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Research Paper

AMINO ACIDS PROFILE IN GONADS OF THE RED SEA FISH *RHABDOSARGUS SARBA* DURING BREEDING SEASON

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Eighteen amino acids were detected in both sexes of *Rhabdosargus sarba*, 10 Essential Amino Acids (EAA) and 8 Non Essential Amino Acids (NEAA), females had higher concentration of EAA (2365 μg/mg) than males (1513 μg/mg); however males had higher concentration of NEAA (2418 μg/mg) than females (1614 μg/mg). In females, the minimum concentration of total EAA (2192.6μg/mg) was in nearly ripe stage but the maximum concentration (2595 μg/mg) was in ripe ovaries. The maximum concentration of total NEAA was during spawning (2584.6 μg/mg) but the minimum concentration (1043.5 μg/mg) was in ripe stage. The EAA Arginine, Histidine, and Tryptophan were significantly higher (P<0.05) in males than females, whereas Lysine, Valine, Leucine, and Methionine were significantly higher (P<0.05) in females than males. The NEAA Aspartate, Proline, and Glutamate were highly significant (P<0.05) in males than females, hwoever Glycine and Threonine were significantly higher in females than males.

Keywords: Sparidae: *Rhabdosargus sarba*, Fish, Amino acids, Breeding season, Gonads, Red Sea

INTRODUCTION

The Sparidae family contains a number of economically important species throughout the world (Nelson, 1994), and is one of the most important fish species in the Red Sea. The family Sparidae contains 35 genera and 112 species, distributed mainly in tropical and temperate waters of the Atlantic, Pacific and Indian oceans (Froese and Pauly, 2005). Arid fishes *Rhabdosargus sarba* are one of the sparidae family.

Fish protein is of very high quality, and contains sufficient amounts of all the essential amino acids required by the body for growth and maintenance of lean muscle tissue. On the basis of needs from diet for growth, Amino Acids (AA) were traditionally classified as nutritionally essential and nonessential, and all nonessential AA can be synthesized adequately by aquatic animals (Li *et al*., 2008). An imbalance in the composition of amino acids in the feed would affect the metabolism of the fish and may also, influence

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the way of fat utilization within fish body. These changes, in turn, can affect the growth, health and quality of the fish that is produced. Previous studies have revealed that amino acid metabolism in the form of yolk formation and degradation is intractably linked to lipid and water transport in the oocytes, embryos and larvae (Wright and Fyhn, 2001). Separate reviews have very recently been compiled that document the importance of amino acids for fish larval growth (Kamler, 2008), digestion (Rønnestad and Morais, 2008), buoyancy (Govoni and Forward, 2008) and thyroid hormone metabolism (Power *et al.,* 2008). Partial exchange of dietary proteins with Free Amino Acid (FAA) in larval red seabream (*Pagrus major*) increases their rate of growth and survival (López-Alvarado and Kanazawa, 1995). A typical marine pelagic teleost egg consists of proteins, FAA, lipids, inorganic ions and glycogen. More than 600 different proteins have been identified during oogenic stages of zebra fish and gilthead seabream (*Sparus aurata*) (Ziv *et al.*, 2008). Despite such impressive numbers of proteins in the developing oocytes of fish, the most abundant and major of amino acid delivery in all fish is vitellogenin (Vtg) (Ziv *et al.*, 2008).

The aim of the study to examine the amino acid profiles in the *S. sarbs* during the spawning season from the Red Sea. It is emphasized that no earlier studies of the fatty and amino acids composition of this species occur.

MATERIALS AND METHODS

Fish Samples

Monthly individual *Rhabdosargus sarba* (L) were obtained from Jeddah fish market Bangalah, Red Sea, Saudi Arabia. The experiments were carried out in the breeding seasons from October 2010 to December 2011. Fishes were transported to the laboratory in ice aquarium, and then the total length, standard length and weight were measured. After that fishes were dissected to determine sex and maturity stage. The testes and ovaries were removed, weighed and thoroughly examined.

Crude Protein (CP) Determination using Kjeldahl Method

The Kjeldahl method is the standard method of nitrogen determination. The procedure consists of: (1) digestion of the sample in sulfuric acid with a catalyst, which results in conversation of nitrogen to ammonia; (2) distillation of the ammonia into a trapping solution; and (3) quantification of the ammonia by titration with a standard solution. A reagent blank and at least one sample of in- house standard are run as check of the correctness of the procedure. If digestion is not complete, make appropriate adjustments. Calculations were determined according to the follow formula:

% Crude Protein = {(ml titrated– blank)(.8756)/(sample wt in grams)(% lab DM)}*100

% Nitrogen = {(ml titrated – blank)(1401)/(sample wt in grams)(% lab DM)}*100

Results are reported as crude protein (CP) as a % of Dry Matter.

Data were analyzed, using one-way ANOVA followed by the *t*-test student for significant differences. The level of significance used was $P>0.05$.

Amino Acid Analyzer

Hydrolyze 0.5 mg sample in 5.0 mL of 6 M HCl. Evaporate to dryness and reconstitute to the same volume with NLeu/azide diluent. Dilute

an aliquot 1,000–2,000x with the NLeu/azide diluent.

