Received: 21 November 2016 | Revised: 27 February 2017 | Accepted: 3 March 2017

DOI: 10.1111/are.13344

ORIGINAL ARTICLE

WILEY Aquaculture Research

Effects of dietary essential amino acid deficiencies on the growth performance and humoral immune response in silvery-black porgy (*Sparidentex hasta*) juveniles

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Funding information
Iran National Science Foundation, Grant/Award Number: 92011610.

Abstract

A 6-week feeding trial was conducted for determining the effects of dietary essential amino acids (EAA) deficiencies on growth performance and non-specific immune responses in silvery-black porgy juveniles (4.7 ± 0.1 g initial weight). Eleven isoproteic (*ca.* 47%) diets were formulated including a control diet containing the optimum quantity of EAA, and ten EAA-deficient diets. All diets contained 36% fish meal and 18.5% crystalline EAA and non-essential amino acids (NEAA) as the main source of dietary proteins. All the EAA and NEAA incorporated in the crystalline amino acids mixture of the control diet simulated the amino acids profile of the fish meal. The other 10 EAA-deficient diets were formulated by the deletion of each of the 10 EAA (crystalline form) from the control diet and replaced by a mixture of NEAA for the adjustment of dietary nitrogen contents. At the end of the experiment, fish fed with threonine-deficient diet showed the lowest survival rate ($p < .05$), whereas growth performance decreased in fish fed all EAA-deficient diets, although the reduction in body growth varied depending on the EAA considered. Plasma total protein decreased in all experimental groups except for fish fed the phenylalanine-deficient diet. Fish fed with arginine- and lysine-deficient diets had the lowest plasma C3, C4, lysozyme, total immunoglobulin and total superoxide dismutase activity ($p < .05$). Present results indicated that lysine, methionine and threonine were the most limiting EAA in terms of growth performance; however, arginine, threonine and lysine were the most limiting EAA for innate immunity responses in silvery-black porgy juveniles.

KEYWORDS
essential amino acids, growth performance, haematology, humoral immunity, Sparidae

1 | INTRODUCTION

The reduction in dietary protein content, as the most expensive ingredient of the diet, will improve fish production by lowering feed costs, optimizing protein utilization and reducing nitrogen excretion. Moreover, the replacement of dietary fish meal (FM) with alternative protein sources such as plant proteins and crystalline amino acids (CAA) in aqua feeds is required for sustainability of aquaculture industry (Gatlin et al., 2007). Thus, determining the dietary essential amino acid (EAA) requirements in a given species is a prerequisite factor for avoiding feed EAA deficiencies and/or imbalances that may affect fish performance and condition (Mambrini & Kaushik, 1994). Moreover, protein and EAA deficiencies have long been recognized to impair immune function and

increase the susceptibility of animals to infectious diseases, as protein malnutrition reduces the concentration of most plasma AA, and these have an important role in the immune response (Li, Yin, Li, Kim & Wu, 2007). However, available data on the effects of protein and EAA deficiencies in health and immune nutrition are relatively scarce in fish (Kiron, 2012; Oliva-Teles, 2012; Trichet, 2010). Essential amino acids are important regulators of key metabolism pathways that are necessary for maintenance, growth, reproduction and immunity in all organisms (Wu, 2009). Several studies in mammals have shown that dietary protein in general or AA in particular has important roles in immune responses by regulating different cellular and molecular mechanisms of the immune system (Li et al., 2007; Wu, 2009). For instance, branched-chain amino acids including valine, leucine and isoleucine are essential for the function of immune cells through their roles in protein synthesis (e.g., antibodies, immunoglobulins and acute phase proteins) (Calder, 2006). Moreover, it has been shown that sulphur AA, including methionine and its derivatives can affect immune responses through the metabolism of glutathione, homocysteine and taurine by changing the redox state of immune cells and ameliorating inflammatory reactions (Grimble, 2006). Aromatic AA (phenylalanine and tyrosine) and tryptophan can also modulate immune responses by regulating the synthesis of tetrahydrobiopterin (a cofactor for nitric oxide synthesis), hormones (catecholamines and thyroid hormones) and neurotransmitters (dopamine, serotonin, *N*-acetylserotonin and melatonin) (Li et al., 2007; Wu, 2009). Furthermore, arginine and lysine have essential role in production and regulation of nitric oxide synthesis, which is an important antimicrobial as well as signalling molecule in the immune system (Bogdan, 2001; Li et al., 2007). On the other hand, threonine is the most abundant AA in mucins, which are required for normal intestinal immune function. Mucins produced by goblet cells also provide a physiological and immunological barrier to a wide range of micro-organisms and foreign substances (Lallès, 2010). In addition, threonine is also a major component of plasma γ-globulins in different animal species (Li et al., 2007). Furthermore, histidine has an important role on the immune function through histidine-rich-glycoproteins and histamine (Poon, Patel, Davis, Parish & Hulett, 2011). In addition, dietary EAA can modulate immune system responses by non-specific cellular (i.e., phagocytosis and respiratory burst activity) and humoral (i.e., lysozyme and alternative complement activity) responses (Li, Mai, Trushenski & Wu, 2009; Cheng, Buentello & Gatlin, 2011; Costas et al., 2011; Pohlenz, Buentello, Mwangi & Gatlin, 2012; Rahimnejad & Lee, 2013, 2014a,b), cytokines production (Feng et al., 2015; Luo et al., 2014; Zhao et al., 2013), intestinal immune activities (Luo et al., 2014; Feng et al., 2015; Ren et al., 2015) and regulation of immune gene expression (Costas et al., 2011; Feng et al., 2015; Giri et al., 2015; Habte-Tsion, Ge, et al., 2015; Luo et al., 2014; Ren et al., 2015; Wen et al., 2014; Zhao et al., 2013) in different fish species. These data clearly indicate that EAA have an important role in the immune system; thus, evaluating the impact of dietary EAA imbalances is worthy of investigation.

Silvery-black porgy (*Sparidentex hasta*) is recognized as a potential species for aquaculture diversification in Persian Gulf and the Oman Sea regions, because of its good adaptation to captivity, rapid growth and high market price (Basurco, Lovatelli & García, 2011). In this context, efforts have been focused on establishing the nutritional requirements for this warm-water species and optimizing diet formulation (Hossain, Al-Abdul-Elah & El-Dakour, 2014; Mozanzadeh, Yavari, Marammazi, Agh & Gisbert, 2015; Mozanzadeh, Marammazi, Yavari et al., 2015; Mozanzadeh, Agh, et al., 2016; Mozanzadeh, Yavari, Marammazi, Agh & Gisbert, 2016; Yaghoubi, Mozanzadeh, Marammazi, Safari & Gisbert, 2016). Moreover, Marammazi, Yaghoubi, Safari, Peres and Mozanzadeh (2017) have recently established the optimum dietary EAA pattern for this species by means of the deletion method. According to the above-mentioned research, the optimal dietary EAA profile for silvery-black porgy juveniles was estimated to be (g/16 g N): arginine 5.3, lysine 6.0, threonine 5.2, histidine 2.5, isoleucine 4.6, leucine 5.4, methionine + cysteine 4.0 (in a diet containing 0.6 cysteine), phenylalanine + tyrosine 5.6 (in a diet containing 1.9 tyrosine), tryptophan 1.0 and valine 4.6. However, to our knowledge there is no available information about the effects of dietary EAA deficiency on physiological responses in this species. Thus, this study aimed to evaluate how EAA deficiencies may affect the growth performance and some humoral immune parameters and to clarify which EAA have the most relevant role in modulating different immune responses in silvery-black porgy.

