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RESEARCH

Comparison of Nutritional Quality of Amino Acid in Elasmobranchii Species: Thornback Ray, *Raja clavata* (Rajidae) and Common Stingray, *Dasyatis pastinaca* (Dasyatidae) with Implications to Human Health

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Abstract
To evaluate the nutritive quality and amino acid profiles of the muscles from two elasmobranch species, Thornback ray (*Raja clavata*) and Common stingray (*Dasyatis pastinaca*), and to assess their potential as dietary sources for human consumption, a comprehensive analysis of their amino acid composition was conducted. Employing Liquid Chromatography Mass Spectrometry (LC-MS/MS), the analysis identified nineteen amino acids in the muscle tissues of both species, encompassing seven essential amino acids, two semi-essential amino acids, and ten non-essential amino acids. Glutamic acid, lysine, and aspartic acid were the most abundant amino acids in both species, while ornithine, cystine, and taurine were the least abundant. Compared to *D. pastinaca*, the amino acid profile of *R. clavata* exhibited slightly lower levels of lysine, leucine, and methionine. However, essential amino acids (ΣEAA), semi-essential amino acids (ΣSEAA), and non-essential amino acids (ΣNEAA) were statistically similar between the species ($p > 0.05$), except for total amino acids (ΣAA) ($p < 0.05$). In terms of protein quality, the amino acid score (AAS), chemical score (CS), essential amino acid index (EAAI), and biological value index (BVI) revealed that both species offer high-quality protein. Isoleucine was identified as the first limiting amino acid, followed by valine, while lysine had the highest protein score for both species. The ratios of ΣEAA/ΣAA (38.03% −39.42%) and ΣEAA/ΣNEAA (72.63% −76.52%) were close to the constitutional rate of essential amino acids and met the minimum recommendation of >60% set by the Food and Agriculture Organization/World Health Organization (FAO/WHO). A 145 g portion of fillet from either species can provide the recommended average daily intake of essential amino acids for a 70 kg adult. These results suggest that both *R. clavata* and *D. pastinaca* possess high nutritional quality and are suitable as healthy dietary sources.

Keywords Elasmobranchii · Amino acid · Nutritional value · Nutritive quality · Human health · Black Sea

Introduction

Thornback ray, *Raja clavata* and common stingray, *Dasyatis pastinaca* are two demersal elasmobranchii rays, found over sandy and muddy bottoms from the shore to about 200 m depth, but are more commonly found in shallow waters (<50 m) (Froese and Pauly 2024). They are distributed throughout the Eastern Atlantic, ranging from southern Norway and the United Kingdom to South Africa. Their presence extends to the Canary Islands, Madeira, the western Baltic Sea, the Mediterranean Sea, the Sea of Marmara and the Black Sea (Froese and Pauly 2024). In Black Sea, these species are taken by trawl, gillnet and purse seiner particularly as bycatch and are sometimes targeted in commercial bottom trawl (Başusta and Başusta 2014). *R. clavata* made up more than 90% of the elasmobranch biomass captured both gill net and commercial bottom trawl fishery off the Sinop Peninsula. But, *D. pastinaca* is a fewer common species, generally showing a low abundance index in comparison to the *R. clavata* (personal observations). According to Bauşta et al. (2016), skate and rays are typically processed as wings and marketed frozen and without skin. They are consumed directly or such as surimi, fish

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protein concentrate, salami and sausage indirectly (Kabasakal 1998; Turan et al. 2007; Başusta and Başusta 2014). In Türkiye, the average annual catch of skate and rays for last decades has been 77.1 ± 26.16 tons (between 0.7 and 196.4 tons), which constitute about 0.029 ± 0.010% (between 0.000 and 0.085%) of the total marine fisheries production of Türkiye (TUIK, 2014–2023) and Turkish people usually do not appreciate due to their shape and taste.

Their domestic consumption remains limited, mainly due to their cartilaginous structure, lack of scales, and the strong ammonia-like odor that develops after capture, which negatively influences consumer preference (Turan et al. 2007; Başusta and Başusta 2014). As a result, these species are predominantly exported in processed forms, such as skinned and frozen wings, or are utilized for value-added products like surimi, fish protein concentrates, and processed meats (e.g., sausages) (Kabasakal 1998; Turan et al. 2007). *R. clavata* is rich in protein (18.58–20.02%), low in lipid content (0.5–3.4%) and the total saturated fatty acids (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) content are varied between 28 and 48%, 12 to 13% and 24 to 30% respectively (Turan et al. 2007; Colakoglu et al. 2011), indicating ray fillets seem to be good protein sources.

Proteins are fundamental macromolecules essential for life, serving critical roles in enzyme and hormone production, immune defense, and tissue repair (Medeiros and Wildman 2019). Their nutritional value is determined by the amino acid (AA) composition, especially the balance of essential amino acids (EAAs), which cannot be synthesized by the human body and must be supplied through diet (FAO/WHO/UNU 2007; Wolfe 2006). Amino acids, the building blocks of proteins, influence biological value and protein synthesis efficiency (Mohanty et al. 2014). The indispensable EAAs include leucine, isoleucine, lysine, phenylalanine, methionine, tryptophan, valine, threonine, arginine, and histidine (Oser 1951, 1959; FAO/WHO, 1973). Although arginine and histidine are sometimes classified as semi-essential amino acids (SEAAs), they remain essential for human adults (Zhuang et al. 2010; Wang et al. 2014; Mitchell and Block 1978). Since amino acids are not stored in the body, a continuous dietary supply is required; excess amino acids are either metabolized or excreted (FAO/WHO 2013; Olu and Adediran 2015). The absence of even a single EAA disrupts protein synthesis, highlighting the importance of ensuring an adequate and balanced intake of both EAAs and non-essential amino acids (NEAAs) to maintain optimal health (FAO/WHO 1991). They can reduce blood cholesterol levels and exhibit antimutagenic properties (Simopoulos 2002). Specific amino acids, including aspartic acid, glycine, and glutamic acid, are instrumental in wound healing processes (Chyun and Griminger 1984). Furthermore, aspartic acid, proline, glycine, and leucine have demonstrated cytotoxic effects against cancer cells (Kim et al. 1999). Additionally, amino acids such as methionine, lysine, tyrosine, histidine, and tryptophan are acknowledged for their antioxidant capabilities (Saito et al. 2003). Methionine, in particular, promotes cell survival and protein synthesis (Belghit et al. 2014), whereas arginine has been shown to enhance disease resistance in fish under external stress conditions (Costas et al. 2011).

