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Profiles of amino acid, fatty acid and nucleic acid in the edible muscles of important commercially crustaceans from Saudi Arabia

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Abstract: The edible crustaceans; shrimps (*Penaeus semisulcatus*) and crabs (*Portunus pelagicus*) are valued commercial crustacean species in Saudi Arabia that are considered important components of the aquatic fauna in many areas of the world. Yet, they consumed by the local population as seafood products without knowing their nutritional quality. So, the present study quantified the concentration of amino acids, fatty acids and nucleic acids to evaluate the nutritive quality of these crustacean species which may be in the future used as nutritional supplements. The results of this study revealed that edible muscles of these marine crustaceans contain nutrients for human good health to prevent nutritional deficiencies and may be used in some pharmaceutical industries to help in treatment of some diseases. Considering the results obtained in the present study in light of nutritional point of view, male and female crabs (*Portunus pelagicus*) are possessing

relatively high amounts of essential, non-essential amino acids, saturated, unsaturated fatty acids, RNA and DNA constituents and highly nutritious compared to both sexes of shrimp species. However, persons who suffer from coronary disease should be careful about the consumption of crab due to its high saturated fatty acids content.

Key words: Edible crustaceans, Amino acids, Fatty acids, Nucleic acids, Nutritive quality

INTRODUCTION

Edible crustaceans' species such as shrimps and crabs are considered major sources of nutritional products for human being and form one of the key points of food chain cycle¹. Shrimps and crabs are considered popular dietary components of world dishes, apart from their delicacy, their edible muscles consist of useful nutrients such as amino acids, fatty acids, protein, lipid, carbohydrate, vitamins and minerals². These biochemical components play an important role in health, growth, development and maintenance of physiological and biochemical activities in human beings³.

Amino acids are one of the most prominent biochemical component of marine crustacean species. Edible muscle of shrimp and crab crustaceans contains high amount of amino acids which are considered main building unit of protein and serve as body builders. The protein quality in edible aquatic organisms is evaluated by estimating the contents of amino acids. Amino acids are represented as source of energy, besides lipids⁴. Furthermore, they play several important role in the biological process of living organism such as regulation of intracellular osmoregulation process⁵, also some amino acids play an important role in the brain as a precursor of neurotransmitters component and involved in nerve conduction as reported previously by Das et al.(2003)⁶. Additionally, they assist minerals and vitamins to do their biological function⁷. In general, each amino acid either essential or non-essential has its own physiological role once it is consumed.

Another important biochemical components in muscle tissues of shrimp and crab species are represented by fatty acids. Fatty acids are the main components of lipids. Generally, fatty acids play an important role in biological process of human body, such as free fatty acids can be oxidized in the liver and muscle to provide energy⁸. Additionally, they are considered structural components of lipid membrane. They are attached to certain intracellular proteins to enhance in turn the ability of these proteins to associate with membranes. Moreover, fatty acids are represented as precursors of some hormones such as prostaglandin which is essential for reproduction and vitellogenesis⁹. They can be used in living organism in defense mechanism against microbial infections and protozoan infection as recorded by Sudhakar et al⁸. (2009). Long-chain fatty acids can be classified into n-3 fatty acids and n-6 polyunsaturated fatty acids. These biochemical components are essential for marine organisms particularly crustaceans^{6,9}. Furthermore, they are beneficial to human health due to their anti-inflammatory properties and their effect to decrease the risk of hypotriglyceridemia, cardiovascular disease, and increase HDL cholesterol^{10,11}. Also, polyunsaturated fatty acids with other nutrients such as vitamins (A, D, E, C) and selenium, zinc and iron affect the activity of the immune system¹². Whereas, saturated fatty acids (C12-C16) increase total and LDL-cholesterol concentrations that has a causal role in the development of cardiovascular disease¹³.

