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ORIGINAL ARTICLE

Protein and amino acid composition of wild caught freshwater crayfish (*Pontastacus leptodactylus*) in the reproductive season

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ABSTRACT

Interest in feeding crayfish under culture conditions has increased in the last few years; however, there is still a lack of information on feeding crayfish according to their nutritional requirements (*i.e.*, protein and amino acid dietary demands of broodstock). In this study, the protein and amino acid composition of abdominal muscle, gonads and hepatopancreas of male and female broodstock crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823) were determined in the reproductive season. The results show that in *P. leptodactylus*, the amount of protein in the ovary was 47.25 mg/g and that it was 35.03 mg/g and 39.36 mg/g in the testes and vasa deferentia, respectively. In males, the values of essential amino acids (EAA) obtained in the abdominal muscle were significantly lower than those obtained from the hepatopancreas, testes and vasa deferentia ($P < 0.05$). In female crayfish, EAA/total amino acids (TAA) were found to be 45.48 % in the ovary, which is significantly higher than that of the abdominal muscle (40.19 %) and hepatopancreas (42.14 %) ($P < 0.05$). The results also show that abdominal valine, threonine, lysine, and histidine were statistically higher in female crayfish than male crayfish ($P < 0.01$). Leucine was the major EAA found in males in abdominal muscle (8.73 %). In conclusion, this study analyzed the protein and amino acid composition of abdominal muscle, hepatopancreas

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and gonad of crayfish in the breeding season in order to understand the protein and amino acid contents of wild caught crayfish and get an idea on the nutritional requirements of *P. leptodactylus* in the reproductive season.

Keywords

Crustacean metabolism, gonad, hepatopancreas, protein composition

Introduction

Examination of the biochemical composition and intermediate metabolism of crayfish reveals the presence of considerable inter- and intraspecific change that makes it difficult to determine a typical metabolic structure (Oliveira *et al.,* 2003; Vinagre *et al.,* 2007). These differences can arise from various factors such as environmental conditions, seasonality, molt cycle stage, sexual maturity, nutritional status and current food (Schirf *et al.,* 1987; Buckup *et al.,* 2008). In addition, the analysis of the biochemical composition differences in different seasons are important for reproductive biology, as it is essential to know how various organs can accumulate and utilize organic reserves to undergo gonad maturation, breeding activities and animal maintenance (Rosa and Nunes, 2003; Buckup *et al.,* 2008).

Gonad growth is vital for the quality of embryos in crayfish because the nutrient needs of the embryos are provided only by egg yolk reserves (Harrison, 1990; Hasek and Felder, 2005). Yolk nutrients are stored during ovarian development and they depend on female body reserves and nutritional intake (Harrison, 1997). The hepatopancreas in crayfish is involved in nutrient absorption, digestion, and accumulation (Sousa and Petriella, 2000; Reppond *et al.,* 2009; McGaw and Curtis, 2013). Studies on the diversity of biochemical composition in different physiological stages of the crayfish hepatopancreas and ovary should be assessed at the biochemical level, to prepare efficient broodstock diets and meet nutritional requirements (Ying *et al.,* 2006).

Crayfish are consumed as a luxury food item in many parts of the world. Furthermore, they are traditionally considered as important food items in some countries such as Sweden and Finland. Due to the economic importance of crayfish, production of crayfish in many regions is of great interest (Wickins and Lee, 2002). There are approximately 15 commercially important crayfish species in the world including *Pontastacus leptodactylus* (Eschscholtz, 1823) (see Holdich, 1993). *Pontastacus leptodactylus* is also known as the narrow-clawed crayfish, the Danube crayfish, Galician crayfish or the Turkish crayfish. It is distributed in eastern Europe, and the Middle East (Köksal, 1988). This species is native to more than 16 countries from Austria in the west, Russia in the north, Greece in the south and Turkey and Iran in the east. In addition, *P. leptodactylus* has been introduced into many countries including Armenia, Belgium, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Switzerland, UK, and Uzbekistan (Skurdal and Taugbøl, 2002; Harlıoğlu, 2004; Gherardi and Souty-Grosset, 2017). Moreover, *P. leptodactylus* is considered as an easy crayfish species to produce via aquaculture (Wickins and Lee, 2002).

