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RESEARCH PAPER

Determination of Essential Amino Acid Changes Related to the Growth of Sea Bass (*Dicentrarchus labrax*)

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Abstract

The aim of this study was to determine the changes in essential amino acids related to the growth of sea bass (*Dicentrarchus labrax*) from the first stocking in cages to the harvesting in production areas at the Aegean and the Black Seas. The highest levels of essential amino acids at the beginning of the trial were in lysine, leucine and threonine, while the highest levels of essential amino acids at the end of the trial at both production area were in lysine, leucine, threonine and isoleucine. The total essential amino acid value of 9.47±0.02 at the beginning of the study exhibited irregular fluctuations between the periods and was 10.45±0.01 and 9.70±0.01 at the Aegean and the Black Seas, respectively at the end of the study. In the study, it was determined that there was a reverse relation between the water temperature and the total essential amino acid values in both production areas, and the observed differences in some amino acids were related to the water parameters and the changes in amino acid metabolism due to the growth of the fish.

Keywords: Sea bass, *Dicentrarchus labrax*, The Aegean Sea, The Black Sea, essential amino acids.

Introduction

Aquaculture allows the supply of fish having high protein level, which has an important place in human nutrition, to the market as well as its contribution to the economy of a country. Societies, awaring of the importance of balanced and healthy diets, frequently consume aquaculture products to meet their animal protein needs; therefore, healthy and high quality aquaculture has gained great importance.

One of the most important issues in healthy and high quality aquaculture is the preparation of diet that is suitable for species and combining well-balanced feed raw materials. Considering that amino acids form starting materials of various substances and are known to have important roles in energy metabolism, especially in the energy metabolism of fish (Ronnestad, 1989), protein sources should be combined in such a manner that they fulfill the amino acid needs of cultured fish and the ration should be in a specific balance for essential amino acids. The lack of a single essential amino acid can cause reductions in protein use and reduce growth performance (Kroeckel, Dietz, Schulz & Susenbeth, 2013).

The net protein needs of the species are determined after defining of their essential amino acid (EAA) needs. Information on essential amino acid requirements for different fish species play an important role in the assessment of feed nutrient value and protein source balance, better production, better protein utilization and lower cost of feed. Protein quality and amino acid compositions of the diet are two important factors affecting fish production (Zakeri, Marammazi & Haghi, 2014).

Amino acids as keys in metabolic pathways, are important for body maintenance, growth, feed intake, nutrient utilization, immunity, behavior, larval metamorphosis, reproduction and resistance to environmental stress and pathogenic organisms in fish (Li, Mai, Trushenski &Wu, 2008; Namulawa, Rutaisire & Britz, 2012). Amino acid analyses have been carried out to determine the protein requirements of fish as well as to reduce phosphorus and nitrogen pollution from waste feed in fish culture area (Namulawa, Rutaisire & Britz, 2012).

This study aimed to determine essential amino acid compositions of sea bass (*Dicentrarchus labrax*) obtained from the same hatchery during the first stocking up to harvesting in two different production areas.

Materials and Methods

The study was conducted between June 2014 and September 2015 in the cage farms in the Aegean

(Muğla-Milas) and the Black Seas (Samsun-Yakakent). In both regions, sea bass obtained from the same hatchery were fed with the same diet during the study starting from the first stocking up to harvesting. In the study, polyetylene plastic cages of 225 mm double pipe with a diameter of 22 m and a depth of 10 m and mesh material with dynema featured. Fish were fed 0.5-2.3% of body weight per day according to water temperature changes.

Fish were chosen in the specified periods by random sampling to represent the stock. Fish were killed with an overdose of an anesthetic substance (MS-222, 200 mg/L) and delivered freshly to the laboratory. Following the biometric measurements of the samples and meat yield calculations, consumable fish parts were separated for biochemical analyses. Amino acid analysis of fish meat was performed according to the Hydrolysis method using Eppendorf LC 3000 amino acid analyzer at TUBITAK Marmara Research Center Food Institute.

Water parameters were determined daily with a field type YSI 556 MPS model multiparameter measurement instrument.

The relationship between essential amino acid values and temperature, salinity and feed amino acid values were done by correlation analysis. The differences between the average values were compared using Tukey's multiple comparison tests and at $P<0.05$ significance levels. One-way variance (ANOVA) analysis and the t-test were performed for the data of the same region and differences between the two regions, respectively after performing tests for normality of the data and equal variances of the groups. All statistical analyzes were performed using SPSS 21 statistical program.