Regarding, nucleic acids; deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) as another important biochemical contents in edible muscle of shrimps and crabs. Nucleic acids are biopolymers consist of

nucleotides. These large biomolecules are essential for all known forms of life. The nucleic acids are found in abundance in all living organisms, where they play an important role in encoding, transmitting and expressing genetic information¹⁴. Furthermore, the estimation of nucleic acids and RNA / DNA ratio of marine organisms are used as indicators of nutritional condition, growth rate, starvation indices, trophic interactions in marine environment and biodiversity changes¹⁵. Besides, RNA/ DNA ratios were used as measurement tool to evaluate the response of marine organisms to climate change as well as for predictions of the effects of anthropogenic activities on marine organisms. Since RNA/ DNA ratio is eco-physiological index of growth rate, secretion, reproduction mechanism, trophic interaction under the environmental conditions¹⁶.

With this background, in the present study an attempt has been made to evaluate and compare the proximate composition (amino acids, fatty acids and nucleic acids) of the edible muscle tissues of the two major commercially important crustaceans species of shrimps (*Penaeus semisulcatus*) and crabs (*Portunus pelagicus*) along Saudi Arabia, Arabian Gulf coasts to evaluate their nutritive quality as well as, to publish as completely as possible information on their nutrients that may play vital role in some pharmaceutical industries and in turn, these marine crustaceans in the future can be used as specific health foods (dietary supplements).

MATERIAL AND METHODS

Collection of Samples: Freshly male and female samples of shrimp species *Penaeus semisulcatus* and blue swimmer crab species *Portunus pelagicus* were captured from the fishermen from Majmaah markets. An attempt was made to collect consistent size ranges of male and female sample for each species. All samples were put in containers containing crushed ice and transported to the laboratory. Samples were washed with distilled water to remove any adhering contamination and drained using filter paper. The male and female samples were segregated. The samples were separated into the external shells and edible muscle portions.

Biochemical Analysis

1. **Quantitative determination of amino acids:** Essential and non-essential amino acids were measured by high performance liquid analyzer¹⁷.
2. **Quantitative determination of fatty acids:** Fatty acid analysis of shrimp and crab muscle tissues were conducted using the direct methyl-esterification method¹⁸.
3. **Quantitative determination of nucleic acids:** The DNA and RNA in the muscle tissues were analysed using the techniques described by Clemmensen¹⁹ (1993). The concentration of nucleic acids was measured through fluorimetry using a spectrofluorometer with an excitation wavelength of 365 nm and an emission wavelength of 590 nm.

Statistical Analysis: The obtained data were used for descriptive statistical analysis consisting of means \pm standard deviation of 5 separated males and 5 females in each test of shrimp and crab species. The data obtained was subjected to statistical analysis in SPSS. In order to test the significance of the differences among the mean values of the present studied species one-way ANOVA test was applied.

RESULTS

(1) Essential amino acids (EAAS) analysis: As shown in **Fig 1**, analysis of edible muscles of both sexes of shrimp and crab species indicated the presence of 10 essential amino acids (EAAS) (arginine, histidine, methionine, leucine, isoleucine, lysine, threonine, valine, phenylalanine and tyryptophan). The most promient EAAS in all studied samples were leucine (7.48 ± 0.39 , 7.54 ± 0.40 mg/100g and 7.42 ± 0.36 , 7.28 ± 0.41 mg/100g for male and female crabs and shrimps respectively) and arginine (7.38 ± 0.38 , 7.16 ± 0.66 mg/100g and 6.60 ± 0.25 , 6.68 ± 0.37 mg/100g for male and female crab and shrimp respectively) comporable with other EAAS. Also, in crab species, lysine had higher level than other EAAS (7.50 ± 0.35 and 7.54 ± 0.27 mg/100 g for male and female respectively).