In this context, fish can play a vital role as it is an important and better source of quality animal proteins that play critical roles in human nutrition, disease prevention and health promotion (Usydus et al. 2009; Mohanty et al. 2014). Fish protein is characterized by a desirable composition of amino acids, which is responsible for some of the taste and flavor of fish (Dogan and Ertan 2017), and has a greater postprandial satiety effect than other sources of animal proteins such as beef and chicken (Uhe et al. 1992; Mohanty et al. 2014). Fish meat is also considered to be a good source of essential amino acids (Kim and Lall 2000; Limin et al. 2006; Adeyeye 2009; Erkan et al. 2010; Ozden and Erkan 2011; Dogan and Ertan 2017). It has been reported that in fish, 50–80% of the non-protein nitrogenous compounds are amino acids, with significant proportions of these AAs being arginine, glutamic acid, lysine, proline, and taurine (Ozden 2005; Dogan and Ertan 2017).

Optimal nutrition and dietary intake extend beyond individual and community levels to exert a global influence (Ma and Lee 2012). Achieving success in this domain necessitates the implementation of coherent and robust strategies for our food systems. Therefore, there is an escalating need to generate and document nutritional information on the diverse varieties and species of food fish available. To the best of our knowledge, there is a few investigations related to amino acids profile of different wild fish species such as anchovy, *Engraulis encrasicolus* (Bilgin et al. 2018), whiting, *Merlangus merlangus* (Bilgin et al. 2019a), and picarel, *Spicara smaris* (Bilgin et al. 2019b) in the Black Sea. Although some studies have been published (Turan et al. 2007; Colakoglu et al. 2011; Tufan et al. 2013; Ayas et al. 2019) about the fatty acid profile and proximate biochemical composition of the examined species; *R. clavata* and *D. pastinaca*, no information was found about the amino acid composition of these species, except for the recent study, which were investigated *R. clavata* in the Norwegian Sea (Kendler et al. 2023). The differences between *R. clavata* and *D. pastinaca* with respect to their meat protein quality are a remarkable key factor to reveal and ensure regulatory nutritional requirements for human health and also consumer preferences. Due, the present study serves to assess food quality between two fish species by comparing amino acid profiles along with amino acid scores (AAS), essential amino acid index (EAAI), chemical scores (CS) and biological value index (BVI) in *R. clavata* and *D. Pastinaca* with reference to these elasmobranchii populations in different areas.

Despite existing studies on the fatty acid profiles and proximate biochemical composition of *R. clavata* and *D. pastinaca* (Turan et al. 2007; Colakoglu et al. 2011; Tufan et al. 2013; Ayas et al. 2019), no data have been reported on their amino acid composition, except for a recent study on *R. clavata* from the Norwegian Sea (Kendler et al. 2023). The present study aims to compare the amino acid composition and protein quality indices (AAS, CS, EAAI, BVI) of these two demersal elasmobranch species from the Black Sea. The findings will provide valuable insights for consumers and nutritionists, as well as contribute to the documentation of high-quality protein sources for both local and global markets.

Materials and methods

Study Location and Sampling

In this study, *R. clavata* individuals ($n=4$) with mean weight of 3.62 ± 0.56 kg (between: 2.6 and 5.2 kg) and mean length of 73.8 ± 4.13 cm (between 64 and 84 cm), and *D. pastinaca* individuals ($n=4$) with mean weight of 6.98 ± 2.10 kg (between 0.9 and 10.4 kg) and a mean length of 73.5 ± 14.4 cm (between 31 and 95 cm) were used for the bioassays and were investigated in terms of variation in amino acid compositions. The mean total length (*t*-test, $P=0.9872$) and mean weight (*t*-test, $P=0.174$) were not significantly different (*t*-test, $P=0.1744$) between the species. Both *R. clavata* and *D. pastinaca* specimens were obtained as by-catch in September 2019 from fishermen fishing around the Sinop peninsula in the Black Sea region of Türkiye. Specimens were obtained in September 2019 from fishermen operating around the Sinop Peninsula in the Black Sea, Türkiye. After dissection, only the edible muscle tissues (pectorals, main body, and tail) were collected, carefully trimmed of skin and cartilage (Tufan et al. 2013). Approximately 250–300 g of muscle tissue from each individual was homogenized to ensure a uniform sample for analysis. The homogenized tissue was then dried at 105 °C for 12 h (AOAC 1995). The homogenized tissue was then dried at 105 °C for 12 h (AOAC 1995).

Amino Acids Analyses

For amino acid analysis, the methodology outlined by Bilgin et al. (2019a) was employed using a LC-MS/MS (liquid chromatography coupled with tandem mass spectrometry) system, which integrates the separation capabilities of liquid chromatography with the highly sensitive and selective mass analysis of a triple quadrupole mass spectrometer. The amino acid analysis was conducted in duplicate utilizing the Agilent Infinity 1260 HPLC system at SUBITAM (Sinop University Scientific and Technological Research Application and Research Center). This system comprises a binary pump, degasser, and autosampler, and is coupled with a 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). For quantification, muscle tissue samples were prepared using Jasem LC-MS/MS amino acid kits (Jasem Laboratory Systems and Solutions, Istanbul, Türkiye). Amino acid concentrations were determined through electrospray ionization (ESI) and multiple reaction monitoring (MRM) techniques. Sample hydrolysis involved treating 0.5 g of tissue with 4 ml of acidic hydrolysis reagent in a screw-capped glass tube, followed by incubation for 24 h at 110 °C. After cooling the sample to room temperature (~24 °C), it was centrifuged using a Hettich Universal 320 desktop air-cooled centrifuge at 4000 rpm (g-force: 3756.48) for 5 min. Subsequently, 10 µL of the supernatant was transferred to a sample vial. As the analysis was based on dry matter content, the hydrolysates were diluted 4000-fold, unlike the 800-fold dilution used for wet samples as described by Bilgin et al. (2019a).

