	1.6±0.1^{a}	1.4±0.0^{ab}	1.4±0.0^{ab}	1.2±0.0^{b}	1.3±0.0^{abc}	1.0±0.0^{c}	1.3±0.0^{abc}	<0.001	<0.001	<0.001
WG (%)	156.1±7.3^{a}	125.9± 2.5^{ab}	128.7±7.6^{ab}	101.6±9.4^{bc}	113.3±5.1^{bc}	78.6±7.8^{c}	101.7± 8.7^{bc}	<0.001	<0.001	0.003
S (%) [d]	100±0.0	97.8± 2.2	100± 0.0	97.8±2.2	97.8 ± 2.2	94.7±2.4	91.1±6.2	0.548	0.086	0.268
FI (g $fish^{-1}$)[e]	36.2±0.0^{a}	32.3± 0.0^{abc}	31.2±0.2^{bc}	30.4±0.2^{c}	31.9± 1.4^{abc}	30.2±0.3^{c}	32.6±1.2^{abc}	0.041	0.29	0.285
FCR[f]	1.4±0.1^{b}	1.5± 0.0^{b}	1.5±0.0^{b}	1.7±0.0^{a}	1.7±0.0^{a}	1.7±0.1^{a}	1.7 ± 0.1^{a}	<0.001	0.09	0.383
PER[g]	1.4±0.0^{a}	1.3±0.0^{abc}	1.4±0.0^{ab}	1.1±0.0^{bc}	1.2±0.0^{abc}	0.9±0.1^{c}	1.0 ± 0.1^{c}	<0.001	0.008	0.465
ADCs of protein[h]	98.2±0.2^{a}	97.9±0.1^{ab}	96.2±0.2^{d}	97.6 ±0.2^{bc}	97.1±0.2^{c}	97.7±0.1^{abc}	97.2 ± 0.2^{c}	0.036	<0.001	<0.001
ADCs of lipid	99.2±0.1^{a}	98.5±0.1^{bc}	97.4±0.1^{e}	98.3±0.1^{cd}	98.0±0.1^{d}	98.4±0.2^{c}	98.1 ± 0.1^{cd}	0.06	<0.001	<0.001
Somatic indices										
K[i]	2.9±0.1	2.8 ± 0.1	2.9±0.02	2.8 ± 0.1	2.9±0.1	2.7 ± 0.1	2.9 ± 0.06	0.529	0.122	0.728
HSI[j]	1.8±0.0	1.7 ± 0.1	1.6±0.1	1.6 ± 0.1	1.9± 0.2	2.0 ± 0.0	1.8 ± 0.3	0.296	0.875	0.269
VSI[k]	6.7±0.7	7.0 ± 0.5	5.1 ± 0.2	6.5 ± 0.7	4.5±0.3	7.8 ± 0.4	4.7 ± 0.7	0.458	0.610	0.324
IPF[l]	1.5±0.7	2.2 ± 0.3	1.89±0.18	2.0 ± 0.3	1.71±0.15	2.8 ± 0.8	1.96 ± 0.43	0.538	0.229	0.791

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

[a] IBW:initial body weight

[b] FBW:final body weight

[c] SGR: specific growth rate = ((ln final weight – ln initial weight) / t) × 100, where t is experimental period = 60 days

[d] S: survival = (number of fish in each group remaining on day 60 / initial number of fish) × 100

[e] Feed Consumption=feed intake/ fish number

[f] FCR : feed conversion ratio = weight gain (g) / feed intake (g)

[g] PER: protein efficiency ratio = weight gain (g) / protein intake (g)

[h] ADC of nutrients (%) = 100 – [100 × (cr_2o_3 in diet / cr_2o_3 in faeces)] × [(% nutrient in faeces / % nutrient in diet)]

[i] K : condition factor = (body weight (g)/ (body length (cm))^3) × 100

[j] HSI : hepatosomatic index = (liver weight (g) / whole body weight (g)) × 100

[k] VSI : viscerosomatic index = (viscera weight (g) / whole body weight (g)) × 100

[l] IPF : intraperitoneal fat = (IPF weight (g) / whole body weight (g)) × 100

Proximate composition and AA profile

In the present study, whole body moisture, protein and ash did not change among dietary treatments (p>0.05; Table 4); however, whole body lipid content was significantly affected by FM substitution with SP and CAA supplementation, as well as their interactions (p<0.05). In this context, the highest whole body lipid content was observed in SP75 (11.4±0.5).