2 | MATERIALS AND METHODS

2.1 | Experimental diets

Eleven isoproteic (*ca.* 47%) and isoenergetic (*ca.* 20.5 MJ/kg) diets were formulated to evaluate the effects of EAA deficiency on growth performance and non-specific humoral immune responses in silvery-black porgy juveniles. Sixty per cent of the dietary protein was provided by intact protein sources (FM and gelatin), whereas the rest was provided by CAA. The CAA mixture was prepared by blends of EAA and NEAA, which were coated with 1% agar in order to delay their absorption and to optimize their use for protein accretion (Mambrini & Kaushik, 1994). All the EAA and NEAA incorporated in the CAA mixture of the control diet simulated the AA profile of FM from *Clupeonella* sp and matched the EAA nutritional requirements for this species as described by Marammazi et al., 2017;. The other 10 experimental diets were formulated by the deletion of each one of the 10 EAA (crystalline form) from the control diet and replaced by a mixture of NEAA to adjust their dietary N content (Peres & Oliva-Teles, 2009; Rollin, Mambrini, Abboudi, Larondelle & Kaushik, 2003). Thus, we were able to get a 40% reduction for each of the tested EAA in each EAA-deficient diet, while all other EAA were in the same proportions in comparison with the control diet (Tables 1 and 2). Feed used in this study was prepared by mixing all dry ingredients for 30 min. Then, fish oil and sufficient distilled water, as well as

TABLE 1 Ingredient composition and proximate analysis of the experimental diets

	Diets										
Dietary ingredients (g/100 g dry diet)	Control	ARG	LYS	THR	HIS	ILE	LEU	MET	PHE	TRP	VAL
Fish meal[a]	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0	36.0
Gelatin[b]	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Wheat meal[c]	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Corn starch[d]	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
Fish oil[e]	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0
Agar[f]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[g]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Mineral premix[h]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
L-arginine	0.85	0	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
L-lysine-HCl	1.15	1.15	0.0	1.15	1.15	1.15	1.15	1.15	1.15	1.15	1.15
L-threonine	0.8	0.8	0.8	0.0	0.8	0.8	0.8	0.8	0.8	0.8	0.8
L-histidine	0.5	0.5	0.5	0.5	0.0	0.5	0.5	0.5	0.5	0.5	0.5
L-isoleucine	0.85	0.85	0.85	0.85	0.85	0.0	0.85	0.85	0.85	0.85	0.85
L-leucine	1.4	1.4	1.4	1.4	1.4	1.4	0.0	1.4	1.4	1.4	1.4
L-methionine	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.0	0.6	0.6	0.6
L-phenylalanine	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.0	0.75	0.75
L-tryptophan	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.0	0.2
L-valine	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.0
NEAA mixture[i]	10.45	11.3	11.85	11.25	10.95	11.3	11.85	11.05	11.2	10.65	11.4
Proximate composition (%)											
Dry matter	92.91	92.98	92.02	92.04	92.03	92.1	91.95	92.5	92.14	91.98	91.97
Protein	46.67	47.75	47.92	46.29	47.53	47.37	47.44	46.87	46.71	48.44	48.17
Crude lipid	20.14	19.51	18.91	20.07	18.66	19.47	19.26	19.23	19.29	19.82	20.16
Crude fibre	1.08	0.76	0.41	0.18	0.12	0.68	0.46	0.43	0.28	0.46	0.36
Ash	6.75	6.07	5.84	5.65	6.01	6.32	6.18	6.24	6.24	6.03	6.31
Gross energy (MJ/kg)[j]	20.92	20.79	20.46	20.61	20.25	20.38	20.42	20.35	20.33	20.51	20.56
NFE[k]	19.14	19.64	19.34	19.59	19.83	18.93	19.06	20.16	19.89	17.68	17.12

Diets were named according to their deficiency in each EAA.

[a]Fish meal (*Clupeonella* sp.); Parskilka Mazandaran, Iran (63.5% crude protein, 17.7% crude lipid).

[b]Gelatin; Beyza feed mill, Shiraz, Iran. (85% crude protein, crude lipid, 4.2).

[c]Wheat meal; Beyza feed mill, Shiraz, Iran.12% crude protein, 3% crude lipid).

[d]Corn starch, Beyza feed mill, Shiraz, Iran. (3.7% crude lipid);

[e]Parskilka Mazandaran, Iran (*Clupeonella* sp.).

[f]Merck, Germany.

[g]Vitamin premix U/kg of premix: vitamin A, 5,000,000 IU; vitamin D3, 500,000 IU; vitamin E, 3,000 mg; vitamin K3, 1,500 mg; vitamin B1, 6,000 mg; vitamin B2, 24,000 mg; vitamin B5, 52,000 mg; vitamin B6, 18,000 mg; vitamin B12, 60,000 mg; Folic acid, 3000 mg; nicotinamide 180,000 mg; antioxidant, 500 mg, career up to1 kg, Damloran pharmaceutical company, Broujerd, Iran.

[h]Mineral premix U/kg of premix: copper: 3,000 mg; zinc: 15,000 mg; manganese: 20,000 mg; iron: 10,000 mg; potassium iodate: 300 mg, career up to 1 kg, Microvit®, Razak Laboratories, Tehran, Iran.

Crystalline amino acids: Merck, Germany, except isoleucine (Sigma-Aldrich, USA).

[i]Non-essential amino acids mixture (% mixture): L-alanine: 13; L-aspartic acid: 20; sodium glutamate: 32; L-glycine: 15; L-serine: 10; and L-proline: 10, Merck, Germany.

[j]Calculated on gross energy values of 23.6 kJ/g proteins, 39.5 kJ/g fat and 17.2 kJ/g carbohydrates (NRC 2011).

[k]Nitrogen-free extract = 100 − (protein + lipid + ash + fibre).

the CAA mixture, were added to form a soft dough that was mechanically extruded to obtain pellets of *ca.* 2 mm in size. Pellets were dried in a convection oven at 25°C and stored in resealable plastic bags at −20°C until their use as described in Marammazi et al. (2017).