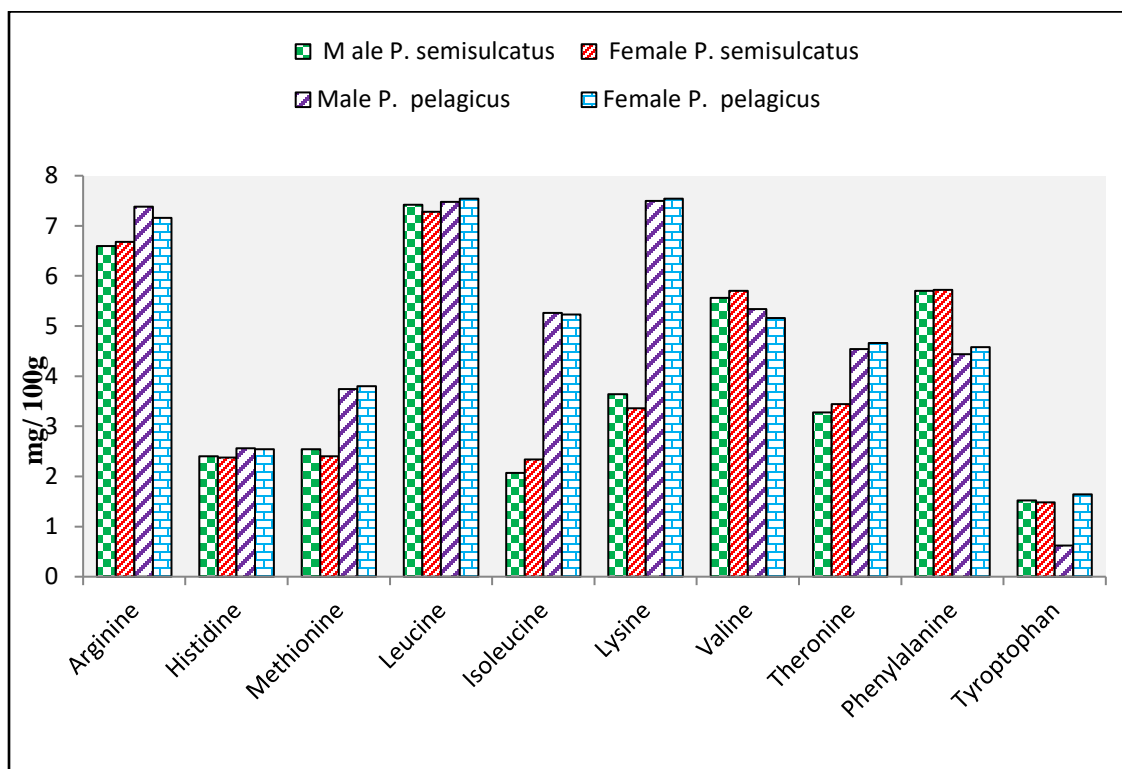


Figure 1: Essential amino acids concentrations (mg/100g) in edible muscles of male and female of *P. semisulcatus* and *P. pelagicus*

Moreover, the recorded data showed that the concentration of all EAAS in crab species were higher than shrimp species except phenylalanine. Statistically significant differences ($p < 0.001$) in the contents of methionine, isoleucine, lysine, theronine and phenylalanine between species of shrimps and crabs were recorded. Whereas, non- significant variations ($P > 0.05$) were observed between males and females of each species. Additionally, the present data illustrated that the contents of EAAS were recorded according to the following order in males *P. semisulcatus*: leucine > arginine > phenylalanine > valine > lysine > threonine > methionine > histidine > isoleucine > tryptophan. While their females showed this order: leucine > arginine > phenylalanine > valine > threonine > lysine > methionine > histidine >

isoleucine > tryptophan. As regarding to crab species these following descending arrangements were detected in males *P. pelagicus* : leucine > lysine > arginine > valine > isoleucine > threonine > phenylalanine > methionine > histidine > tyroptophan. whereas, in female *P. pelagicus* : leucine and lysine > arginine > isoleucine > valine > threonine > phenylalanine > methionine > histidine > tyroptophan was detected.

(2) Nonessential amino acids (EAAS) analysis: Regarding with NEAAS, edible muscle tissues of all studied samples contained five NEAAS (glycine, proline, serine, cysteine, and alanine). (**Fig. 2**). It was observed that the highest average concentrations of proline were recorded in edible muscles of male and female shrimp (4.14 ± 0.60 and 4.30 ± 0.32 mg/ 100g respectively) and both sexes of crab species (5.82 ± 0.44 and 5.86 ± 0.48 mg/ 100g in male and female respectively) compared to other NEAAS. Statically, non-significant variations was recorded in the levels of NEAAS between both sexes of each species of shrimps and crabs. Whereas, significant differences in proline and serine concentrations was observed among studied crustaceans species ($p < 0.0001$). Furthermore, the arrangement of NEAAS was recorded according to the following orders; in male *P. semisulcatus*: proline > serine > cysteine > alanine > glycine. Whereas in females: proline > serine > cysteine > glycine > alanine. In crab species, both sexes had this descending order of NEAAS: proline > alanine > cysteine > glycine > serine.

