

Review

# Macronutrient Requirements of Silvery-Black Porgy (*Sparidentex hasta*): A Comparison with Other Farmed Sparid Species

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**Abstract:** Silvery-black porgy (*Sparidentex hasta*) is recognized as one of the most promising fish species for aquaculture diversification in the Persian Gulf and the Oman Sea regions. In this regard, *S. hasta* has received considerable attention, and nutritional studies focused on establishing the nutritional requirements for improving diet formulation have been conducted during recent years. Considering the results from different dose–response nutritional studies on macronutrient requirements conducted in this species, it can be concluded that diets containing ca. 48% crude protein, 15% crude lipid, 15% carbohydrates and 20 KJ g<sup>−1</sup> gross energy are recommended for on-growing *S. hasta* juveniles. In addition, the optimum essential amino acid profile for this species (expressed as g 16 g N<sup>−1</sup>), should be approximately 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. Moreover, the optimum dietary n-3 long chain polyunsaturated fatty acids and soybean lecithin are recommended to be 0.8% and 6%, respectively. The maximum replacement of fish meal with soy protein is recommended to be between 16.5% and 27.3%. In addition, different vegetal oil sources are also recommended for partial and almost complete replacement of fish oil in diets. Although the nutritional requirements in terms of macronutrients have been established under laboratory conditions, the analysis of the available literature indicate that future studies need to be conducted using a more holistic approach under intensive farming conditions in which different nutrients or additives need to be tested under different rearing conditions for refining nutrient requirements in this species.

**Keywords:** protein sources; lipids; fish meal substitution; essential amino acids; essential fatty acids; sobaity sea bream

## 1. Introduction

Due to their aquaculture potential, several new sparid species have been considered in recent years as potential candidates for aquaculture diversification in the world, taking advantage of their easy adaptation to captivity and the use of available production technology (i.e., husbandry and rearing protocols, diets, production facilities, etc.) similar to those of well-established aquaculture species such as the gilthead seabream (*Sparus aurata*) and red seabream (*Pagrus major*) [1]. Among different finfish species from the Persian Gulf and the Oman Sea regions, the silvery-black porgy,

*Sparidentex hasta* is recognized as one of the most promising fish species for aquaculture diversification, because of its good adaptation to captivity, rapid growth (a growth rate of ca.  $1.6 \text{ g day}^{-1}$  during the grow-out phase) and high market price [2]. This species is a shoreline surface carnivorous fish, foraging on small fishes and invertebrates. In this sense, the pattern of digestive enzymes activities in *S. hasta* is consistent with the overall pattern of digestive enzyme activities in other carnivorous fish species with high protease and lipase, but low amylase activities [3]. This species is generally found in brackish and marine waters in tropical areas (water temperature *preferendum* of 28–30 °C), and from shallow to moderate water depths (depth range: 1–50 m). This silvery fish has a tender lean flesh and a rich flavor, making it a table delicacy and a symbol of celebration during traditional events in Arabian countries [4]. Due to its great aquaculture potential, the larviculture and farming techniques for this species have been rapidly developed during the last three decades in the Southern seashores of Iran and Arabian countries. *S. hasta* are normally raised to a market size of 450 g or higher (750–1000 g) in sea cages and the average rearing period to market size is around 18 months [2].

The intensive culture of *S. hasta* started in Bahrain in 1995 (production = 4 t). Since then, other countries from the Persian Gulf like Qatar, Kuwait, Saudi Arabia and the United Arab Emirates have started culturing this species. In 2013, an estimated 500 t of *S. hasta* were produced in this region [5]. However, in 2014, *S. hasta* production significantly decreased, because of the introduction of barramundi, *Lates calcalifer* and *S. aurata* as exotic new aquaculture species [5]. In recent years, this species has been regularly propagated in the Mariculture Research Station of the South Iranian Aquaculture Research Center (Sarbandar, Iran). Juvenile fish have regularly been released into the Persian Gulf for restocking purposes in order to increase stock numbers to support the conservation and fisheries of this species, as well as transferred to sea cages for their intensive culture.

Sparids have species-specific nutritional requirements because of their different feeding habits; thus, studies on the nutritional requirements of each candidate sparid species for aquaculture diversification is fundamental for a proper evaluation of their true potential value for the aquaculture industry [6]. In this regard, *S. hasta* has received considerable attention from the scientific community in order to develop its intensive culture, and, consequently, this attention has been coupled with a considerable investment in nutritional studies focused on establishing the nutritional requirements of this species, as well as improving diet formulation. Thus, the present work aims to review recent findings in different aspects of the nutritional requirements in macronutrients during the on-growing production phase of *S. hasta*, information that would be compared to that available from other sparid species and discussed in terms of diet formulation.

## 2. Protein

### 2.1. Protein/Energy (P/E) Ratio

Energy and protein requirements of fish are dependent on their growth potential and demand for maintenance regardless of the species considered [6]. Since energy and protein requirements are closely linked, the optimal balance between the supply of dietary non-protein energy and protein should be determined. In fact, if other nutrients that can act as energy sources are present in adequate amounts, the efficiency with which protein is used for growth may increase, due to a protein-sparing effect, and excretory losses of nitrogen decrease. In addition, improper protein/energy (P/E) ratios not only result in an increase in fish production costs, but also may result in a reduction of feed intake and the utilization of other nutrients [7]. In this regard, the P/E is a more rational way of expressing protein requirement than the dietary crude protein needs [7]. Regardless of this fact, most of the authors still present this information as crude protein requirements, values that generally range from 27% (of feed dry matter (DM)) in omnivorous species such as white seabream, *Diplodus sargus* [8] to 55% in carnivorous species such as *S. aurata* [9] (Table 1). Energy requirements for fish maintenance mainly depend on body size and temperature; thus, it is proportional to the metabolic body weight of the animal [10]. In addition, it has also been reported that sparid species have the ability to utilize

the non-protein energy and effectively spare protein for growth purposes [2]. In recent years, three studies have been conducted for determining P/E ratio in *S. hasta* juveniles. In these studies, fish meal (FM) and fish oil (FO) were used as the main dietary protein and lipid sources. Azhdari et al. [11] conducted a 3 × 2 factorial study on *S. hasta* juveniles (initial body weight (BW<sub>i</sub>) = 37.0 ± 0.2 g) for eight weeks at 23 °C using diets that contained three protein (35%, 40% and 45% DM) and two energy levels (17 and 19 KJ g diet<sup>-1</sup>). Fish fed with diets containing 40% crude protein and 19 KJ g diet<sup>-1</sup> had a better growth (specific growth rate (SGR) = 0.7% BW day<sup>-1</sup>) than fish in the other experimental groups. Marammazi et al. conducted a 4 × 3 factorial experiment in which fish (BW<sub>i</sub> = 28.0 ± 0.1 g) were fed to satiation on diets with increasing levels of protein (45%, 50%, 55% and 60% DM) at three energy levels (20, 22 and 24 KJ g diet<sup>-1</sup>) for eight weeks at 22 °C [12]. The best growth (SGR = 0.9% BW day<sup>-1</sup>) was seen when dietary protein and P/E ratio were ca. 50% and 22.7 KJ g<sup>-1</sup>, respectively. Among the other experimental groups, Hossain et al., who gave commercial feeds to juvenile *S. hasta* (BW<sub>i</sub> = 51.4 ± 0.6 g) for six months at 25 °C, reported that the best growth (SGR = 0.8% BW day<sup>-1</sup>) was obtained on a diet with ca. 48.8% and 23.4 KJ g diet<sup>-1</sup> [13]. All of these studies were conducted under suboptimal water temperatures (~28 °C), which may explain the lower growth rates found in comparison to values (SGR ~1.5% BW day<sup>-1</sup>) reported in other nutritional studies conducted on this warm-water species [14]. Since protein and energy demands change as fish grow, diets with different P/E need to be tested for different phases of the grow-out period in order to find formulations suitable for fish of different sizes.