Although crayfish production is mostly carried out by extensive and semi-intensive stocking methods, the intensive stocking method is mainly used for the production of juveniles (Harlıoğlu and Farhadi, 2017). Therefore, there has been an increasing demand for crayfish feed production in recent years in the aquaculture sector. However, despite the rapid development of crayfish aquaculture in recent years, the improvement of a formulated diet has not kept pace, even though there is a need to feed the species well for successful aquaculture. Nutrients and proteins constitute a huge percentage of total aquaculture expenditure and are the most economically important components of aquaculture diets (Garza de Yta *et al.,* 2012).

A number of authors have identified optimum dietary protein demands for crayfish species (Cortes-Jacinto *et al.,* 2003; Saoud *et al.,* 2012). Nevertheless, there are no reports recording the required amino acid demands of these same crayfish species. The aim of this research is to describe the characteristics and variation in biochemical composition, including protein and amino acid content, in abdominal muscle and hepatopancreas of both sexes of *P. leptodactylus*. In addition, the same composition in ovaries, testes, and vasa deferentia of male *P. leptodactylus* during the reproductive season is described in order to understand their protein and amino acid content and to get an idea on the nutritional requirements in the reproductive season.

Materials and Methods

Crayfish samples

Crayfish specimens were obtained from the Keban Dam Lake (38°48′22″N 38°45′14″E) population of *P. leptodactylus* by a commercial fisherman in the reproductive season in January 2018. They were immediately transported to the laboratory on ice. The males and females of *P. leptodactylus* attain sexual maturity in their third year at a body length of about 77–80 mm. They reproduce only once a year and the reproductive season for this species starts when the water temperature declines in the fall. Mating takes place in January and egg-laying is completed in 4–6 weeks in the Keban Dam Lake population of *P. leptodactylus*. The stage 1 juveniles emerge in late May–June depending on the temperature fluctations for the year (Harlıoğlu *et al.,* 2012a; 2012b; 2013; 2017).

The crayfish samples were weighed to an accuracy of 0.001 g with a precision balance and the total length from the tip of the rostrum to the tip of the telson was measured to the nearest 0.001 mm. Abdominal muscle, gonad and hepatopancreas were dissected and stored at -30 °C until analyzed. The analyses outlined below were carried out in the biology department of the Life Science Faculty of Firat University.

In crayfish, the determination of ovarian developmental stages depends on the gonadosomatic index, color and shape, histological structure and relative proportion of cellular types, and the location and content of these cells. Therefore, four ovarian developmental stages are distinguished: proliferation, previtellogenic, vitellogenic, and mature (Üniş and Erkan, 2012). In the present study, female crayfish in the fourth stage of ovarian development (mature stage) with a large, and full, olive-green, Y-shaped ovarian structure were used. The morphological differentiation of the gonads in male crayfish are divided into 5 stages, from stage A to stage E. The testes of male crayfish differentiate in stage A, develop and enlarge in stage B, begin spermatogenesis in stage C, and become sexually mature in stages D and E. In the late stage C, mature spermatozoa can be observed in several lobules. In late stage D, spermatozoa are released from lobules to the vas deferens. The features of the testes in stage E are the same as in stage D (Taketomi *et al.*, 1996). In the present study, male crayfish with mature swollen testes at stages D-E were used.

In this study, forty female (average total length and weight: 97 ± 3.8 mm and 33.9 ± 4.2 g, respectively, mean ± SD) and forty male (average total length and weight: 110 ± 4.6 mm and 39.9 ± 5.4 g, respectively, mean ± SD) *P. leptodactylus* were used.

Protein analysis

After washing the tissue samples with 0.9 % NaCl, the wet weights were determined and then homogenized in 10 mL of 50 mM Tris-20 mM EDTA (pH = 7.4) buffer mixture. After this, the samples were centrifuged at 4 °C for 9 minutes at 9000 rpm. After centrifugation, 50 µL of the supernatant was taken and total protein was measured according to the Lowry *et al.* (1951) method.

