



The Effects of Different Commercial Feeds and Seasonal Variation on Fillet Amino Acid Profile of Sea Bream (*Sparus aurata*) and Sea Bass (*Dicentrarchus labrax*)

Mustafa YILDIZ^{1*} and Samuel OFORI-MENSAH²

¹ Istanbul University, Department of Aquaculture, Fisheries Faculty, Istanbul, Turkey

² Istanbul University, Department of Aquaculture, Institute of Graduate Studies in Science and Technology, Istanbul, Turkey

E-mail: musstar@gmail.com

Abstract

This study determined the influence of marine fish feeds on the amino acid (AA) composition in fillet of sea bream and sea bass at different seasons. AA profile of cultured fish fillets mirrored dietary amino acids (correlation ranged from 0.77 to 0.91). Slight variations ($P > 0.05$) existed in protein and AA profiles in cultured and wild sea bream and sea bass. Main essential amino acids (EAA) were arginine, leucine and lysine while alanine, aspartic acid, glutamic acid and glycine were the main non-essential amino acids (NEAA) for both winter and summer in fish filets. EAA/NEAA ratio ranged from 0.96 to 1.10 and 1.02 to 1.08 in summer and winter respectively for cultured and wild sea bream. In sea bass, the range was from 0.90 to 1.16 in summer and from 0.96 to 1.19 in winter. The study revealed that the EAA composition of fish filets was affected by feeds and seasons ($P < 0.05$). EAA/NEAA ratios indicated that both captured and cultured sea bream and sea bass filets are sources of well-balanced protein and/or AA in all seasons for humans.

Keywords: commercial feeds, cultured, wild, sea bream, sea bass, flesh quality, amino acid profiles, seasons

Introduction

Flesh quality of cultured fish species is becoming the focus of the development of aquaculture and fish feed industry. Fish feed quality is important for optimizing the nutrition structure of food for humans (de Francesco *et al.*, 2004; Grigorakis, 2007). The most important factor affecting growth performances of fish and feed cost is the dietary protein (Lovell, 1989). The protein quality of food depends on its ability to provide all the essential amino acids (EAA) required by the organism. It is well known that fish is a perfect protein source due to its ability to supply all the dietary EAA requirements of humans. However, the composition of amino acids (AA) of fish flesh and the main component of proteins, are affected by factors such as species, size, food resources, fishing season, salinity and temperature (Wesselinova, 2000; Özyurt & Polat, 2006). In general, feed consumption increases with the water temperature. The variations of water temperature have been reported to alter the protein requirements of some fishes. However, a change in water temperature affects feed intake much more than the protein requirement (Wilson, 2002; Kaushik & Seiliez, 2010).



Fishes, like other animals, are known to require all the EAA in their diet for maximum growth (Wilson, 2002; Berge *et al.*, 2002; Kaushik & Seiliez, 2010). However, the requirements of various fish species for individual EAA are significantly different (NRC, 2011; Wilson, 2002; Kaushik and Seiliez, 2010).

The requirements for all 10 EAA have been determined for several commercially important species such as rainbow trout, channel catfish, blue tilapia, common carp, milkfish, red drum and yellowtail (Ogino, 1980; Moon & Gatlin, 1991; Alam *et al.*, 2002; NRC, 2011) but are still not clear for sea bream and sea bass (NRC, 2011). Sea bream and sea bass require the same EAA like other fishes, and data available for other species having similar food habits and ecological requirement may be used as standards. Previous studies have been interested on dietary EAA profiles for optimum growth of sea bream (Kaushik, 1998; Marcouli *et al.*, 2004; Kaushik & Seiliez, 2010) and sea bass (Kaushik, 1998; Peres & Oliva-Teles, 2006; Kaushik & Seiliez, 2010). The fillet EAA pattern of a given fish is considered to be a representative of the dietary EAA requirement of that fish species (Wilson & Cowey, 1985; Mambrini & Kaushik, 1995; Kaushik, 1998). Similarly, Wilson & Poe (1985) reported that there was a positive correlation between dietary EAA requirement pattern and the whole-body EAA pattern in channel catfish.

In recent years, there is limited information about the impacts of AA profile of commercial fish feeds on the flesh of cultured marine fish species from commercial farms around the world. The substitution of traditional fish feed ingredients with alternative sources as a way of reducing production costs (Bell *et al.*, 2004; Izquierdo *et al.*, 2005; Pratoomyot *et al.*, 2010) while boosting aquaculture production has led to safety and quality concerns of products (Frewer *et al.*, 2005). Such feeds have been found to change body composition and slaughter quality (Cowey, 1993; Hillestad & Johnsen, 1994; Vergara, *et al.*, 1999; Kim *et al.*, 2012). Therefore, it is the desire of consumers to be able to differentiate among aquaculture products based on their nutritional value (Valente *et al.*, 2011). Sea bream and sea bass are the most important and commercially successful marine food fish cultured in Turkey (TURKSTAT, 2015). The present study is a continuation of studies that have examined seasonal changes of body proximate composition for sea bream (Yildiz *et al.*, 2006) and sea bass (Yildiz *et al.*, 2007), seasonal changes in fatty acid (Yildiz *et al.*, 2008) and mineral composition in sea bream and sea bass (Yildiz, 2008) but not amino acid composition of fish fillets. Very little is known on the dietary AA impact on fillet and muscle free AA in sea bream and sea bass, especially at market sizes (Gomez-Requeni *et al.*, 2004). It is the reason why Grigorakis (2007) to recommend further research on the muscle AA of commercially sized sea bream and sea bass. Therefore, the objective of the present work was to determine the effects of the commonly used commercial marine fish feeds in Turkey on the AA composition in fillets of sea bream and sea bass at different seasons. The study also included comparisons between AA profile of cultured and wild fish fillets.

