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Biochemical changes of *Litopenaeus vannamei* and *Fenneropenaeus indicus* in the different stages of WSSV infection

Ramachandran Shalini, Abdul Razack Nazar, Mohideen Abdul Badhul Haq, Selvaraju Shanker*

Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai-608 502, Tamilnadu, India

PEER REVIEW

Peer reviewer

Dr. Akbar John, Department of biotechnology, Faculty of science, International Islamic University Malaysia, Kuantan, Pahang, Malaysia.
Tel: +60109019245
E-mail: akbarjohn50@gmail.com

Comments

This is a written study in which the authors studied the difference in the proximate composition and fatty acid profile of *L. vannamei* and *F. indicus* infected with different stages of WSSV. The results are interesting that the healthy shrimps are nutritionally rich than the WSSV affected shrimps.
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ABSTRACT

Objective: To find out the difference in the proximate composition and fatty acid profile of both the species of shrimp *Litopenaeus vannamei* (*L. vannamei*) and *Fenneropenaeus indicus* (*F. indicus*) infected with different stages of white spot syndrome virus (WSSV).
Methods: Standard methods were followed by estimating the proximate composition and fatty acid analysis. Each fish specimens were beheaded, eviscerated and filleted manually. The tissue samples were oven dried at 67 °C for 24 h. Then the samples were grounded finely with pestle and mortar. The saponified samples were cooled at room temperature for 25 min. They were acidified and methylated by adding 2 mL 54% 6 mol/L HCL in 46% aqueous methanol and incubated at 80 °C for 10 min in water bath. Following the base wash step, the fatty acid methyl esters were cleaned in anhydrous sodium sulphate and then transferred into gas chromatograph sample vial for analysis. Fatty acid methyl esters were separated by gas chromatograph.
Results: The proximate composition was higher in the both control tissue than the three (low, moderate, severe) infected ones. For *L. vannamei* and *F. indicus,* the carbohydrates are 5.07% and 6.18%, and the proteins are 25.01% and 22.17%, respectively. Lipid level recorded was little higher in the shrimps maintained and showed severe sign of WSSV infection than the control and the fatty acid profile result revealed that saturated fatty acids and monounsaturated fatty acid was in higher [48.72% (Severe) & 16.87% (low)] *L. vannamei*. In the polyunsaturated fatty acid, *F. indicus* was 40.47% (low).
Conclusions: Our study showed that the healthy shrimps are nutritionally rich than the WSSV affected shrimps.

KEYWORDS

Litopenaeus vannamei, Fenneropenaeus indicus, WSSV, Fatty acids, Proximate composition

1. Introduction

Seafoods are important source of nutrients in the human diet[1]. Crustaceans such as shrimps, crabs and lobsters, are good sources of amino acids, protein and other nutrients. Aquaculture emerged worldwide in the last two decades[2]. World shrimp aquaculture is producing now well over four million tons. Shrimp muscle is an excellent source of protein[3]. Shrimp is one of the most popular species as it is a part of traditional meal for almost every nation, rich in protein and minerals[4] and is very low in fat and calories, making it a very healthy choice of food. Shrimps have very low saturated fat, which is the fat that raises cholesterol levels in the body. Minerals are essentials in shrimp nutrition. Its biochemical composition may be affected by several factors such as species, environmental factors, size, age, natural diet and feed composition[5] and it changes seasonally[6-9]. The biochemical composition also varied in shells and flesh of *Fenneropenaeus indicus* (*F. indicus*)[10]. Recently, it is found that the decrease in

*Corresponding author: Selvaraju shanker, Ph.D, Research scholar, CAS in Marine Biology, Annamalai University, Parangipettai, India.
Tel: +91-9003450353
E-mail: shanker.sel@gmail.com
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nutritional value of *Metapenaeus monoceros* is due to the effect of organochlorine pesticide[11]. There are changes in the proximate chemical composition, fresh mass, water content, ash content, organic constituents, lipid and protein contents and energy levels of penaeid prawn, *Penaeus monodon* (*P. monodon*) during different reproductive stages[12]. Nevertheless, dietary requirements of important mineral elements are known for selected species of shrimps such as *Penaeus japonicus, F. indicus*, and *P. monodon*. There are many inorganic elements in the body of shrimps associated with the skeletal structure and biochemicals involved in vital physiological functions. The knowledge of the biochemical composition of any edible organism is extremely important as the nutritive value is reflected in the biochemical contents. The absence of essential micronutrients in the diet may lead to deficiency diseases. Thus it is necessary to understand the dietary requirements during the feed formulation.