2.2 | Fish maintenance and experimental design

This study was conducted at the Mariculture Research Station of the South Iranian Aquaculture Research Center (SIARC) (Sarbandar, Iran). Four hundred ninety-five silvery-black porgy juveniles (initial body

TABLE 2 Amino acids profile of the experimental diets (n = 1), g/100 g diet

	Diets										
	Control	ARG	LYS	THR	HIS	ILE	LEU	MET	PHE	TRP	VAL
Arginine	2.56	1.73	2.5	2.58	2.48	2.45	2.62	2.54	2.55	2.46	2.68
Lysine	2.15	2.17	1.29	2.15	2.14	2.18	2.18	2.14	2.2	2.15	2.15
Threonine	1.90	1.95	1.92	1.12	1.86	1.8	1.78	1.8	1.72	1.98	2
Histidine	1.15	1.2	1.1	1.24	0.66	1.08	1.02	1.3	1.24	1.2	1.2
Isoleucine	2.07	2.1	2.12	2.12	2.24	1.24	2.02	2.08	2.16	2.22	2.3
Leucine	2.43	2.38	2.32	2.36	2.39	2.42	1.48	2.45	2.3	2.40	2.34
Methionine	1.38	1.40	1.44	1.32	1.38	1.34	1.46	0.79	1.36	1.38	1.41
Cysteine[a]	0.27	0.25	0.32	0.31	0.29	0.28	0.28	0.25	0.22	0.26	0.27
Phenylalanine	1.84	1.86	1.80	1.85	1.89	1.92	1.88	1.81	1.11	1.9	1.9
Tyrosine	0.88	0.87	0.85	0.92	0.87	0.82	0.81	0.83	0.85	0.85	0.87
Tryptophan[a]	0.45	0.48	0.46	0.43	0.45	0.44	0.47	0.42	0.44	0.26	0.42
Valine	2.13	2.15	2.16	2.19	2.08	2.13	2.18	2.14	2.13	2.12	1.28
Alanine	3.62	3.75	3.72	3.68	3.69	3.71	3.76	3.78	3.75	3.79	3.8
Aspartic acid	5.5	5.62	5.66	5.65	5.61	5.68	5.58	5.6	5.59	5.67	5.61
Glutamic acid	7.8	7.9	7.88	7.85	7.83	7.80	7.85	7.84	7.84	7.84	7.92
Glycine	5.00	5.16	5.33	5.15	5.10	5.16	5.26	5.11	5.14	5.04	5.18
Proline	2.34	2.26	2.25	2.28	2.32	2.38	2.36	2.36	2.34	2.37	2.33
Serine	2.04	2.12	2.21	2.12	2.09	2.12	2.18	2.10	2.11	2.06	2.13

Diets were named according to their deficiency in each EAA.
[a]These amounts were calculated based on the ingredient cysteine and tryptophan composition.

weight, BW_i = 4.7 ± 0.1 g; mean ± *SD*) produced at the same hatchery were used in this trial. Fish were randomly distributed into 33 cylindrical polyethylene tanks (250 L of volume) at the density of 15 tank^{-1} and acclimated for 2 weeks before the onset of the nutritional trial, period during which a commercial diet (Biomar, France; size 2 mm; 54% crude protein, 18% crude lipid, 10% ash, 1% fibre; 25.4 MJ/kg digestible energy) was progressively replaced by the control diet. Tanks were supplied with filtered running seawater (1 L/min; salinity = 48.0 ± 0.5 ‰) and maintained at a temperature of 28.9 ± 1.5°C under natural photo-thermal conditions (30°32′N, 49°20′E). Average for dissolved oxygen and pH was 6.8 ± 0.4 mg/L and 7.6 ± 0.2 respectively. Triplicate groups of fish were handfed one of the above-mentioned experimental diets to visual satiation three times per day (0800, 1300 and 1800 hours) for 42 days. Uneaten feed was siphoned out 1 hr after feeding and weighed after being dried in convection oven (45°C for 24–36 hr) to determine feed intake values.

2.3 | Sampling and growth performance and feed efficiency calculations

At the end of the trial, fish were fasted for 24 hr before being anaesthetized (2-phenoxyethanol at 0.5 ml/L; Merck, Schuchardt, Germany) and individually weighed (BW_f). In addition, six specimens from each replicate were anaesthetized with 2-phenoxyethanol and blood was collected from the caudal vein with heparinized syringes (500–700 μl per specimen) and pooled together (each pool contained the blood from three fish). A 1 ml aliquot of blood was used for the analysis of haematological parameters, and two additional 1 ml aliquots were centrifuged (4,000 *g*, 10 min, 4°C) and plasma separated. The vials containing plasma samples were then transferred into liquid nitrogen and stored at −80°C until further analysis.

The following formulae were used to evaluate body growth performance and feed utilization parameters:

$$\text{body weight gain (WG, \%)} = [(BW_f - BW_i)/BW_i] \times 100;$$
$$\text{specific growth rate (SGR, \%)} = [(\ln BW_f - \ln BW_i)/t] \times 100,$$

where t is experimental period (42 days); survival (S, %) = (number of fish in each group remaining on day 42/initial number of fish) × 100; feed intake (FI) = total feed intake (g)/number of fish and feed conversion ratio (FCR, %) = (feed intake (g)/weight gain (g)).

2.4 | Chemical analyses

Proximate analyses of ingredients and diets were determined using standard methods (AOAC 2005). Dry matter 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 petroleumbenzene using the

Soxhlet method (Barnstead/Electrothermal, UK). Fibre content was analysed with a fibre 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 hr. The gross energy values calculated based on the proximate composition of samples using 23.6, 39.5 and 17.2 kJ/g for proteins, fat and carbohydrates respectively (NRC 2011). The amino acid composition of experimental diets and fish was determined after acid hydrolysis (6 N, 110°C, 24 hr) of freeze-dried samples (Freeze dryer, Operon, OPRFDU 7012, Korea). 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 with a fluorescence detector (RF-530, Knauer, Germany). Peak identification and integration was performed by the software Waters Empower 2 (Milford, MA) using an AA standard H (Pierce, USA) as an external standard. Levels of tryptophan and cysteine could not be measured because of the susceptibility of these two AA.

2.5 | Haematological and plasma biochemical analyses

Haematocrit (%; Hct), haemoglobin concentration (Hb; g/dl) and the number of red blood cells (RBC) and white blood cells (WBC) were assessed according to methods described by Blaxhall and Daisley (1973). In this regards, Hct was measured by microcentrifugation method and determination of the percentage of packed cell volume after centrifuging in standard heparinized microhaematocrit capillary tubes (3,500 *g*, 10 min). Haemoglobin concentration was spectrophotometrically assayed by cyanmethemoglobin method. Red blood cell and WBC were counted manually using an improved Neubauer haemocytometer after diluting blood samples by adding modified Dacie's fluid for RBC and Turk solution for WBC. The mean cell volume (MCV) was calculated according to Lewis, Bain and Bates (2001): MCV (fl) = Hct (%)/RBC ($\times 10^6$) $\times$ 10. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein were analysed by means of an autoanalyzer (Technicon RA-1000, Technicon Instruments, NewYork, NY, USA) using commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran).

2.6 | Plasma non-specific immunological parameters

Lysozyme activity was measured using the turbidimetric method described by Ellis (1990) that measures the lytic activity of plasma against lyophilized *Micrococcus lysodeikticus* (Sigma, St Louis, MO, USA). The optical density was measured after 15 and 180 s at 670 nm, and results are given as lysozyme μg/ml. Levels of complement components 3 (C3) and 4 (C4) were measured according to the method described by Tang, Wu, Zhao and Pan (2008) using the kit from Pars Azmon (Tehran, Iran). In brief, plasma samples were automatically mixed with an antibody provided by the test kit and then, an antigen-antibody complex was formed. The change in absorbance was read at 340 nm over a fixed-time interval (15 min), which is directly proportional to the complement C3 and C4 concentrations in the sample. Plasma total immunoglobulin (Ig) was measured using the method described by Siwicki et al. (Siwicki, Anderson & Rumsey, 1994). Primary separation of immunoglobulins from the plasma was achieved by precipitation with polyethylene glycol (PEG) and the resulting supernatant analysed. To perform the assay, 100 μl of plasma was combined with 100 μl 12% PEG and incubated at room temperature for 2 hr in continuous agitation. Following the incubation time, the mixture was centrifuged (400 *g*, 10 min at room temperature) and total protein concentration in the supernatant determined using the biuret method (Kingsley, 1942). Total immunoglobulin value for individuals was calculated from the total protein value less the quantity of protein in the supernatant. Total superoxide dismutase (t-SOD) activity in plasma was measured by its ability to inhibit superoxide anion reduction in nitrobluetetrazolium (NBT) generated by xanthine-xanthineoxidase reaction using a commercial kit (Sigma-Aldrich, Switzerland) according to the manufacturer's instructions. One unit of SOD activity was defined as the amount of enzyme necessary to produce a 50% inhibition of the NBT reduction rate measured at 550 nm. All immunological parameters were measured in triplicate (methodological replicates) using a microplate scanning spectrophotometer (PowerWave HT, BioTek®, USA).