Materials and methods

Materials

Most commercial feeds used in growing sea bream and sea bass are extruded feeds A (6–8 mm, Camli yem, Izmir, Turkey); B (Kilic yem, Mugla, Turkey); C (EcoBio yem, Izmir, Turkey); and D (Trouvit feed, Skretting, Turkey). Farmed gilthead sea bream, *Sparus aurata* (average mass, 391.4 ± 23.7 g) and sea bass, *Dicentrarchus labrax* (average mass, 406.4 ± 26.3 g) and commercial feed samples were obtained from four fish farms on the Aegean coast of Turkey according to feed used during the summer and winter seasons. These farms produce



about 70% of sea bream and sea bass. Wild sea bream (average mass, 325.2 ± 16.4 g) and sea bass (average mass, 312.8 ± 32.6 g) were caught in the same region and seasons. The average seawater temperature was 27 ± 0.19 °C (summer) and 14 ± 0.15 °C (winter) for farmed and wild fish. During the experiment, water salinity was about 35–38‰. Fish were fed at approximately 0.8 and 1.8% of body weight per day in winter and summer respectively. Feed and fish samples, sea bream ($n = 10$) and sea bass ($n = 10$) were obtained from each fish farm, seasonally. Similarly, sea bream ($n = 10$) and sea bass ($n = 10$) were caught in the farm area. Fish samples were killed and packaged in black nylon bags (packed into an insulated polystyrene box with dry ice) and then transported to the laboratory in a freezer box. The samples were stored at -80 °C until analysis.

Protein and amino acid analysis

Fillets of fish were randomly divided into three homogeneous groups with each group blended to homogenize. Protein was determined as total nitrogen (N) using a semi-automatic Kjeldahl (Gerhardt Vapodest, 45s) technique and multiplying N by 6.25 (AOAC, 1995). The preparation of samples for analysis of amino acids followed the methodology of the AOAC (2000): Acid digestion (HCl 6 N, 24 h) was performed in sealed glass tubes under a nitrogen atmosphere (110 °C). Excess acid from the hydrolysate was removed by flash evaporation under reduced pressure. The analysis was carried out using an Eppendorf Biotronic LC 3000 Amino acid Analyzer (Eppendorf- Biotronic, Hamburg, Germany). The resulting peak areas of samples were calculated relative to the peak of the external standard. The AA mixed standards were from Sigma–Aldrich Inc. (Saint Louis, USA). The results were given as means of triplicate. The same procedures were used in analyzing the protein and AA profile of the commercial feeds.

Data analysis

Data are presented as means \pm SE. Differences between the feed and fillets groups were tested using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences among feed type, season and fillets groups were tested using two-way ANOVA. The relation between dietary and fillet amino acids levels were also investigated using Pearson's coefficient correlation for sea bream and sea bass. The significance level was set at $P < 0.05$. Analyses were conducted using the SPSS 16.0 software package.

Results and Discussion

Amino acid content of commercial feeds

The average of total protein content and AA composition of commercial feeds sampled did not differ between the two investigated seasons ($P > 0.05$). Hence, average of these parameters at the two seasons is presented in Table 1. The average total protein level in the commercial feeds was $45.3 \pm 0.54\%$. These results are in agreement with the previous optimal findings 45% to 46% for sea bream (Santinha *et al.*, 1996; Oliva-Teles, 2000) and (43% to 45% for sea bass diets (Perez *et al.*, 1997; Kaushik, 2002). Due to the high costs of protein sources in the market, protein is the main limiting nutrient to formulate cost-effective diets for fish (da Silva *et al.*, 2014). Currently NRC (2011) estimates the optimal level of crude protein for juvenile sea bass on dry-matter basis at 40%.



All the AA essential for growth were found in the sampled feeds. However, the AAs in the commercial feeds were dominated by arginine, leucine, aspartic acid and glutamic acid. Levels of some AAs such as arginine, methionine, tryptophan, valine and alanine in the all feeds were similar ($P > 0.05$). Phenylalanine and leucine contents in the feeds B and C respectively, were the highest ($P < 0.05$). Isoleucine and threonine contents in the feed D, were lowest ($P < 0.05$). However, the requirement levels of only five EAA (arginine, lysine, methionine + cystine, tryptophan and threonine) have been determined for sea bass and sea bream. The known EAA requirements of sea bass and sea bream are given in Table 2.

The results of the EAA in the commercial feeds used in this study (Table 1) in comparison with the above concentrations (Table 2) suggest similar dietary AA requirements for sea bream and sea bass. Concentrations of lysine in commercial feeds used in the present study were within suggested values for the requirements of sea bass and sea bream. However, arginine, threonine and tryptophan concentrations in the commercial feeds tested in our study were markedly higher than the requirements in cultured sea bass or sea bream (Table 1). Although there is some information on the dietary requirements of EAA, the requirements keep on changing (Table 2). This might be due to the use of different dietary sources and different methods by different researchers in establishing optimum levels. Irrespective of this, available information shows dietary requirements for AA in the diets of sea bream and sea bass. The quality of proteins in diets of fish affects their dietary protein requirement (Sanchez-Muros *et al.*, 2003; Gomez-Requeni *et al.*, 2004) together with factors such as size of fish, composition of diet and environment (Grigorakis, 2007; NRC, 2011). The supply of adequate levels of amino acids with a balanced profile through feeding is essential for their maximal accretion and growth in fish subsequently (El-Mowafi *et al.*, 2010). The quantitative EAA amounts (Table 1) in the commercial feeds in the current study meet dietary requirements of many cultured carnivorous fish species (Ogino, 1980; Moon and Gatlin, 1991; Alam *et al.*, 2002; NRC, 2011) as shown in Table 2.