RESULTS

Amino Acids

Table 1 shows the free amino acids composition of *R.* In both sexes 10 Essential Amino Acids (EAA) and 8 Non Essential Amino Acids (NEAA) were detected. Females had a higher concentration of EAA (2365.25) than males (1513.30), however in males; NEAA was higher (2418.92) as compared with females (1614.16).

Table 1: Essential (EAA) and Non Essential (NEAA) Amino Acids Compositions During the Spawning Cycle of Male and Female *R.sarba*

	Amino Acid µg/mg	Male	Female	P<0.05
EAA				
1	Arginine	319.58	131.7	0.015*
2	Lysine	66.873	280.25	0.316*
3	Threonine	119.71	130.8867	0.407
4	Valine	85.533	133.9933	0.025*
5	Isoleucine	65.66	77.87	0.131
6	Leucine	187.68	1103.57	0.083*
7	Methionine	76.286	210.17	0.042*
8	Histidine	276.3	131.7233	0.016*
9	Phenylalanine	64.173	73.14667	0.171
10	Tryptophan	251.5	91.94333	0.039*
Total		1513.295	2365.253	
NEAA				
11	Alanine	432.68	344.35	0.171
12	Glycine	218.27	409.4667	0.044*
13	Serine	280.49	445.7267	0.141
14	Proline	252.03	78.25667	0.020*
15	Glutamate	233.01	92.57667	0.041*
16	Aspartate	875.15	114.01	0.019*
17	Cystine	23.24	17.56	0.663
18	Tyrosine	104.05	112.2167	0.037*
Total		2418.92	1614.163	

Note: * Values Are Significantly Different (P<0.05).

EAA and NEAA In Female During Spawning Season

Relationships between each amino acids and ovarian stages during the breading season are presented in Table 2. The maximum content of EAA (2595 μg/mg) was recorded in January (ripe stage) and the minimum concentration of the total EAA (2192.6 μg/mg) was in nearly ripe stage during December. By February, when the spawning began the total content of EAA decreased to 2307.7 μg/mg.

It is obvious that leucine, lysine , methionine and tryptophan were the main constituents of EAA in nearly ripe and ripe stages and their maximum concentrations were in ripe ovaries during January (Luc, 1336.1 ; Lys.,328.3; Meth., 251.3

Table 2: Essential (EAA) and Non Essential (NEAA) Amino Acids Compositions (%) During the Seasonal Spawning Cycle of Female *R.sarba*

	Amino Acid μg/mg	January (Ripe)	February (Spawning)
EAA			
Arginine	52.3	83.8	259
Lysine	287.2	328.3	225.3
Threonine	89.4	124.2	179.1
Valine	78.3	84.6	239.1
Isoleucine	50.9	52.6	130
Leucine	1157.6	1336.1	817
Methionine	235.1	251.3	144.1
Histidine	122.8	136.9	135.5
Phenylalanine	48	66.2	104.8
Tryptophan	71	131	73.8
Total	2192.6	2595	2307.7
NEAA			
Alanine	123.4	157.2	752.5
Glycine	195.1	222.8	810.6
Serine	445.1	539.4	352.7
Proline	54.9	83.1	96.7
Glutamate	53.2	47.2	177.4
Aspartate	101.7	106.8	133.6
Cystine	8.3	29.2	15.3
Tyrosine	61.8	29.1	245.8
Total	1043.5	1214.8	2584.6

and Try., 131 μg/mg, respectively). The total content of these amino acids increased from 1043.5 μg/mg in December (nearly ripe stage) to 2584.6 μg/mg in February (spawning stage). Also, it can be seen that the lowest content of NEAA was cystine, which ranged between 8.3-15.3 μg/mg from December to February. Alanine, glycine and serine were the major NEAA and spawning stage at February had the maximum content of NEAA (2584.6 μg/mg) and the minimum content (1043.5 μg/mg) was in ripe stage during December.

EAA and NEAA in Male During Spawning Season

Table 3 shows the amino acids composition of testis in *R. sarba* during the spawning seasons.

Table 3: Essential (EAA) and Non Essential (NEAA) Amino Acids Compositions During the Seasonal Spawning Cycle of Male *R. sarba*

	Amino Acid μg/mg	January (Ripe)	February (Spawning)
EAA			
Arginine	277.4	323.5	357.9
Lysine	94.8	54.2	51.6
Threonine	147.6	96.8	114.7
Valine	92.5	73.8	90.3
Isoleucine	90.1	56.4	50.5
Leucine	146.5	193.3	223.2
Methionine	83.3	64.1	81.4
Histidine	276.3	318.8	288.9
Phenylalanine	69.9	53.6	68.9
Tryptophan	447.1	169.8	137.8
Total	1725.5	1404.3	1465.2
NEAA			
Alanine	219.1	524.2	554.1
Glycine	318.1	170.5	166.3
Serine	509.4	170.2	161.9
Proline	665.2	39.1	51.7
Glutamate	231.4	217.3	250.3
Aspartate	421.3	1101.8	1102.3
Cystine	38.9	7.5	23.3
Tyrosine	124.5	94.6	93.1
Total	2527.9	2325.2	2403