Following hydrolysis, calibration standards, sample preparations and the HPLC elution protocol were performed according to Bilgin et al. (2019a) as follows: specifically, 50 µL of either the standard or diluted hydrolysate was transferred into a sample vial. Then, 50 µL of a labeled stable isotope mixture was added as an internal standard, followed by the addition of 700 µL of Reagent-1. The vial was swirled for 5 s to ensure thorough mixing. The HPLC system was configured to inject 3 µL of the prepared sample into the Jasem analytical column, designed for amino acid analysis and maintained at 30 °C. Chromatographic separation was achieved using Jasem's mobile phases A and B with gradient elution at a flow rate of 0.7 mL/min. The HPLC elution protocol was as follows: the gradient began at 22% mobile phase A and was held for 1 min, then increased to 78% mobile phase A over 3 min and maintained at this concentration for 0.5 min. The column was then equilibrated at 22% mobile phase A for an additional 3 min, resulting in a total run time of 7.5 min. Mass spectrometric detection was conducted using an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive ion mode. Optimal mass spectrometer settings included a drying gas temperature of 150 °C, a drying gas flow rate of 10 L/min, a nebulizer pressure of 40 psi (noting that nebulization occurs at atmospheric pressure, not in a vacuum), and a capillary voltage of 2000 V (+). Detection was performed in positive ESI mode, with amino acids and internal standards (IS) identified as protonated ions (m/z = [M+1]+). Quantification of the targeted amino acids was achieved by evaluating the peak area ratio of each amino acid relative to its corresponding internal standard.

Calculation of Amino Acid Scores, Chemical Scores, Essential Amino Acid Index and Biological Value Index

The nutritive value of protein in the samples was determined using the amino acid scores (AAS), the essential amino acid index (EAAI), the biological value index (BVI) and the chemical measure of protein quality known as chemical score (CS).

For the estimation of nutritional values and availabilities of the amino acids analysed, they have been multiplied by 0.625 according to Jones (1941) and FAO/WHO (1973), thereafter AAS and the CS have been performed using equations of Oser (1959), FAO/WHO (1973) and Mitchell and Block (1978), also provided later by Saavedraa et al. (2017), Taha et al. (2019) and Oztekin et al. (2020) as follows:

Amino Acid Score (AAS)

The amino acid score of the protein was calculated according to the equation of FAO/WHO (1973) as follows:

$$\text{Amino Acid Score, AAS (\%)} = [(\text{AAFBP, \%}) / (\text{AAFW, \%})],$$

where, AA_{FW} = the level of amino acid per protein of the whole egg (dry weight) according to the reference composition of FAO/WHO (1973) standard in per cent as given in Table 2.

AA_{FBP} is the concentration level of amino acid per test protein (FBP: edible fish body protein, dry weight) in per cent, and calculated as follows:

AA_{FBP} = essential amino acid x 0.625,

where, 0.625 is the nitrogen-to-protein conversion factor also called Jones factor (Jones 1941; FAO/WHO, 1973).

Chemical Score (CS)

The chemical score was calculated from the chemical analysis of the essential amino acids of fish according to the formula of Mitchell and Block (1978) as follows:

$$\text{Chemical Score, CS} = [(\text{AAFBP})/(\text{AAEGG})],$$

where, AA_{EGG} shows the concentration of amino acid per protein in whole egg protein reference composition in per cent as provided in Table 2.

Essential Amino Acids Index (EAAI)

The EAA index defined as the geometric mean of “the egg ratios”, i.e., the ratios of the essential amino acids in a protein relative to their respective amounts in whole egg protein (Oser 1959). Based on the amino acids’ composition of the two fish species and of that of the chicken egg, the EAAI was calculated using following equation (Oser 1959), which was also recently reported by Zhuang et al. (2010), Wang et al. (2014), Ibrahim (2015), Jiang et al. (2017), Taha et al. (2019) and Oztekin et al. (2020):

$$EAA\ Index\ (\%) = \left(\sqrt[n]{100 \times \frac{Lys_P}{Lys_S} \times 100\frac{Tryp_P}{Tryp_S} \times \cdots \times 100 \times \frac{His_P}{His_S}}\right)$$

where, n = the number of amino acids, p = tested food protein; and s = the standard protein (whole egg protein). Oser (1959) also suggested that, on occasions when certain amino acid values are not available, they could be omitted, provided that the appropriate ‘n’ is not used. Here to calculate EAAI values, we used seven EAAI values including isoleucine, leucine, lysine, methionine + cystine, phenylalanine + tyrosine, threonine and valine given in Table 2.

Biological Value Index (BVI)

A high degree of correlation between essential amino acid indexes and biological values for 77 comparisons was reported by Oser (1959). The biological value index was estimated using the empirical equation suggested by Oser (1959) and also addressed recently by Ibrahim (2015), Olu and Adediran (2015) and Oztekin et al. (2020):

$$BVI = [((1.09) \times (EAAI)) - (11.73)]$$

Statistical Analysis

In order to compare the differences between two species, *D. pastinaca* and *R. clavata*, data were statistically analyzed by simple t-tests using PAST version 1.75b software (Hammer et al. 2001). Data are expressed as means ± standard error, and a *p*-value of less than 0.05 was considered statistically level in this study.