Amino acid content of sea bream

The protein and AA concentrations in the fillets of seasonally obtained cultured and wild sea bream are shown in Tables 3 and 4. Fish fed commercial feeds A and B had comparatively the highest levels ($P < 0.05$) of fillet proteins in both summer and winter. Fish fed feeds C and D had similar fillet protein level as the wild fish in summer ($P > 0.05$). In winter, fish group from the wild had the least level of fillet protein compared to cultured sea bream ($P < 0.05$). In culture, Velázquez *et al.* (2004) found that sea bream feeds during the highest temperature of the day in winter. Protein accumulation in tissues during growth is the result of protein synthesis and breakdown (Fauconneau & Arnal, 1985) with dietary sources providing such proteins. In the wild, sea bream may not locate the right feed and/or do not feed sufficiently due to changes in prey abundance and/or unfavourable conditions during winter (Love, 1988). Cultured fishes, however, are fed complete diets with optimum protein levels throughout the year hence have a more stable fillet composition (Haard, 1992; Cowey, 1993). Generally, the protein levels in fillets of cultured fish groups were higher in the winter season. Low water temperatures due to winter slow fish metabolic rates and reduce energetic requirements together with a constraint on activity (Brett, 1979; Huusko *et al.*, 2007; Jonsson & Jonsson, 2011). Therefore, relatively little amounts of proteins consumed are catabolized while the rest are synthesized once the required AA are present. This leads to the accumulation of proteins in the fillet.



In the current study, arginine, leucine and lysine were the main EAA whereas alanine, aspartic acid, glutamic acid and glycine were the main NEAA in the fillets of the cultured and wild sea bream in summer and winter seasons. The levels of the EAA such as histidine, methionine, tryptophan were lower ($P < 0.05$) than the other EAA. In general, the AA concentrations in the fillets of cultured and wild sea bream were similar in summer and winter. However, amounts of histidine in the B and C groups; lysine in the A, B and C groups; methionine and tryptophan in the C groups and wild fish; prolin in the A group and serin in the B and C groups were significantly lower in winter than in summer.

Fillet amino acid composition of sea bream was affected by feed type and season (two-way ANOVA, $P < 0.05$). A positive relationship existed between feeds and fish fillets; for feed A (Pearson's correlation coefficient, $r = 0.73$ and 0.87 in summer and winter respectively), B ($r = 0.80$ in summer and 0.86 in winter), C ($r = 0.64$ in summer and 0.84 in winter), and D ($r = 0.85$ in summer and 0.81 in winter) at $P < 0.01$. Although dietary protein levels were similar during both seasons (Tables 1, 3 and 4), the AA profile of the feeds differed. The main purpose of dietary protein and/or AA supply is for increasing whole body protein accumulation (Kaushik & Seiliez, 2010). Dietary sources have been implicated in impacting on fillet and muscle free AAs (Mente *et al.*, 2003; Yamamoto *et al.*, 2005). Similarly, a positive correlation was reported between dietary EAA/NEAA ratios and muscle free AAs (Gomez-Requeni *et al.*, 2003). After a study on juvenile gilthead sea bream, Gomez-Requeni *et al.* (2004) reported that dietary plant protein increased free AAs in muscles. These studies confirm that feeds can impact the AA profile of fish fillet including that of sea bream.

Amino acid content of sea bass

Protein levels and AA profile of the fillets of wild and cultured sea bass are shown in Tables 5 and 6 for summer and winter respectively. In both seasons, protein levels ranged from 18.12 to 21.28 g/100 g protein. Protein levels in fillet of cultured fish were similar ($P > 0.05$) whereas the level was significantly low ($P < 0.05$) in the wild fish in the summer. Fish metabolism increases as a result of the increasing water temperature leading to a relatively higher feed consumption (Wilson, 2002; Kaushik & Seiliez, 2010; Sandblom *et al.*, 2014). For cultured sea bass, fish are fed with the right amount of feed with optimum protein levels while residing in a restricted area. This may lead to a relatively higher retention of proteins in fillets as fish do not have to expend energy in search for feed like the wild fish. However, wild sea bass have to constantly search for feed ingredients and prey over wider area to satisfy their nutritional needs. Fish are known to prefer protein to carbohydrate for energy metabolism (Cowey & Luguët, 1983; Weber & Haman, 1996). This may be the reason for relatively low accumulation of proteins in their fillets as noticed in the current study. In the winter protein levels were similar in the fillets of both the cultured fish and the wild fish ($P > 0.05$). The main activator of metabolism is temperature and as such, variations of temperature can influence changes in AAs (Gómez-Milán *et al.*, 2011). In winter, water temperatures are not that low in the Aegean region. Although these temperatures are not ideal for sea bass, they do not impair feeding, especially in the wild. These conditions coupled with the reduced and/or slowed metabolism in fish might have led to the accumulation of proteins in their fillets with a resultant slightly increased protein levels in fillets in winter compared to summer.