2.7 | Statistical analysis

Data were analysed using SPSS version 15.0 (Chicago, IL, USA). All data are presented as means $\pm$ *SEM*. Equality of variances was tested with Levene's test and normality with Shapiro–Wilk's test. Arcsine transformations were conducted on all percentage data to achieve homogeneity of variance before statistical analysis. One-way ANOVA analysis of variance was performed at a significance level of .05 following the confirmation of normality and homogeneity of variance. Tukey's procedure was used for multiple comparisons.

3 | RESULTS

Survival, growth performance and feed utilization were significantly affected by dietary EAA deficiencies (Table 3; $p < .05$). Survival rates in fish fed the control and threonine (1.12% DM)-deficient diets were the highest (100%) and the lowest (81.1 $\pm$ 1.1%) ones, respectively, whereas the other groups showed intermediate values ($p < .05$). Growth performance parameters such as BW_f, WG and SGR significantly decreased in all groups fed EAA-deficient diets, but the extent of growth reduction depended on the EAA considered ($p < .05$). In particular, fish fed lysine-, threonine- and methionine-deficient diets (1.29%, 1.12% and 0.79% DM respectively) showed the largest reduction in growth performance ($p < .05$); however, dietary deficiencies in arginine, isoleucine and phenylalanine (1.73%, 2.02% and 1.11% DM respectively) resulted in the lowest growth reduction in comparison with the control diet ($p < .05$). Regarding

TABLE 3 Growth, survival and feed utilization of *S. hasta* juvenile fed different experimental diets at the end of growth trial (mean ± *SE*, *n* = 3)

	Diet										
	Control	ARG	LYS	THR	HIS	ILE	LEU	MET	PHE	TRP	VAL
BW_i (g)[a]	4.6 ± 0.1	4.7 ± 0.1	4.7 ± 0.0	4.7 ± 0.0	4.8 ± 0.0	4.6 ± 0.0	4.7 ± 0.1	4.7 ± 0.0	4.6 ± 0.0	4.6 ± 0.0	4.8 ± 0.1
BW_f (g)[b]	12.8 ± 0.5^{a}	12.0 ± 0.6^{ab}	7.8 ± 0.3^{d}	7.9 ± 0.3^{d}	9.2 ± 0.5^{cd}	10.4 ± 0.4^{bc}	9.9 ± 0.2^{c}	8.8 ± 0.4^{cd}	10.1 ± 0.3^{bc}	9.2 ± 0.2^{cd}	10.0 ± 0.4^{c}
WG (%)[c]	177.0 ± 5.9^{a}	156.3 ± 4.4^{b}	66.9 ± 2.0^{g}	68.4 ± 1.5^{fg}	93.3 ± 5.4^{de}	124.2 ± 1.9^{c}	112.9 ± 3.1^{cd}	87.6 ± 1.0^{ef}	120.0 ± 2.6^{c}	98.3 ± 1.3^{de}	109.7 ± 7.9^{bcd}
SGR (% body weight/day)[d]	2.4 ± 0.1^{a}	2.2 ± 0.1^{a}	1.2 ± 0.1^{f}	1.2 ± 0.0^{f}	1.6 ± 0.1^{de}	1.9 ± 0.2^{b}	1.8 ± 0.0^{bc}	1.5 ± 0.1^{e}	1.9 ± 0.0^{b}	1.6 ± 0.0^{cde}	1.8 ± 0.1^{cde}
S (%)[e]	100.0 ± 0.0^{a}	94.4 ± 2.9^{ab}	90.0 ± 4.2^{ab}	81.1 ± 1.1^{b}	89.4 ± 2.4^{ab}	95.6 ± 2.9^{a}	95.6 ± 2.9^{a}	95.7 ± 3.0^{a}	95.0 ± 2.9^{a}	94.4 ± 2.9^{ab}	97.8 ± 2.2^{a}
FI(g/fish)[f]	11.4 ± 0.4^{a}	10.6 ± 0.5^{abc}	8.8 ± 0.3^{fg}	8.3 ± 0.1^{g}	9.2 ± 0.3^{efg}	10.5 ± 0.4^{abcd}	9.9 ± 0.1^{bcde}	9.5 ± 0.2^{cdef}	9.2 ± 0.3^{efg}	9.4 ± 0.2^{defg}	10.7 ± 0.3^{ab}
FCR[g]	1.4 ± 0.1^{e}	1.5 ± 0.1^{de}	2.9 ± 0.2^{a}	2.6 ± 0.0^{ab}	2.1 ± 0.2^{bcd}	1.8 ± 0.1^{bcd}	1.9 ± 0.0^{bcd}	2.3 ± 0.1^{abc}	1.7 ± 0.06^{cde}	2.1 ± 0.0^{bcd}	2.1 ± 0.2^{bcd}

Diets were named according to their deficiency in each EAA. A different superscript in the same row denotes statistically significant differences ($p < .05$).

[a]BW_i, initial body weight.

[b]BW_f, final body weight.

[c]WG: weight gain = $[(BW_f - BW_i)/BW_i] \times 100$.

[d]SGR: specific growth rate = $[(\ln BW_f - \ln BW_i)/t] \times 100$, where t is experimental period = 42 days.

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

[f]FI: feed intake = total feed intake (g)/number of fish.

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

feed utilization parameters, lysine- and threonine-deficient (1.29% and 1.12% DM respectively) diets had the most negative effects on FI and FCR values ($p < .05$). No major differences were found in the whole body AA profile of silvery-black porgy juveniles fed different experimental diets; in particular, the reduction in a specific EAA in the diet did not result in its correspondent decrease in the organism (data not shown).

Haematological parameters were significantly affected by dietary EAA deficiencies (Table 4; $p < .05$). Thus, fish fed with lysine-, threonine- and phenylalanine-deficient diets (1.29%, 1.12% and 1.11% DM respectively) had lower RBC counts than the control group, whereas fish fed with arginine-, isoleucine- and tryptophan-deficient (1.73%, 1.24% and 0.26% DM respectively) diets had higher RBC counts than the control group ($p < .05$). Fish fed with lysine-, threonine- and phenylalanine-deficient (1.29%, 1.12% and 1.11% DM respectively) diets had the lowest Hb content, whereas silvery-black porgy fed with the control diet and arginine-, histidine-, methionine-, tryptophan- and valine-deficient diets showed the highest Hb levels ($p < .05$), while the other groups had intermediated values. Mean cell volume values were highest and lowest in fish fed with lysine- and isoleucine-deficient diets respectively, whereas the other groups showed intermediate values ($p < .05$). There were not significant differences in WBC and Hct values among fish fed different dietary treatments ($p > .05$).