Derivation of amino acids and analysis by gas chromatography

Derivation of amino acids with N-(t-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) gives rise to the simultaneous silylation of the amino- and carboxyl groups in a solitary step with modifications to the procedure as declared by Buch *et al.* (2006).

Each sample protein was hydrolyzed with 6 M HCl (% 0.5 phenol) at the 110 °C for 24 hours and each sample was spiked with 1 mL of the internal standard working solution (1.5 µg) and evaporated to complete dryness. Then, 10 µL of dimethylformamide and 60 µL of MTBSTFA were added and the vial was sealed with a polytetrafluoroethylene-lined cap. Finally, the sample was heated to 70 °C for 20 min to achieve the chemical derivation of amino acid and the derivatives were analyzed by GC–MS.

For the analysis of amino acid derivatives, a Shimadzu gas chromatograph (2010 plus) modified for glass-capillary work and a flame ionization detector (FID) were used. Amino acid derivatives were separated on a 20 m Supelco Slb 5 ms capillary column (Supelco, Sigma, 0.25 mm ID 0.25 µm film thickness) operating with helium carrier gas (45 cm/sec) under the following temperature program: from 120 to 150 °C at 120 °C/min (5 min hold), to 240 °C at 7 °C/min and finally to 285 °C at 20 °C/min (18 min hold). The temperature of the injector and detector was kept constant at 240 °C and 300 °C, respectively. The identification of amino acid derivatives was based on comparison of their FID chromatogram and retention times with those of an authentic reference.

Statistical analysis

The data were analyzed statistically by using a one-way analysis of variance (ANOVA) and Duncan's new multiple range test (SPSS 16.0). Levene's test was employed to check the homogeneity of the data. The level of significance for all analyses was determined at $P < 0.05$. Data were subject to ANOVA independent t test for determining significant differences between protein and amino acid means in the different tissues of male and female crayfish. The level of statistical significance were reported as $P > 0.05$, $P < 0.05$, $P < 0.01$ and $P < 0.001$. All data were presented as means ± standard deviation.

Results

In this study, it was determined that the protein content of female abdominal muscle was 40.19 % and the protein content of male abdominal muscle was 40.11 %. The amount of protein was greatest in the ovary (47 %). In addition, the amount of protein was higher in the vasa deferentia (39 %) than in the testes (35 %). It was also determined that the amount of protein content in the ovary was significantly higher than that of the testes and vasa deferentia ($P < 0.001$). The protein content of the female hepatopancreas was significantly higher than that of males ($P < 0.05$) (Tab. 1).

Amino acid composition (%, wet weight) in the studied organs of male and female *P. leptodactylus* are shown in Tab. 2 and Tab. 3, respectively. Statistical comparison of amino acid concentration (%, wet weight) in abdominal muscle, hepatopancreas, ovary-testes, ovary-vasa deferentia between male and female *P. leptodactylus* are given in Tab. 4.

Eighteen amino acids, ten EAA and eight non-essential amino acids (NEAA) were determined. The principal among the EAA were leucine and valine, whereas those among the NEAA were aspartic acid, serine and cysteine. Leucine concentration was considerably ($P < 0.05$) higher in the hepatopancreas, testes and vasa deferentia than in the abdominal muscle. Aspartic acid was significantly ($P < 0.05$) higher in testes and vasa deferentia than in the abdominal muscle and hepatopancreas in male crayfish. The total amount of EAA and TAA were higher ($P < 0.05$) in the hepatopancreas, testes and vasa deferentia than in the abdominal muscle. Similarly, leucine, isoleucine and glycine were higher ($P < 0.05$) in the hepatopancreas, testes and vasa deferentia than in the abdominal muscle. The content of the amino acid threonine was the highest amount (6.07 %) in the vasa deferentia. The ratio of EAA/TAA in hepatopancreas (42.24 %) and testes (41.88 %) was significantly higher ($P < 0.05$) than was observed in the vasa deferentia (40.17 %) and abdominal muscle (39.14 %).