Like sea bream, AA concentrations in the fillets of the cultured sea bass were affected by feed type and season (two-way ANOVA, $P < 0.05$). Further, there was a positive correlation between feed and fish fillets. For feed A,



$r = 0.78$ and 0.77 in summer and winter respectively; $r = 0.88$ in summer and 0.91 in winter for feed B, $r = 0.77$ for both summer and winter for feed C; and $r = 0.82$ in summer and 0.83 in winter for feed D with the relationships being significant ($P < 0.01$). The EAA compositions in fillets were mainly made up of arginine, leucine, and lysine in the summer whereas arginine, isoleucine, leucine and lysine constituted the main EAA in winter. Alanine, aspartic acid, glutamic acid, and glycine were the main NEAA for both summer and winter seasons. The levels of histidine, methionine and tryptophan were low in comparison to the other EAA in fish fillets. In general, although slight variations existed in total EAA in fillets of cultured and wild sea bass groups, the levels were similar. Likewise, the ratio of EAA and NEAA were also similar between the groups (Tables 5 and 6). The cultured fish have the right amount of protein and amino acid levels in their fillets. Feed (artificial or natural) do not only fulfill the nutritional needs of fish but also contribute to determining their growth and flesh quality (Izquierdo *et al.*, 2003). With this in mind, the main purpose of dietary protein/AA supply is for increasing the accretion of protein/AA in body or fillets (Kaushik & Seilez, 2010). The commercial feeds used in the current study had the right amounts of proteins and AA (Table 1) in balanced proportions throughout the culture seasons. Hence these feeds are able to supply fish with the required protein and AA requisite for growth. Similarly, wild fish are able to find prey and food materials to supply their nutritional requirements for both seasons. For this reason, wild fish have similar protein and AA levels in their fillets.

Some studies on sea bream (Mongile *et al.*, 2014; Cardinal *et al.*, 2011; Valente *et al.*, 2011) and sea bass (Özyurt and Polat, 2006; Tibaldi *et al.*, 2015) reported glutamic acid, glycine, histidine, and isoleucine as the main AA as found in the present study. Glutamic acid, aspartic acid, alanine, and glycine are known to be related to the characteristic flavour of fish flesh (Ruiz-Capillas & Moral, 2004). Results from the current study shows that these amino acids were in abundance in the fillets of sea bream and sea bass. Therefore, fish produced from this study had high flesh quality in terms of flesh proteins and AAs and agrees with previous studies on the current subject.

In the current study, the ratio EAA/NEAA ranged from 0.96 to 1.10 in summer (Table 3) and from 1.02 to 1.08 in winter (Table 4) in fillets of both cultured and wild sea bream. In sea bass, the range was from 0.90 to 1.16 in summer (Table 5) and from 0.96 to 1.19 for winter (Table 6) in fish fillets of the cultured and wild fish. Özyurt & Polat (2006) reported that the ratio of EAA to NEAA for wild sea bass were 0.75 and 0.77 for winter and summer respectively. According to other studies, the ratio of EAA to NEAA was 0.71 for cod *Gadus morhua* (Jhaveri *et al.*, 1984) and mullet *Mugil cephalus* (Iwasaki & Harada, 1985); 0.74 for herring *Clupea pallasii*; and 0.75 for chum salmon *Oncorhynchus keta* (Iwasaki and Harada, 1985). Similar ratios were obtained by Cardinal *et al.* (2011) and Valente *et al.* (2011) in fillets of sea bream from other Mediterranean Regions in Europe. The EAA/NEAA ratio in the studies aforementioned showed a well-balanced AA. This implies that in the current study, the fillets of sea bream and sea bass have high protein and EAA levels.

In comparison with other studies in marine and carnivorous fish (Alasalvar *et al.*, 2002; Özyurt & Polat, 2006; Velázquez *et al.*, 2006; Fuentes *et al.*, 2010; Salama *et al.*, 2013), the levels of proteins and AAs found in fillets of sea bream and sea bass of the present study typifies fillets of very good quality. Researches on protein and AAs dietary requirements for humans continue to be an actively carried out (FAO, 2007; DRI, 2005; Elango *et al.*, 2012) being a few examples. From these studies, the level of protein and AA found in the fillets of sea bream and sea bass from the current study meet the human dietary requirements of protein and/or AAs. This implies



fillets of sea bream and sea bass produced in the Aegean Region of Turkey are good dietary sources of proteins and AAs for human consumption and nutrition.

Finally, during the current study, no significant differences were noticed among protein and AA profiles of cultured and wild sea bream and sea bass flesh. There were no differences because commercial feeds given to cultured fish supplied their dietary proteins and AAs requirements for growth and consequently enabled the accumulation of these in their fillets. Due to the ever changing sources of dietary ingredients in recent years, similar studies should be done periodically to ascertain the influence of commercial feeds on fillet proteins and AA profiles of cultured marine fish.

Conclusion

The commercial feeds produced in Turkey meet the optimum dietary requirements of proteins and/or AA requirements of sea bream and sea bass. Dietary AAs influenced the fillet EAA levels in sea bream and sea bass. The fillet quality of sea bream and sea bass cultured is similar to those from the wild. The abundance of the AAs glutamic acid, aspartic acid, alanine, and glycine shows that sea bream and sea bass produced in this study have good flavor. The fillet composition of proteins and/or AAs of sea bream and sea bass produced from the Aegean Region in Turkey compares favourably with other areas of production from the Mediterranean Region and slightly superior. For a fish species to be considered ideal for human protein needs, it should fulfill the nutritional protein and AA requirements. Therefore sea bass and sea bream produced from culture and capture fisheries are ideal dietary sources of nutritional protein and AA as they meet the human dietary requirements of protein and/or AA.