Results

The amino acid composition of *R. clavata* and *D. pastinaca* is presented in Table 1. Nineteen amino acids were detected in the muscle tissues of both species, comprising seven essential amino acids (EAAs), two semi-essential amino acids (SEAAs), and ten non-essential amino acids

Table 1 Amino acid groups, composition and ratios of Thornback ray, *Raja clavata* and common stingray, *Dasyatis Pastinaca* (dry weight basis, means ± SE)

		Dasyatis pastinaca	*Raja clavata*
Total length, TL/ Weight, kg		(TL: 73.5 ± 14.41 cm) (6.98 ± 2.10 kg)	(TL: 73.8 ± 4.13 cm) (3.62 ± 0.56 kg)
EAA (g/100 g)			
Isoleucine	Ile	2.35 ± 0.029^a (3.10)	2.72 ± 0.001^b (3.34)
Leucine	Leu	5.40 ± 0.029^a (7.11)	5.81 ± 0.017^b (7.14)
Lysine	Lys	8.92 ± 0.008 (11.75)	8.78 ± 0.037 (10.79)
Methionine	Met	2.21 ± 0.050 (2.91)	2.14 ± 0.110 (2.63)
Phenylalanine	Phe	3.49 ± 0.000 (4.59)	3.45 ± 0.087 (4.25)
Threonine	Thr	4.00 ± 0.009 (5.26)	4.30 ± 0.103 (5.29)
Valine	Val	3.57 ± 0.018^a (4.70)	3.73 ± 0.029^b (4.59)
∑EAA (g/100 g)		29.92 ± 0.592 (39.43)	30.92 ± 0.580 (38.03)
NEAA (g/100 g)			
Alanine	Ala	4.91 ± 0.016^a (6.47)	5.03 ± 0.020^b (6.18)
Aspartic acid	Asp	6.84 ± 0.004 (9.01)	6.57 ± 0.156 (8.07)
Cystine*	Cys	0.47 ± 0.022 (0.62)	0.49 ± 0.004 (0.60)
Glutamic acid	Glu	10.55 ± 0.004^a (13.90)	11.72 ± 0.060^b (14.40)
Glycine	Gly	4.50 ± 0.531^a (5.93)	6.16 ± 0.433^b (7.57)
Ornithine	Orn	0.28 ± 0.005 (0.37)	0.25 ± 0.002 (0.31)
Proline	Pro	2.80 ± 0.026^a (3.69)	3.96 ± 0.015^b (4.86)
Serine	Ser	3.99 ± 0.010 (5.26)	4.03 ± 0.024 (4.95)
Taurine	Tau	2.13 ± 0.127 (2.81)	1.89 ± 0.077 (2.32)
Tyrosine*	Tyr	2.64 ± 0.074 (3.48)	2.51 ± 0.009 (3.08)
∑NEAA (g/100 g)		39.12 ± 0.672 (51.53)	42.60 ± 0.740 (52.36)
SEAA (g/100 g)			
Arginine	Arg	4.59 ± 0.056^a (6.05)	5.67 ± 0.144^b (6.97)
Histidine	His	2.28 ± 0.047 (3.01)	2.14 ± 0.076 (2.63)
∑SEAA(g/100 g)		6.88 ± 0.944 (9.06)	7.80 ± 1.444 (9.60)
∑AA(g/100 g)		75.92 ± 0.416^a	81.36 ± 0.448^b
Ratio (%)			
∑EAA/∑AA		39.42	38.03
∑NEAA/∑AA		51.52	52.37
∑SEAA/∑AA		9.06	9.60
∑EAA/∑NEAA		76.52	72.63

Abbreviations: EAA, essential amino acids; NEAA, non-essential amino acids; SEAA, semi-essential amino acids; ∑EAA, total essential amino acids; ∑NEAA, total non-essential amino acids; ∑SEAA, total semi-essential amino acids; ∑AA, total amino acids. *: conditionally EAA. Values in parentheses indicate the percentage of amino acid

(NEAAs). Among all amino acids, *R. clavata* exhibited slightly higher levels of EAAs, NEAAs, and SEAAs compared to *D. pastinaca*, with the exceptions of lysine, methionine, and phenylalanine among EAAs; aspartic acid, cysteine, ornithine, taurine, and tyrosine among NEAAs; and histidine among SEAAs. Nevertheless, statistical analysis revealed no significant differences ($p > 0.05$) between the species for the majority of EAAs, SEAAs, or NEAAs, with the exceptions of isoleucine, leucine, and valine among EAAs; alanine, glutamic acid, glycine, and proline among NEAAs; and arginine among SEAAs, which were found to be significantly higher ($P < 0.05$) in *R. clavata* compared to *D. pastinaca*.

R. clavata contained high levels of glutamic acid (11.72 ± 0,060 g/100 g), followed by lysine, aspartic acid, glycine, leucine, arginine, alanine, threonine, serine, proline, valine, phenylalanine, isoleucine, tyrosine, histidine, methionine, taurine, cystine in a decreasing trend, with the lowest level of ornithine contents. This content order is generally similar for *D. pastinaca*, with the highest value being glutamic acid (10.55 ± 0,004 g/100 g), followed by a decreasing trend as lysine, aspartic acid, leucine, alanine, arginine, glycine, threonine, serine, valine, phenylalanine, proline, tyrosine, isoleucine, histidine, methionine, taurine, cystine, and the lowest value being ornithine (Table 1). As an interesting result, the composition of the most and least three abundant amino acids in both *R. clavata* and *D. Pastinaca* species consisted of the same amino acids. Namely, both *R. clavata* and *D. pastinaca* contained higher levels of glutamic acid, lysine and aspartic acid, and lower levels of ornitine, cystine and taurine (Table 1).

Overall, the levels of total amino acids, total essential amino acids, total semi-essential amino acids, and total non-essential amino acids were slightly higher in *R. clavata* compared to *D. pastinaca*. However, no significant differences ($p > 0.05$) were observed for the ΣEAA, ΣSEAA, and ΣNEAA between the species. In contrast, ΣAA was significantly higher in *R. clavata* compared to *D. pastinaca* ($p < 0.05$). The ratio of total essential amino acids to total amino acids (ΣEAA/ΣAA) was 39.42% for *D. pastinaca* and 38.03% for *R. clavata*. The ratio of ΣNEAA to ΣEAA was slightly higher in *R. clavata* (52.37%) compared to *D. pastinaca* (51.52%). Conversely, the ratio of ΣEAA to ΣNEAA was lower in *R. clavata* (72.63%) than in *D. pastinaca* (76.52%).