Silvery-black porgy juveniles fed with a well-balanced EAA profile diet (control group) showed the highest levels of the non-specific immunological plasmatic parameters; however, all immune parameters significantly were reduced in fish fed EAA-deficient diets (Table 5; $p < .05$). Except for fish fed with the phenylalanine-deficient (1.11% DM) diet, total plasmatic proteins decreased in fish fed with the other EAA-deficient diets ($p < .05$). Silvery-black porgy fed with arginine- and lysine-deficient (1.73% and 1.29% DM respectively) diets had the lowest plasma C3, C4 and total Ig concentrations with values that were 62, 63 and 80% lower than in fish fed the control diet ($p < .05$) respectively. Furthermore, the lowest plasma lysozyme and SOD activities were also observed in silvery-black porgy fed with arginine- and lysine-deficient diets and their activities were 77% and 88.6% lower than those recorded in fish fed the control diet ($p < .05$). In addition, plasma ALT and AST were highest in fish fed arginine-deficient diet and lowest in fish fed with tryptophan- and valine-deficient diets.

4 | DISCUSSION

In the present study, silvery-black porgy juveniles fed the threonine-deficient diet (1.12% DM) had the lowest survival rates, which may be attributed to some physiological and metabolic dysfunctions in this experimental group. In this context, it has been reported that threonine is involved in many physiological and biochemical processes, including somatic growth, feed efficiency, digestive and absorptive, gene expression regulation, antioxidant and immune functions in different fish species (Gao Feng et al., 2013;

TABLE 4 Hematological parameters of *S. hasta* juvenile fed different experimental diets at the end of growth trial (mean ± *SE*, *n* = 3)

	Diets										
	Control	ARG	LYS	THR	HIS	ILE	LEU	MET	PHE	TRP	VAL
RBC ($\times 10^6$ μl)	1.8 ± 0.0^{c}	2.5 ± 0.1^{a}	1.1 ± 0.1^{d}	1.2 ± 0.1^{d}	1.7 ± 0.0^{c}	2.2 ± 0.0^{a}	2.1 ± 0.0^{b}	1.4 ± 0.1^{cd}	1.3 ± 0.1^{d}	2.5 ± 0.1^{a}	1.7 ± 0.1^{c}
WBC ($\times 10^3$ μl)	13.9 ± 0.7	14.2 ± 0.4	12.9 ± 0.4	13.8 ± 0.8	12.8 ± 0.3	12.4 ± 0.3	13.4 ± 0.2	13.5 ± 0.1	12.2 ± 0.6	13.2 ± 0.6	13.5 ± 0.2
Hct (%)	32.0 ± 1.0	35.3 ± 0.9	28.7 ± 0.7	27.7 ± 0.7	29.3 ± 2.6	28.3 ± 2	32.7 ± 0.9	33.7 ± 1.7	30.3 ± 0.9	35.7 ± 3.5	33.0 ± 1.0
Hb (g/dl)	4.8 ± 0.2^{a}	4.7 ± 0.2^{a}	3.2 ± 0.1^{c}	3.3 ± 0.2^{c}	4.9 ± 0.1^{a}	4.1 ± 0.1^{b}	4.5 ± 0.1^{ab}	4.8 ± 0.1^{a}	3.0 ± 0.1^{c}	5.1 ± 0.1^{a}	5.1 ± 0.1^{a}
MCV (fl)	178.6 ± 7.2^{bcd}	143.1 ± 6.5^{cd}	251.3 ± 7.8^{a}	236.7 ± 15.3^{ab}	174.8 ± 18^{bcd}	128.6 ± 9.7^{d}	159.7 ± 4.7^{cd}	248.7 ± 25.7^{a}	238.9 ± 16.5^{ab}	142.7 ± 16.4^{cd}	191.7 ± 6.6^{abc}

Diets were named according to their deficiency in each EAA. A different superscript in the same row denotes statistically significant differences ($p < .05$).

TABLE 5 Plasma immunological parameters of *S. hasta* juvenile fed different experimental diets at the end of growth trial (means ± *SE*, *n* = 3)

	Dietary treatments										
	Control	ARG	LYS	THR	HIS	ILE	LEU	MET	PHE	TRP	VAL
Total protein (g/L)	29.0 ± 3.0^{a}	16.1 ± 4.0^{c}	14.0 ± 1.0^{e}	14.3 ± 0.7^{e}	14.5 ± 0.5^{e}	14.6 ± 1.0^{e}	15.5 ± 0.2^{d}	12.5 ± 0.2^{f}	30.5 ± 1.0^{a}	17.3 ± 0.8^{b}	18.0 ± 0.7^{b}
Total Ig (μg/ml)	442.0 ± 55.0^{a}	170.0 ± 35.0^{e}	165.0 ± 28.0^{e}	228.0 ± 48.0^{d}	249.0 ± 39.0^{c}	253.0 ± 32.0^{c}	256.0 ± 25.0^{c}	260.0 ± 20.0^{c}	270.0 ± 20.0^{b}	267.0 ± 20.0^{b}	253.0 ± 32.0^{c}
C_3 (μg/ml)	480.0 ± 70.0^{a}	186.0 ± 45.0^{e}	181.0 ± 21.0^{e}	251.0 ± 37.0^{d}	274.0 ± 33.0^{c}	278.0 ± 60.0^{c}	282 ± 20.0^{c}	268.0 ± 20.0^{c}	298.0 ± 42.0^{b}	294.0 ± 30.0^{b}	275.0 ± 20.0^{c}
C_4 (μg/ml)	340.0 ± 42.0^{a}	68.0 ± 25.0^{e}	63.0 ± 20.0^{e}	126.0 ± 20.0^{d}	147.0 ± 30.0^{c}	151.0 ± 14.0^{c}	154.0 ± 10.0^{c}	156 ± 18.0^{c}	168.0 ± 33.0^{b}	171.0 ± 20.0^{b}	151.0 ± 20.0^{c}
Lysozyme (μg/ml)	4.1 ± 0.2^{a}	1.0 ± 0.2^{e}	0.9 ± 0.2^{e}	1.8 ± 0.2^{d}	2.0 ± 0.4^{c}	2.2 ± 0.3^{c}	2.1 ± 0.4^{c}	2.1 ± 0.2^{c}	2.3 ± 0.2^{b}	2.2 ± 0.2^{b}	2.0 ± 0.2^{c}
SOD (U/mg protein)	1.1 ± 0.1^{a}	0.1 ± 0.0^{e}	0.1 ± 0.0^{e}	0.2 ± 0.0^{d}	0.3 ± 0.1^{c}	0.3 ± 0.1^{c}	0.3 ± 0.1^{c}	0.3 ± 0.0^{c}	0.5 ± 0.1^{b}	0.2 ± 0.0^{d}	0.2 ± 0.0^{d}
AST (U/mg protein)	2.7 ± 0.3^{g}	8.6 ± 0.6^{a}	8.1 ± 0.9^{b}	4.2 ± 0.5^{d}	3.7 ± 0.2^{e}	3.5 ± 0.0^{e}	3.6 ± 0.5^{e}	3.1 ± 0.3^{f}	5.9 ± 0.3^{c}	1.9 ± 0.1^{h}	2.2 ± 0.1^{h}
ALT (U/mg protein)	2.6 ± 0.2^{e}	7.5 ± 0.5^{a}	6.9 ± 0.8^{b}	3.6 ± 0.1^{d}	3.1 ± 0.3^{d}	3.0 ± 0.1^{d}	3.1 ± 0.3^{d}	2.6 ± 0.1^{e}	5.5 ± 0.5^{c}	1.4 ± 0.3^{f}	1.6 ± 0.4^{f}

Diets were named according to their deficiency in each EAA. A different superscript in the same row denotes statistically significant differences ($p < .05$).