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Table 1. Average protein content (% of dry weight basis) and amino acid composition (g 100 g⁻¹ dry diet) of the different commercial feed samples¹

Protein and amino acids	Feeds ²			
	A	B	C	D
Protein (%)	45.32±0.53	44.94±0.67	45.06±0.51	46.05±0.43
<i>Essential amino acids (EAA)</i>				
Arginine	7.01±0.15	6.53±0.19	7.19±0.28	7.23±0.15
Histidine	1.46±0.07 ^b	1.68±0.06 ^{ab}	1.87±0.05 ^a	1.81±0.04 ^a
Isoleucine	4.32±0.14 ^a	4.36±0.12 ^a	4.44±0.14 ^a	3.87±0.11 ^b
Leucine	7.97±0.15 ^b	8.00±0.12 ^b	8.63±0.11 ^a	7.95±0.27 ^b
Lysine	4.81±0.24 ^{ab}	5.39±0.15 ^a	4.35±0.25 ^b	5.42±0.16 ^a
Methionine	1.82±0.08	1.70±0.08	1.92±0.15	1.83±0.09
Phenylalanine	4.62±0.13 ^b	5.40±0.15 ^a	4.68±0.06 ^b	4.77±0.17 ^b
Threonine	3.93±0.11 ^a	3.97±0.15 ^a	4.14±0.21 ^a	3.07±0.16 ^b
Tryptophan	1.83±0.13	1.67±0.09	1.81±0.04	1.71±0.07
Valine	3.73±0.17	4.12±0.02	3.98±0.07	4.12±0.21
Total EAA	41.50±0.1 ^{4b}	42.82±0.10 ^a	43.01±0.14 ^a	41.78±0.14 ^{ab}
<i>Nonessential amino acids (NEAA)</i>				
Alanine	5.85±0.20	6.27±0.27	6.39±0.33	5.47±0.19
Aspartic acid	8.28±0.14 ^{ab}	9.78±0.30 ^a	9.55±0.24 ^a	7.79±0.17 ^b
Glutamic acid	13.85±0.45 ^a	12.87±0.42 ^{ab}	12.59±0.37 ^{ab}	11.54±0.20 ^b
Glycine	4.72±0.13 ^b	6.10±0.11 ^a	5.79±0.18 ^a	4.88±0.14 ^b
Proline	4.32±0.14 ^b	5.19±0.13 ^a	5.18±0.18 ^a	4.50±0.19 ^b
Serine	3.69±0.09 ^a	2.97±0.13 ^b	3.27±0.16 ^{ab}	2.93±0.14 ^b
Tyrosine	3.18±0.10 ^b	4.09±0.16 ^a	3.51±0.08 ^{ab}	3.36±0.04 ^b
Total NEAA	43.89±0.18 ^b	47.27±0.22 ^a	46.28±0.22 ^a	40.47±0.15 ^c
EAA/NEAA	0.95	0.91	0.93	1.03

¹Protein and amino acid compositions are presented as means ± S.E.M. of six replicates for two seasons. Results in each row with different superscript letters were significantly different ($P < 0.05$).

²All feed groups were produced by different commercial companies in Turkey. The feeds were used by different sea fish farms in Aegean region, Turkey.

**Table 2.** Available data on dietary EAA requirements of sea bass and sea bream

Amino acids	Dietary requirement (g/16 g N)	Species	References
Arginine	4.1	Sea bass	Tibaldi et al. (1994)
	3.9-4.6		Tibaldi et al. (1993, 1994)
	7.5		NRC (2011)
	5	Sea bream	Luquet & Sabaut (1974)
	5.55		Peres & Oliva-Teles (2009)
Histidine	1.89	Sea bream	Peres & Oliva-Teles (2009)
Isoleucine	2.55	Sea bream	Peres & Oliva-Teles (2009)
Leucine	4.75	Sea bream	Peres & Oliva-Teles (2009)
Lysine	4.8	Sea bass	Tibaldi & Lanari (1991)
	2.2		NRC (2011)
	5-5.13	Sea bream	Luquet & Sabaut (1974); Peres & Oliva-Teles (2009)
Methionine + cystine	4.4	Sea bass	Luquet & Sabaut (1974); Peres & Oliva-Teles (2009)
	4.99	Sea bass	Tulli et al. (2010)
		Sea bream	Oliva-Teles (2000)
Methionine	2.6	Sea bream	Peres & Oliva-Teles (2009)
Phenylalanine + tyrosine	5.76	Sea bream	Peres & Oliva-Teles (2009)
Threonine	1.2	Sea bass	NRC (2011)
	2.3-2.6		Tibaldi & Tulli (1999)
	2.98	Sea bream	Peres & Oliva-Teles (2009)
Tryptophan	0.5	Sea bass	Tibaldi et al. (1993)
	0.6	Sea bream	Luquet & Sabaut (1974)
	0.75		Peres & Oliva-Teles (2009)
Valine	4.7	Sea bass	NRC (2011)
	3.21	Sea bream	Peres & Oliva-Teles (2009)