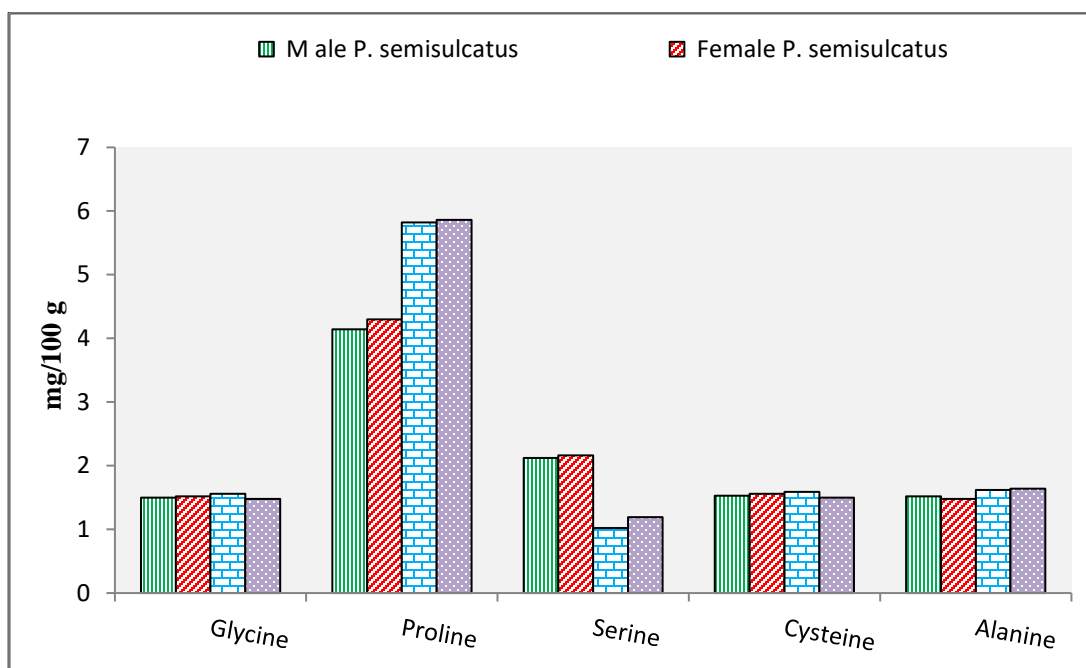


Figure. 2: Non-essential amino acids (NEAAS) concentrations (mg/100g) in edible muscles of male and female of *P. semisulcatus* and *P. pelagicus*

(3) Total amino acids and EAAS/ NEAAS ratio: To compare the concentration of amino acids in edible muscle tissues between studied crustacean species, the ratio of EAAS/ NEAAS was estimated. The present data declared that the contents of EAAS in edible muscles of all studied samples showed an increase than NEAAS (**Fig. 3**). It was recorded that the edible muscles of male and female crabs had

maximum values of total EAAS, NEAAS and EAAS/ NEAAS ratio comparable with shrimps, where significant difference ($p < 0.05$) in EAAS was detected.