Table 1. Protein content (%, wet weight) of abdomen muscle, hepatopancreas and gonads during the reproductive season in male and female *Pontastacus leptodactylus* and statistical comparison of protein content in the ovary and testes, ovary and vasa deferentia, hepatopancreas and abdomen muscle between male and female of *P. leptodactylus*.

Protein (%)	Abdomen muscle	Hepatopancreas	Ovary	Testes	Vasa deferentia
Female	40.19±2.04	36.89±2.78	47.25±2.75	–	–
Male	40.11±1.62	31.73±2.56	–	35.03±0.34	39.36±1.57
Statistical comparison of protein content	**Ovary – Testes**	**Ovary – Vasa Deferentia**	**Male and female hepatopancreas**	**Male and female abdomen Muscle**	
Protein	***	***	*	ns	

Level of statistical significance (ANOVA, t-test; ns= $P > 0.05$; *= $P < 0.05$; ***= $P < 0.001$)

Table 2. Amino acid composition in the abdomen muscle, hepatopancreas, testes and vasa deferentia (%, wet weight) during the reproductive season in male *Pontastacus leptodactylus.*

Amino acids (%)	Abdomen muscle	Hepatopancreas	Testes	Vasa deferentia
EAA †				
Valine	7.44±0.32^{a}	6.92±0.33^{bc}	7.07±0.18^{ab}	6.60±0.25^{c}
Leucine	8.73±0.80^{b}	9.94±0.64^{a}	9.84±0.35^{a}	9.71±0.31^{a}
Isoleucine	4.58±0.30^{b}	5.10±0.28^{a}	5.34±0.10^{a}	5.14±0.08^{a}
Threonine	3.72±0.40^{c}	3.30±0.47^{c}	5.17±0.23^{b}	6.07±0.17^{a}
Methionine	2.53±0.79^{a}	1.20±0.59^{b}	2.22±0.16^{a}	1.96±0.29^{a}
Phenylalanine	6.35±0.69^{bc}	7.37±0.50^{a}	6.96±0.32^{ab}	6.09±0.18^{c}
Lysine	0.78±0.08^{b}	4.51±0.48^{a}	0.78±0.01^{b}	1.06±0.2^{b}
Histidine	1.19±0.25^{c}	1.08±0.23^{c}	2.28±0.35^{a}	1.71±0.33^{b}
Arginine	1.38±0.29^{a}	1.35±0.39^{a}	1.77±0.45^{a}	1.22±0.35^{a}
Tryptophan	0.12±0.04^{c}	0.57±0.18^{a}	0.31±0.04^{bc}	0.44±0.14^{ab}
ΣEAA †	36.85±0.31^{b}	41.39±1.58^{a}	41.80±0.61^{a}	40.01±1.07^{a}
NEAA ‡				
Tyrosine	6.27±1.32^{a}	5.58±0.18^{ab}	5.56±0.44^{ab}	5.12±0.21^{b}
Alanine	8.17±1.01^{a}	7.20±0.63^{b}	7.73±0.61^{a}	8.69±0.32^{a}
Glycine	5.27±0.91^{b}	6.91±0.54^{a}	6.41±0.31^{a}	6.78±0.27^{a}
Serine	9.96±1.83^{a}	8.36±0.30^{b}	8.29±0.96^{b}	9.95±0.12^{a}
Proline	1.03±0.15^{a}	1.16±0.29^{a}	1.06±0.67^{a}	0.70±0.35^{a}
Aspartic acid	15.80±0.98^{b}	16.45±0.94^{b}	18.40±0.58^{a}	17.94±0.24^{a}
Glutamic acid	1.56±0.44^{a}	1.57±0.18^{a}	1.07±0.80^{ab}	0.44±0.23^{b}
Cysteine	9.28±0.48^{a}	9.31±0.70^{a}	9.45±0.64^{a}	9.93±0.42^{a}
ΣNEAA ‡	57.36±2.97^{ab}	56.56±1.18^{b}	58.01±0.56^{ab}	59.58±0.32^{a}
TAA §	94.21±3.28^{b}	97.96±2.38^{a}	99.82±0.32^{a}	99.60±0.76^{a}
EAA/TAA(%)	39.14±1.05^{b}	42.24±0.82^{a}	41.88±0.57^{a}	40.17±0.77^{b}