Habte-Tsion, Ge, et al., 2015, Habte-Tsion, Liu, et al., 2015, Habte-Tsion, Ren, et al., 2015, Habte-Tsion, Ren, Liu, Xie, et al., 2015, Habte-Tsion et al., 2016). Moreover, along with arginine- (1.73% DM) and lysine (1.29% DM)-deficient diets, all immunological parameters were lower in fish fed the threonine-deficient diet than in the other groups, which indicated its significant role in fish health. It has been reported that threonine has the main role in maintaining intestinal integrity and immune function, cellular and humoral non-specific immune responses (lysozyme, complement components and respiratory burst activities) and the regulation of immune genes such as tumour necrosis factor-α, copper–zinc SOD and mammalian target of rapamycin (Gao et al., 2014; Habte-Tsion, Ge, et al., 2015; Li, Mai, Trushenski & Wu, 2009; Ren et al., 2015). Protein synthesis and protein accretion are a key component of the processes involved in growth response (Anthony, Reiter, Anthony, Kimball & Jefferson, 2001). In this study, the dietary deficiency of the tested EAA resulted in lower growth performance in comparison with the control group as a consequence of a reduction in the FI and protein synthesis. Generally, deficiency of most EAA in fish leads to failure or loss of appetite which result in a reduced FI and weight gain, as well as lower disease resistance (Cowey, 1979; Wilson, 2002). On the other hand, it has been reported that dietary deficiency in one or more EAA results in the deamination of other AA in the liver, which leads to an increased excretion of nitrogenous compounds and inefficient protein synthesis and growth performance in fish (Von der Decken & Lied, 1993). In fact, imbalances in the dietary AA profile tend to lead to an increase in the oxidation of other EAA and NEAA present at normal levels in the feed, which result in reduced protein utilization (Rønnestad, Conceição, Aragão & Dinis, 2000). Moreover, the reduction in each EAA from the diet may trigger antagonism effects among different EAA such as arginine/lysine, leucine/isoleucine or methionine/cysteine, which in turn may result in a limitation in the intestinal AA uptake and consequently, restrict protein synthesis and growth performance. In the present study, fish fed lysine-, threonine- and methionine (1.29%, 1.12% and 0.79% DM respectively)-deficient diets showed the poorest growth performance results with BW_f values that were 39.0%, 38.3% and 31.3% lower in comparison with the control diet, which indicated that these EAA were the most limiting EAA in terms of somatic growth performance in silvery-black porgy juveniles. These EAA are also the most common limiting EAA in plant protein sources (Gatlin et al., 2007), which should be considered when formulating well-balanced environmental-friendly and cost-effective diets for this carnivorous and warm-water species. However, EAA deficiency did not affect the AA profile of the whole body indicating that the magnitude effects of the single reduction in an EAA from the control diet are more dependent of the EAA profile of the diet. Overall, the results of this section indicated that dietary EEA deficiencies can depress growth performance and feed utilization by affecting FI, protein synthesis, increasing EAA oxidation and inducing antagonism effects among different EAA.

Haematological parameters may be used as valuable biological indicators of nutrition, stress and the overall health condition in fish (Grant, 2015). On the other hand, proliferation, differentiation and maturation of haematopoietic cells in fish are regulated by EAA-dependent cytokines that are mainly produced by WBC (Secombes, Hardie & Daniels, 1996). According to Mozanzadeh, Yaghoubi, Yavari, Agh and Marammazi (2015), the normal haematological values in healthy silvery-black porgy range from 1.7 to 2.2 ($\times 10^6$ μl), 126 to 192 (fl), 8.8 to 14.5 ($\times 10^3$ μl), 2.8 to 6 (g/dl) and 25 to 34 (%) for RBC, MCV, WBC, Hb and Hct respectively. In the present study, except for RBC and MCV, all values reported for WBC, Hb and Hct were within the normal range reported for healthy silvery-black porgy. However, RBC numbers and MCV were significantly affected by different EAA-deficient diets, indicating that erythropoiesis in silvery-black porgy juveniles was sensitive to changes in the dietary EAA profile. Moreover, fish fed lysine-, threonine-, methionine- and phenylalanine (1.29%, 1.12%, 0.79% and 1.11% DM respectively)-deficient diets had the higher and lower MCV and RBC counts values, respectively, than normal values reported in healthy silvery-black porgy juveniles (Mozanzadeh, Yaghoubi, et al., 2015). These results indicate macrocytic anaemia in the above-mentioned groups, which maybe occurred as the consequence of disorders in hemopoietic tissues function (kidney and spleen) in these groups.

The activity of certain transaminases like AST and ALT is known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as a link between carbohydrate and protein metabolism under altered physiological conditions (Chen et al., 2015). Both enzymes are extensively investigated in fish stress and health studies and are commonly recognized as a valuable tool for tissue damage detection (Olsen, Sundell, Mayhew, Myklebust & Ringø, 2005; Welker & Congleton, 2003). Increments in AST and ALT plasmatic levels generally indicate hepatic and myocardial cells damage and/or their abnormal function (Yamamoto, 1981), whereas a reduction in plasma aminotransferases may indicate a renal malfunction (Ray, Nanda, Chatterjee, Sarangi & Ganguly, 2015). In the present study, fish fed the arginine-deficient diet (1.7% DM) had the highest plasma ALT and AST levels, which might be attributed to hepatic damage, as well as metabolic disorders in this experimental group. In this context, moderate dietary arginine levels (2.2 to 3.2% DM) led to a decrease in serum ALT and AST levels in comparison with lower (1.8%) or higher (3.4%) dietary arginine levels in black sea bream (*Sparus macrocephalus*) (Zhou et al., 2010) and red sea bream (*Pagrus major*) (Rahimnejad & Lee, 2014a) respectively. Other studies have also demonstrated that an increase in serum ALT or AST as a result of liver malfunction in different fish species fed EAA-deficient diets (Gao et al., 2014; Habte-Tsion, Ge, et al., 2015; Li, Lai, Li, Gong & Wang, 2016; Rahimnejad & Lee, 2014b). Fish fed tryptophan (0.26% DM)- and valine (1.28% DM)-deficient diets had lower plasma ALT and AST levels than the control group, which might be as a consequence of kidney malfunction (Ray et al., 2015). Total plasma or serum protein concentrations have been used as broad clinical indicators of health, stress and nutritional condition in fish (Riche, 2007). The liver parenchymal cells are the major source of most the plasma proteins including albumin, fibrinogen, coagulating factors and most of α- and β-globulins (Riche, 2007). In the present study, except for

fish fed the phenylalanine-deficient diet (1.11% DM), all groups fed with EAA-deficient diets had lower plasma protein levels than the control group, suggesting malnutrition as a consequence of a failure in plasma protein synthesis in the liver in the above-mentioned groups (Bernet, Schmidt, Wahli & Burkhardt-Holm, 2001). These results were in line with the results of plasma total Ig that also decreased in fish fed with EAA-deficient diets, which is indicate a humoral immunodeficiency in these groups (Li et al., 2007; Wu, 2009).