**Table 3.** Average protein content (% of wet weight basis) and amino acid composition (g 100 g⁻¹ protein) in fillets of sea bream, *Sparus aurata* in summer¹

Protein and amino acids	Cultured fish groups ²				Wild fish
	A	B	C	D	
Protein (%)	20.53±0.22 ^a	20.14±0.25 ^a	19.55±0.30 ^b	19.17±0.71 ^b	19.56±0.42 ^b
<i>Essential amino acids (EAA)</i>					
Arginine	5.02±0.10 ^{ab}	4.76±0.07 ^b	5.54±0.04 ^a	5.12±0.07 ^{ab}	5.18±0.12 ^{ab}
Histidine	2.54±0.03 ^a	2.03±0.06 ^{ab}	2.36±0.02 ^a	2.04±0.03 ^{ab}	1.96±0.01 ^b
Isoleucine	4.34±0.09 ^{ab}	4.87±0.05 ^a	4.36±0.11 ^{ab}	3.45±0.08 ^b	3.59±0.05 ^b
Leucine	6.44±0.12 ^c	8.06±0.09 ^a	6.77±0.07 ^c	8.01±0.10 ^a	7.12±0.06 ^b
Lysine	8.36±0.15 ^b	9.35±0.10 ^a	9.18±0.08 ^a	8.18±0.12 ^b	8.76±0.08 ^{ab}
Methionine	2.00±0.03 ^{ab}	1.74±0.02 ^b	2.31±0.03 ^a	1.73±0.02 ^b	2.18±0.04 ^a
Phenylalanine	3.51±0.05 ^b	4.33±0.04 ^a	3.44±0.03 ^b	4.14±0.03 ^a	4.20±0.02 ^a
Threonine	4.49±0.07	4.28±0.04	4.61±0.10	4.17±0.04	4.39±0.08
Tryptophan	1.78±0.01 ^b	2.55±0.02 ^a	2.60±0.02 ^a	1.97±0.03 ^b	2.32±0.04 ^{ab}
Valine	3.76±0.07 ^b	4.68±0.05 ^a	3.90±0.04 ^b	3.66±0.02 ^b	3.76±0.03 ^b
Total EAA	42.24±0.07 ^c	46.65±0.05 ^a	45.07±0.05 ^a	42.47±0.05 ^c	43.46±0.05 ^{bc}
<i>Nonessential amino acids (NEAA)</i>					
Alanine	6.49±0.08 ^{bc}	7.21±0.13 ^a	6.82±0.15 ^{ab}	5.55±0.10 ^c	6.17±0.08 ^{bc}
Aspartic acid	8.90±0.13 ^{bc}	8.56±0.10 ^c	9.46±0.07 ^b	10.51±0.07 ^a	9.68±0.10 ^b
Glutamic acid	10.97±0.13 ^b	11.73±0.17 ^a	11.95±0.12 ^a	9.63±0.17 ^c	10.05±0.11 ^c
Glycine	5.76±0.04 ^a	4.33±0.02 ^b	4.20±0.06 ^b	5.55±0.03 ^a	4.83±0.03 ^{ab}
Proline	4.68±0.02 ^a	3.98±0.01 ^b	4.26±0.04 ^{ab}	3.82±0.03 ^b	4.03±0.00 ^b
Serine	3.80±0.02 ^a	3.63±0.04 ^a	3.79±0.01 ^a	3.77±0.03 ^a	3.16±0.02 ^b
Tyrosine	2.68±0.01 ^c	3.06±0.03 ^{bc}	2.67±0.03 ^c	4.55±0.02 ^a	3.54±0.04 ^b
Total NEAA	43.28±0.06 ^c	42.5±0.07 ^{bc}	43.15±0.07 ^a	43.38±0.06 ^a	41.46±0.05 ^c
EAA/NEAA	0.96	1.10	1.04	0.98	1.05

¹Protein and amino acid compositions are presented as means ± S.E.M. of three replicates. Results in each row with different superscript letters were significantly different ($P < 0.05$).

²Fish A, B, C and D were cultured by different fish farms, respectively. These fish were fed feeds A, B, C and D, respectively.

**Table 4.** Average protein content (% of wet weight basis) and amino acid composition (g 100 g⁻¹ protein) in fillets of sea bream, *Sparus aurata* in winter¹