The whole body AA composition of fish fed experimental diets is reported in Table 5. The entire AA assessed in this study except for valine was affected by dietary treatments (p<0.05). The lowest levels of histidine, isoleucine, lysine, methionine, phenylalanine and taurine were observed in fish fed the SP75 diet. Whole body threonine concentration increased; however, concentrations of taurine decreased with increasing dietary SP level (p<0.05). The supplementation of dietary Lys did not have any effect on whole body Lys concentrations; however, dietary Met supplementation had significant effect on the whole body Met concentrations (p=0.006). The whole body arginine, lysine, threonine, aspartic acid, glutamic acid and tyrosine were only affected by FM substitution rather than CAA supplementation or their interactions (p>0.05).

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Table 4: Composition of whole body proximate (% of wet weight) 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)*		
	Initial	FM	SP45	SP45^{+}	SP60	SP60^{+}	SP75	SP75^{+}	FM Substitution	CAA Supplementation	Interactions
Moisture	62.7 ± 1.0	67.1± 1.0	66.2± 1.5	68.7±1.6	68.9 ± 1.1	69.2 ± 1.5	65.9 ± 0.9	67 ± 1.9	0.25	0.277	0.593
Crude protein	17.7 ± 0.3	18.1± 0.5	18.7± 0.9	18.2 ± 1.2	17.9 ± 0.3	17.8 ± 0.8	17.5 ± 0.5	18.7 ± 1.1	0.821	0.747	0.704
Crude lipid	10.8 ± 0.2	9.9± 0.2^{ab}	10± 0.7^{ab}	7.9 ± 0.6 ^{b}	8.0 ± 0.4^{b}	8.4 ± 0.2 $_{b}$	11.7± 0. 5^{a}	9.4 ± 0.6^{b}	<0.001	0.005	0.024
Ash	5.9 ± 0.1	4.9 ± 0.1	5.2 ± 0.2	5.3 ± 0.3	5.3 ± 0.2	4.7 ± 0.2	4.9 ± 0.1	4.9 ± 0.2	0.284	0.272	0.263

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

Table 5: Amino acids profile of whole body (% of amino acids) 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 Substitution	CAA Supplementation	Interactions
Essential amino acid										
Arginine	7.4±0.4^{ab}	7.2±0.3^{bc}	7.4±0.2^{bc}	8.3±0.4^{a}	6.4±0.1^{c}	6.4±0.2^{c}	8.3±0.4^{a}	<0.001	0.805	0.891
Histidine	2.7±0 ^{a}	2.6±0^{a}	2.6±0 ^{a}	2.6±0.1^{a}	2.5±0 ^{b}	2.4±0 ^{b}	2.7±0 ^{a}	<0.001	0.026	0.813
Isoleucine	3.2±0.1 ^{a}	3.3±0.4^{a}	3.9±0.1^{a}	3.5 ± 0 ^{a}	3.5±0.1^{a}	2.3±0.6^{b}	3.5±0.1^{a}	0.042	0.017	0.097
leucine	6.9±0.3^{ab}	7 ± 0.3 ^{ab}	7.5±0.2 ^{a}	7.5±0 ^{a}	6.7±0.1^{b}	7.3±0.3^{ab}	7.1±0.2^{ab}	0.395	0.354	0.054
Lysine	8.1±0.2^{abc}	7.9±0.2^{bc}	7.7±0.1^{c}	8.2±0.2^{ab}	8±0.1 ^{abc}	7.6±0.1^{c}	8.5±0 ^{a}	0.004	0.226	0.145
Methionine	2.7± 0.2 ^{a}	1.9±0.2 ^{b}	2.2±0.1^{b}	1.9±0.1^{b}	2.1±0.1^{b}	1.3±0.2^{c}	1.9±0.2 ^{b}	0.09	0.006	0.074
Phenylalanine	3.8± 0.1 ^{c}	3.8±0.2 ^{c}	4±0^{bc}	4.6±0.2^{ab}	4.7±0.1^{a}	3.5±0.3^{c}	4.7±0.1 ^{a}	0.003	0.002	0.02
Threonine	3.7± 0.1 ^{c}	3.9±0.7^{c}	3.8±0.5^{c}	4.6±0.5^{b}	4.6±0.3^{b}	5.4±0.6^{a}	5.2±0.1^{ab}	<0.001	0.554	0.177
Valine	4.3± 0.4	3.7± 0.3	4.2±0.1	3.8±0.1	4.5±0.1	4 ± 0.4	4 ± 0	0.355	0.067	0.893
Non-essential amino acid composition										
Aspartic	9.5±0.8 ^{ab}	10.3±0.3^{ab}	10±0.7^{ab}	10.9±0.5^{ab}	11.3±0.1^{a}	8.7 ± 1 ^{b}	8.9±0.7 ^{b}	0.01	0.909	0.845
Glutamic	14.8±0.5^{a}	14.2±0.3^{a}	14±0.6^{a}	15.7±0.6^{a}	16.1±0.1^{a}	11.9±1.4^{b}	11.4±0.3^{b}	<0.001	0.843	0.799
Serine	4.1±0.3^{b}	6±0.9^{a}	4.3±0.4^{b}	4.3±0.5^{b}	2.7±0.3 ^{c}	4.6±0.2^{ab}	4.8±0.2 ^{ab}	0.006	0.009	0.091
Glycine	11.4±0.7^{ab}	10.5±1.5^{b}	10.0±0.4^{b}	12.1±0.9 ^{a}	10.9±0.5^{b}	10.3±0.7^{b}	10.3±0.1^{b}	0.777	0.313	0.115
Taurine	1.2±0.1 ^{a}	0.7±0.1^{b}	0.6±0.1^{bc}	0.5±0 ^{bc}	0.6±0.1^{bc}	0.4± 0 ^{c}	0.5±0.1 ^{bc}	0.066	0.366	0.439
Alanine	7.1± 0.2 ^{c}	7.5±0.1 ^{b}	7.6±0^{b}	6.2±0.1 ^{b}	7.0±0.1^{c}	8.1± 0.1 ^{a}	7.9±0.2 ^{ab}	<0.001	0.04	0.005
Tyrosine	2.9± 0.1 ^{b}	2.9 ± 0.1^{b}	3.1±0.1^{b}	2.2±0^{c}	2.3±0^{c}	3.5± 0.3 ^{a}	3.8± 0.1 ^{a}	<0.001	0.129	0.535
Total EAA	42.7±1.5^{bc}	41.3±2.5^{c}	44±0.6^{abc}	45±1.3 ^{ab}	43.7±0.4^{abc}	40.3±1.4^{c}	46.5±0.6^{a}	0.021	0.137	0.369
Total NEAA	56.3±1.5^{a}	56.7±2.3^{a}	54.1±0.7^{b}	53.7±1.1^{bc}	55.5±0.6 ^{b}	56.1±1.4^{a}	51.9±0.7^{c}	0.016	0.235	0.095