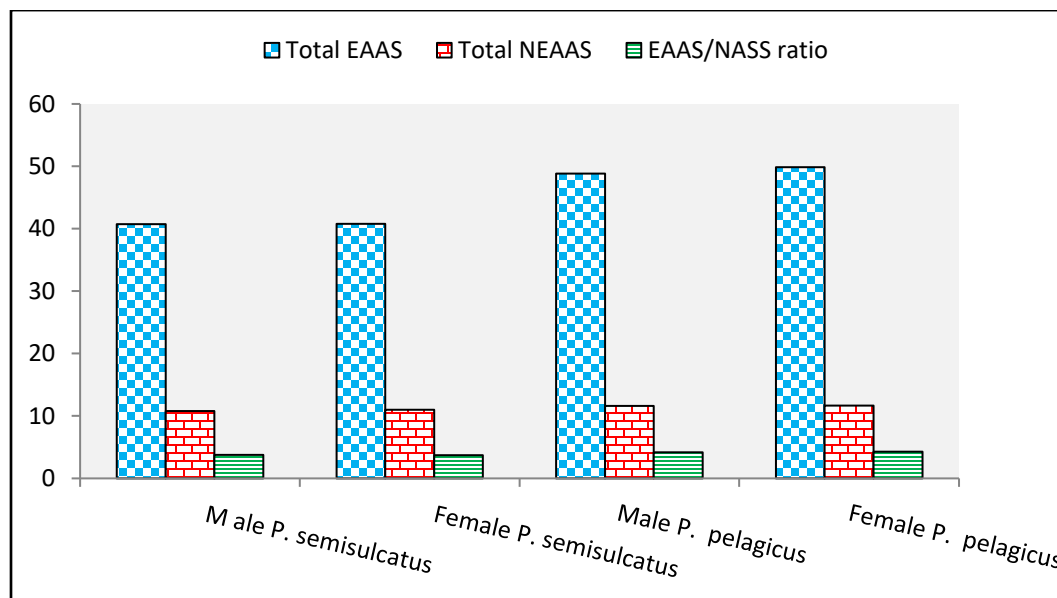


Figure. 3: Total amino acids level (mg/100g) and EAAS/NEAAS ratio in in edible muscles of male and female of *P. semisulcatus* and *P. pelagicus*.

(4) Fatty acids analysis

4.1 Saturated fatty acids analysis: The present results in **Fig. 4** detected that the edible muscle tissues of male and female *P. semisulcatus* and *P. pelagicus* contained four saturated fatty acids (lauric acid, myristic acid, palmitic acid and stearic acid). Both sexes of crabs had higher levels of saturated fatty acids compared to that of males and females of shrimps. Statistically, this increase was significant ($p < 0.0001$). On the other hand no significant differences ($p > 0.05$) were detected between sexes of each species. The concentrations of saturated fatty acids had this decreasing order: in male shrimps; stearic acid > myristic acid > lauric acid > palmitic acid and in their females stearic acid > myristic acid > palmitic acid > lauric acid. While, in both sexes of crab species, this order is recorded: palmitic acid > lauric acid > stearic acid > myristic acid.

4.2 Unsaturated fatty acids analysis: As shown in **Fig. 5**, the edible portions of all studied samples had five unsaturated fatty acids (two monounsaturated fatty acids; palmitoleic and oleic acids and three polyunsaturated fatty acids; linoleic, linolenic and arachidonic acids). As in saturated fatty acids non-significant variations in unsaturated fatty acids level were recorded between males and females of each species. However, the edible muscles of both sexes of crab species had significant increase ($p < 0.0001$) with respect to concentrations of all determined unsaturated fatty acids except linolenic acid i.e., male and female crabs had lower level of linolenic acid (0.22 ± 0.01 and 0.120 ± 0.01 mg /100 g for males and females respectively) comparable to shrimp samples (0.28 ± 0.14 and 0.28 ± 0.17 mg/100 g for males and females respectively), this decrease is significant ($P < 0.05$). Moreover, these following descending orders were recoded; in both sexes of shrimp species: arachidonic acid > palmitoleic acid > oleic acid > linoleic acid > linolenic acid, whereas in muscles of male and female crabs this order is observed:

arachidonic acid > oleic acid > palmitoleic acid > linoleic acid > linolenic acid and oleic acid > arachidonic acid > palmitoleic acid > linoleic acid > linolenic acid respectively. To compare the fatty acid contents in muscle portions in studied species, it was observed that all crab samples had significant elevation ($p < 0.001$) in total saturated and unsaturated fatty acids (**Fig. 4 & Fig. 5**).