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Results

Enviromental Parameters

During the study period, the average annual water temperatures were 20.16±0.17°C and 14.95±0.30°C (F=4.944, p=0.036); the average oxygen levels were 9.33±0.36 ve 9.75±0.04 mg/lt (F=1.388, p=0.304); the average pH were 8.49±0.04 and 8.71±0.04 (F=15.338, p=0.017) and the average salinity were ‰35.42±0.07 and 16.20±0.11 (F=23.654, p=0.000) in the Aegean Sea and the Black Sea, respectively.

Essential Amino Acid Composition of Diets

In the study, commercial feed which were produced in the same factory with the same properties were used for feeding fish (Table 1).

The highest baseline essential amino acid values in starting diet were in leucine (3.78±0.08) and arginine (3.46±0.10) whereas the highest final values were in isoleucine (8.04±0.05) and leucine (3.32±0.05). At the end of the study, all amino acids except for isoleucine decreased in feed analyzes and the value of isoleucine increased from 2.10±0.02 to 8.04±0.05. The feed ΣEAA value increased from 21.89±0.35 at the beginning of the study to 23.27±0.04 at the end of the study (F=15.080, p=0.06).

Essential Amino Acid Values of Fish Muscle Tissue

Essential amino acid values of fish sampled from the Aegean Sea and the Black Sea during the study were given in Table 2.

Methionine (0.79±0.01), leucine (2.21±0.01), isoleucine (1.60±0.01), phenylalanine (1.18±0.01), valine (1.09±0.01) and ΣEAA (10.64±0.01) values of the fishes obtained from the Black Sea Region reached the highest value in the november-february period. The highest lysine level (3.14±0.06) was

Table 1. The Essential Amino Acid composition of feed used in the experiment (g/100g)

Essential Amino Acids (g/100g)	Initial Feed	Final Feed	p	F
Methionine	$1.31±0.05^b$	$0.80±0.01^a$	0.010	3.440
Lysine	$2.95±0.08^b$	$2.35±0.04^a$	0.020	1.426
Leucine	$3.78±0.08^b$	$3.32±0.04^a$	0.036	1.075
Isoleucine	$2.10±0.02^a$	$8.04±0.05^b$	0.000	0.968
Phenylalanine	$2.26±0.05^b$	$1.89±0.02^a$	0.017	2.084
Valine	$2.47±0.11^b$	$1.87±0.03^a$	0.031	1.848
Threonine	$2.39±0.10^b$	$1.81±0.06^a$	0.039	0.505
Histidine	$1.14±0.06^a$	$0.93±0.04^a$	0.106	0.269
Arginine	$3.46±0.10^b$	$2.67±0.05^a$	0.019	0.953
ΣEAA (Total Essential Amino Acids)	$21.89±0.35^a$	$23.27±0.04^a$	0.060	3.184

Values in the same row with different superscripts are significantly different (P<0.05).

Table 2. Essential amino acid values of fish sampled from the Aegean and the Black Seas