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

Discussion

Fish survival was not affected by experimental diets, which is in line with the results reported in other sparid species fed PP based diets (Biswas *et al.*, 2007; Hernández *et al.*, 2007; Zhou *et al.*, 2011). Growth performance significantly decreased by replacement of FM with SP. In this context, it has been reported that the replacement of a large fraction of FM with PP sources tended to decrease growth performance in many marine carnivorous fish species. This reduction in growth performance is the consequence of the low palatability, low protein

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digestibility, low availability of EAA and minerals, and also due to the presence of the ANFs in SP, which led to a feed intake reduction (Glencross *et al*., 2007; Lim and Lee, 2009; Song *et al*., 2014; Trushenski *et al*., 2014; García-Ortega *et al*., 2015). On the other hand, the results of the current study also showed that fish fed those diets with high levels of FM substitution and supplemented with CAA had slightly higher growth performance than the fish fed diets without CAA supplementation (but not significant). The supplementation of the Lys and Met in PP based diets has been commonly recommended to compensate the dietary deficiency of EAA and improve growth performance in different fish species (Mambrini *et al*., 1999; Deng *et al*., 2006; Silva *et al*., 2009). Decreasing FI and PER and increasing FCR in fish fed experimental diets regarding to FM diet might be related to lower protein quality of SP, as it has been reported by Ye *et al.* (2011) in Japanese flounder *Paralichthys olivaceus*.

Similarly apparent digestibility coefficient of protein observed in this study, it has also been reported that ADCs of protein significantly decreased with increasing SP levels in diets for Japanese flounder (Deng *et al*., 2006), European sea bass *Dicentrarchus labrax* (Tibaldi *et al*., 2006) and sharpsnout seabream *Diplodus puntazzo* (Hernández *et al*., 2007). Non-starch polysaccharides and other ANFs such as phytic acid, α-galactosides, alkaloids, amphiphilic globulins, trypsin inhibitors, antilipase and anticolipase factors in SP may lead to a decrease in bioavailability and ADCs of nutrients (Francis *et al.*, 2001; Gatlin *et al.*, 2007). On the other hand, fish fed CAA supplemented diets had lower ADCs of nutrients than the other groups. In contrast to the results of the current study, Zhang *et al.* (2016) reported that supplementation of crystalline Met and Lys in SP-based diets did not affect ADCs of proteins in Japanese sea bass. The divergence between the present study and the above-mentioned study of Zhang *et al.* (2016) may be due to differences in digestibility of feed ingredients between both studies. Similar to our results in somatic parameters, replacement of FM by PP did not affect HSI, VSI and IPF values in other sparid species like gilthead seabream *Sparus aurata* (Gómez-Requeni *et al*., 2004) and red seabream *Pagrus major* (Kader *et al*., 2012). However, substitution of FM by fermented soybean meal significantly increased the VSI as the consequence of lipid deposition in visceral cavity, but it did not affect the HSI or K in juvenile black seabream *Acanthopagrus schlegelii* (Azarm and Lee, 2014). These results indicated that the effects of replacement of FM by PP on somatic parameters might depend on the source and processing techniques of PP, which affect lipid and ANFs content in PP sources (Gatlin *et al*., 2007). Increasing whole body lipid content, especially in fish fed the SP75 diet was attributed to the lower whole body taurine concentrations, which may stimulate lipogenesis as it has been recently reported in Atlantic salmon *Salmo salar* (Espe *et al*., 2010). In addition, high dietary PP contents generally lead to an