Since the diseases of cultured shrimp includes infectious (viral, bacterial, and fungal) and non-infectious diseases caused by environmental hindrances, nutritional imbalance, toxicants and genetic factors[13]. Among the differentially expressed genes found in this study, several had been previously reported and found to involve in shrimp response against white spot syndrome virus (WSSV), such as C-type lectin and hemocyanin[14]. White spot syndrome, one of the most serious viral diseases among cultured shrimps in the world[15], has emerged in cultured kuruma shrimp (*P. japonicus*) in Japan in 1993. Present work was undertaken to determine the proximate composition and fatty acid profile of the whole body/tissues of *Litopenaeus vannamei* (*L. vannamei*) and *F. indicus* infected during different phases (low, moderate and severe) of WSSV infection.

2. Materials and methods

2.1. Experimental setup

Equal number of shrimps of both the species (10 animals per tank) was maintained in separate tanks. Four different tanks (control, low, moderate and severe) were for each species. The control tank was fed with the normal feed and the other tanks were fed with WSSV infected shrimp tissue (oral method) to enhance WSSV infection. The level of infection was monitored regularly.

2.2. Preparation of sample

The shrimps were fed regularly till the infection ranges were attained. The tissue samples from all the three stages were collected for the proximate composition and fatty acid analysis.

2.3. Estimation of protein

Folin-Ciocalteu phenol method of Lowry *et al.* was adopted for the estimation of total proteins in the tissue[16]. The dry tissue sample weighing 10 mg was thoroughly homogenized with 1 mL of deproteinizing agent (10% TCA) by keeping the tubes in ice. All samples were centrifuged for 20 min at 3 000 r/min. The precipitate obtained was used for protein estimation. The precipitate was dissolved in 2 mL 1 mol/L NaOH and to 1 mL of this solution, freshly prepared 5 mL alkaline reagent was added. This was kept at room temperature for 10 min, after which 0.5 mL of 1 mol/L Folin-Ciocalteu reagent was added and mixed rapidly. A standard stock solution was prepared using bovine serum albumin crystals at a concentration of 25 mg/5 mL NaOH. Different dilutions in the range of 0.25 to 2.50 mg/mL were prepared from this stock solution, the alkaline reagent and Folin-Phenol reagent was added as in the case of tissue samples. A blank was prepared with 1 mL 1 mol/L NaOH and treated the same way as earlier mentioned. All the test tubes were kept for 30 min at room temperature, the blue colour developed, and the optical density (OD) was evaluated against the blank at 660 nm:

$$\%\ \text{Composition of protein} = \frac{\text{Standard value} \times \text{OD of sample}}{\text{Weight of tissue}} \times 100$$

2.4. Estimation of carbohydrate

For the estimation of total carbohydrate content, the procedure of Dubois *et al.* using phenol-sulphuric acid was followed[17].

About 5 mg of oven-dried tissue was taken for carbohydrate analysis. The tissue was taken in a test tube and 1 mL of phenol (5%) and 5 mL concentrate H_2SO_4 were added in quick succession. The tubes were kept for 30 min at 30 °C and the OD of the colour developed was measured at 490 nm against the blank. D-glucose was used as a standard and it had an OD value of 0.1 carbohydrates as calculated by using the formula:

$$\%\ \text{Composition of carbohydrate} = \frac{\text{Standard value} \times \text{OD of sample}}{\text{Weight of tissue}} \times 100$$

2.5. Estimation of lipid

The chloroform-methanol extraction procedure of Floch *et al.* was used for extracting lipid from the various body parts[18]. The lipid content was estimated gravimetrically by following method. The lipid was extracted from 500 mg of powdered oven-dried tissue with 5 mL of chloroform: methanol (2:1) mixture added. The mixture was filtered by a micro filter. This extract was taken in a pre-weighed beaker and oven dried. Beaker was reweighed with lipid. The

difference in weight was taken as total lipid content and the percentage was calculated as follows:

$$\% \text{ Composition of lipid} = \frac{\text{Weight of lipid}}{\text{Weight of tissue}} \times 100$$

2.6. Estimation of calcium

To the 5 g of wet tissue samples, mixture of hydrochloric acid, nitric acid and perchloric acid (HCl, HNO_3-$HClO_4$) at a ratio of 10:5:1 was added for digestion at 300 °C. The digests were filtered suitably and aspirated in digital flame photometer. The obtained values were expressed in mg/100 g. The level of calcium was estimated by following the method of Guzman and Jimenez[19].