**Table 1.** Comparison between the optimum dietary protein/energy (P/E) ratios in the on-growing stage of different sparid species. The table includes fish body weight range tested, dietary protein and energy range tested and optimum dietary protein and P/E ratios in different sparid species [8,9,11–13,15–34].

Species	Body Weight Range (g)	Protein Range Tested (%)	Energy Range Tested (MJ kg <sup>-1</sup> )	Optimum Protein (%)	Optimum P/E Ratio (g Protein MJ <sup>-1</sup> )	Ref.
<i>Sparus aurata</i>	10–64	40–55	22	45	20.5	[15]
	0.8–3.3	35–65	20	55	27.5	[9]
	5.5–30	42–58	20–21	46	23	[16]
	70	35–53	23	44–47	19–20.5	[17]
<i>Dentex dentex</i>	2.5–15	40–55	21–22	50	22–25	[18]
	10–37	50–57	22	50	22.7	[19]
	90–262	39–51	22	43	19.5	[19]
	17–25	44–59	21	49	23.3	[20]
	91.7–288	38–43	22	43	19.5	[21]
<i>Diplodus cervinus</i>	7.7–20	25–55	20–21	45	22.5	[22]
<i>Diplodus vulgaris</i>	6–20	5–55	22	36	16.4	[23]
<i>Diplodus sargus</i>	3.6–12	35–50	18–20	35	17.5	[24]
	22–36	6–49	21	27	13	[8]
<i>Diplodus puntazzo</i>	0.6–10	25–45	19–20	45	22.1	[25]
	49–113	15–55	18–22	45	20.5–25	[26]
<i>Pagellus bogaraveo</i>	23–62	20–60	20	40	20	[27]
<i>Pagrus major</i>	1.6–1.8	27–52	-	52	-	[28]
	0.3–1	41–60	18–22	60	33.1	[29]
<i>Pagrus auratus</i>	30–90	21–56	15–21	56	26.4	[30]
<i>Pagrus pagrus</i>	3–22	40–65	20	50	25	[31]
<i>Acanthopagrus berda</i>	8.4–90	20–50	20.1	42	12.7	[32]
<i>Acanthopagrus schlegeli</i>	13–56	32–48	16	41.4	26.3	[33]
<i>Acanthopagrus latus</i>	12–18	45–65	20–24	56	22.9	[34]
Hybrid ( <i>A. schlegeli</i> ♂ × <i>P. major</i> ♀)	0.3–1	41–60	18–22	55	28.3	[29]
<i>Sparidentex hasta</i>	27–45	45–60	20–24	50	22.7	[12]
	37–53	35–45	17–19	40	21	[11]
	51–205	48–58	21–24	48	23.4	[13]

## 2.2. Essential Amino Acids

Protein and amino acids should be supplied in sufficient amounts to meet fish dietary requirements in order to maximize fish growth and economic return. An absolute requirement for 10 essential amino acids (EAA) (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) has been demonstrated in most fish species studied to date [7].

Essential amino acids are key molecules for building proteins, as well as important regulators of key metabolic pathways including cell signaling, appetite stimulation, growth and development regulation, energy utilization, immunity, osmoregulation, ammonia detoxification, antioxidative defense, metamorphosis, pigmentation, gut development, necessary for plastic purposes, neuronal development, stress responses, reproduction and suppression of aggressive behavior in aquatic animals [35,36]. Thus, the evaluation of EAA requirements of a given species is particularly important for formulating nutritionally balanced and economically sustainable feeds [35]. Regardless of the importance of EAA in fish nutrition, there is a paucity of information on the EAA requirements in sparids (Table 2).

**Table 2.** Comparison between the optimum essential amino acid requirements of different sparid species and the experimental approach used for their determination.

Species	<i>Sparidentex hasta</i>	<i>Sparus aurata</i>	<i>Sparus aurata</i>	<i>Sparus aurata</i>	<i>Pagrus major</i>	<i>Pagrus major</i>	<i>Dentex dentex</i>
Experimental Approach	Deletion Method	Deletion Method	Ideal Protein	EAA Increment	Ideal Protein	Reference amino acid pattern	EAA Increment
ARG	5.3	5.6	5.4	3.1	3.5	3.5	3.7
HIS	2.5	1.9	1.7	3.5	1.4	1.3	1.3
ILE	4.6	2.6	2.6	1.1	2.2	2.6	2.5
LEU	5.4	4.8	4.5	5.3	4.2	3.9	4.1
LYS	6.0	5.1	5.0	5.1	4.4	5.4	4.7
MET and CYS	4.0	2.6 *	2.4	2.4 *	2.2	1.3 *	2.2
PHE and TYR	5.6	5.8	2.9	3.2	4.1	2.4	4.1
THR	5.2	3.0	2.8	1.4	1.8	2.3	2.4
TRP	1.0	0.8	0.6	0.9	0.6	1.3	0.5
VAL	4.6	3.2	3.0	2.7	2.5	2.7	2.8
Reference	[37]	[38]	[39]	[40]	[41]	[42]	[43]

Abbreviations: ARG: arginine; HIS: histidine; ILE: isoleucine; LYS: lysine; LEU: leucine; MET: methionine; CYS: cysteine; PHE: phenylalanine; TYR: tyrosine; THR: threonine; TRP: tryptophan; VAL: valine; \* Methionine alone.

To evaluate the EAA requirements in *S. hasta* ( $BW_i = 4.7$  g), Marammazi et al. conducted a six-week study at 29 °C based on the amino acids (AA) deletion method [37]. This experimental method is based on the principle that a change in N retention due to the partial deletion of each EAA in the diet serves to determine the ideal dietary EAA profile [44]. Eleven isonitrogenous (ca. 75.2 g N kg<sup>-1</sup>) and isoenergetic (ca. 20.5 MJ kg<sup>-1</sup>) diets containing 36% FM, 18.5% a blend of essential non-essential crystalline amino acids (CAA) mixtures and 11% FO were formulated as described by Marammazi et al. [37]. In the control diet, 60% of dietary N was provided by intact protein and 40% by CAA, whereas the other ten experimental diets were formulated by the deletion of 40% of each of the 10 EAA (crystalline form) from the control diet, and replaced by a mixture of non-essential CAA in order to adjust the dietary N content. At the end of the trial, final body weight ( $BW_f$ ) in all the EAA-deficient experimental groups was lower than that of the control group, ranging from a 6.3% of reduction in BW in fish fed the arginine-deficient diet to a 39.4% of reduction in BW in those animals fed the lysine-deficient diet (Table 3). Considering the linear response between EAA intake and N gain and assuming that each EAA is equally limiting, the optimum EAA profile for *S. hasta* juveniles, expressed as g 16 g N<sup>-1</sup>, was estimated to be: 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 [37]. Essential amino acid requirements among species would be essentially related to their overall protein requirements [45]. However, comparison between patterns of EAA requirements estimated for different sparid species show considerable specificity of EAA requirements among species, as it is shown in Table 2.

**Table 3.** Growth in body weight (g) and specific growth rate (SGR) and feed utilization parameters of *S. hasta* fed essential amino acid-deficient diets during the on-growing stage [37].

