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Biochemical Transition Effects of Fungi Fouling Disease in *Penaeus Monodon* Shrimp

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A B S T R A C T

Penaeus monodon (black tiger shrimp) is one of the most cultured shrimp species in India. Biochemical compositions of such shrimp species play a major role in recent decades. The biochemical composition is an index to assess the nutritional quality of food sources. The present work endeavor is to analyze total protein, carbohydrates, total lipids, amino acids, fatty acids, vitamins and minerals from muscle tissues of both *Gilbertella persicaria* fungal fouling infected and healthy shrimp, *P. monodon*. The study animal was captured by cast net operation from cultured grow-out shrimp pond located in Tamil Nadu. The study revealed maximum amounts of nutritional status was observed in healthy shrimp *P. monodon* (total protein 49.15%, carbohydrate 16.53%, total lipid 2.37%) tissues compared with *G. persicaria* fungal fouling infected shrimp (total protein 31.15%, carbohydrate 11.05%, total lipid 1.04%) were recorded. These results indicate novel report on nutritional aspect of fungal fouling pathogen *G. persicaria* which has significantly contributed in biochemical composition reduction of *P. monodon*.

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INTRODUCTION

Sea foods products encompass as important sources of nutrients in the human diet which are simply digestible because of the less connective tissue (Yanar and Çelik, 2006). Apart from their delicacy, crustaceans such as shrimps, crabs and lobsters clinch high amount of proteins, lipids and vitamins with wide assortment of minerals without much fat, cholesterol, sodium, metals and low concentration of carbohydrates (González-Félix *et al.*, 2002). Generally, people who consume seafood routinely in their diet are less likely to develop heart diseases, diabetes, arthritis, bronchial, asthma and psoriasis (Chiu and Ni, 2014).

Compared to all other sea foods, shrimps are one among delicious seafood in the world. More than 50 countries have developed shrimp farming, India is one among those leading countries which harvest and export shrimps (Puga-lopez *et al.*, 2013). India by virtue of its 7000 kms long coastline has tremendous potential in terms of marine foods resources (Vivekanandan *et al.*, 2005). (Shalini, 2013) reported that healthy shrimps are nutritionally rich than shrimps infected with white spot syndrome virus (WSSV). In Year 2012-2013 many fungal fouling *P. monodon* shrimps predominantly infected by phytopathogenic fungi *G. persicaria* were found in grow out farms at Vellapallam coastal areas of Tamil Nadu. It was first time novel report of phytopathogenic fungi *G. persicaria* infection on *P. monodon* (Karthikeyan and Gopalakrishnan, 2014).

Nevertheless, this *P. monodon* being consumed in all countries, no substantial studies have been reported so far in comparison of nutritive values between infected and healthy shrimps. Hence, the present study was aimed to compare the proximate composition of phytopathogenic fungi *G. persicaria* infected and healthy shrimps, *P. monodon* through estimation of major biochemical components such as total protein, carbohydrate, total lipid, vitamins and minerals in the whole body tissue.

MATERIALS AND METHODS

Study Area

Vellapallam (Lat.10^{0}30'55.65"N; Long.79^{0}50'37.04"E) is a coastal area located at Nagapattinam district of Tamil Nadu, India. A total of 133 shrimp grow out farms were in working form at this area between Year 2012 and 2013.

Sample Collection

The shrimps, *P. monodon* weighing of about 17 - 30 g were sampled monthly between January 2012 and March 2013 by cast net operation. The fungal fouling infected (Figures 1) and healthy *P. monodon* were collected from grow-out pond and brought to the laboratory at 4 ^{o}C for further studies. The shrimp's carapace was removed, and tissues were dried at 50 ^{o}C in hot air oven for 24 hrs. Further, the dried meat was powdered and used for estimation of total protein, carbohydrate, total lipid, amino acids, fatty acids, vitamins and minerals.

Estimation of protein

The crude protein amount mg/g in the tissue was estimated by kjeldahl method (AOAC, 1997).

Estimation of carbohydrate

The total carbohydrate amount was estimated by (DuBois *et al.*, 1956) method using Phenol - Sulphuric acid.