In fish, the humoral immune components such as lysozyme, complement and immunoglobulins play an important role in the non-specific and specific immunity and in the defence against microbial pathogens (Magnadóttir, Lange, Gudmundsdottir, Bøgwald & Dalmo, 2005). It is well known that complement system has a primary role in the innate immunity of fish, as it can recognize and opsonize foreign organisms as well as facilitate chemotaxis by phagocytes, and C3 and C4 complements are essential for activating all complement pathways (Boshra, Li & Sunyer, 2006; Kiron, 2012). In the present study, plasma C3 and C4 significantly decreased in fish fed EAA-deficient diets. However, the magnitude of the decrease in complement activity differed depending on the EAA considered. In particular, these components C3 and C4 levels were lowest in fish fed arginine- and lysine-deficient diets (1.73% and 1.29% DM respectively), which might be attributed to the impaired liver function observed in these groups, as levels of ALT and AST indicated. This hypothesis was supported by the fact that the liver is the main source of complement proteins; thus, hepatic damage, as indicated by ALT and AST levels, might therefore affect negatively on the complement system (Holland & Lambris, 2002). Comparable results were reported for various fish species as a consequence of deficiencies in dietary arginine, threonine, methionine, tryptophan, leucine, isoleucine, valine and phenylalanine (Feng et al., 2015; Habte-Tsion, Ge, et al., 2015; Jiang et al., 2015; Kuang et al., 2012; Luo et al., 2014; Wen et al., 2014; Zhao et al., 2013).

Immunoglobulins are the primary humoral component of the acquired immune system in fish, which are produced by B-cell lymphocytes after being stimulated by antigens (Magnadóttir et al., 2005). In this nutritional trial, fish fed EAA-deficient diets had lower plasma total Ig levels than the control group, as EAA are involved in the Ig synthesis (Kiron, 2012). This result indicated that a dietary EAA deficiency may result in lymphocytes malfunction through disordering antibody synthesis, because EAA have main role in protein synthesis in immune cells as it has been postulated by Calder (2006) and Li et al. (2007). Furthermore, fish fed arginine- and lysine-deficient diets (1.73% and 1.29% DM respectively) had the lowest plasma Ig levels, which might be linked to a decrease in inducible nitric oxide (NO_i) synthase activity. Arginine is the sole precursor for NO_i (Buentello & Gatlin, 1999), and lysine can modulate the entry of arginine into leucocytes for NO_i synthesis by sharing the same transport systems with arginine (Wu, 2009; Wu & Meininger, 2002). In this context, Batra et al. (2007) reported an association between NO_i synthase and serum total Ig. Nitric oxide, which is mainly produced by macrophages, as a signalling molecule has the essential role in differentiation, proliferation and apoptosis of immune cells as well as the production of cytokines and antibodies (Bogdan, 2001; Li et al., 2007).

Lysozyme is one of the non-specific defence mechanisms that is widely distributed throughout the body and mainly synthesized in the liver, kidney, skin, macrophages and gill (Kiron, 2012). Under current experimental conditions, fish fed EAA-deficient diets had lower lysozyme activity than the control group, which was in accordance with results reported in other fish species fed diets with different EAA deficiencies (Feng et al., 2015; Jiang et al., 2015; Kuang et al., 2012; Luo et al., 2014; Rahimnejad & Lee, 2014a,b; Tang et al., 2009; Wen et al., 2014; Zhao et al., 2013). This result suggested that EAA-deficient diets may result in a decrease in plasma lysozyme as a consequence of disorder in distribution of EAA toward its main producers, namely hepatocytes and neutrophils. Furthermore, the reduction in each EAA from diet may cause or trigger antagonism effects especially among BCAA and also arginine and lysine, which may result in intestinal AA uptake limitation and consequently, restrict protein synthesis in immune cells. The antagonism effects among BCAA, as well as lysine and arginine, are well studied in terrestrial animals, but poorly described in fish (NRC 2011).

Superoxide dismutase, which plays an important role in the self-defence and immune systems, belongs to the main antioxidant defence pathways in response to oxidative stress (Fridovich, 1995; Lin, Pan, Luo & Luo, 2011). In several studies in different fish species, the activity of SOD for detoxifying superoxide anion is associated with the respiratory burst activity of macrophages, which represents an indirect indicator for the non-specific cellular immune responses (Buentello, Reyes-Becerril, de Jesús Romero-Geraldo & de Jesús, 2007; Lin et al., 2011; Sun, Yang, Ma & Lin, 2010; Yeh, Chang, Chang, Liu & Cheng, 2008). The results of the present study showed that SOD activity was lower in silvery-black porgy juveniles fed with EAA-deficient diets than the control group, which might be because of the down-regulation of SOD gene expression and/or non-specific cellular immune response suppression. In this context, Li et al. (2015) reported that increasing dietary phenylalanine levels up to 9.1 g/kg led to an increase in SOD activity in the intestine of grass carp (*Ctenopharhyngodon idella*). Phenylalanine directly can perform as hydroxyl radical scavenger and as precursor for tyrosine, which in turn is the precursor for dopamine and thyroxine, and can increase the cell surface-SOD activity and extracellular SOD protein expression, indirectly (Li et al., 2015; Takano, Tanaka, Kawabe, Moriyama & Nakamura, 2013). Moreover, Habte-Tsion, Ge, et al. (2015) reported that deficient or excess levels of dietary threonine upregulated Cu/Zn-SOD mRNA levels in the intestine of blunt snout bream (*Megalobrama amblycephala*); however, intestinal SOD activity did not changed by dietary threonine levels. Other studies in different fish species also showed that dietary methionine, isoleucine and tryptophan increased the activity of antioxidant enzymes by increasing the gene expression of antioxidant enzymes and related signal molecules (Kuang et al., 2012; Wen et al., 2014; Zhao et al., 2013). In summary, results of haematological and plasma immunological parameters assessed in this study illustrated the critical role of EAA