Growth Performance	Diets											
	Control	ARG	LYS	THR	HIS	ILE	LEU	MET	PHE	TRP	VAL	SEM
BW <sub>f</sub> (g) <sup>a</sup>	12.8 <sup>a</sup>	12.0 <sup>ab</sup>	7.8 <sup>d</sup>	7.9 <sup>d</sup>	9.2 <sup>cd</sup>	10.4 <sup>bc</sup>	9.9 <sup>c</sup>	8.8 <sup>cd</sup>	10.1 <sup>bc</sup>	9.2 <sup>cd</sup>	10.0 <sup>c</sup>	0.48
SGR (% BW day <sup>-1</sup> ) <sup>b</sup>	2.39 <sup>a</sup>	2.24 <sup>a</sup>	1.21 <sup>f</sup>	1.24 <sup>f</sup>	1.56 <sup>de</sup>	1.92 <sup>b</sup>	1.80 <sup>bc</sup>	1.50 <sup>e</sup>	1.87 <sup>b</sup>	1.60 <sup>cde</sup>	1.76 <sup>cde</sup>	0.12
FI (g fish <sup>-1</sup> ) <sup>c</sup>	11.4 <sup>a</sup>	10.6 <sup>abc</sup>	8.8 <sup>fg</sup>	8.3 <sup>g</sup>	9.2 <sup>efg</sup>	10.5 <sup>abcd</sup>	9.9 <sup>bcde</sup>	9.5 <sup>cdef</sup>	9.2 <sup>efg</sup>	9.4 <sup>defg</sup>	10.7 <sup>ab</sup>	0.17
FCR <sup>d</sup>	1.40 <sup>e</sup>	1.45 <sup>de</sup>	2.87 <sup>a</sup>	2.58 <sup>ab</sup>	2.12 <sup>bcd</sup>	1.83 <sup>bcde</sup>	1.89 <sup>bcde</sup>	2.32 <sup>abc</sup>	1.68 <sup>cde</sup>	2.06 <sup>bcde</sup>	2.08 <sup>bcde</sup>	0.08
PER <sup>e</sup>	1.59 <sup>a</sup>	1.54 <sup>ab</sup>	0.78 <sup>f</sup>	0.86 <sup>ef</sup>	1.07 <sup>cdef</sup>	1.22 <sup>bcd</sup>	1.17 <sup>cde</sup>	0.96 <sup>def</sup>	1.32 <sup>abc</sup>	1.08 <sup>cdef</sup>	1.09 <sup>cdef</sup>	0.05

Abbreviations: SEM: standard error mean. <sup>a</sup> BW<sub>f</sub> : final body weight; <sup>b</sup> SGR: specific growth rate =  $(\ln \text{ final weight} - \ln \text{ initial weight})/t \times 100$ ; where t is experimental period = 42 days; <sup>c</sup> FI: feed intake = total feed intake (g)/number of fish; <sup>d</sup> FCR: feed conversion ratio = feed intake (g)/weight gain (g); <sup>e</sup> PER: protein efficiency ratio = weight gain (g)/protein intake (g).

Regardless of the impact of EAA on growth performance, a recent study from Yaghoubi et al. has evaluated the effects of EAA deficiencies on the humoral immune response in *S. hasta* juveniles [46]. The results of this study showed that humoral immune responses including plasma total protein, total immunoglobulins, lysozyme, complement and superoxide dismutase activities were significantly reduced in fish fed with each of the above-mentioned EAA-deficient diets. Moreover, these authors concluded that arginine, threonine and lysine were the most limiting EAA for humoral immune responses in *S. hasta* juveniles, considering the variation of the above-mentioned parameters in comparison to the control group.

### 2.3. Alternative Protein Sources

One of the major bottlenecks of the sustainability of aquaculture is finding economically viable and environmentally friendly alternative lipid and protein sources for FM and FO substitution to meet the increasing global demands of aquafeeds [47]. Furthermore, reduction of marine resources in aquafeeds is more challenging for carnivorous fish, rather than for omnivorous or herbivorous species [6,48]. When considering FM alternatives in aquafeeds, different aspects such as price, protein content, amino acid profile, digestibility, EAA deficiencies or imbalances, anti-nutritional factors (ANFs) and palatability must be addressed [6,49,50]. Previous studies have indicated that ca. 20–60% FM and in extreme scenarios up to 100% FM could be replaced by plant proteins (PP) when crystalline AA supplemented in diets for marine carnivorous fish species without a significant effect on growth performance and feed efficiency parameters [51–54]. Nevertheless, not all species necessarily respond equally to dietary FM replacements and although these generalizations may be used as a benchmark, effects of dietary alternative protein sources should be evaluated on a case by case basis. Thus, several studies have been carried out to evaluate the potential of different feedstuffs as alternative to FM in diets for sparids [2]. For example, high levels of FM substitution (50–75%) [55–57] or total replacement of FM [58] have been achieved in *S. aurata* by using combinations of PP concentrates and EAA supplementation without affecting growth performance or fillet quality traits. In contrast, Sitjà-Bobadilla et al. reported that replacement of dietary FM with blends of PP led to a decrease in growth performance, feed utilization and reduced plasma alternative complement activity in *S. aurata* fed diets with above 75% PP level [59]. In other sparid species, the maximum dietary FM replacement with different PP sources without compromising physiological and growth performances has been reported to be 15% in blackspot seabream, *Pagellus bogaraveo* [60]; 25% in common dentex, *Dentex dentex* [61]; 30% in black seabream, *Acanthopagrus schlegelii* [62]; 50% in two-banded seabream, *Diplodus vulgaris* [63]; 60% in *Pagrus auratus* [64]; 68% in sharpnose seabream, *D. puntazzo* [65]; and 100% in *P. major* [52] (Table 4).

**Table 4.** Alternative protein sources tested in diets for on-growing stage of different sparid species. The table includes fish body weight range tested, dietary alternative protein sources and additives tested as well as optimum dietary fish meal replacement in different sparid species.

Species	Weight Range (g)	Dietary Protein (%)	Alternative Protein Sources	Additives	Optimum FM Replacement (%)	Optimum Incorporation Level in Diet (%)	Ref.
<i>Sparus aurata</i>	5.5–29	44	PSM	-	20	35	[66]
	8–33	47	CGM	-	60	40	[67]
	42–165	45	LSM	-	30	40	[68]
	15–80	46	SBM	-	15	30	[69]
	180–346	46	BM	-	-	5	[70]
	18–95	47	SBM	-	34	30	[71]
	174–372	49	PPC + RPC	-	60	38	[72]
	26–93	45	SPC	DL-Met	40	39	[73]
<i>Dentex dentex</i>	39–105	50	SPC	taurine	25	25	[61]
	40–98	50	SBM	Met	40	37	[74]
<i>Diplodus vulgaris</i>	11.5–18	40	SBM	-	43	43	[63]
	6–15	40	CGM	-	30	24.5	[75]
<i>Diplodus puntazzo</i>	48–118	45	SBM	-	68	60	[65]
<i>Pagellus bogaraveo</i>	75–97	47	RPC	-	64	35	[76]
<i>Pagrus major</i>	280–830	48	PBPM	-	100	59	[77]
	53–112	48	PBPM	-	70	41	[77]
	280–750	48	CGM	-	70	36	[78]
	53–88	48	CGM	-	30	15	[78]
	24–62	48	SBM	Phytase	38	30	[79]
	7.3–61	49	DSM	FS, SM, KM	50	31	[52]
	1.4–27.7	50	FSP	FS, SM, KM	40	24	[80]
<i>Pagrus auratus</i>	77–168	50	SBM + PBPM	-	50	40	[81]
	14.7–41	36	CM	-	40	40	[64]
<i>Pagrus pagrus</i>	2.2–15.2	48	SBP+SPC	Lys + Met	34.5–45	27	[82]
<i>Acanthopagrus schlegeli</i>	1.2–5.8	44	FSP	-	40	24	[83]
	7.9–47.5	42	PPI-PPC-ETPP	-	16	-	[84]
<i>Acanthopagrus latus</i>	2.5–6.9	50	FSP	-	30	30	[85]
<i>Oblada melanura</i>	1.5–6.6	44	SBM	-	28	34	[86]
<i>Sparidentex hasta</i>	16.7–42.7	50	SBM + ISP	-	27.3	20	[87]