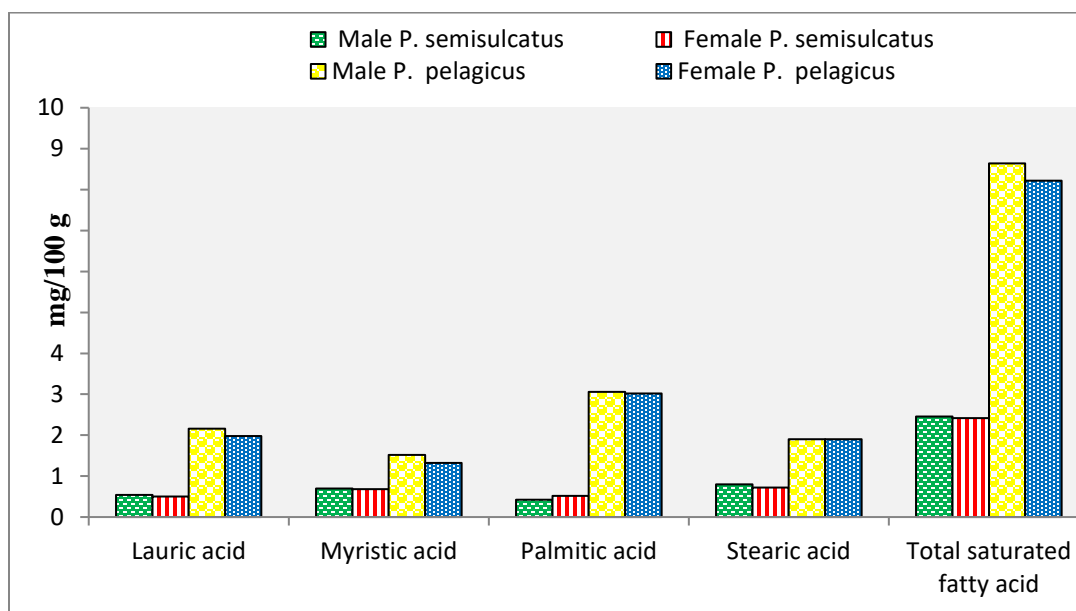


Figure. 4: Saturated fatty acids concentration (mg/100g) in in edible muscles of male and female of *P. semisulcatus* and *P. pelagicus*.

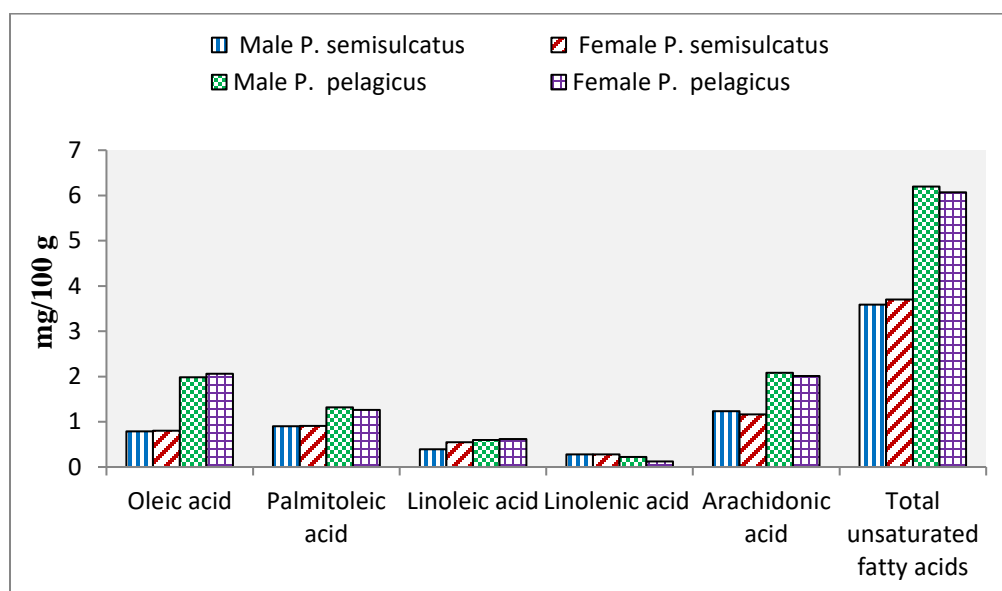


Figure 5: Unsaturated fatty acids concentration (mg/100g) in in edible muscles of male and female of *P. semisulcatus* and *P. pelagicus*.