Protein and amino acids	Cultured fish groups ²				Wild fish
	A	B	C	D	
Protein (%)	21.58±0.18 ^a	21.32±0.45 ^a	20.36±0.25 ^b	19.30±0.32 ^c	19.16±0.20 ^c
<i>Essential amino acids (EAA)</i>					
Arginine	5.56±0.02 ^a	4.94±0.04 ^b	5.38±0.03 ^a	5.23±0.21 ^{ab}	5.12±0.07 ^b
Histidine	2.27±0.06 ^a	1.38±0.03 ^b	1.55±0.02 ^b	2.33±0.012 ^a	1.98±0.04 ^{ab}
Isoleucine	4.41±0.01 ^a	4.64±0.01 ^a	4.97±0.08 ^a	3.37±0.08 ^b	4.45±0.08 ^a
Leucine	7.57±0.04 ^b	7.76±0.03 ^b	8.99±0.10 ^a	7.78±0.10 ^b	8.05±0.10 ^b
Lysine	7.32±0.10 ^c	7.76±0.06 ^b	7.84±0.04 ^b	8.67±0.11 ^a	8.48±0.06 ^a
Methionine	2.04±0.01 ^a	2.20±0.01 ^a	1.67±0.06 ^b	2.23±0.05 ^a	1.67±0.12 ^b
Phenylalanine	3.69±0.03 ^b	3.84±0.02 ^b	4.45±0.01 ^a	3.89±0.03 ^b	4.71±0.03 ^a
Threonine	4.09±0.01	4.10±0.03	4.33±0.03	4.16±0.01	4.07±0.04
Tryptophan	1.98±0.02 ^b	2.43±0.06 ^a	1.89±0.00 ^b	2.45±0.02 ^a	1.85±0.10 ^b
Valine	3.93±0.03 ^b	4.12±0.01 ^b	5.12±0.04 ^a	4.14±0.04 ^b	4.24±0.02 ^a
Total EAA	42.86±0.03 ^c	43.17±0.03 ^{bc}	46.19±0.04 ^a	44.25±0.06 ^b	44.62±0.07 ^b
<i>Nonessential amino acids (NEAA)</i>					
Alanine	6.50±0.01 ^b	6.77±0.05 ^b	7.34±0.06 ^a	5.70±0.03 ^c	6.43±0.05 ^b
Aspartic acid	8.27±0.06 ^c	11.04±0.03 ^a	10.57±0.12 ^a	10.73±0.18 ^a	9.91±0.07 ^b
Glutamic acid	10.59±0.06 ^b	9.80±0.01 ^c	11.13±0.09 ^{ab}	9.64±0.07 ^c	11.57±0.13 ^a
Glycine	5.02±0.01 ^{ab}	5.34±0.01 ^a	5.75±0.05 ^a	4.97±0.09 ^b	5.65±0.01 ^a
Proline	2.90±0.03 ^b	3.19±0.07 ^{ab}	3.26±0.01 ^a	3.63±0.04 ^a	3.35±0.03 ^a
Serine	3.14±0.03 ^a	2.86±0.01 ^a	2.46±0.03 ^b	3.09±0.03 ^a	2.97±0.01 ^a
Tyrosine	3.23±0.16 ^b	3.26±0.04 ^b	3.99±0.02 ^a	4.01±0.02 ^a	3.71±0.05 ^{ab}
Total NEAA	39.65±0.05 ^c	42.26±0.03 ^b	44.50±0.05 ^a	41.77±0.07 ^{bc}	43.59±0.05 ^a
EAA/NEAA	1.08	1.02	1.04	1.06	1.02

¹Protein and amino acid compositions are presented as means ± S.E.M. of three replicates. Results in each row with different superscript letters were significantly different ($P < 0.05$).

²Fish A, B, C and D were cultured by different fish farms, respectively. These fish were fed feeds A, B, C and D, respectively.

**Table 5.** Average protein content (% of wet weight basis) and amino acid composition (g 100 g⁻¹ protein) in fillets of sea bass, *Dicentrarchus labrax* in summer¹

Protein and amino acids	Cultured fish groups ²				Wild fish
	A	B	C	D	
Protein (%)	19.54±0.39 ^a	19.10±0.21 ^a	19.43±0.40 ^a	19.26±0.08 ^a	18.12±0.32 ^b
<i>Essential amino acids (EAA)</i>					
Arginine	5.43±0.08 ^a	5.12±0.06 ^{ab}	4.98±0.04 ^b	5.23±0.06 ^a	5.32±0.03 ^a
Histidine	1.72±0.01 ^b	1.88±0.01 ^{ab}	1.91±0.03 ^a	2.09±0.02 ^a	1.71±0.02 ^b
Isoleucine	4.77±0.05 ^b	5.39±0.03 ^a	3.56±0.04 ^c	5.47±0.05 ^a	4.20±0.05 ^{bc}
Leucine	7.02±0.06 ^c	9.80±0.10 ^a	8.18±0.10 ^b	9.98±0.12 ^a	9.72±0.07 ^a
Lysine	8.46±0.09 ^b	7.88±0.04 ^c	8.09±0.05 ^{bc}	9.48±0.09 ^a	8.28±0.07 ^{bc}
Methionine	2.31±0.03 ^{ab}	2.72±0.02 ^a	2.34±0.02 ^{ab}	1.88±0.01 ^b	1.96±0.03 ^b
Phenylalanine	3.74±0.02 ^c	4.24±0.02 ^{bc}	5.31±0.04 ^a	4.82±0.03 ^{ab}	4.47±0.02 ^b
Threonine	4.97±0.06 ^a	4.87±0.05 ^a	3.76±0.03 ^c	5.14±0.04 ^a	4.63±0.02 ^{bc}
Tryptophan	1.78±0.00 ^b	2.10±0.01 ^a	2.24±0.03 ^a	1.86±0.00 ^{ab}	1.97±0.02 ^{ab}
Valine	4.26±0.03 ^{bc}	4.71±0.04 ^b	3.14±0.05 ^c	5.42±0.02 ^a	4.75±0.03 ^b
Total EAA	44.46±0.04 ^c	48.71±0.04 ^b	43.51±0.04 ^c	51.37±0.04 ^a	47.01±0.10 ^b
<i>Nonessential amino acids (NEAA)</i>					
Alanine	7.33±0.06 ^{ab}	7.49±0.08 ^{ab}	6.98±0.03 ^b	7.75±0.05 ^a	6.84±0.03 ^b
Aspartic acid	9.77±0.10 ^a	9.48±0.06 ^a	8.85±0.08 ^b	11.06±0.09 ^a	9.24±0.05 ^{ab}
Glutamic acid	14.36±0.16 ^a	12.66±0.013 ^b	11.29±0.10 ^c	10.95±0.12 ^c	9.14±0.08 ^d
Glycine	5.56±0.04 ^c	4.45±0.01 ^d	6.81±0.07 ^b	7.60±0.03 ^a	6.24±0.05 ^{bc}
Proline	4.18±0.02 ^a	3.43±0.01 ^b	3.06±0.04 ^c	3.69±0.03 ^b	3.20±0.00 ^{bc}
Serine	3.45±0.03 ^a	3.03±0.02 ^b	3.15±0.01 ^b	2.95±0.02 ^b	2.76±0.03 ^c
Tyrosine	4.75±0.04 ^a	3.14±0.01 ^b	2.88±0.03 ^b	4.69±0.03 ^a	3.02±0.04 ^b
Total NEAA	49.40±0.06 ^a	43.68±0.02 ^b	43.02±0.05 ^b	48.69±0.05 ^a	40.44±0.04 ^c
EAA/NEAA	0.90	1.12	1.01	1.06	1.16