Abbreviations: BM: Blood meal; CGM: corn gluten meal; DSM: dehulled soybean meal; ETPP: enzyme treated poultry protein; FM: fish meal; FS: fish soluble; FSP: fermented soy protein; ISP: isolated soy protein; KM: krill meal; LSM: lupine seed meal; Lys: lysine; Met: Methionine; PBPM: poultry by product meal; PP: pea protein; PPC: pea protein concentrate; PPI: pea protein isolate; PSM: pea seed meal; concentrate; RPC: rice protein concentrate; SBM: soybean meal; SPC: soy protein concentrate; SM: squid meal.

Alternative protein sources tested in diets for on-growing stage of different sparid species. The table includes fish body weight range tested, dietary alternative protein sources and additives tested as well as optimum dietary fish meal replacement in different sparid species.

Regarding *S. hasta* juveniles ( $BW_i = 16.7 \pm 0.1$  g), Yaghoubi et al. conducted a two-month nutritional trial to determine the amount of soy products (SP), including soybean meal and isolated soy protein, which could be used for replacing FM in diets without reducing growth performance [14]. In this sense, six isoproteic (ca. 50%) and isoenergetic (ca. 22.4 MJ kg<sup>-1</sup>) diets were formulated in which FM was replaced by 15% (SP15), 30% (SP30), 45% (SP45), 60% (SP60) and 75% (SP75) of soy products. Feed intake (FI), SGR, feed utilization (FCR) significantly decreased by increasing dietary SP supplementation (Table 5).

**Table 5.** Growth in body weight (g), specific growth rate (SGR), feed utilization parameters and apparent digestibility coefficients (ADC) of protein and lipid of *S. hasta* fed different experimental diets in which fish meal partially replaced by soybean protein during the on-growing stage (mean  $\pm$  standard error,  $n = 3$ ) [14].

Growth Performance	Diets					
	FM	SP15	SP30	SP45	SP60	SP75
BW <sub>f</sub> (g) <sup>a</sup>	42.7 $\pm$ 1.2 <sup>a</sup>	42.6 $\pm$ 0.4 <sup>a</sup>	38.6 $\pm$ 0.2 <sup>b</sup>	37.8 $\pm$ 0.6 <sup>bc</sup>	34.3 $\pm$ 1.1 <sup>cd</sup>	33.9 $\pm$ 0.6 <sup>d</sup>
SGR (% BW day <sup>-1</sup> ) <sup>b</sup>	1.6 $\pm$ 0.1 <sup>a</sup>	1.6 $\pm$ 0.1 <sup>a</sup>	1.4 $\pm$ 0.0 <sup>b</sup>	1.4 $\pm$ 0.0 <sup>b</sup>	1.2 $\pm$ 0.0 <sup>c</sup>	1.0 $\pm$ 0.0 <sup>d</sup>
FI (g fish <sup>-1</sup> ) <sup>c</sup>	36.2 $\pm$ 0.0 <sup>a</sup>	35.4 $\pm$ 0.0 <sup>b</sup>	33.9 $\pm$ 0.0 <sup>c</sup>	32.3 $\pm$ 0.0 <sup>d</sup>	30.4 $\pm$ 0.2 <sup>e</sup>	30.2 $\pm$ 0.3 <sup>e</sup>
FCR <sup>d</sup>	1.4 $\pm$ 0.1 <sup>a</sup>	1.4 $\pm$ 0.0 <sup>a</sup>	1.6 $\pm$ 0.1 <sup>b</sup>	1.5 $\pm$ 0.0 <sup>b</sup>	1.7 $\pm$ 0.0 <sup>c</sup>	1.7 $\pm$ 0.1 <sup>c</sup>
ADC of protein (%) <sup>e</sup>	98.2 $\pm$ 0.2 <sup>a</sup>	98.3 $\pm$ 0.1 <sup>a</sup>	98.1 $\pm$ 0.1 <sup>a</sup>	97.9 $\pm$ 0.1 <sup>ab</sup>	97.6 $\pm$ 0.2 <sup>b</sup>	97.7 $\pm$ 0.1 <sup>b</sup>
ADC of lipid (%)	99.2 $\pm$ 0.1 <sup>a</sup>	98.8 $\pm$ 0.1 <sup>ab</sup>	98.9 $\pm$ 0.1 <sup>ab</sup>	98.5 $\pm$ 0.1 <sup>b</sup>	98.3 $\pm$ 0.1 <sup>b</sup>	98.4 $\pm$ 0.2 <sup>b</sup>

<sup>a</sup> BW<sub>f</sub>: final body weight; <sup>b</sup> SGR: specific growth rate =  $[(\ln \text{ final weight} - \ln \text{ initial weight})/t] \times 100$ ; where  $t$  is experimental period = 60 days; <sup>c</sup> FI: feed intake = total feed intake (g)/number of fish; <sup>d</sup> FCR: feed conversion ratio = feed intake (g)/weight gain (g); <sup>e</sup> ADCs of nutrients =  $100 - [100 \times (\text{Cr}_2\text{O}_3 \text{ in diet}/\text{Cr}_2\text{O}_3 \text{ in feces})] \times [(\% \text{ nutrient in feces}/\% \text{ nutrient in diet})]$ .

According to the broken-line regression method using data from FCR and weight gain (WG) variables, the maximum replacement of FM with SP in *S. hasta* diets was estimated to be between 16.5% and 27.3%, which indicated a low tolerance of this species to SP. Moreover, digestibility of dry matter, protein and lipid decreased by increasing dietary SP supplementation. These authors concluded that some ANFs in SP might also potentially have led to a decrease in the bioavailability and apparent digestibility coefficients (ADCs) of nutrients, as previously described in other species [49,87]. Using mixtures of alternative protein sources that may complement each other in terms of EAA composition, inclusion of feed attractants and exogenous enzymes, as well as dietary supplementation with protein hydrolysates and limiting EAAs are adequate strategies for replacing FM protein with alternative protein sources [6]. However, the use of the above-mentioned nutritional strategies in terms of ingredient selection and diet formulation needs to be further tested in this species.

### 3. Lipid

Fats and oils extracted from animals and plants are important sources of energy that are well utilized by most fish species, but they also provide essential fatty acids (EFA), fat soluble vitamins, phospholipids and cholesterol required by fish for normal growth and development and the maintenance of health [88]. The fats and oils used as lipid and energy sources in fish feeds are more expensive and less readily available than carbohydrate-based energy sources, and this is particularly the case for fish oils (FOs) [89]. The current trend of high-lipid diet use has been shown to induce undesirable increase in fat deposition, as well as physiological symptoms, such as susceptibility to autoxidation and tissue lipid peroxidation, and/or it might also lead to a decrease feed consumption and growth reduction [7,90]. Regarding sparids, the optimum dietary lipid levels were recommended to be ca. 22% in *S. aurata* [91], ca. 15% in *P. major* [28], 15% in *P. pagrus* [31], 17% in *D. dentex* [18] and 18% in *D. sargus* [92]. Regarding *S. hasta*, there is no available study that determined the absolute dietary lipid requirement in this species; however, Mozanzadeh et al. [93] recommended to include lipids between 15% and 20% taking into account the results from a study about the effects of dietary carbohydrate to lipid ratios, as illustrated in the next section.