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increase in the whole body lipid deposition levels in other marine carnivorous fish species, which may be also due to the high carbohydrate and/or low taurine and carnitine levels in PP sources (Lim *et al*., 2004; Zhou *et al*., 2005; Martínez-Llorens *et al*., 2009). Consumption of carbohydrate-rich PP based diets tends to stimulate lipogenic enzymes such as glucose-6-phosphate dehydrogenase, which have main role in lipogenesis through pentose phosphate metabolic pathway (Kamalam *et al*., 2013). On the other hand, supplementation of Lys and Met reduced lipid deposition. Met and Lys as the precursors for biosynthesizing of carnitine may have an important role in mitochondrial lipid β-oxidation (Harpaz, 2005). Carnitine plays a key role in lipid metabolism by the transportation of fatty acids from cytoplasm to mitochondrial matrix for β-oxidation through carnitine shuttle system (Dayanand *et al*., 2011). Thus, supplementation of Lys and Met might be increased the biosynthesis of carnitine that resulted in an increase in lipid β-oxidation and lower whole body lipid content in fish fed CAA supplemented diets. There is a close relationship between dietary AA supply, their bioavailability and concentrations in different tissues (Gómez-Requeni *et al*., 2004). In the present study, the whole body AA composition mainly affected by FM substitution with SP rather than CAA supplementation. It has been reported that CAA may not rapidly be taken up by tissues in comparison with protein-bound AA and tissues preferentially use CAA as energy sources by donating their carbon chain during the oxidation process rather than deposit them (Nunes *et al*., 2014). The lowest levels of histidine, isoleucine, lysine, methionine, phenylalanine and taurine were observed in fish fed the SP75 diet, which may be linked to the low bioavailability and/or digestibility of these AAs in SP. In this study, whole body taurine decreased with increasing FM substitution, which in line with the results reported in other marine fish species, fed diets with PP sources (Deng *et al*., 2006; Valente *et al*., 2011). The supplementation of dietary Met had significant effect on whole body Met concentration. These results suggested that Met might accumulate in the whole body when the total dietary methionine supply exceeds its requirements. In this context, Mambrini *et al.*, (1999) reported dietary supplementation of Met led to an increase in plasma Met in rainbow trout *Oncorhynchus mykiss.* On the other hand, a taurine-sparing effect was not observed in fish fed diets with Met supplementation, which suggested that silvery-black porgy did not have sufficient ability to convert Met to taurine, as it has also been reported in other marine carnivorous fish (El-Sayed, 2014; Salze and Davis, 2015). Thus, taurine supplementation would be necessary for optimal growth performance and physiological functions in silvery-black porgy juveniles, as well as in other marine carnivorous fish species when they fed PP based diets, since it plays a critical role in osmoregulation, membrane

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protection, antioxidative defenses, detoxification mechanisms and enhancement of bile salts production (El-Sayed, 2014; Salze and Davis, 2015). Marine fish species have negligible ability of taurine synthesis due to the absence of or low cysteine sulphinate decarboxylase activity through the trans-sulphuration pathway from sulphur AA (El-Sayed, 2014). In this context, the inclusion of high levels of PP in red seabream diet resulted in poorer growth performance, because of low or inability of this species to synthesize taurine, and supplementation of 0.5 to 1% taurine was required for their optimum performance (Takagi *et al*., 2011). Similarly, it also reported that, dietary supplementation of 0.2% taurine improved growth rate, feed efficiency and lipid metabolism in common dentex (*Dentex dentex*) juveniles fed SP-based diets (Chatzifotis *et al*., 2008).

In conclusion, the replacement of FM by SP with supplementing Met and Lys slightly improved growth performance in silvery-black porgy juveniles, which suggested that ANFs rather than Met and Lys deficiency were limited when SP substituted FM in this species. The low growth performance associated with low ADCs of nutrients may be because of presence of ANFs such as phytic acid and enzymes inhibitors in the SP. In addition, whole body taurine levels decreased with increasing dietary SP, and taurine sparing effect was not observed in fish fed Met-supplemented diets, indicating a negligible capacity in this species of taurine synthesis. The results of this study suggest that the anti-nutritional factors in SP rather than Met and Lys deficiency might limit FM substitution with SP in silvery-black porgy juveniles.

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