Estimation of total lipid

The total lipid content was estimated by (Floch, 1956) method using chloroform-methanol extraction procedure.

Estimation of amino acids

For amino acid analysis, the tissue samples were finely powdered and estimated through HPLC (Merck, Hitachi L-7400) by adopting the method of (Baker and Han, 1994).

Fatty acid analysis

For fatty acids analysis, samples were homogenized with chloroform: methanol (2:1 v/v) mixture and were extracted using (Bligh and Dyer, 1959) method. After extraction, the fats were esterified with 1% H_2SO_4 and fatty acid methyl esters. The identification and quantification of fatty acids was done using Gas chromatography (Hewlett Packard 5890 model).

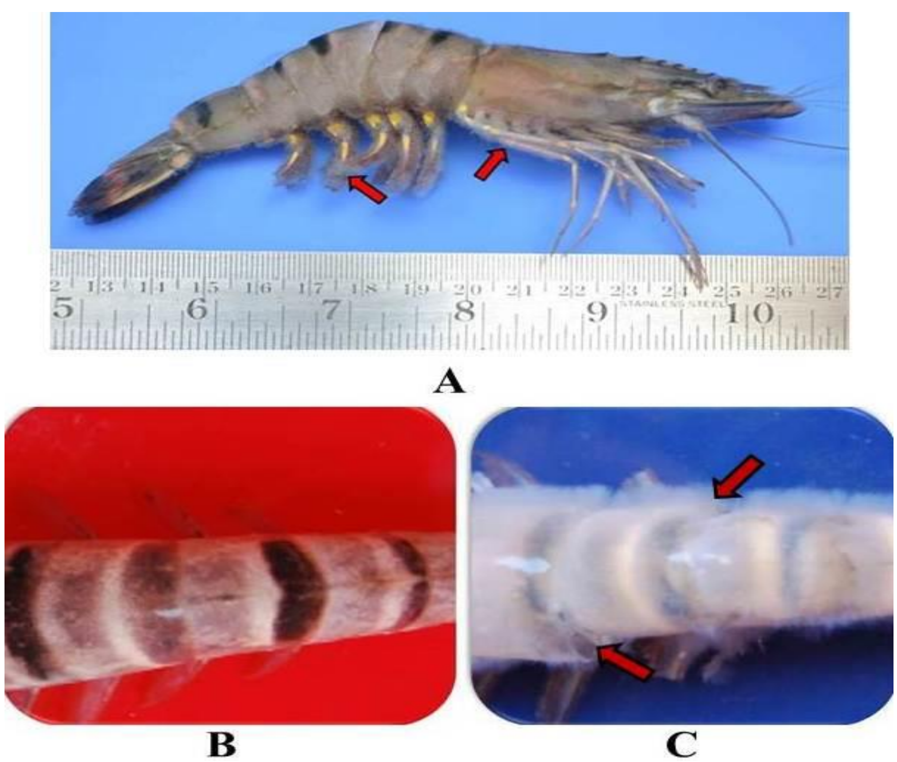

Figure 1. (A)Photograph reveals that the fungal fouled on *P. monodon* shrimp (B) Close up view of healthy *P. monodon* shrimp (C) Close up view of fungal fouled on *P. monodon* shrimp.

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Estimation of vitamins

The fat-soluble vitamins such as A, D, E and K and water-soluble vitamins namely B1, B2, B6, B12 and C were analyzed through HPLC (Merk Hitachi L-74000) method explained by (Sadasivam, 1996). The folic acid content was estimated by calorimetric method of (Sethi, 1997). Further the pyridoxine, panthothenic acid and vitamin B12 were estimated by USP NF 2000 Asian edition (Srilatha *et al.*, 2013) methods.

Estimation of Minerals

The minerals were estimated by (Guzmán and Jiménez, 1992) method.

RESULTS

In present study the proximate composition of tissues of both healthy and fungal infected shrimps were examined. As a result, the protein contents were found to be higher (49.15 and 31.15%) followed by carbohydrate (16.53 and 11.5%) and lipid (2.37 and 1.04%) in healthy and infected shrimps, respectively. The percentage composition of essential and non - essential amino acids in healthy shrimp tissues were presented in Table 1).