Note: Within rows, values (mean±SD) followed by the different letters are significantly different ($P < 0.05$). † essential amino acids, ‡ nonessential amino acids, § total amino acids

The results revealed that in female *P. leptodactylus*, the amount of threonine (8.77 %), leucine (8.53 %) and valine (7.64 %) were higher in the ovaries. Threonine concentration in ovaries was higher ($P < 0.05$) than abdominal muscle and hepatopancreas. Methionine was the highest ($P < 0.05$) amount (2.48 %) in the abdominal muscle. In contrast, there were not significant ($P > 0.05$) differences in valine, leucine, phenylalanine, histidine and arginine content among the essential amino acids between abdominal muscle, hepatopancreas and ovaries. The ovaries contained a higher content of EAA than abdominal muscle and the hepatopancreas statistically, but this difference was not-significant ($P > 0.05$) (Tab. 3). In addition, aspartic

acid and alanine were significantly higher ($P < 0.05$) in abdominal muscle compared to hepatopancreas and ovaries. Serine content in ovaries was higher than that of abdominal muscle and hepatopancreas ($P < 0.05$). Compared to the abdominal muscle and hepatopancreas the ovaries contained significantly higher levels ($P < 0.05$) of EAA/TAA (45.48 %). It was 42.14 % for hepatopancreas and 40.19 % for abdominal muscle. EAA in abdominal muscle was statistically higher in female crayfish than in male crayfish ($P < 0.01$) (Tab. 4). In the present study, it was determined that the amount of threonine, lysine and tryptophan, which are among the EAA, were significantly higher in the ovary than those of the testes ($P < 0.001$). Furthermore, it was also determined that the amount of threonine, lysine and tryptophan in the ovary was significantly higher than the amount in the vasa deferentia ($P < 0.001$ for threonine, $P < 0.01$ for lysine and $P < 0.05$ tryptophan). However, it was found that the amount of leucine, isoleucine and methionine in the vasa deferentia was significantly higher than the amount in the ovary ($P < 0.01$) (Tab. 4).

Table 3. Amino acid composition in the abdominal muscle, hepatopancreas, ovary (%, wet weight) during the reproductive season in female *Pontastacus leptodactylus.*

Amino acids (%)	Abdomen muscle	Hepatopancreas	Ovary
EAA[†]			
Valine	6.67±0.16[a]	5.68±0.49[a]	7.64±0.89[a]
Leucine	9.64±0.70[a]	8.81±0.84[a]	8.53±0.32[a]
Isoleucine	4.94±0.17[a]	4.24±0.43[b]	4.66±0.14[ab]
Threonine	5.53±0.47[b]	3.67±0.98[c]	8.77±0.38[a]
Methionine	2.48±0.30[a]	1.20±0.31[b]	1.32±0.17[b]
Phenylalanine	7.10±0.08[a]	6.32±1.97[a]	5.81±0.26[a]
Lysine	0.32±0.13[c]	6.83±0.72[a]	3.75±0.97[b]
Histidine	1.85±0.12[a]	1.52±0.34[a]	1.85±0.24[a]
Arginine	1.40±0.86[a]	0.83±0.13[a]	1.33±0.26[a]
Tryptophan	0.16±0.03[b]	1.21±0.47[a]	0.78±0.22[a]
ΣEAA[†]	40.12±0.76[a]	40.35±4.85[a]	44.47±2.01[a]
NEAA[†]			
Tyrosine	5.55±0.85[a]	5.94±1.22[a]	5.96±0.68[a]
Alanine	8.65±0.91[a]	6.65±0.97[b]	6.61±0.42[b]
Glycine	6.17±1.18[a]	5.37±1.38[a]	5.31±0.23[a]
Serine	8.44±0.79[b]	8.23±0.98[b]	10.57±0.88[a]
Proline	0.98±0.82[a]	0.67±0.26[a]	1.22±0.78[a]
Aspartic acid	18.25±0.32[a]	15.07±0.50[b]	13.42±0.41[c]
Glutamic acid	1.38±0.49[a]	1.46±0.37[a]	0.90±0.07[a]
Cysteine	10.25±0.55[a]	11.69±2.50[a]	9.25±0.25[a]
ΣNEAA[‡]	59.71±0.82[a]	55.13±1.03[b]	53.27±0.98[c]
TAA[§]	98.83±0.14[a]	97.75±2.36[a]	95.48±5.21[a]
EAA/TAA (%)	40.19±0.78[b]	42.14±2.88[b]	45.48±1.14[a]