#### 3.1. Carbohydrate/Lipid (CHO/L) Ratio

Carbohydrates are valued ingredients in aquafeeds because of their low cost, high availability, and reduction of the dietary protein and lipid catabolism for energy yielding processes [7]. The ability of CHO utilization by fish differs considerably among species [94]. The appropriate dietary inclusion level of digestible CHO has been established between a recommended 20% maximum for carnivorous species to 40% for warm water omnivorous fish species [95]. In this context, the maximum dietary

CHO that different sparid species can tolerate without showing any physiological disorder and growth impairment has been reported to be ca. 20% (raw corn starch) in yellowfin seabream, *A. latus* [96]; 25% ( $\beta$ -starch) in *P. major* [97]; 28% (maltodextrin) in *D. dentex* [21]; 40% (raw and pro-gelatinized corn starch) in *S. aurata* [98]; and 42% (dextrin) in *D. sargus* [99]. Thus, optimizing of the dietary CHO/L ratio is considered to be beneficial not only for improving fish quality, but also for sparing the consumption of protein and fat as energy sources. In *S. hasta* juveniles ( $BW_i = 14.6 \pm 0.1$  g), four isonitrogenous (ca. 48%) diets containing four different lipid levels (10%, 15%, 20% and 25%) and CHO/L ratios (0.3, 0.6, 1.1 and 1.8) were tested in triplicate groups for eight weeks at 29 °C [93]. Growth performance and FI were not affected by different dietary CHO/L ratios, which indicated that when protein supply was equal among experimental diets; different non-protein energy sources might not have a direct effect on FI or growth performance (Table 6).

**Table 6.** Growth in body weight (g), specific growth rate (SGR), feed utilization parameters and apparent digestibility coefficients (ADC) of protein and lipid of *S. hasta* fed different experimental diets containing different CHO/L ratios during the on-growing stage (mean  $\pm$  standard error,  $n = 3$ ) [93].

Growth Performance	CHO/L Ratios			
	0.3	0.6	1.1	1.8
$BW_f$ (g) <sup>a</sup>	36.6 $\pm$ 0.5	35.7 $\pm$ 0.5	35.0 $\pm$ 0.1	35.0 $\pm$ 0.9
SGR (% BW d <sup>-1</sup> ) <sup>b</sup>	1.7 $\pm$ 0.1	1.6 $\pm$ 0.1	1.6 $\pm$ 0.0	1.6 $\pm$ 0.1
FCR <sup>c</sup>	1.5 $\pm$ 0.0	1.7 $\pm$ 0.1	1.5 $\pm$ 0.0	1.7 $\pm$ 0.1
FI (g fish <sup>-1</sup> ) <sup>d</sup>	31.4 $\pm$ 1.5	28.8 $\pm$ 1.1	31.4 $\pm$ 0.5	33.5 $\pm$ 0.9
ADC of protein (%) <sup>e</sup>	97.5 $\pm$ 0.7 <sup>a</sup>	93.9 $\pm$ 1.5 <sup>ab</sup>	95.8 $\pm$ 1.0 <sup>ab</sup>	89.8 $\pm$ 2.3 <sup>b</sup>
ADC of lipid (%)	98.9 $\pm$ 0.1 <sup>a</sup>	97.4 $\pm$ 0.5 <sup>ab</sup>	97.9 $\pm$ 0.7 <sup>ab</sup>	94.5 $\pm$ 1.4 <sup>b</sup>

<sup>a</sup>  $BW_f$ : final body weight; <sup>b</sup> SGR: specific growth rate =  $[(\ln \text{ final weight} - \ln \text{ initial weight})/t] \times 100$ ; where  $t$  is experimental period = 56 days; <sup>c</sup> FI: feed intake = total feed intake (g)/number of fish; <sup>d</sup> FCR: feed conversion ratio = feed intake (g)/weight gain (g); <sup>e</sup> ADCs of nutrients =  $100 - [100 \times (\text{Cr}_2\text{O}_3 \text{ in diet}/\text{Cr}_2\text{O}_3 \text{ in feces})] \times [(\% \text{ nutrient in feces}/\% \text{ nutrient in diet})]$ .

Regarding diet digestibility, fish fed with a 1.8 CHO/L diet had the lowest ADCs of protein and lipid, which was attributed by the authors of the study to the high inclusion of corn starch in this diet, since corn starch has lower ADCs than other cereal meals [7,100]. Thus, considering the results from a wide range of production and physiological parameters evaluated, the study from Mozanzadeh et al. [93] concluded that diets with lipid content between 15% and 20% and CHO/L ratios between 0.6 and 1.1 were optimal for *S. hasta* juveniles, whereas higher ratios may result in hyperglycemia and immune suppression, and lower CHO/L ratios may lead to oxidative stress and liver dysfunction. Comparative dietary CHO/L ratios in *S. hasta* juveniles and other sparid species are presented in Table 7.

**Table 7.** Comparison between the optimum dietary carbohydrates to lipids (CHO/L) ratios reported for the on-growing stage of different sparid species. The table includes fish body weight range tested, dietary protein and lipid range tested and optimum dietary lipid and CHO/L ratios in different sparid species.

Species	Body Weight Range (g)	Optimum Dietary Protein (%)	Lipid Range Tested (%)	Carbohydrate Range Tested (%)	Optimum Dietary Lipid (%)	Optimum CHO/L Ratio	Ref.
<i>Sparus aurata</i>	42–142	47	15–21	17–22	21	0.8	[101]
	68–400	48	15–28	15–28	22	0.6–0.9	[92]
	70	44–47	19–27	10–21	21–22	0.7	[17]
<i>Dentex dentex</i>	2–14	50	12–22	7–34	12–17	1–1.8	[18]
	91.7–288	43	16–24	4–28	16	1.8	[21]
	9.8–65	43	20–27	19–27	23	1	[102]
<i>Pagrus pagrus</i>	3–22	50	15	7–38	15	1.8	[31]
	15–74	50	10–20	19–33	15	1.6	[31]
<i>Pagrus auratus</i>	30–90	56	64–305	68–480	14	1	[30]
<i>Diplodus sargus</i>	11–23	28	12–16	46–66	12–16	3–4.3	[103]
	41–77	38–42	12–18	3–28	12–18	-	[93]
	17–36	45	9–24	11–19	9	2.2	[104]
	0.6–10	45	8–12	28–56	12	2.4	[25]
<i>Diplodus puntazzo</i>	3.4–145	45	10–15	17–25	10	2.5	[105]
<i>Diplodus vulgaris</i>	3.6–12	35	10–15	15–39	15	2.2	[24]
<i>Pagellus bogaraveo</i>	37.6–94	50	6–10	10–19	10	1.4	[106]
<i>Acanthopagrus berda</i>	10–56	42	15–30	10–25	20	1	[107]
<i>Acanthopagrus latus</i>	4.9–22	45	5–17	0.5–28	13–15	0.3–0.7	[96]
<i>Sparidentex hasta</i>	14.5–36	50	10–25	7–18	15–20	0.6–1.1	[94]