¹Protein and amino acid compositions are presented as means ± S.E.M. of three replicates. Results in each row with different superscript letters were significantly different (P < 0.05).

²Fish A, B, C and D were cultured by different fish farms, respectively. These fish were fed feeds A, B, C and D, respectively.

Table 6. Average protein content (% of wet weight basis) and amino acid composition (g 100 g⁻¹ protein) in fillets of sea bass, *Dicentrarchus labrax* in winter¹

Protein and amino acids	Cultured fish groups ²				Wild fish
	A	B	C	D	
Protein (%)	20.80±0.24	21.24±0.42	20.96±0.40	20.85±0.33	21.28±0.18
<i>Essential amino acids (EAA)</i>					
Arginine	5.08±0.08 ^{ab}	5.64±0.13 ^a	5.26±0.07 ^a	4.82±0.06 ^b	5.25±0.10 ^a
Histidine	1.56±0.03 ^a	1.53±0.03 ^a	1.97±0.06 ^a	1.74±0.01 ^a	0.85±0.07 ^b
Isoleucine	5.32±0.07 ^a	4.56±0.01 ^b	5.03±0.10 ^a	5.14±0.03 ^a	4.88±0.12 ^{ab}
Leucine	9.11±0.01 ^a	7.67±0.05 ^c	8.25±0.05 ^b	9.54±0.08 ^a	8.37±0.11 ^b
Lysine	9.88±0.47 ^a	7.25±0.20 ^c	8.64±0.03 ^b	7.46±0.10 ^c	7.61±0.10 ^c
Methionine	2.42±0.03 ^a	2.05±0.03 ^b	2.15±0.06 ^b	2.27±0.10 ^{ab}	2.57±0.20 ^a
Phenylalanine	4.70±0.01 ^a	3.83±0.03 ^b	4.80±0.04 ^a	4.78±0.06 ^a	4.55±0.13 ^a
Threonine	4.63±0.37 ^b	3.65±0.03 ^c	4.93±0.12 ^a	5.24±0.03 ^a	4.41±0.11 ^b
Tryptophan	2.35±0.20	2.22±0.13	2.40±0.08	2.53±0.02	2.36±0.07
Valine	4.77±0.03 ^a	4.00±0.01 ^b	5.07±0.03 ^a	4.24±0.04 ^b	4.53±0.03 ^{ab}
Total EAA	49.82±0.13 ^a	42.40±0.07 ^c	48.50±0.06 ^a	47.76±0.05 ^b	45.38±0.10 ^{bc}
<i>Nonessential amino acids (NEAA)</i>					
Alanine	7.00±0.07 ^a	5.81±0.06 ^b	6.85±0.06 ^a	7.19±0.12 ^a	6.65±0.05 ^{ab}



Aspartic acid	10.45±0.07 ^b	8.09±0.01 ^c	10.39±0.05 ^b	11.60±0.09 ^a	10.18±0.11 ^b
Glutamic acid	14.22±0.17 ^a	8.40±0.03 ^c	13.44±0.02 ^{ab}	12.31±0.11 ^b	13.86±0.12 ^a
Glycine	6.04±0.07 ^a	4.49±0.03 ^c	5.18±0.02 ^b	6.32±0.08 ^a	6.25±0.12 ^a
Proline	3.55±0.07 ^a	2.99±0.04 ^b	3.51±0.04 ^a	3.25±0.10 ^{ab}	3.82±0.10 ^a
Serine	3.62±0.04 ^a	2.33±0.23 ^c	2.97±0.06 ^{ab}	2.55±0.09 ^b	2.78±0.07 ^{ab}
Tyrosine	4.24±0.03 ^a	3.46±0.10 ^b	4.43±0.07 ^a	4.13±0.02 ^a	3.87±0.02 ^{ab}
Total NEAA	49.12±0.07 ^a	35.57±0.07 ^c	46.77±0.05 ^b	47.35±0.09 ^b	47.41±0.08 ^b
EAA/NEAA	1.01	1.19	1.04	1.01	0.96

¹Protein and amino acid compositions are presented as means ± S.E.M. of three replicates. Results in each row with different superscript letters were significantly different ($P < 0.05$).

²Fish A, B, C and D were cultured by different fish farms, respectively. These fish were fed feeds A, B, C and D, respectively.

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