Note: Within rows, values (mean±SD) followed by the different letters are significantly different ($P< 0.05$). [†] essential amino acids, [‡] nonessential amino acids, [§] total amino acids

Discussion

Nutrition is one of the most crucial factors affecting maturation of captive and wild crustacean broodstocks. In this study, it was shown that the amount of protein in male and female *P. leptodactylus* gonads was higher than that in the hepatopancreas. Similarly, Xu *et al.* (2014) declared that during ovarian development of *Charybdis* (*Charybdis*) *japonica* (A. Milne-Edwards, 1861), there was a significant increase in protein content in the ovaries, accompanied by a reduction in proteins in the hepatopancreas. This suggests that energy stocks are passed to the gonad from the hepatopancreas.

Table 4. Statistical comparison of amino acid concentration (%, wet weight) in abdomen muscle, hepatopancreas, ovary-testes, ovary-vasa deferentia between male and female *Pontastacus leptodactylus.*

Amino acids (% wet weight)	Abdomen muscle	Hepatopancreas	Ovary-Testes	Ovary-Vasa deferentia
Valine	**	**	ns	ns
Leucine	ns	ns	**	**
Isoleucine	ns	*	***	**
Threonine	**	ns	***	***
Methionine	ns	ns	***	**
Phenylalanine	ns	ns	**	ns
Lysine	**	**	***	**
Histidine	**	ns	ns	ns
Arginine	ns	*	ns	ns
Tryptophan	ns	*	**	*
ΣEAA[†]	**	ns	*	**
Tyrosine	ns	ns	ns	ns
Alanine	ns	ns	*	***
Glycine	ns	ns	**	***
Serine	ns	ns	*	ns
Proline	ns	*	ns	ns
Aspartic acid	**	ns	***	***
Glutamic acid	ns	ns	ns	*
Cysteine	ns	ns	ns	*
ΣNEAA[‡]	ns	ns	***	***

Level of statistical significance (ANOVA, t-test) (*P* value): ns: (*P*> 0.05); * :(*P*< 0.05); ** :(*P*< 0.01); ***:(*P*< 0.001); [†] essential amino acids, [‡] nonessential amino acids

It was reported that ovaries had more protein, fat, cholesterol and amino acids than testes in the brown crab *Cancer pagurus* Linnaeus, 1758 (see Barrento *et al.,* 2010). Non-essential amino acids (NEAA), namely glutamic acid, aspartic acid and glycine dominated the protein content in all tissues of *Ca. pagurus*. Similarly, in that study, 47 % protein in the ovary and 35 % in the testes were determined and it was found that non-essential amino acids were in higher amounts than essential amino acids compared to male and female crayfish. Aspartic acid, serine, cysteine, alanine, tyrosine were determined to be dominant in *Ca. pagurus*.