5. Nucleic acid analysis: The estimation of DNA and RNA (**Fig. 6**) illustrated that both sexes of shrimp and crab species contained higher amount of RNA as comparable with DNA. Furthermore, it was observed that all crab samples had high levels of DNA and RNA than shrimp samples ($P < 0.0001$). Furthermore, in each species non-significant difference in DNA level between males and females was recorded. Whereas, females of each shrimp and crab species had higher RNA levels than their males. Statically, this increase is significant ($p < 0.05$).

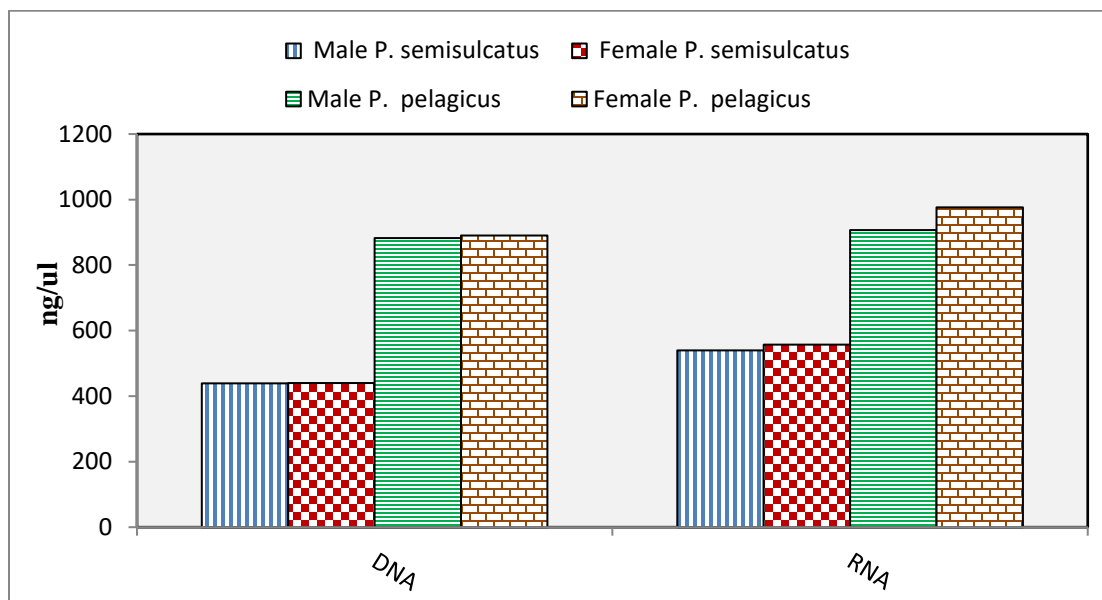


Figure. 6: Deoxy-ribonucleic acid (DNA) and Ribonucleic acid (RNA) concentration (ng/ul) in edible muscles of male and female of *P. semisulcatus* and *P. pelagicus*.

DISCUSSION

The analysis of proximate composition of edible muscles of both sexes of shrimp and crab samples in the present study detected the presence of 10 EAAS and 5 NEAAS. Furthermore, the recorded data showed that both male and female crabs species had higher EAAS level than both sexes of shrimp species where significant increase was recorded. So, both sexes of crabs are declared superior over both sexes of shrimps in terms of nutritive value. Additionally, the present results are supported the findings of the previous results which indicated that the edible muscle tissues of crustaceans species contain considerable amounts of the amino acids, such as cysteine, histidine, arginine, leucine, phenylalanine, proline, serine, tyrosine etc^{7,20,21,22}. In recent years, studies have shown that the marine crustaceans can be used as antioxidant agents that play a critical role in body protection by scavenging active oxygen and free radicals and neutralizing lipid peroxides as reported by (Maitraie et al., 2009)²³ because they contain a variety of substances having antioxidant activity including thiol-containing amino acids like methionine and cysteine which reduce oxidative stress and replenish the levels of antioxidants. Also, methionine and cysteine had stimulative activities on liver function and enzymes²⁴. Additionally, the edible muscles of

shrimps and crabs contained tyroptophan which is an essential amino acid that is needed to maintain optimum health and it is also represented the nucleus of the important neurotransmitter serotonin. Tryptophan is required for the production of niacin (vitamin B3)²⁵. Furthermore, the ratios of EAAS / NEAAS in the present study ranged from 3.70 to 4.27 (in female shrimps and crabs respectively). This ratio is greater than which was recorded by other studies in marine crustacean organisms^{7, 26,27}. On this background, the muscles of both sexes of these crustaceans species in the present study may in the future play an important role in some pharmaceutical industries and may be used as a specific functional supplements.