Table 1. Essential and non-essential amino acid of the tissue of healthy shrimp.

EAA	% of amino acids	NEAA	% of amino acids
Phenylalanine	6.91	Glycine	3.86
Lysine	3.24	Serine	8.65
Histidine	7.13	Glutamic acid	6.03
Methionine	4.06	Cystine	2.45
Leucine	14.18	Glutamine	4.68
Threonine	2.96	Alanine	0.74
Isoleucine	6.32	Proline	1.94
Valine	4.18	Asparagine	11.03
Tryptophan	3.84	Tyrosine	2.96
-	-	Aspartic acid	0.24
-	-	Arginine	2.42

The results revealed that the total essential and non-essential amino acids in the healthy shrimp healthy tissues were about 55.84 and 45.0%, respectively on dry matter basis. Similarly, the percentage composition of essential and non-essential amino acids of infected shrimp was found to be about 32.21 and 52.24 %, respectively (Table 2).

Among the essential amino acids, methionine (4.06 and 0.00%), valine (4.18 and 0.31%), phenylalanine (6.91 and 5.36%), tryptophan (3.84 and 0.08%), leucine (14.18 and 2.16 %) and lysine (3.24 and 3.15%) composition were found to be lower in infected shrimps compared to healthy shrimps. Some essential amino acids such as histidine (8.46 and 7.13%), isoleucine (9.26 and 6.32%), and threonine (3.45 and 2.96%) composition were increased considerably in infected shrimps compared to healthy shrimps respectively.

Table 2. Essential and non-essential amino acid in the tissue of infected shrimp.

EAA	% of amino acids	NEAA	% of amino acids
Phenylalanine	5.36	Glycine	7.54
Lysine	3.15	Serine	2.18
Histidine	8.46	Glutamic acid	12.54
Methionine	0.00	Cystine	6.13
Leucine	2.16	Glutamine	3.62
Threonine	3.43	Alanine	2.18
Isoleucine	9.26	Proline	6.13
Valine	0.31	Asparagine	5.51
Tryptophan	0.08	Tyrosine	1.51
-	-	Aspartic acid	0.39
-	-	Arginine	4.51

The non - essential amino acids such as serine (8.64 and 2.18%) glutamine (4.68 and 3.62%) asparagine (11.03 and 5.51%) and tyrosine (2.96 and 1.51%) were found to be lower on dry matter basis in fungal infected and healthy shrimps respectively. In addition, there are some non - essential amino acids viz. glycine (3.84 and 7.54%), glutamic acid (6.03 and 12.54%), cysteine (2.45 and 6.13%), alanine (0.74 and 2.18%), proline (1.94 and 6.13%), aspartic acid (0.24 and 0.39%), and arginine (2.42 and 4.51%) whose compositions were significantly increased in infected dry matter than healthy shrimps. Hence results obtained from present study clearly indicates that, protein content of fungal infected shrimp was comparatively lower than the healthy shrimp due to the *G. persicaria* which completely inhibits the transcription process initiation.

A total of 7 different fatty acids including three saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA) were found in fungal infected and healthy shrimps P. monodon. The percentage availability of SFA, MUFA and PUFA contents in healthy and fungal infected tissues were about 57.25 and 53.07, 14.96 and 13.60 and 25.63 and

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18.57 % respectively (Table 3). The trivia of vitamins in healthy and infected tissues of shrimp P. monodon were presented in (Table 4). Among the vitamins reported for both healthy and infected tissues, Pyridoxin (B6) (26.78 and 18.87) were seemed to be in higher levels followed by Riboflavin (B2) (0.13 and 0.12).