The total EAA content were 1725.5, 1404.3 and 1465.2 μg/mg in nearly ripe, ripe and spawning stage, respectively. In NEAA, the total contents were 2527.9, 2325.2 and 2403 μg/mg in the former stages, respectively. It is obvious that Arg and His were the major EAA in the three mature stages of testis during the three months of the spawning season. Also, the main constituents of NEAA were Proline (665.2 μg/mg), Serine (509.4 μg/mg) and Aspartate (421.3 μg/mg) which were found in nearly ripe stage (December). In ripe and spawning stages during January and February months aspartate (1101.8, 1102.3 μg/mg) and alanine (524.2, 554.1 μg/mg) were the major content of NEAA.

DISCUSSION

The present results revealed that there was an increase in the total EAA reaching maximum value at January (ripe ovaries) in female and December (nearly ripe testes) in male. Similar results have been reported for *Oreochromos spilurus* by Baloubid (2005). This increase may be related to the importance of these EAA in the development that accelerates the growth of ripe yolks eggs in the ovaries and activates spermatogenesis and spermiogenesis process in testes (Zeitoun *et al*., 1977). In Female, the most abundant EAA were Leucine (1103.6 μg/g), Lysine (280.3 μg/g) and Methionine (210.2 μg/g). These results are in accordance with Abou-Shabana (2004) for *Oreochromis nilotica* and Wahbi *et al*. (2004) for *Lithognathus mormyrus* (Sparidae). The higher contents of Leucine may be also related to the energy requirement and phosphorylation as mentioned by Foster and Ogata (1998) and Kim and Lall (2000).

The present results also showed that , the total NEAA content increased progressively to reach its maximum concentration at spawning ovary (February) in female and nearly ripe testes (December) in male. The present data however is in agreement with Srivastava *et al.* (1991) who reported that the energy utilized during embryogenesis comes from the catabolism of body stores. The previous results sustain the present result concerning the declination of the total mean of NEAA in the different maturity stages. In Female of *R. sarba*, the dominated non-essential amino acids were Serine (445.7 μg/mg), Glycine (409.5 μg/mg) and Alanine (344.4 μg/mg).). Serine amino acid in the present study reached the maximum value (Table 2) in ripe ovaries followed by decreasing at spawning stage .The decrease in serine content may be related to the ovulation process as reported by Coffman and Goetz (1998).

However, in Male the maximum contents of EAA were arginine (319.6 μg/g), histidine (276.3 μg/g) and tryptophan (251.5 μg/g). In particular, it is known that arginine is the dominant component of promatines, which are important components of spermatozoa (Lewis *et al*., 2004). Arginine contents were significantly ($P< 0.05$) higher in the testis than in the ovary of *R. sarba* as observed in the present study. Arginine is very important for energy metabolism in aquatic animals (Tsuchiya, 1962). The higher amount of arginine in the testis than in the ovary suggested that spermatozoa need arginine as an energy medium more than the ovary does, since spermatozoa swim actively at fertilization. Arginine can stimulate the release of various hormones such as insulin, growth hormone, and glucagon in fish (Mommsen *et al.*, 2001). Jobgen *et al*. (2006) and Yao *et al*. (2008) reported that arginine plays a crucial role in regulating endocrine and reproductive functions.

Also the results showed that the high content of NEAA in the testes of *R. sarba* were Asparate, Alanine, Serine, Proline and Glycine (Table 1). Asparate, Alanine and Serine increased to its maximum value at ripe stage, while Proline and Glycine reached to the maximum value at nearly ripe stage in January (Table 3). Similar findings were reported for *Oreochromis spilurus* by (Baloubid, 2005) .This is consistent with the result obtained by Osako *et al*. (2007) .This maximum content was related to the maximum requirement of energy for the gonads and rebuilding as reported by Kim (1997) which concluded that Rainbow trout was found to utilize either a dispensable amino acid mixture or alanine alone as energy source. Alanine can stimulate feeding response of certain fishes (Shamushaki *et al.*, 2007). This data disagree with that of Wahbi *et al.* (2004) who reported that alanine level was low in *Lithognathus Mormyrus* testes.

CONCLUSION

We have found that 10 EAA and 8 NEAA were detected in male and female of *Rhabdosargus sarba*, and females had a higher concentration of EAA than males however NEAA was higher in males. We have defined in females, the minimum concentration of total EAA and NEAA were in nearly ripe stage (December) but the maximum concentration of total EAA and NEAA were in ripe ovaries (January) and during spawning stage (February), respectively. We have confirmed that the EAA Arginine, Histidine, and Tryptophan were significantly higher in males than females, whereas Lysine, Valine, Leucine, and Methionine were significantly higher in females than males. The NEAA Aspartate, Proline, and Glutamate were highly significant in males than females; however Glycine and Threonine were significantly higher in females than males.

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