EAA		Initial (May)	June-September	Sep.-November	Nov.-February	February-April	April-June	Final (September)
Methionine	Aegean Sea	0.65 ± 0.01^{d}	0.51 ± 0.01^{ax}	0.57 ± 0.01^{by}	0.58 ± 0.01^{bcx}	0.63 ± 0.01^{dx}	0.64 ± 0.01^{dx}	0.61 ± 0.01^{bcy}
	Black Sea	0.65 ± 0.01^{d}	0.57 ± 0.01^{bcy}	0.46 ± 0.01^{ax}	0.79 ± 0.01^{ey}	0.62 ± 0.01^{cdx}	0.64 ± 0.02^{dx}	0.51 ± 0.01^{abx}
Lysine	Aegean Sea	2.52 ± 0.05^{b}	2.37 ± 0.01^{bx}	2.32 ± 0.01^{bx}	3.33 ± 0.01^{cy}	1.80 ± 0.09^{ax}	1.82 ± 0.08^{ax}	3.18 ± 0.01^{cy}
	Black Sea	2.52 ± 0.05^{c}	2.81 ± 0.01^{dy}	3.14 ± 0.06^{ey}	1.51 ± 0.01^{ax}	1.78 ± 0.01^{bx}	1.74 ± 0.07^{abx}	2.89 ± 0.01^{dex}
Leucine	Aegean Sea	1.59 ± 0.01^{c}	1.21 ± 0.01^{ax}	1.55 ± 0.04^{cy}	1.53 ± 0.01^{bcx}	1.62 ± 0.01^{dx}	1.67 ± 0.01^{dx}	1.45 ± 0.01^{by}
	Black Sea	1.59 ± 0.01^{b}	1.31 ± 0.01^{ay}	1.32 ± 0.01^{ax}	2.21 ± 0.01^{cy}	1.60 ± 0.01^{bx}	1.56 ± 0.02^{bx}	1.28 ± 0.01^{ax}
Isoleucine	Aegean Sea	0.71 ± 0.02^{b}	0.65 ± 0.01^{ax}	0.97 ± 0.01^{dy}	1.15 ± 0.01^{fx}	0.90 ± 0.01^{dx}	0.88 ± 0.02^{cx}	1.04 ± 0.01^{ey}
	Black Sea	0.71 ± 0.01^{b}	0.65 ± 0.01^{ax}	0.78 ± 0.01^{cx}	1.60 ± 0.01^{gy}	0.88 ± 0.01^{ex}	0.84 ± 0.01^{dx}	0.93 ± 0.01^{fx}
Phenylalanine	Aegean Sea	0.82 ± 0.01^{b}	0.61 ± 0.01^{ax}	0.78 ± 0.01^{by}	0.77 ± 0.01^{bx}	0.84 ± 0.02^{bx}	0.81 ± 0.01^{bx}	0.79 ± 0.04^{by}
	Black Sea	0.82 ± 0.01^{b}	0.66 ± 0.01^{ay}	0.65 ± 0.01^{ax}	1.18 ± 0.01^{cy}	0.83 ± 0.01^{bx}	0.84 ± 0.01^{bx}	0.68 ± 0.01^{ax}
Valine	Aegean Sea	0.77 ± 0.01^{b}	0.64 ± 0.01^{ax}	0.64 ± 0.01^{ax}	0.77 ± 0.01^{bx}	0.90 ± 0.02^{cx}	0.94 ± 0.02^{cx}	0.90 ± 0.01^{cy}
	Black Sea	0.77 ± 0.01^{c}	0.65 ± 0.01^{bx}	0.61 ± 0.01^{ax}	1.09 ± 0.01^{ey}	0.91 ± 0.01^{dx}	0.89 ± 0.01^{dx}	0.78 ± 0.01^{cx}
Threonine	Aegean Sea	1.15 ± 0.01^{b}	0.99 ± 0.02^{ax}	1.54 ± 0.02^{cy}	1.17 ± 0.01^{bx}	0.99 ± 0.01^{ax}	1.00 ± 0.01^{ay}	0.95 ± 0.01^{ax}
	Black Sea	1.15 ± 0.01^{e}	1.05 ± 0.01^{bcx}	1.07 ± 0.02^{cdx}	1.90 ± 0.01^{fy}	0.98 ± 0.01^{abx}	0.94 ± 0.01^{ax}	1.14 ± 0.01^{dey}
Histidine	Aegean Sea	0.64 ± 0.01^{ab}	0.58 ± 0.01^{bcx}	0.48 ± 0.01^{dy}	0.52 ± 0.01^{cdy}	0.49 ± 0.02^{dx}	0.48 ± 0.01^{dx}	0.66 ± 0.01^{ax}
	Black Sea	0.64 ± 0.01^{c}	0.64 ± 0.01^{cy}	0.39 ± 0.01^{bx}	0.28 ± 0.01^{ax}	0.43 ± 0.01^{bx}	0.46 ± 0.02^{bx}	0.71 ± 0.01^{cy}
Arginine	Aegean Sea	0.61 ± 0.01^{c}	0.82 ± 0.01^{dx}	0.26 ± 0.01^{bx}	0.09 ± 0.01^{ax}	0.61 ± 0.01^{cx}	1.36 ± 0.01^{ex}	0.87 ± 0.01^{dy}
	Black Sea	0.61 ± 0.01^{b}	0.81 ± 0.02^{bx}	0.69 ± 0.01^{by}	0.09 ± 0.01^{ax}	1.30 ± 0.02^{cb}	1.32 ± 0.02^{cx}	0.79 ± 0.01^{bx}
ΣEAA	Aegean Sea	9.47 ± 0.02^{c}	8.38 ± 0.01^{ab}	9.10 ± 0.11^{bx}	10.42 ± 0.02^{dx}	9.50 ± 0.01^{cx}	9.60 ± 0.03^{cy}	10.45 ± 0.01^{dy}
	Black Sea	9.47 ± 0.01^{c}	6.34 ± 0.01^{ax}	9.11 ± 0.09^{bx}	10.64 ± 0.01^{dx}	9.32 ± 0.01^{bcx}	9.22 ± 0.02^{bcx}	9.70 ± 0.01^{cx}

The difference between the values expressed with different exponential letters in same row (a, b) and same column (x, y) ($p<0.05$).

detected in september-june period whereas the highest arginine level (1.32±0.02) was detected during april-june period and the highest level of histidine (0.71±0.01) was detected in the final period.