Comparative analyses of AAS, CS, EAAI and BVI between *D. pastinaca* and *R. clavata* (dry weight basis, mg/g N) are given in Table 2. The amino acid score, which is an indicator of nutritional quality, was found higher or equal to "1.00" for lysine (1.64), phenylalanine + tyrosine (1.01) and threonine (1.00) in *D. pastinaca*. Similarly, except for phenylalanine + tyrosine, lysine (1.61) and threonine (1.08) were found more than "1.00" in *R. clavata*. However, the AASs of isoleucine and valine has been calculated at the lowest level in both species, these two amino acids were identified as the first limiting amino acid (isoleucine) and the second limiting amino acid (valine) for protein available in the species (Table 2).

In both species, the CS values were determined as lower than "1.00" for isoleucine, leucine, methionine + cystine, phenylalanine + tyrosine, threonine and valine, with only exceptions for the CS values of lysine, which were found to be higher than "1.00".The essential amino acid index, representing the entire essential amino acid spectrum of a protein, and the biological value index for *D. pastinaca* was

Table 2 Comparative analyses of amino acid score (AAS), chemical score (CS), essential amino acid index (EAAI) and biological value index (BVI) between *Dasyatis Pastinaca* and *Raja clavata* (dry weight basis, mg/g N), calculated based on data given in Table 1

	AA_{FBP}*		Standards		*D. pastinaca*		*R. clavata*	
	D. pastinaca	*R. clavata*	F/W	WEP	AAS	CS	AAS	CS
Isoleucine	1.47	1.70	2.50	3.31	**0.59**[a]	0.44	**0.68**[a]	0.51
Leucine	3.38	3.63	4.40	5.34	0.77	0.63	0.83	0.68
Lysine	5.58	5.49	3.40	4.41	**1.64**	**1.26**	**1.61**	**1.24**
Methionine + Cystine	1.68	1.64	2.20	3.86	0.76	0.43	0.75	0.43
Phenylalanine + Tyrosine	3.83	3.73	3.80	5.65	1.01	0.68	0.98	0.66
Threonine	2.50	2.69	2.50	2.92	1.00	0.86	1.08	0.92
Valine	2.23	2.33	3.10	4.10	**0.72**[b]	0.54	**0.75**[b]	0.57
Total (W_{EAA}, mg/g N)	20.66	21.21						
EAAI	**64.93**	**67.51**						
BVI	**59.04**	**61.85**						

Abbreviations: F/W (FAO/WHO) standard; WEP (whole egg protein) standard; AAFBP, fish body protein amino acids

* Converted fish body protein amino acids (AAFBP) by multiplying EAA values in Table 1 with 0.625, according to Jones (1941); FAO/WHO (1973)

[a] the first limiting amino acid

[b] the second limiting amino acid

determined to be lower than in *R. clavate* (Table 2). Moreover, The EAAIs were determined to be higher than the "60" value for *D. pastinaca* (64.93) and *R. clavata* (67.51). Furthermore, the weight basis total contribution of amino acids as per protein in *D. pastinaca* (W_{EAA} = 20.66 mg/g N) was estimated as slightly lower than in *R. clavata* (W_{EAA} = 21.21 mg/g N).

Discussion

The amino acid composition is a critical determinant of protein nutritional quality, with the overall value of a protein source being largely contingent upon both the presence and concentration of amino acids, particularly essential amino acids (Jiang et al. 2017; Oztekin et al. 2020). Therefore, the evaluation of dietary protein quality should incorporate a separate and detailed assessment of dietary amino acids (FAO/WHO/UNU 2007; FAO/WHO 2013; Brestenský et al. 2019). Previous studies have indicated that variations in amino acid profiles may be influenced by a range of factors, including dietary quality and feeding behavior (Skog et al. 2003; Fernandez-Jover et al. 2008; Öztekin et al. 2020), environmental conditions and seasonal changes (Ozden and Erkan 2011; Wang et al. 2014; Manthey-Karl et al. 2016), as well as the age and size of fish (McLean et al. 2024). Additionally, species-specific differences contribute to this variation (Ozden and Erkan 2011; Wang et al. 2014; Manthey-Karl et al. 2016; Dogan and Ertan 2017; Kendler et al. 2023; McLean et al. 2024). These factors collectively account for the discrepancies observed in amino acid compositions across various studies. Table 3 elucidates the variability in amino acid compositions among different fish species, reflecting influences from marine environments, aquaculture systems, and dietary patterns across various geographical regions. The data presented in Table 3 reveal that the amino acid profiles of fish species from disparate regions can exhibit significant differences. For instance, the amino acid profile of anchovy (*E. encrasicolus*) from the Black Sea is characterized by the sequence "glutamic acid > aspartic acid > lysine > leucine > alanine," whereas anchovies from multiple seas available in Istanbul markets display a profile of "lysine > leucine > arginine > glutamic acid > aspartic acid." Similarly, the amino acid profile of whiting (*M. merlangus*) from the Black Sea is "glutamic acid > aspartic acid > lysine > leucine > alanine," while *M. merlangus* sold in Istanbul markets exhibits a profile of "lysine > leucine > arginine > glutamic acid > aspartic acid." This variability is also observed in *R. clavata* from the Black Sea. Specifically, the amino acid profile of *R. clavata* in our study was "glutamic acid > lysine > aspartic acid > glycine > leucine," whereas *R. clavata* captured from the Norwegian Sea demonstrated a profile of "glutamic acid > aspartic acid > lysine > leucine > alanine."