In addition, the studies include detection of 5 macro and 2 trace minerals in healthy and infected shrimps. The macro minerals in both healthy and infected shrimps were in the order of calcium (345.6 and 156.4 mg/g), potassium (345.6 and 103.4 mg/ g), sodium (234.5 and 145.5 mg/g), magnesium (134.7 and 145.3 mg/g), copper (4.23 and 1.33 mg/g) zinc (14.1 and 0.33 mg/g) and iron (6.96 and 7.01 mg/g) respectively. Hence the study concludes that, macro minerals such as calcium, sodium and potassium were found to be higher in healthy shrimp tissues (Table 5).

Table 3. Fatty acid profile of healthy and infected shrimp tissue.

S. No	Fatty acids	Carbon atom (n)	Healthy tissue	Infected
		Saturated Fatty Acids		
1	Palmitic acid	C16:0	16.22	14.88
2	Margaric acid	C17:0	23.78	18.04
3	Stearic acid	C18:0	17.25	20.15
	Total		57.25	53.07
		Monounsaturated Fatty Acids		
4	Oleic acid		14.96	13.6
	Total		14.96	13.6
		Polyunsaturated Fatty Acids		
5	Linolenic acid	C18:3	14.65	11.16
6	α – Linolenic acid	C18:3	9.46	5.38
7	Stearidonic acid	C18:4	1.52	2.03
	Total		25.63	18.57
	Grand Total		97.84	85.54

Table 4. The vitamins in healthy and infected tissue of *P. monodon*.

S. No	Vitamins	Healthy tissue (mg / g)	Infected tissue (mg / g)
1	Thiamine B1	1.08	0.22
2	Riboflavin B2	0.13	0.12
3	Pyridoxin (B6)	26.78	18.87
4	Cobalamin (B12)	1.63	0.88
5	Ascorbic acid (C)	0.23	0.21

Table 5. The minerals in the tissue of infected and healthy shrimp.

S. No	Minerals	Healthy tissue (mg / g)	Infected tissue (mg / g)
	Macro		
1	Calcium	345.6	156.4
2	Sodium	234.5	145.5
3	Potassium	345.6	103.4
4	Copper	4.23	1.33
5	Magnesium	134.7	145.3
	Micro		
6	Iron	6.96	7.01
7	Zinc	14.1	0.33

DISCUSSION

Foods obtained from sea have been a high quality protein source for hundreds of years (Okuzumi, 2000). Among five basic groups, sea foods belong to the same category as meat, poultry, eggs, dried beans and peas as major sources of protein (Dean, 1990). Shrimps are enormously good protein source, yet very low in fat and calories, making them a healthy choice of food (Sriket *et al.*, 2007). In general, the biochemical composition of any organisms is acknowledged to vary with season, size of animal, stages of maturity and availability of food, temperature, etc. (Ravichandran, 2009). Fungal infections are most commonly occurring phenomenon in crustacean species of marine environment (Khoa *et al.*, 2005). In general, black gill circumstance in shrimps caused by *Fusarium* species develops to "blackened gill" condition thereby leading to death of affected individuals (Khoa, 2005).

In present study, proximate compositions of amino acids, fatty acids, vitamins and minerals of both *G. persicaria* infected and healthy shrimps *P. monodon* were investigated. As a result, the protein content was higher than fat and carbohydrate in healthy and infected tissues of *P. monodon* (49.15 and 31.15%). Likewise, (Narasimhan, 2013)(Narasimhan et al., 2013) reported that the amount of protein contents was higher in muscle (149.00 ± 0.65 mg/g) followed by gills (89.46 ± 1.26 mg/g) and intestine (114.40 ± 0.97 mg/gm) of *P. monodon*. Agreeing to the above results, the protein content in tissues of healthy and infected of *P. monodon* was higher with significant variations. Moreover, the carbohydrate contents in healthy and infected *P. monodon* tissues indicate that the healthy shrimps (16.53%) contains higher carbohydrate levels than infected shrimps (11.5%) tissues owing to fungal load enhancement in their circulatory system. The prospect of high levels of total carbohydrates might be due to transport of glucose and carbohydrate from hepatopancreas and muscles to hemolymph (Zeng *et al.*, 2013). Commonly the carbohydrate levels increased in infected or stressed animals. (Hall and Ham, 1998) reported that significant elevation of blood glucose levels (carbohydrate) in *P. monodon* occurs during stress conditions. In both healthy and infected shrimp *P.monodon* tissues the lipid levels achieved are 2.37 and 1.04 % respectively. Likewise, (Zeng *et al.*, 2013) have reported that lipid levels are higher in the mid gut due to intake of additional diet. In healthy shrimp tissues, essential amino acids such as leucine (9.78%) and tryptophan (0.12%) were found in higher and lower levels.