In decapod crabs the muscles are the major protein reserve sites and amino acid levels in muscles are ten times higher than those found in vertebrates (Silva-Castiglioni *et al.,* 2007). Abdel–Salam (2014) determined sixteen amino acids (nine EAAs and seven NEAAs) in the muscles of some crustaceans (*Erugosquilla massavensis* (Kossmann, 1880); *Portunus* (*Portunus*) *pelagicus* (Linnaeus, 1758); *Penaeus semisulcatus* De Haan, 1844; *Metapenaeus monoceros* (Fabricius, 1798); and *Penaeus indicus* H. Milne Edwards, 1837)). Whereas in the present study, ten EAAs and eight NEAAs were observed in the amino acid analysis of *P. leptodactylus* muscle.

It was found that among the 17 amino acids in female mud crab, *Scylla paramamosain* Estampador, 1950, glutamate had the highest concentration in the ovarian tissues (> 49 mg/g), in the hepatopancreas (> 20 mg/g) and in the muscle tissues (> 88 mg/g) throughout all ovarian maturation stages (Wu *et al.,* 2020). However, in the present study, leucine was the most dominant amino acid in the abdominal muscle, hepatopancreas and gonads, in both male and female crayfish. Similarly, leucine was found to be the most dominant amino acid in the female marine crab, *Ch.* (*C.*) *japonica* by Xu *et al.* (2014).

Amino acids are the formative molecules of proteins. Besides their function in protein synthesis amino acids are also used to create a range of cell structures that are essential elements and serve as

an energy source (Bhavan *et al.*, 2010). In this study, high levels of EAA such as valine, leucine, isoleucine, threonine and phenylalanine were found in male and female gonads. Leucine is an amino acid that produces ketones. Soundarapandian *et al.* (2014) reported that leucine, isoleucine and valine support healing of the muscle tissue, skin and bones and is recommended for people recovering from surgery, lowering blood sugar levels and helping to increase growth hormone production. In the present study, lysine was 3.75 % in female crayfish ovary, 0.78 % and 1.06 % in the male testes and the vasa deferentia, respectively. On the other hand, tryptophan was 0.78 % in female crayfish ovary and 0.31 % and 0.44 % in the male testes and vasa deferentia, respectively. Similarly, Soundarapandian *et al.* (2014) noted that in *Charybdis* (*Charybdis*) *natator* (Herbst, 1794), lysine and tryptophan were higher in berried females than males. Bhavan *et al.* (2010) found that because tryptophan is the precursor for the neurotransmitter serotonin, it is important in brain function and metabolism, which has a great influence on the feeding behavior of the prawn *Macrobrachium rosenbergii* (De Man, 1879).

Threonine helps maintain the proper protein balance in the human body. It is important for the formation of collagen, elastin and tooth enamel, helps liver and lipotropic function when combined with aspartic acid and methionine and prevents fat accumulation in the liver. In general, it helps with metabolism and assimilation. It has already been reported in various crabs and shrimps (Soundarapandian *et al.,* 2014). In the present study, in female crayfish, the amount of threonine in the ovaries was statistically higher than that of abdominal muscle and hepatopancreas. Similarly, in male crayfish, it was statistically higher in the vasa deferentia and testes than abdominal muscle and hepatopancreas ($P < 0.05$). Xu *et al.* (2014) investigated the amino acid profiles of the ovaries and hepatopancreas of the female marine crab, *Ch.* (*C.*) *japonica*. They found that the amount of threonine in the ovary in the mature period was significantly higher than the immature and spawning periods ($P < 0.05$). In a different study on amino acid profiles of the ridged swimming crab *Ch.* (*C.*) *natator*, Soundarapandian *et al.* (2014) observed that threonine was maximized in berried females. These results support the established idea that threonine is important in reproduction for ovary, vasa deferentia and testes development in crayfish and crabs. Vitellogenin can be synthesized both in the hepatopancreas and ovaries, and this location varies between crustacean species. Harrison (1990) stated that variations in the dietary amino acid demands of the broodstock of various species can be understood from the amino acid profiles of lipovitellin. During vitellogenesis the significant actions of proteins in growth and maturation are mediated in the alteration of the proximate structure of the ovaries and hepatopancreas.