To the best of our knowledge, this study represents the first investigation into the amino acid composition of *D. pastinaca* and the second analysis of *R. clavata*, following the initial study conducted in the Norwegian Sea (Kendler et al. 2023). The present study identified the highest concentrations of essential amino acids (EAAs) such as lysine, leucine, threonine, and valine, as well as non-essential amino acids (NEAAs) including glutamic acid, aspartic acid, glycine, and alanine in the muscle tissue of both species. Significant differences ($p < 0.05$) were observed in the levels of leucine, valine, glutamic acid, alanine, and glycine between the two fish species. Consistent with the findings of Kendler

Table 3 The five most abundant amino acids in muscle of different fish species from different geographical areas

Species	Origin	Five most abundant amino acids (AAs)	Ref.
Hippoglossus hippoglossus	Aquaculture	Glutamic acid > aspartic acid > lysine > leucine > arginine	1
Paralichthys olivaceus	Aquaculture	Glutamic acid > aspartic acid > lysine > leucine > arginine	1
Pseudosciaena crocea	Xiamen Bay of China	Glutamic acid > aspartic acid > lysine > leucine > arginine	2
Lateolabrax japonicas	Xiamen Bay of China	Glutamic acid > aspartic acid > lysine > leucine > arginine	2
Pagrosomus major	Xiamen Bay of China	Glutamic acid > aspartic acid > lysine > leucine > arginine	2
Seriola dumerili	Xiamen Bay of China	Glutamic acid > aspartic acid > lysine > leucine > arginine	2
Hapalogenys nitens	Xiamen Bay of China	Glutamic acid > aspartic acid > leucine > lysine > arginine	2
Zeus faber	Aegean Sea	Glutamic acid > aspartic acid > lysine > leucine > alanine	3
Scorpaena porcus	Marmara Sea	Proline > phenylalanine > glutamic acid > lysine > leucine	3
Merluccius merluccius	Marmara Sea	Proline > phenylalanine > glutamic acid > lysine > leucine	3
Lophius piscotorius	Marmara Sea	Proline > glutamic acid > phenylalanine > lysine > leucine	3
Trachinus draco	Marmara Sea	Proline > phenylalanine > glutamic acid > lysine > leucine	3
Engraulis encrasicolus	Market in Istanbul, Türkiye	Lysine > leucine > arginine > glutamic acid > aspartic acid	4
Pomatomus saltatrix	Market in Istanbul, Türkiye	Lysine > leucine > arginine > glutamic acid > aspartic acid	4
Sarda sarda	Market in Istanbul, Türkiye	Lysine > leucine > arginine > glutamic acid > aspartic acid	4
Murlus surmelutus	Market in Istanbul, Türkiye	Lysine > leucine > arginine > glutamic acid > aspartic acid	4
Merlangius merlangus	Market in Istanbul, Türkiye	Lysine > leucine > arginine > glutamic acid > aspartic acid	4
Engraulis engrasicolus	Black Sea	Glutamic acid > aspartic acid > lysine > leucine > arginine	5
Merlangus merlangus	Black Sea	Glutamic acid > aspartic acid > lysine > leucine > alanine	6
Spicara smaris	Black Sea	Glutamic acid > aspartic acid > lysine > leucine > alanine	7
Raja clavata	Norwegian Sea	Glutamic acid > aspartic acid > lysine > leucine > alanine	8
Raja clavata	**Black Sea**	**Glutamic acid > lysine > aspartic acid > glycine > leucine**	**9**
Dasyatis pastinaca	**Black Sea**	**Glutamic acid > lysine > aspartic acid > leucine > alanine**	**9**

Ref., Reference; 1, Kim and Lall (2000); 2, Limin et al. (2006); 3, Ozden and Erkan (2011); 4, Erkan et al. (2010); 5, Bilgin et al. (2018); 6, Bilgin et al. (2019a); 7, Bilgin et al. (2019b); 8, Kendler et al. (2023); 9, present study

et al. (2023), which identified glutamic acid and aspartic acid as the most abundant NEAAs and lysine, leucine, and threonine as the predominant EAAs in muscle tissue, our results for *R. clavata* in the Black Sea align generally with these observations. Lysine levels were high in both species, with *R. clavata* at 8.78 ± 0.037 g/100 g and in *D. pastinaca* at 8.92 ± 0.008 g/100 g. The FAO/WHO/UNU (2007) recommends a daily lysine intake of 30 mg/kg body weight. Both *R. clavata* and *D. pastinaca* exceed this requirement, making them excellent sources of lysine, which is crucial for immune function, calcium absorption, collagen synthesis (Wu 2009), enhance muscle development and overall nutritional quality. Leucine was present at 5.81 ± 0.017 g/100 g in *R. clavata* and 5.40 ± 0.029 g/100 g in *D. pastinaca*, while isoleucine was found at 2.72 ± 0.001 g/100 g and 2.35 ± 0.029 g/100 g, respectively. Leucine is vital for muscle protein synthesis and activates the mammalian target of rapamycin (mTOR) signaling pathway (Medeiros and Wildman 2019). The FAO/WHO suggests a daily leucine requirement of 39 mg/kg body weight. Both *D. pastinaca* and *R. clavata*'s higher leucine content may offer advantages for muscle development and repair. Additionally, leucine is a key amino acid that activates the mechanistic target of rapamycin pathway, which is crucial for muscle protein synthesis, making two species as a potentially effective protein source (Wolfe 2001). Threonine levels were 4.30 ± 0.103 g/100 g in *R. clavata* and 4.00 ± 0.009 g/100 g in *D. pastinaca*. Threonine is essential for mucosal integrity and immune function (Medeiros and Wildman 2019). FAO/WHO recommends a daily threonine intake of 15 mg/kg body weight. Both species provide sufficient threonine to meet daily nutritional needs. The essential amino acid profiles of *R. clavata* and *D. pastinaca* highlights the significant nutritional potential of these species and when compared with the existing literature, the results show that both species are rich sources of essential amino acids and therefore valuable for human nutrition.

The present study exhibited that the consumable muscular tissue of *R. clavata* contains substantial concentrations of certain NEAAs. For instance, glutamic acid and aspartic acid were found in the highest concentrations of 11.72 ± 0.060 g/100 g and 6.57 ± 0.156 g/100 g, respectively and also this order was similar in the consumable muscular tissue of *D. pastinaca* with concentrations of 10.55 ± 0.004 g/100 g and 6.84 ± 0.004 g/100 g, respectively. Furter, glutamic acid was determined as the most abundant non-essential amino acid in both fish species. This amino acid plays a pivotal role in transamination reactions and is crucial for synthesizing key molecules such as glutathione. Moreover, glutamic acid significantly influences amino acid metabolism, metabolic regulation, tissue damage prevention, antioxidant activity enhancement, protein synthesis, wound healing, and immune system support (Mohanty et al. 2014). Its relevance extends to various health conditions, including obesity, diabetes, cardiovascular diseases, cancer, and inflammatory disorders (Mohanty et al. 2014). Additionally, glutamic acid functions as a neurotransmitter in the central nervous system and is involved in ammonia detoxification (Brosnan and Brosnan 2006). The high glutamic acid content in these fish species underscores their potential as valuable dietary sources for health benefits.