Examining the essential amino acid values of fish obtained from the Aegean Sea, it was determined that, different from those obtained from the Black Sea, the highest baseline values were in metionin (0.65±0.01), the highest april-june period values were in leucine (1.67±0.01), arginine (1.36±0.01) and valine (0.94±0.02), the highest february-april period values were in phenylalanine (0.84±0.02) and the highest final period values were in histidine (0.66±0.01) and ΣEAA (10.45±0.01).

In the examples taken from both seas, isoleucine values between june-september period, valin values in september-november period, arginine values in november-february period and ΣEAA values in june-september period reached the minimum levels.

At the end of the study, the essential amino acids values of fish sampled from the Aegean Sea and the Black Sea for methionine, lysine, leucine, isoleucine, phenylalanine, valine, threonine, histidine, arginine and ΣEAA were 0.61±0.01 and 0.51±0.01, 3.18±0.01 and 2.89±0.01, 1.45±0.01 and 1.28±0.01, 1.04±0.01 and 0.93±0.01, 0.79±0.04 and 0.68±0.01, 0.90±0.01 and 0.78±0.01, 0.95±0.01 and 1.14±0.01, 0.66±0.01 and 0.71±0.01, 0.87±0.01 and 0.79±0.01, 10.45±0.01 and 9.70±0.01, respectively.

The highest essential amino acid rate was at the winter period (november-february) at the Black Sea ($F=607.714$, $p=0.000$), whereas it was at the end of the trial for the Aegean Sea and the differences between the groups were significant ($F=206.462$, $p=0.000$). For both production regions, difference in ΣEAA between periods was significant for september and june.

The relationship between essential amino acid values and water temperature-salinity values and the essential amino acid values of fish harvested from both production sites and the feed at the end of the trial were given in Table 3 and Figure 1, respectively.

When the relationship between the essential amino acid values of the fish and the temperature values was examined, negative medium relation with metionin ($F=11.700$, $p=0.002$) leucine ($F=19.212$, $p=0.000$), isoleucine ($F=13.323$, $p=0.001$), phenylalanine (17.387, $p=0.000$), valine ($F=6.593$, $p=0.017$), threonine ($F=6.751$, $p=0.016$) and ΣEAA ($F=3.187$, $p=0.087$), positive weak relation with arginine ($F=0.714$, $p=0.406$) and positive strong relation with histidine ($F=36.827$, $p=0.000$) were determined. When the relationship between the essential amino acid values of the fish and the salinity values was examined, negative weak relation with threonine ($F=0.980$, $p=0.346$) and positive weak and medium relation with the other amino acids values were found. When amino acid values in the fish and the feed were examined, positive medium relation with threonine ($F=1.673$, $p=0.265$) and phenylalanine ($F=2.474$, $p=0.191$), negative medium relation with valine ($F=1.753$, $p=0.256$) and histidine ($F=2.602$, $p=0.182$), positive strong relation with ΣEAA ($F=2.983$, $p=0.159$), metionin ($F=5.036$, $p=0.088$) and leucine ($F=7.877$, $p=0.048$), negative very strong relation with lysine ($F=13.300$, $p=0.022$) and arginine ($F=25.419$, $p=0.006$) and positive very stron relation with lysine ($F=13.300$, $p=0.022$) and arginine ($F=25.419$, $p=0.006$) were detected.

Discussions

The aim of this study was to determine the changes in essential amino acids related to the growth of sea bass from the first stocking in cages to the harvesting in production areas at the Aegean and the Black Seas.

At the end of the study, the differences between

Table 3. Relationship between essential amino acid values in fish and temperature, salinity and feed amino acid values

EAA (g/100g)	Temperature (°C)			Salinity (‰)			Feed EAA (g/100g)		
	PC	p	F	PC	p	F	PC	p	F
Methionine	-0,572	0.002	11.700	0.409	0.187	2.009	0.747	0.088	5.036
Lysine	0.369	0.076	3.473	0.111	0.731	0.125	-0.877	0.022	13.300
Leucine	-0.667	0.000	19.212	0.372	0.234	1.606	0.814	0.048	7.877
Isoleucine	-0.597	0.001	13.323	0.448	0.145	2.505	0.941	0.005	31.133
Phenylalanine	-0.648	0.000	17.387	0.273	0.390	0.808	0.618	0.191	2.474
Valine	-0.464	0.017	6.593	0.534	0.074	3.990	-0.552	0.256	1.753
Threonine	-0.469	0.016	6.751	-0.299	0.346	0.980	0.543	0.265	1.673
Histidine	0.778	0.000	36.827	0.048	0.882	0.023	-0.628	0.182	2.602
Arginine	0.170	0.406	0.714	0.108	0.739	0.117	-0.934	0.006	25.419
ΣEAA	-0.342	0.087	3.187	0.541	0.069	4.137	0.654	0.159	2.983