### 3.2. Requirements of n-3 Long Chain Polyunsaturated Fatty Acids

Long chain polyunsaturated fatty acids from the n-3 series (n-3 LC-PUFAs) play important and diverse roles in different physiological and biochemical cellular processes, including cell synthesis, neural development, endocrine function and control, ionic regulation, immune function and reproduction [89]. It is generally considered that marine fish have limited or no capacity for converting linolenic acid (LNA; 18:3n-3) into n-3 LC-PUFAs, mainly eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [89,108]. In sparids, inadequacy of LNA to support fish growth was attributed to an inefficiency of conversion of LNA into n-3 LC-PUFAs [109]. For example, the impairment in the desaturase/elongase pathway in *S. aurata* was described to be at the level of  $\Delta 5$ -desaturase [110]. Therefore, some exogenous n-3 LC-PUFA need to be provided in marine fish diets for supporting proper growth and good feed efficiency [89]. However, dietary n-3 LC-PUFA requirements vary depending on the stage of development and fish species, which are correlated with their ecology and metabolism [108]. In this context, most of the studies conducted in marine carnivorous fish species so far established an optimal dietary n-3 LC-PUFA requirement of 0.5%–2% of dietary DM [7]. Regarding sparids, the minimum requirement in dietary n-3 LC-PUFAs (DHA + EPA) required for optimal growth and development in juveniles has been reported to be about 1.0 in *S. aurata* [111], 1.3 in silver seabream, *Rhabdosargus sarba* [112] and 3.7 in *P. major* [113]. In *S. hasta*, the optimum dietary level of n-3 LC-PUFA in juveniles ( $BW_i = 13.3 \pm 0.1$ g) was determined in a nutritional study using five isonitrogenous (ca. 50%) and isolipidic (ca. 15%) semi-purified diets containing graded levels of n-3 LC-PUFAs (0.1, 0.6, 1.2, 1.9 and 4.2%) that were tested during eight weeks at 29 °C [114]. Specific growth rate, WG and FCR improved by increasing the dietary LC-PUFA content from 0.1% to 1.2%, and remained stable at higher n-3 LC-PUFA levels (Table 8). According to broken-line regression analysis, the minimum n-3 LC-PUFA requirements in *S. hasta* juveniles fed a diet containing 15% of lipids were estimated to be between 0.6% and 0.8% with a DHA/EPA and n-3/n-6 ratios of 2.0 and 0.8, respectively.

**Table 8.** Growth in body weight (g), specific growth rate (SGR), feed conversion ratio (FCR) of *S. hasta* fed diets with graded n-3 LC-PUFA during the on-growing stage (mean  $\pm$  SEM,  $n = 3$ ) [114].

Growth Performance	Dietary n-3 LC-PUFA Content (% Dry Weight)				
	0.1	0.6	1.2	1.9	4.2
BW <sub>f</sub> (g) <sup>a</sup>	20.4 $\pm$ 1.1 <sup>c</sup>	26.3 $\pm$ 0.9 <sup>b</sup>	29.1 $\pm$ 1.7 <sup>a</sup>	28.3 $\pm$ 0.8 <sup>a</sup>	30.5 $\pm$ 0.5 <sup>a</sup>
SGR (% BW day <sup>-1</sup> ) <sup>b</sup>	0.7 $\pm$ 0.1 <sup>c</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	1.4 $\pm$ 0.1 <sup>a</sup>	1.4 $\pm$ 0.1 <sup>a</sup>	1.5 $\pm$ 0.1 <sup>a</sup>
FCR <sup>c</sup>	2.3 $\pm$ 0.5 <sup>a</sup>	1.6 $\pm$ 0.1 <sup>ab</sup>	1.4 $\pm$ 0.2 <sup>c</sup>	1.5 $\pm$ 0.1 <sup>c</sup>	1.3 $\pm$ 0.1 <sup>c</sup>
HSI (%) <sup>d</sup>	1.6 $\pm$ 0.2 <sup>a</sup>	1.3 $\pm$ 0.1 <sup>ab</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	1.1 $\pm$ 0.1 <sup>b</sup>	1.1 $\pm$ 0.1 <sup>b</sup>

<sup>a</sup> BW<sub>f</sub>: final body weight; <sup>b</sup> SGR: specific growth rate =  $[(\ln \text{ final weight} - \ln \text{ initial weight})/t] \times 100$ ; where  $t$  is experimental period = 56 days; <sup>c</sup> FCR: feed conversion ratio = feed intake (g)/weight gain (g); <sup>d</sup> HSI: hepatosomatic index = liver weight (g)/body weight (g)  $\times$  10.

Besides the effects of the absolute dietary total n-3 LC-PUFA levels on physiological and biochemical processes, the ratio of DHA to EPA also exerts the same significant influences on several physiological parameters [115–121]. In this context, most of the studies conducted in marine carnivorous fish species so far, established an optimum dietary DHA/EPA ratio between 1 and 2 [119–124]. To determine the optimum dietary DHA to EPA ratio in *S. hasta* juveniles ( $BW_i = 20.1 \pm 0.1$  g), an eight-week feeding experiment was conducted by Mozanzadeh et al. [125]. Thus, triplicate groups of fish were reared at 18.7 °C and handfed at satiation by one of the five isonitrogenous (ca. 50%) and isolipidic (ca. 15%) diets that were formulated with graded ratios of DHA/EPA (0.2, 0.4, 0.9, 2.0, and 3.3). No statistically significant differences were observed in growth (SGR and WG) and feed utilization parameters among different treatments, whereas, in other sparid species, the best growth performance in juvenile *P. major* [126] and juvenile *S. aurata* [127] was reported to occur at a DHA/EPA ratio of 0.5. However, in the study from Mozanzadeh et al. [125], SGR and FCR

values in *S. hasta* juveniles were not affected by different dietary DHA/EPA ratios, possibly reflecting species-specific differences with regard to *P. major* [113] and *S. aurata* [126]. These results in *S. hasta* suggested that either fatty acid (DHA or EPA) was able to meet the essential fatty acid requirements, if sufficient total n-3 LC-PUFAs were supplied in the diet. Thus, the former authors concluded that growth performance and feed utilization of *S. hasta* juveniles were not influenced by dietary graded ratios of DHA/EPA.

### 3.3. Phospholipids

Phospholipids (PLs) have been demonstrated to significantly affect survival, growth, proper skeletal development and/or resistance to stress in several fish species. As important part of cell membranes, PLs play a major role in maintaining the structure and function of membranes, as well as in the formation of cell organelles [127,128]. PLs also improve intestinal absorption of lipids by acting as emulsifiers in the chyme, as well as stimulating lipoprotein synthesis in enterocytes and lipid transport, reducing lipid accumulation in the intestine. In addition, it has been suggested that PLs may improve diet quality by reducing the leaching of water-soluble micronutrients, acting as antioxidants, as well as feed attractants and providing choline, inositol, phosphorus and EFA that are essential for fish growth [127,128]. The optimal level of dietary PL supplementation depends on the species, developmental stage, culture conditions, and PL source [127]. In this regard, soybean lecithin (SBL) due to its market availability and relatively stable composition has been commercially used as a convenient source of PLs in aquafeeds, although some studies dealing with larvae have used marine PLs sources [127]. However, SBL contains elevated linoleic acid (18:2n-6) levels and does not contain LC-PUFAs, which characterize marine PLs.