Our findings in the present study are the first attempt to compare the total EAA and total NEAA content between *R. clavata* and *D. pastinaca*, and there is no significant difference between the two species. That is, both total EAA and total NEAA content are similar in the two species. However, this content was slightly higher in *R. clavata* (total EAA = 30.92 ± 0.580 g/100 g; total NEAA = 42.60 ± 0.740 g/100 g) than in *D. pastinaca* (total EAA = 29.92 ± 0.592 g/100 g; (total NEAA = 39.12 ± 0.672 g/100 g). On the other hand, total AAs content including semi-essential amino acids (arginine and histidine), which is a critical factor in evaluating the overall protein quality (FAO/WHO, 1991) of these species, was found to be significantly higher in *R. clavata* (total AA = 81.36 ± 0.448 g/100 g) compared to *D. pastinaca* (total AA = 75.92 ± 0.416 g/100 g), indicates that *R. clavata* had better amino acid content than *D. pastinaca* in terms of evaluating the overall protein quality. However, the similar ratio of ΣEAA, ΣSEAA, and ΣNEAA between the two species indicate that their protein quality does not differ substantially. According to the FAO/WHO/UNU (2007) report, the recommended daily protein intake for an adult human is 0.66 g/kg. Of this protein, approximately 27.27% (0.18 g/kg) should come from essential amino acids (EAAs), while the remaining 72.72% (0.48) can be non-essential amino acids (NEAAs). Furthermore, considering the reference values provided by the FAO for essential amino acids (namely histidine, isoleucine, leucine, lysine, methionine + cystine, phenylalanine + tyrosine, threonine, tryptophan, and valine), the total daily requirement for essential amino acids in an adult human is reported to be 0.184 g/kg per day (WHO/FAO/UNU, 2007). To put this into context, based on the FAO's specified value of 0.184 g/kg per day, a 70 kg adult would need to consume approximately 12.88 g of essential amino acids daily. Therefore, the findings of the present study suggest that for a 70 kg adult to meet their total EAA needs, consuming approximately 145.87 g (for *D. Pastinaca*) or 142.95 g (for *R. clavata*) of fish meat would be sufficient for maintaining a healthy diet. The percentage of total essential amino acids to total amino acids for *R. clavata* (∑EAA/∑AA = 38.03%) was slightly lower than in *D. pastinaca* (∑EAA/∑AA = 39.42%). This rate is lower compared to values reported in previous studies, such as 54.5% for seabass (Baki et al. 2015), 57.59%% for anchovy (Bilgin et al. 2018), 55.93% for whiting (Bilgin et al. 2019a) and 52.44% for picarel (Bilgin et al. 2019b) from the Black Sea and with the ratio given for eggs (50%) according to FAO/WHO guidelines (1973, 1991). A slightly lower ratio of total essential amino acids to total non-essential amino acids was observed in *R. calavata* (∑EAA/∑NEAA = 72.63%) and *D. pastinaca* (∑EAA/∑NEAA = 76.52%), which were above the reference values of 60% recommended by FAO/WHO (1991) report, indicating that these species can be regarded as high-quality sources of dietary protein (Pinto et al. 2007). The weight basis total contribution of amino acids as per protein in *D. pastinaca* (W_{EAA} = 20.66 mg/g N) was slightly lower than in *R. clavata* (W_{EAA} = 21.21 mg/g N), and the W_{EAA} contents of these species was found to be slightly lower than the value stated in the FAO/ WHO (1991) pattern (21.90 mg/g N), but were slightly higher than previously reported values for farmed axillary seabream (18.54 mg/g N), farmed ussuri catfish (18.07 mg/g N), and wild ussuri catfish (19.70 mg/g N) (Wang et al. 2014; Oztekin et al. 2020).

Nutritive Value of Protein

In the context of human nutrition, fish muscle tissue plays a significant role as a protein source (Mohanty et al. 2014). Considering that amino acid composition provides an important criterion for the nutritional quality of protein, amino acid score (AAS) are commonly used in the assessment of protein quality. In addition to AAS, other parameters frequently used in the assessment of the nutritional quality level of a protein are chemical score (CS), essential amino acid index (EAAI) and biological values index (BVI).

Amino Acid Score (AAS)

Based on the FAO/WHO (1973) reference amino acid contents, some AAS values of *R. clavata* and *D. Pastinaca*, including isoleucine, valine, leucine, and methionine + cystine were calculated to be lower than '1' (< 1.00), except for lysine, threonine and phenylalanine + tyrosine (AAS ≥ 1). This indicates that, the amino acids levels in these two ray species provide good nutritional quality and could be considered as a favourable protein source. Furthermore, when evaluated in terms of amino acid scores, both *R. clavata* and *D. pastinaca* revealed isoleucine as the "first limiting amino acid" and valine as the "second limiting amino acid". These identified limiting amino acids play a crucial role in constructing a healthy diet (Jiang et al. 2017).

Comparison of AAS, CS, EAAI and BVI for anchovy (Bilgin et al. 2018); whiting (Bilgin et al. 2019a) and picarel (Bilgin et al. 2019b) from the Black Sea given in Table 4.