TABLE 6 Physiological parameter changes in *S. hasta* juvenile

	Diets									
	ARG	LYS	THR	HIS	ILE	LEU	MET	PHE	TRP	VAL
WG (%)	Δ = −11.7 (↓)	Δ = −62.2 (↓)	Δ = −61.4 (↓)	Δ = −42.3 (↓)	Δ = −29.8 (↓)	Δ = −36.2 (↓)	Δ = −50.5 (↓)	Δ = −32.2 (↓)	Δ = −44.5 (↓)	Δ = −38.0 (↓)
S (%)	Δ = −5.6 (=)	Δ = −10.0 (=)	Δ = −18.9 (↓)	Δ = −10.6 (=)	Δ = −4.4 (=)	Δ = −4.4 (=)	Δ = −4.3 (=)	Δ = −5.0 (=)	Δ = −5.6 (=)	Δ = −2.2 (=)
FI (g/fish)	Δ = −1.2 (=)	Δ = −2.6 (↓)	Δ = −3.1 (↓)	Δ = −2.2 (↓)	Δ = −0.9 (↓)	Δ = −1.5 (↓)	Δ = −1.9 (↓)	Δ = −2.2 (↓)	Δ = −2.0 (↓)	Δ = −0.7 (=)
FCR	Δ = +0.1 (=)	Δ = +1.5 (↑)	Δ = +1.2 (↑)	Δ = +0.7 (↑)	Δ = +0.4 (↑)	Δ = +0.5 (↑)	Δ = +0.9 (↑)	Δ = +0.3 (=)	Δ = +0.7 (↑)	Δ = +0.6 (↑)
RBC ($\times 10^6$ μl)	Δ = +0.7 (↑)	Δ = −0.7 (↓)	Δ = −0.6 (↓)	Δ = −0.1 (=)	Δ = +0.6 (↑)	Δ = +0.3 (↑)	Δ = −0.4 (=)	Δ = –0.5 (↓)	Δ = +0.7 (↑)	Δ = −0.1 (=)
WBC ($\times 10^3$ μl)	Δ = +0.3 (=)	Δ = −1.0 (=)	Δ = −0.1 (=)	Δ = −1.1 (=)	Δ = −1.5 (=)	Δ = −0.5 (=)	Δ = −0.4 (=)	Δ = −1.7 (=)	Δ = −0.7 (=)	Δ = −0.4 (=)
Hct (%)	Δ = +3.3 (=)	Δ = −3.3 (=)	Δ = −4.3 (=)	Δ = −2.7 (=)	Δ = −3.7 (=)	Δ = +0.7 (=)	Δ = +1.7 (=)	Δ = −1.7 (=)	Δ = +3.7 (=)	Δ = +1.0 (=)
Hb (g/dl)	Δ = −0.1 (=)	Δ = −1.6 (↓)	Δ = −1.5 (↓)	Δ = +0.1 (=)	Δ = −0.7 (↓)	Δ = −0.3 (=)	Δ = 0.0 (=)	Δ = −1.8 (↓)	Δ = +0.3 (=)	Δ = +0.3 (=)
Total protein (g/L)	Δ = −12.9 (↓)	Δ = −15.0 (↓)	Δ = −14.7 (↓)	Δ = −14.5 (↓)	Δ = −14.4 (↓)	Δ = −13.5 (↓)	Δ = −16.5 (↓)	Δ = +1.5 (=)	Δ = −11.7 (↓)	Δ = −11.0 (↓)
Total Ig (μg/ml)	Δ = −272.0 (↓)	Δ = −277.0 (↓)	Δ = −214.0 (↓)	Δ = −193.0 (↓)	Δ = −189.0 (↓)	Δ = −186.0 (↓)	Δ = −182.0 (↓)	Δ = −172.0 (↓)	Δ = −175.0 (↓)	Δ = −167.0 (↓)
C3 (μg/ml)	Δ = −294.0 (↓)	Δ = −299.0 (↓)	Δ = −229.0 (↓)	Δ = −206.0 (↓)	Δ = −202.0 (↓)	Δ = −198.0 (↓)	Δ = −212.0 (↓)	Δ = −182.0 (↓)	Δ = −186.0 (↓)	Δ = −205.0 (↓)
C4 (μg/ml)	Δ = −272.0 (↓)	Δ = −277.0 (↓)	Δ = −214.0 (↓)	Δ = −193.0 (↓)	Δ = −189.0 (↓)	Δ = −186.0 (↓)	Δ = −184.0 (↓)	Δ = −172.0 (↓)	Δ = −169.0 (↓)	Δ = −189.0 (↓)
Lysozyme (μg/ml)	Δ = −3.2 (↓)	Δ = −3.2 (↓)	Δ = −2.4 (↓)	Δ = −2.1 (↓)	Δ = −2.0 (↓)	Δ = −2.0 (↓)	Δ = −2.1 (↓)	Δ = −1.9 (↓)	Δ = −1.9 (↓)	Δ = −2.2 (↓)
SOD (U/mg protein)	Δ = −0.9 (↓)	Δ = −0.9 (↓)	Δ = −0.8 (↓)	Δ = −0.8 (↓)	Δ = −0.8 (↓)	Δ = −0.8 (↓)	Δ = −0.8 (↓)	Δ = −0.5 (↓)	Δ = −0.8 (↓)	Δ = −0.9 (↓)
AST (U/mg protein)	Δ = +5.8 (↑)	Δ = +5.4 (↑)	Δ = +1.5 (↑)	Δ = +0.9 (↑)	Δ = +0.8 (↑)	Δ = +0.9 (↑)	Δ = 0.4 (=)	Δ = +3.1 (↑)	Δ = −0.8 (↓)	Δ = −0.6 (↓)
ALT (U/mg protein)	Δ = +4.9 (↑)	Δ = +4.4 (↑)	Δ = +1.1 (↑)	Δ = +0.6 (↑)	Δ = +0.5 (↑)	Δ = +0.6 (↑)	Δ = +0.1(=)	Δ = +2.9 (↑)	Δ = −1.1 (↓)	Δ = −0.9 (↓)
AST/ALT	Δ = +0.1 (↑)	Δ = +0.1 (↑)	Δ = +0.1 (↑)	Δ = +0.1 (↑)	Δ = +0.1 (↑)	Δ = +0.1 (↑)	Δ = +0.1 (↑)	Δ = −0.0 (=)	Δ = +0.3 (↑)	Δ = +0.3 (↑)

Δ is the difference of the measured parameters in each treatment comparing with control. (↑), (↓) and (=) show increasing, decreasing and no significant differences, respectively, in each treatment comparing with control. Diets were named according to their deficiency in each EAA.

on hemopoietic tissues, as well as in the liver and kidney function. In addition, these results indicated that dietary EAA deficiencies or imbalances can disturb humoral immune responses by affecting protein synthesis in immune cells or may disorder the production of cellular signalling molecules like NO_i.

In conclusion, the results of the current study demonstrated that dietary EAA deficiencies resulted in a significant reduction in growth performance and humoral immune suppression. The results of the plasma non-specific enzymes suggested that liver health and function were drastically affected by dietary deficiencies of EAA, especially arginine and lysine. Furthermore, WG decreased 62.2%, 61.4% and 50.5% in fish fed lysine-, methionine- and threonine-deficient diets, respectively, which indicate these EAA are the most limited EAA for somatic growth in silvery-black porgy juveniles (Table 6). However, all humoral immune parameters were assessed in this study significantly decreased in fish fed with arginine-, threonine- and lysine-deficient diets in comparison with other groups, suggesting these EAA are the most limited EAA for humoral immunity in silvery-black porgy juvenile (Table 6). On the other hand due to the lysine-arginine antagonism, the deficiency in each of them in diet may have some antagonism provoking effect on immune responses.

ACKNOWLEDGMENT

This article is extracted from the project recorded under code number of 92011610 and financially supported by Iran National Science Foundation.

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How to cite this article: Yaghoubi M, Torfi Mozanzadeh M, Marammazi JG, Safari O, Gisbert E. Effects of dietary essential amino acid deficiencies on the growth performance and humoral immune response in silvery-black porgy (*Sparidentex hasta*) juveniles. *Aquac Res*.2017;48:5311–5323. https://doi.org/10.1111/are.13344