There is scarce information about the PLs requirements in juveniles from different sparid species, whereas most of the available data is from larvae due to the importance of this source of lipids on proper larval growth, development and quality [127]. Regarding sparids, the optimum quantitative and qualitative PL requirements of *P. major* and *S. aurata* larvae have been reported to be about 5% and 9%, respectively [129,130]. For estimating the optimum levels of dietary PLs in *S. hasta* juveniles ( $BW_i = 37.9 \pm 0.2$  g), an eight-week feeding trial was conducted using four isonitrogenous (ca. 50%), isoenergetic (ca. 18.5 MJ kg<sup>-1</sup>) and isolipidic (ca. 20%) diets containing graded levels of SBL (0%, 3%, 6% and 9%) [131]. Fish meal was used as the main protein source, whereas diets were supplemented with SBL at the expense of FO as the main source of dietary lipid. Growth performance in fish fed SBL-supplemented diets was significantly higher in comparison with the control group (SBL 0%). In this context, fish fed with control and 6% SBL diets had the lowest and highest SGR and WG, respectively, whereas the other groups showed intermediate values (Table 9).

**Table 9.** Growth response of *S. hasta* juvenile fed diets with graded soybean lecithin (SBL) (means  $\pm$  SEM,  $n = 3$ ) [131].

Growth Performance	Diets (% SBL)			
	Control	SBL3	SBL6	SBL9
BW <sub>f</sub> (g) <sup>a</sup>	74.1 $\pm$ 1.6 <sup>b</sup>	77.2 $\pm$ 4.7 <sup>ab</sup>	86.2 $\pm$ 3 <sup>a</sup>	82.6 $\pm$ 6.9 <sup>ab</sup>
SGR (% body weight day <sup>-1</sup> ) <sup>b</sup>	1.2 $\pm$ 0.1 <sup>b</sup>	1.3 $\pm$ 0.1 <sup>ab</sup>	1.5 $\pm$ 0.1 <sup>a</sup>	1.4 $\pm$ 0.2 <sup>ab</sup>
FCR <sup>c</sup>	1.5 $\pm$ 0.1	1.6 $\pm$ 0.2	1.3 $\pm$ 0.1	1.4 $\pm$ 0.2
PER <sup>d</sup>	1.3 $\pm$ 0.1	1.3 $\pm$ 0.1	1.5 $\pm$ 0.1	1.4 $\pm$ 0.2

<sup>a</sup> BW<sub>f</sub>: final body weight; <sup>b</sup> SGR: specific growth rate = [(ln final weight – ln initial weight)/t]  $\times$  100; where t is experimental period = 56 days; <sup>c</sup> FCR: feed conversion ratio = feed intake (g)/weight gain (g); <sup>d</sup> PER: protein efficiency ratio = weight gain (g)/protein intake (g).

However, FI, FCR and PER did not vary among groups. These results may be explained by the growth enhancing effect of PLs, especially phosphatidylcholine, which might increase the efficiency of lipid digestion and absorption [127]. Similar results were also reported in rainbow trout, *Oncorhynchus*

*mykiss* [132] and Atlantic salmon, *Salmo salar* [133] juveniles. Moreover, the authors reported that dietary SBL did not have significant effects on hematological parameters (i.e., hemoglobin, hematocrit, RBCs, and WBCs) in *S. hasta* juveniles. The results of this study concluded that dietary supplementation 6% SBL improve growth performance in *S. hasta* juveniles.

### 3.4. Alternative Lipid Sources

Marine fish have absolute requirements for LC-PUFAs and to date, FO is the only competitive commercial source of these FAs. However, static world capture fisheries, natural environmental impacts such as El Niño events, and competition for using marine derivatives products in the terrestrial animal feed industries and human nutrition have also led to inflated FO prices and demands. In addition, the progressive growth of aquaculture industry, as well as using energy-dense diets in carnivorous fish production have further exacerbated this problem [89,134]. If EFA requirements are met, a large fraction (60–75%) of FO can be substituted with vegetable oils (VO) or terrestrial animal fats (i.e., beef tallow, poultry fat, lard) without significantly affecting growth and FCR [89,135,136]. Regarding sparids, the maximum replacement of dietary FO with different VO sources has been reported to be about 70% in *S. aurata* [137]; 80% in *A. schlegeli* [138]; and 100% in *D. puntazzo* [139], *P. major* [140] and *P. bogaraveo* [76] (Table 10).

**Table 10.** Dietary alternative lipid sources tested in on-growing stage of different sparid species. The table includes fish body weight range tested, dietary alternative lipid sources and optimum dietary fish oil replacement in different sparid species.

Species	Weight Range (g)	Dietary Lipid (%)	Alternative Lipid Sources	Optimum FO Replacement (%)	Optimum Incorporation Level in Diet (%)	Ref.
<i>Sparus aurata</i>	14.7–349	14	SO	48	6.8	[141]
	16–270	22	LO, RO, PO	60	10	[142]
	115–260	19	SO, RO	69	11	[137]
	265–450	21	EO	50	10	[143]
	4–24	18	SFO, LO	50	4	[144]
<i>Diplodus sargus</i>	6.6–16.7	16	SO, CO, HO	100	10	[145]
<i>Diplodus puntazzo</i>	15–73	21	LO, SO	100	16	[139]
<i>Pagellus bogaraveo</i>	75–103	16	LO	100	7.5	[76]
<i>Pagrus major</i>	3.6–52	15	CO	100	10	[140]
	4.9–49.3	12	SO	40	4	[146]
<i>Pagrus auratus</i>	28.4–92	10	CO, SO	100	10	[147]
<i>Acanthopagrus schlegeli</i>	20.3–84	15	SO	80	7.2	[148]
<i>Sparidentex hasta</i>	1.1–4.4	12	SO, LO	100	8	[148]
	14.5–36	20	CO, SFO	100	10	[149]

Abbreviations: CO: canola oil; EO: echium oil; HO: hazelnut oil; LO: linseed oil; PO: palm oil; RO: rapeseed oil; SFO: sunflower oil; SO: soybean oil.

In *S. hasta* juveniles ( $BW_i = 14.6 \pm 0.1$  g), Mozanzadeh et al. conducted an eight-week feeding trial to evaluate the effects of partial (50%) or total (100%) replacement of dietary FO with alternative lipid sources [149]. In this regard, seven isonitrogenous (ca. 50%) and isolipidic (ca. 20%) experimental diets were formulated and named as follows: FO (fish oil), CO (canola oil), SO (sunflower oil), T (tallow), FC (fish oil + canola oil, 50:50), FS (fish oil + sunflower oil, 50:50) and FT (fish oil + tallow, 50:50). The partial or total replacement of dietary FO by VO sources (CO and SO) did not compromise growth performance or feed efficiency parameters compared to the control diet (Table 11). However, fish fed the T and FT diets had lower WG and SGR than the other groups. These results were attributed to the lower concentration of PUFAs and the higher levels of saturated fatty acids (SFAs) in tallow in comparison to the other tested oil sources. Similar results have been reported in other sparid species such as *S. aurata* fed a diet in which FO was replaced by beef tallow and corn oil [150]. Moreover, ADCs for protein and lipid were lowest in fish fed the T diet, which was attributed to the lower growth

performance in this group. In this context, Martins et al. [151] and Karalazos et al. [152] have also reported that the use of tallow led to a decrease in ADCs of nutrients in Atlantic halibut, *Hippoglossus hippoglossus* and *Salmo salar*, because of the high level of SFAs in this lipid source. The adverse effects of SFAs on nutrient utilization and growth performance might be attributed to the negative effects of SFAs on energy, lipid and fatty acid apparent digestibility, which were linked to higher melting point of SFAs than MUFAs and PUFAs that make them less digestible [89]. From the above results, Mozanzadeh et al. [149] stated that VO sources (canola and sunflower oils) were recommended for total and partial replacement of FO in *S. hasta* diets, which may be explained by the content of crude lipids (4–6%) in FM that would assure the dietary minimum amount of EFAs for proper somatic growth. However, tallow was not advisable as an alternative lipid source in this species, regardless of the levels of lipids included in FM.