In crayfish, the hepatopancreas is the largest gland. It has a significant role for the absorption and storage of nutrients and can synthesize digestive enzymes for food digestion. The stored nutrients in the hepatopancreas are transported to muscles, gonads and other tissues during the growth and reproductive stages (Sousa and Petriella, 2000; Reppond *et al.,* 2009; McGaw and Curtis, 2013). The present study shows that the amount of lysine in the hepatopancreas in male and female crayfish was statistically higher ($P < 0.05$) than the other tissues. Furthermore, Soundarapandian *et al.* (2014) found that lysine was greatest in the edible parts of muscle tissues in berried females of *Ch.* (*C.*) *natator.*

Similar to threonine, lysine was also reported in various crabs and shrimps. Lysine has an important role in protein structure. It is envolved in epigenetic regulation by means of histone modification (Dambacher *et al.,* 2010). Lysine has also been noted to play a key role in other biological processes including; structural proteins of connective tissues, calcium homeostasis, and fatty acid metabolism (Shoulders *et al.*, 2009). In addition to these, lysine has been shown to be a precursor for carnitine, which transports fatty acids to the mitochondria, where they can be oxidized for the release of energy (Flanagan *et al.*, 2010). Therefore, the findings of the present study and of Soundarapandian *et al.* (2014) reveal that lysine is related to energy production and transfer in crabs and crayfish during their reproductive period.

The biochemical constituents in the muscle of mature male and female *M. rosenbergii* captured from natural environments were investigated by Bhavan *et al.* (2010). They found that male prawns had lower

essential amino acids and total protein than females and suggested that changes in muscle content between male and female *M. rosenbergii* reflect variation in gender growth and energy demands for body care in the mature phase. Similarly, in our research, total essential amino acids were determined to be higher in the abdominal muscle of females compared to that of males (40.12 % female versus 36.85 % male). On the other hand, the proportion of total protein in the abdominal muscle between male and female crayfish was found to be at the same approximate level (40.11 mg/g in males and 40.19 mg/g in females).

In the spawning stage of the female marine crab *Ch.* (*C.*) *japonica,* the content of EAA/TAA (%) in ovaries and hepatopancreas were 42.78 % and 40.62 %, respectively (Xu *et al.,* 2014). Similarly, we also found that the content of EAA/TAA (%) in the ovaries and hepatopancreas was 45.48 % and 42.14 %, respectively.

There is no information in the literature on the differences in the amino acid profile in the vasa deferentia and testes of crayfish to compare with the findings of this study. However, the proteomic profiling of the signal crayfish *Pacifastacus leniusculus* (Dana, 1852) spermatophore was investigated by Niksirat *et al.* (2014). They identified and compared the gamete proteins in male and female *P. leniusculus* by using in-gel digestion, mass spectrometry, and Mascot search. They identified 150 proteins in the spermatophore. The proteins were divided into nine categories including cell defence, cell signaling and cytoskeleton. Moreover, Niksirat *et al.* (2014) stated that several proteins having possible roles in gametogenesis, capacitation, acrosome reaction, and fertilization were identified. In addition to Niksirat *et al.* (2014), in a study on the protein modification in the post-mating spermatophore of *P. leniusculus,* Niksirat *et al.* (2016) stored the spermatophores of crayfish on the body of the female after mating for a period before fertilization. They then compared the post-mating protein profile and pattern of protein tyrosine phosphorylation of *P. leniusculus* spermatophore to that of the freshly ejaculated spermatophore and found substantial differences. Niksirat *et al.* (2014) found that the male gamete of *P. leniusculus* undergoes molecular modification during post-mating storage on the body of the female including changes in the level of protein expression and protein tyrosine phosphorylation.

This study presents data on the male and female crayfish *P. leptodactylus* in relation to the protein and amino acid profiles of abdominal muscle, hepatopancreas and gonad in the reproductive season. The results of this study are important in determining the amino acid and protein needs in the diet preparation for male and female broodstocks of *P. leptodactylus* because there is a remarkable correlation between dietary amino acid demands of a species and amino acid forms in all body tissue.

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