Similarly, the nonessential amino acids such as glutamate (8.15%) and alanine (0.13%) were found in higher and lower levels respectively. As a result, one of major essential amino acid "methionine" was found to be not present in amino acid profile of infected shrimp. Methionine (α- amino β-methylmercaptobutyrate, symbol met or shortly M) is one of essential amino acid cannot be synthesized by humans and other animals available only in food sources having positively charged sulfur groups (and is thus a sulfonium ion), is a powerful methylating agent essential as a donor of active methyl group. Also it is involved in important biochemical metabolisms, formation of methionyl tRNA, *N*-formylmethionyl tRNA, *S*- adenosylmethionine homocysteine, cysteine, creatine, phosphatidyl choline, methylated derivatives of DNA, RNA, proteins, catechol amines and carnitine (Mudd and Poole, 1975) (Hoshino *et al.*, 2004; Jain, 2005; Murray, 1998). At rare conditions diminished levels of methionine inhibits protein synthesis. Inability to absorb methionine from gut leads to methionine malabsorption syndrome (Murray, 1998). (Sriket *et al.*, 2007) stated that arginine was the most abundant amino acids while leucine, isoleucine and proline were prime in all shrimps and prawns. (Simpson *et al.*, 1998) have found that there is a high level of glycine, proline, arginine, and valine amino acids in fresh *Pandalus borealis* shrimp meat. Hence this study clearly divulges that, the fouling infection by a phytopathogenic fungi *G. persicaria* might drastically reduced the methionine production in shrimp which in turn affects the host's protein synthesis.

The present study also reveals that in healthy tissues of *P. monodon* three saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA) were reported. The percentage availability of SFA, MUFA and PUFA content in healthy shrimp tissues were about 57.25, 14.96 and 25.63%, whereas in infected shrimp tissues it was about 53.07, 13.6 and 18.57 % respectively. Similarly, (Puga-lopez *et al.*, 2013) reported that low SFA and MUFA amounts were found in WSSV infected *L. vannamei* shrimp tissues. Moreover this study implies that maximum amount of oleic acid (14.96 %) reported in healthy shrimp tissues likely equivalent to linoleic acid level traces present in the WSSV infected shrimp tissue when compared to healthy *L.vannamei* (Zeng et al., 2013). Further experimental studies showed, vitamin B_6 dominance in both healthy (26.78 mg/100g) and infected tissues (18.87 mg/100g), whereas vitamin B_2 were found at lower levels in both healthy and infected tissues (0.13 and 0.12 mg/100g) correspondingly. A total of 5 macro and 2 trace minerals were reported in infected as well as healthy shrimp tissues. Among macro minerals reported calcium (345.6 mg/g), potassium (345.6 mg/g), sodium (234.5 mg/g) and copper (0.45 mg/g) were observed at higher levels in healthy tissues compared to infected shrimps. (Ravichandran, 2009) reported in shell and flesh the sodium (29 and 38.6mg/100g, potassium (24.3 mg/100g and 33.2 mg/100g), phosphorus (82.4 and 91.5 mg/100g) levels of shrimp *P. indicus*.

Though the *P. monodon* shrimp possesses significant nutritive values as well found to be a good source of protein and Omega-3 and 6 polyunsaturated fatty acids, the fungal fouling pathogen *G. persicaria* significantly contributed in reduction of its biochemical composition. Conclusively, the infection of *G. persicaria* inhibits the synthesis of initiating codon amino acid "methionine" which is responsible for protein synthesis. Hence, the results revealed that continuous *G. persicaria* infection leads to stunted growth at shrimps grow out farms resulting in less survival rate and poor marketability.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors contributed equally to this research work.

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