**Table 11.** Growth in body weight (g), specific growth rate (SGR), feed utilization parameters and apparent digestibility coefficients (ADC) of protein and lipid of *S. hasta* fed experimental diets in which fish oil partially and totally replaced by different alternative lipid sources during the on-growing stage (mean  $\pm$  SEM,  $n = 3$ ) [149].

Growth Performance	Diets *						
	FO	CO	SO	T	FC	FS	FT
BW <sub>f</sub> (g) <sup>a</sup>	35.7 $\pm$ 0.5 <sup>a</sup>	34.1 $\pm$ 0.6 <sup>ab</sup>	34.0 $\pm$ 0.9 <sup>ab</sup>	32.2 $\pm$ 0.7 <sup>b</sup>	33.6 $\pm$ 0.3 <sup>ab</sup>	35.9 $\pm$ 0.4 <sup>a</sup>	32.6 $\pm$ 0.4 <sup>b</sup>
SGR (% BW day <sup>-1</sup> ) <sup>b</sup>	1.6 $\pm$ 0.1 <sup>a</sup>	1.5 $\pm$ 0.0 <sup>ab</sup>	1.5 $\pm$ 0.0 <sup>ab</sup>	1.4 $\pm$ 0.0 <sup>b</sup>	1.5 $\pm$ 0.0 <sup>ab</sup>	1.6 $\pm$ 0.0 <sup>a</sup>	1.4 $\pm$ 0.0 <sup>b</sup>
FI (g fish <sup>-1</sup> ) <sup>c</sup>	35.5 $\pm$ 2.1	33.0 $\pm$ 2.6	31.0 $\pm$ 1.2	30.1 $\pm$ 1.0	30.2 $\pm$ 2.0	34.2 $\pm$ 3.0	30.6 $\pm$ 1.6
FCR <sup>d</sup>	1.7 $\pm$ 0.1 <sup>ab</sup>	1.7 $\pm$ 0.0 <sup>ab</sup>	1.6 $\pm$ 0.1 <sup>b</sup>	1.7 $\pm$ 0.0 <sup>ab</sup>	1.6 $\pm$ 0.0 <sup>b</sup>	1.6 $\pm$ 0.0 <sup>b</sup>	1.8 $\pm$ 0.0 <sup>a</sup>
ADC of protein (%) <sup>e</sup>	93.5 $\pm$ 2.5 <sup>ab</sup>	91.1 $\pm$ 1.7 <sup>bc</sup>	97.7 $\pm$ 1.3 <sup>a</sup>	87.2 $\pm$ 2.0 <sup>c</sup>	94.2 $\pm$ 1.5 <sup>ab</sup>	89.9 $\pm$ 2.6 <sup>bc</sup>	92.4 $\pm$ 1.8 <sup>abc</sup>
ADC of lipid (%)	97.2 $\pm$ 0.5 <sup>ab</sup>	96.3 $\pm$ 1.3 <sup>ab</sup>	98.9 $\pm$ 0.6 <sup>a</sup>	94.6 $\pm$ 0.6 <sup>b</sup>	98.7 $\pm$ 0.7 <sup>a</sup>	98.3 $\pm$ 0.4 <sup>a</sup>	96.0 $\pm$ 1.4 <sup>ab</sup>

\* Diet abbreviations: FO: fish oil; CO: canola oil; SO: sunflower oil; T: tallow; FC: fish oil + canola oil; FS: fish oil + sunflower oil; FT: fish oil + tallow; <sup>a</sup> BW<sub>f</sub>: final body weight; <sup>b</sup> SGR: specific growth rate = [(ln final weight – ln initial weight)/t]  $\times$  100; where t is experimental period = 56 days; <sup>c</sup> FI: feed intake = total feed intake (g)/number of fish; <sup>d</sup> FCR: feed conversion ratio = feed intake (g)/weight gain (g); <sup>e</sup> ADCs of nutrients = 100 – [100  $\times$  (Cr<sub>2</sub>O<sub>3</sub> in diet/Cr<sub>2</sub>O<sub>3</sub> in feces)]  $\times$  [(% nutrient in feces/% nutrient in diet)].

#### 4. Conclusions and Future Research

Considering the information presented from the above-mentioned studies and comparing that with other sparid species, it can be concluded that diets containing ca. 48% crude protein, 15% crude lipid, 15% carbohydrates and 20 KJ g<sup>-1</sup> gross energy may be recommended for on-growing *S. hasta*. The optimum EAA profile for *S. hasta* juveniles, expressed as g 16 g N<sup>-1</sup>, is as follows: 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. Moreover, the optimum dietary n-3 LC-PUFAs was recommended to be 0.8% of diets. The maximum replacement of FM with SP was between 16.5% and 27.3%. In addition, VO sources are recommended for total and partial replacement of FO in *S. hasta* diets. Future studies should use the more modern “omics” techniques such as transcriptomics, proteomics, metabolomics and lipidomics instead of the traditional single dose–response growth or biochemical methods, so that a more holistic picture will emerge to give us a better understanding on the nutrient requirement and utilization by *S. hasta*, as have been conducted in other sparid species [153,154]. Finally, future experiments need to be designed under a more holistic approach, in which different variables (i.e., levels of macro- or micronutrients, additives, and immunostimulats) need to be tested, individually or combined, under different rearing conditions (i.e., stocking densities, feeding rates and cycles, water temperatures and oxygen levels) in order to provide a more realistic effect of diet formulation of fish performance under culture conditions.

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### Abbreviations

The following abbreviations are used in this publication:

ADCs	Apparent digestibility coefficient
ANFs	Anti-nutritional factors
ARG	Arginine
BM	Blood meal
CGM	Corn gluten meal
CHO	Carbohydrates
CO	Canola oil
CYS	Cysteine
DHA	Docosahexaenoic acid
DSM	Dehulled soybean meal
E	Energy
EO	Echium oil
EAA	Essential amino acids
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
ETPP	Enzyme treated poultry meal
FI	Feed intake
FM	Fish meal
FS	Fish soluble
FSP	Fermented soy protein
FO	Fish oil
HIS	Histidine
HO	Hazelnut oil
HSI	Hepatosomatic index
ILE	Isoleusine
ISP	Isolated soy protein
KM	Krill meal
L	Lipids
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acids
LEU	Leucine
LNA	Linolenic acid
LO	Linseed oil
LSM	Lupine seed meal
LYS	Lysine
MET	methionine
MUFA	Monounsaturated fatty acids
P	Protein
PBPM	Poultry by product meal
PER	Protein efficiency ratio
PHE	Phenylalanine
PLs	Phospholipids
PO	Palm oil
PP	Pea protein
PPC	Pea protein concentrate
PPI	Pea protein isolate

PSM	Pea seed meal
RPC	Rice protein concentrate
SBM	Soybean meal
SBL	Soybean lecithin
SFA	Saturated fatty acids
SFO	Sunflower oil
SM	Squid meal
SO	Soybean oil
SGR	Specific growth rate
T	Tallow
THR	Threonine
TRP	Tryptophane
TYR	Tyrosine
VAL	Valine
WG	Weight gain

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