PC: Pearson Correlation

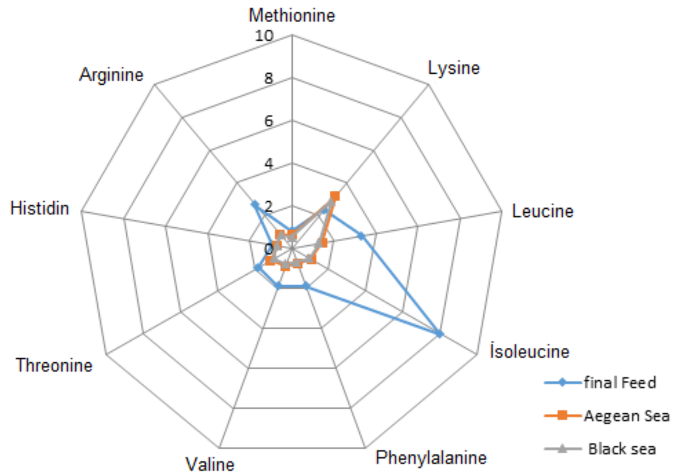

Figure 1. Essential amino acid values (mg/100) of fish and feed at the end of the trial.

the fish produced from the Aegean Sea and the Black Sea in terms of essential amino acid values were determined. When the essential amino acid values obtained in both production areas were examined, they were higher than the values obtained from the studies of Thebault, Alliot and Pastoureaud (1985), Tibaldi, Lanari, Ballestrazzi, Tulli and Pinosa (1993), Tibaldi, Tulli and Lanari (1994), Tibaldi and Tulli (1999), Özden and Erkan (2008), Baki, Gönener and Kaya (2015; 2015) and Çimagil (2016) and lower than the values obtained from the study of Erdem, Baki and Samsun (2009). In the other studies it was indicated that the values of methionine and lysine which are indicator for maximum growth, sufficient and healthy nutrition with phenylalanine which has an important role in the amino acids biosynthesis are important for fish (Polat, 1999; Aragao et al. 2004; Wu, 2013; Furuya, Michelato, Salaro, Da Cruz & Barrivera-Furuya, 2015). Methionine, lysine and phenylalanine values were higher in in the Aegean Sea in this study.

At the end of the study, ΣEAA were 10.45±0.01 and 9.70±0.01 in the Aegean Sea and the Black Sea, respectively. Considering the total amino acid compositions in the study, sea bass from the Aegean Sea had higher amino acid values. In the studies done on sea bass, ΣEAAs were 28% Aegean Sea (Özden & Erkan, 2008), 13.776 g/100 g in Muğla (the Aegean Sea), 10.538 g/100 g in Sinop (the Black Sea) (Erdem, Baki & Samsun, 2009) and 7.36 g/100 g in Muğla (the Aegean Sea) (Baki, Gönener & Kaya, 2015). The reason for different results may be due to using different feed and fish.

It is stated that the amino acid composition of fish should be evaluated together with the amino acid profile of the feed (Lochmann, Goodwin, Lochmann, Stone & Clemment, 2007). In the present study, high correlation in methionine, lysine, leucine, isoleucine and arginine values in feed and fish meat was detected. Other studies also reported a high correlation between the essential amino acid values in the feed and the fish (Tacon & Cowey, 1985, Mambrini & Kaushik, 1995). Although the effect of the values in the diet on the essential amino acid composition in fish is pointed out, it was stated that temperature (Wilson & Halver, 1986; Sivaloganathan, Walford, Ip & Lam, 1998; Costas, Aragao, Ruiz-Jarabo, Vargas-Chacoff & Arjona, 2012) and salinity values (Venkatachari, 1974; Balocco, Boge & Roche, 1993) from the environmental conditions are also effect these values. In the study, it was determined that the correlation between the essential amino acid values in fish and salinity values was low, while the temperature changes had a higher correlation. In this study carried out in the Aegean Sea and the Black Sea with different physico-chemical properties, it was determined that the difference between the determined essential amino acid values arised from the environmental factors and water temperature changes affected the essential amino acid composition of fish.

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