Table 4 Comparison of amino acid scores, chemical scores, essential amino acid indexes and biological values for different fish species (dry weight basis, mg/g N), calculated by Us based on the results reported essential amino acid values for anchovy, *Engraulis encrasicolus* (Bilgin et al. 2018); whiting, *Merlangus Merlangus* (Bilgin et al. 2019a) and picarel, *Spicara Smaris* (Bilgin et al. 2019b) from the black sea

	AA_{FBP}			Standards		Anchovy		Whiting		Picarel	
	Anchovy*	Whiting*	Picarel*	F/W	WEP	AAS	CS	AAS	CS	AAS	CS
Isoleucine	2.75	2.43	2.38	2.50	3.31	**1.10**[b]	0.83	0.97	0.73	0.95	0.72
Leucine	5.33	4.40	4.20	4.40	5.34	1.21	1.00	1.00	0.82	0.96	0.79
Lysine	**6.85**	**5.63**	**5.25**	3.40	4.41	**2.01**	**1.55**	**1.65**	**1.28**	**1.54**	**1.19**
Methionine + Cystine	2.70	2.15	2.13	2.20	3.86	1.23	0.70	0.98	0.56	0.97	0.55
Phenylalanine + Tyrosine	5.13	4.58	3.90	3.80	5.65	1.35	0.91	1.20	0.81	1.03	0.69
Threonine	2.85	2.20	2.10	2.50	2.92	1.14	0.98	**0.88**[b]	0.75	**0.84**[b]	0.72
Valine	3.00	2.65	2.73	3.10	4.10	**0.97**[a]	0.73	**0.86**[a]	0.65	**0.88**[a]	0.66
Total (mg/g N)	28.60	24.03	22.68								
EAAI	92.58	77.58	74.06								
BVI	89.18	72.83	68.99								

Abbreviations: AAS, amino acid scores; CS, chemical scores; EAAI, essential amino acid indexes; BVI, biological values; F/W, FAO/WHO standard; WEP, whole egg protein standard; AAFBP, fish body protein amino acids

* Since the water content in fish muscle tissue is normally reported as about 75% (Sankar et al. 2010), the values reported in wet weight were converted to dry weight values by multiplying by 4, which corresponds to a water content of 75%. Thereafter, converted fish body protein amino acids (AA_{FBP}) by multiplying reported essential amino acid values (dry weight basis, mg/g N) with 0.625, according to Jones (1941) and FAO/WHO (1973)

[a] the first limiting amino acid

[b] the second limiting amino acid

Examining AAS results from the earlier studies involving *E. engrasicolus*, *M. merlangus* and *S. smaris*; lysine emerged as the amino acid with the highest value above 1.00. Our study's findings align with these results (Bilgin et al. 2018, 2019a, b). Furthermore, similar to the results of this study, valine was reported as the first limiting amino acid score calculated in previous studies for anchovy, whiting and picarel, with high economic value that are caught commercially from the Black Sea and for farmed (0.77), wild (0.76) mongolian redfin (*Chanodichthys mongolicus*) (Jiang et al. 2017). When comparing the amino acid profiles of *R. clavata* and *D. pastinaca* with the earlier reports and the FAO/WHO standards, it is clear that both species offer significant nutritional benefits. The high levels of lysine and glutamic acid align with the FAO/WHO's emphasis on the importance of these amino acids in global nutrition. However, the lower levels of isoleucne and valine underscore the need for dietary diversity to ensure a balanced intake of all essential amino acids.

Chemical Score (CS)

In the present study, lysine was found to have the highest value in terms of chemical score (CS) for both *R. clavata* (1,61) and *D. pastinaca* (1.64), while the CS compositions of other essential amino acids were calculated to be close to each other in both species, which was in agreement with earlier reports, conducted in the Black Sea (Table 4). According to the FAO/WHO reports proteins with low CS values for specific amino acids may require dietary complementation with other protein sources rich in these limiting amino acids to achieve balanced nutrition. However, the high chemical score for lysine in both rays, suggests that this amino acid is sufficiently present and could compensate for the deficiencies of other limiting amino acids if consumed adequately in the diet.

Essential Amino Acid Index (EAAI) and Biological Values Index (BVI)

EAAI and BVI are two critical biochemical indicators used to evaluate protein quality (Oser 1959). Higher values of these indicators suggest that the species can serve as more effective dietary protein sources for human nutrition. The values of these two indices for *R. clavata* were higher than those for *D. pastinaca*. However, the EAAI and BVI values determined for both species were lower than those reported for anchovy, whiting, and picarel in previous studies conducted in the Black Sea (Table 4), with the order of these indices being anchovy > whiting > picarel > *R. clavata* > *D. pastinaca*. The FAO/WHO considers EAAI to be a key measure of protein quality, with values above 60 indicating high biological value, while Schaafsma (2000) noted that an EAAI above 60 reflects high bioavailability. In this study, the calculated EAAI values for both *R. clavata* and *D. pastinaca* exceeded the FAO/WHO reference value of 60, indicating that these species provide high-quality protein capable of meeting or surpassing dietary requirements for essential amino acids (EAAs).

Conclusion

The comprehensive analysis of amino acid profiles in *R. clavata* and *D. pastinaca* had been revealed important insights into their nutritional value and potential as dietary protein sources. It can be demonstrated that both species contain a broad spectrum of amino acids, with essential amino acids such as lysine, leucine, and threonine being particularly notable, and suggests that a 145-gram fillet of either species can fulfill the recommended daily intake of these essential amino acids for a 70 kg adult. The amino acid score (AAS) and chemical score (CS) evaluations indicate that both fish species provide high-quality protein, with lysine and threonine showing particularly high scores. *R. clavata* exhibited a slightly higher essential amino acid index (EAAI) and biological value index (BVI) compared to *D. pastinaca*, suggesting a slightly better overall protein quality. Despite this, both species met or exceed FAO/WHO recommended levels for essential amino acids, indicating their substantial contribution to dietary protein needs. Overall, the amino acid profiles of both *R. clavata* and *D. pastinaca* reinforce their potential as high-quality protein sources that can support various health benefits and contribute effectively to human nutritional needs. However, ecosystem-based sustainable fishing of these species must be ensured. Protecting their stocks, managing fishing pressure, and maintaining biodiversity are critical for both ecosystem health and long-term food security (Pauly et al. 2002). Future research should focus on the changes in amino acid content of these fish species under different processing and storage conditions. Additionally, further studies are needed to investigate the long-term effects of these amino acid profiles on human health and to assess potential allergenic properties.

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Declarations

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