The fatty acid profile of muscle in both sexes of shrimp and crab species in the present study indicated the the presence of four non-essential (saturated) fatty acids and five essential (unsaturated) fatty acids. Generally, saturated fatty acids increases low density lipoprotein (LDL) cholesterol concentration which in turn increasing cardiovascular disease (CVD) risk. On the other hand, unsaturated fatty acids result in positive reduction of blood pressure via reduction of total and LDL cholesterol²⁷. The essential fatty acids cannot be produced within the human body, so they must be obtained through diet due to they are necessary for human health. According to the current study shrimps are partially useful in prevention of CVD than crabs due to although crabs had higher unsaturated fatty acids than shrimps, but their total concentrations of unsaturated fatty acids were lower comparable to their total levels of saturated fatty acids. Furthermore, crabs contained less amount of linolenic acid ($p < 0.05$) than shrimps. linolenic acid (Omega 3) is the most important polyunsaturated fatty acid and is very important in human diet. In human body, linolenic acid can convert to eicosapentaenoic acid (an omega-3 polyunsaturated fatty acid)²⁹. Furthermore, linolenic acid and linoleic acid (Omega 6) that play important functions for man health such as lower the amount cholesterol and triglycerides, reduce inflammation, reduce the production of cytokines which involved in the inflammatory response associated with atherosclerosis, inhibit thickening of the arteries, stimulate the secretion of leptin; a hormone that helps regulate food intake and improves the body's ability to respond to insulin and help to prevent cancer cell growth²⁴. Recommendations of American heart association and National institute for health and clinical excellence showed that seafood consumption is very useful for myocard infarction following patients^{30,31}. High unsaturated fatty acids can develop nervous system and play a key role in prevention of CVD³².

The analysis of DNA and RNA in edible muscles of shrimp and crab species in the present study declared that edible muscle tissues of both male and female crabs had higher contents of DNA and RNA than that of both sexes of shrimps. Also, in all studied samples, amount of DNA was found to be almost equal, whereas all females had higher levels of RNA in their muscles comparable with their males, where significant increase was recorded. Several authors reported that, the DNA content assumed to be constant in normal somatic cells of crustacean species^{4, 33, 34}. Regarding, the elevation of RNA contents in the present study, this finding is in agreement with results of previous studies^{4,33, 34,33} in different crustaceans species. The elevation of RNA content might be attributed to the activity of protein synthesis and better growth³⁴, due to the level of RNA in any cell reflects the cell involvement in protein synthesis and protein production³⁴. Thus the recorded data in the present study suggest that the protein synthesis in female crustaceans is more pronounced compared to their males. Furthermore, the variation in RNA muscle constituents between male and female crustaceans reflects the differences in sexual development and their energy requirements to maintain the physiology of the body during the adult stage⁴. The variations in DNA and RNA levels in shrimps and crab species might be attributed to food availability, or other physical factors.

CONCLUSION

The present study was carried out as an attempt to provide holistic nutritional evaluation of amino acids, fatty acids and nucleic acids in two crustacean species shrimps (*Penaeus semisulcatus*) and crabs (*Portunus pelagicus*). The present results indicated that shrimps and crabs used in this study could be employed as an alternative dietary supplement or may be used in some pharmaceutical industries due to their edible muscles contained 10 EAAS, 5 NEAAS, 4 saturated fatty acids and 5 unsaturated fatty acids. Furthermore, edible portions of both sexes of studied crustaceans contained higher RNA levels than DNA concentrations. The male and female crabs are declared superior over both sexes of shrimps in terms of nutritive value. Whereas, shrimps are partially useful in prevention of CVD than crabs due to their muscles contained less level of saturated fatty acids. Hence, the results of the present study opens the door to carry out further studies on the mode of action, the antioxidant properties and characterization of the active components of marine crustaceans.

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