2.7. Estimation of phosphorus and iron

The tissue samples collected were stored in pre-cleaned polythene contains and were later aspired in an inductively coupled plasma spectrophotometer (ICP) after calibrating the instrument with appropriate blank and series of known standards for the minerals (phosphorus and iron). The phosphorus and iron levels were estimated by following the method of Topping[20].

2.8. Calorific content estimation

The calorific content was calculated from the biochemical composition by using the calorific equivalents of 5.65 kcal/g for protein, 4.45 kcal/g for lipid and 4.10 kcal/g for carbohydrate as suggested by Brett and Groves and expressed in dry weight basis[21].

2.9. Estimation of moisture

A known quantity of the wet tissue was dried in a hot air oven at a constant temperature of 60 °C until the wet tissue was dried completely. The moisture content was estimated by subtracting the dry weight of the sample from the total weight. The percentage of moisture was calculated[22].

2.10. Analysis of fatty acids

For fatty acid analysis, each fish specimens were beheaded, eviscerated and filleted manually. The tissue samples were oven dried at 67 °C for 24 h. After that, the samples were grounded finely with pestle and mortar. The preparation and analysis of fatty acid methyl esters (FAMEs) from these fish tissues were performed[23]. A total of 50 mg of tissue samples were added to 1 mL of 1.2 mol/L NaOH in 50% aqueous methanol with glass beads (3 mm dia) in a screw-cap tube and then incubated at 100 °C for 30 min in a water bath. The saponified samples were cooled at room temperature for 25 min, and they were acidified and methylated by adding 2 mL 54% 6 mol/L HCL in 46% aqueous methanol and incubated at 80 °C for 10 min in water bath. After rapid cooling, methylated FAs were extracted with 1.25 mL 50% methyl-test butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the bottom phase removed with a pasteur pipette. Top phase was washed with 3 mL 0.3 mol/L NaOH. After mixing for 5 min, the top phase was removed cleaned in anhydrous sodium sulphate and then transferred in to gas chromatograph sample vial for analysis. FAMEs were separated by gas chromatograph (HP 6890 N, Agilent Technologies, USA). FAMEs profiles of the tissues were identified by comparing the commercial Eucary database with MIS Software package (MIS Ver. No. 3.8, Microbial ID. Inc., Newark, Delaware).

3. Results

3.1. Proximate composition of L. vannamei and F. indicus

Proximate compositions of both species are given in Tables 1 and 2. Higher levels of protein, carbohydrates and lipids were found in the control rather than the infected tissues.

Table 1
Changes in the proximate composition of the tissues of *L.vannamei* exposed to WSSV.

Proximate composition	Low	Moderate	Severe	Control
Protein (%)	22.10±0.02	23.53±0.02	18.07±0.02	25.01±0.02
Carbohydrates (%)	6.23±0.02	4.23±0.02	3.030±0.023	5.07±0.02
Lipid (%)	1.32±0.03	0.01±0.03	1.770±0.025	0.011±0.020
Moisture (%)	70.01±0.02	71.03±0.02	70.01±0.02	71.00±0.02
Calcium (mg/100 g)	277.00±0.02	266.00±0.02	267.00±0.02	279.00±0.03
Phosphorus (mg/100 g)	213.06±0.03	215.03±0.02	203.04±0.02	215.06±0.02
Iron (mg/100 g)	4.30±0.02	3.71±0.54	3.48±0.02	4.53±0.02
Calorific value (kcal/g)	65.71±0.54	64.170±0.025	63.39±0.53	68.460±0.017

Table 2
Changes in the proximate composition in the tissues of *F. indicus* exposed to WSSV.

Proximate composition	Low	Moderate	Severe	Control
Protein (%)	21.07±0.02	21.63±0.02	17.14±0.02	22.17±0.02
Carbohydrates (%)	6.10±0.02	3.39±0.59	4.86±0.24	6.18±0.02
Lipid (%)	2.03±0.02	1.88±0.02	1.21±0.02	2.13±0.02
Moisture (%)	72.07±0.02	72.42±0.60	73.88±0.02	72.18±0.02
Calcium (mg/100 g)	279.03±0.02	265.18±0.02	266.40±0.02	273.30±5.73
Phosphorus (mg/100 g)	214.10±0.02	209.83±0.64	203.13±0.02	251.39±0.53
Iron (mg/100 g)	4.54±0.02	3.38±0.54	3.64±0.02	4.72±0.02
Calorific value (kcal/g)	67.48±0.02	65.17±0.02	64.07±0.02	67.68±0.02

3.2. Fatty acid profiles of L. vannamei and F. indicus

Degree of saturates ranged between 43.47%-48.72% in the shrimps affected with severe condition in both the species, whilst monoenes ranged between 10.80%-16.84%, and PUFA varied from 36.12%-40.47% shrimps with low sign of WSSV infection. A uniform trend of saturates, monoenes, PUFA and

other FA profile levels were found as 42.30%–45.53%, 10.94%–16.46%, 35.80%–39.99% and 0.59%–0.69% respectively (Tables 3 and 4) in *L. vannamei* and *F. indicus* during different stages of WSSV infectivity.

Table 3
The presentage of fatty acid profile of different stages of infested *L.vannamei* tissue (%).

Carbon Chain	Fatty acid	Low	Moderate	Severe	Control
C10:0	Capric acid	1.10	0.19	0.16	1.02
C11:0	Undecyclic acid	1.43	0.57	0.64	0.50
C12:0	Lauric acid	1.64	0.19	0.17	0.72
C13:0	Tri decyclic acid	1.04	1.16	1.84	1.03
C14:0	Myristic acid	1.79	2.54	2.61	2.52
C15:0	Penta decyclic acid	2.51	1.50	1.04	1.21
C16:0	Palmitic acid	21.16	21.59	21.99	20.05
C17:0	Margaric acic	3.05	3.95	3.97	4.64
C18:0	Stearic acid	6.10	7.56	8.09	8.02
C19:0	Nonadecyclic acid	1.09	1.79	1.81	2.03
C20:0	Arachidic acid	0.19	1.10	0.97	0.14
C21:0	Heneicosanoic acid	1.11	1.17	0.97	1.24
C22:0	Pehenic acid	1.19	1.56	1.64	1.12
C23:0	Tricosanic acid	0.59	0.17	0.93	0.13
C24:0	Lignoceric acid	1.27	1.59	1.89	1.16
Σ of SFAs	45.26	46.63	48.72	45.53	
C14:1ε−7	Myristolec acid	1.11	1.13	0.16	–
C16:1ε−7	Palmitoleic acid	1.97	1.21	1.31	1.19
C18:1ε−5	Cis−5−Octadecenoic acid	0.33	0.14	0.17	0.51
C18:1ε−7	Cis−7−Octadecenoic acid	0.54	0.61	0.62	0.91
C18:1ε−9	Oleic acid	7.19	5.13	6.10	8.17
C19:1ε−8	Nonadecenoic acid	1.09	1.02	1.91	1.91
C20:1ε−5	Cis−5− Eicosenoic acid	–	0.54	–	0.93
C20:1ε−7	Cis−7− Eicosenoic acid	0.19	–	0.51	0.11
C20:1ε−9	Cis−9− Eicosenoic acid	1.31	1.92	1.07	1.21
C20:1ε−11	Trans − 11− Eicosenoic	0.67	0.61	–	0.13
C22:1ε−7	Cis−7− Docosenoic acid	0.54	0.59	0.69	0.19
C24:1ε−3	Cis−3− Tetrasenoic acid	–	0.17	1.11	0.19
C24:1ε−6	Cis−6− Tetrasenoic acid	1.93	1.05	–	–
C24:1ε−9	Trans−9−Tetrasenoic acid	–	1.50	1.77	1.01
Σ of MUFAs	16.87	15.62	15.42	16.46	
C18:2ε−6	Linoleic acid	4.01	3.55	3.19	4.59
C18:3ε−3	Alfa linolenic acid	4.19	3.99	3.13	3.17
C18:3ε−6	Gamma linolenic acid	1.17	2.51	2.99	0.50
C18:4ε−3	Stearidonic acid	6.17	5.59	7.01	5.19
C20:2ε−6	Eicosadienoic acid	2.17	1.19	1.08	20.13
C20:3ε−6	Dihomogamma linolenic	5.50	6.17	6.13	4.19
C20:4ε−6	Arachidonic acid	1.17	1.09	1.01	2.08
C20:5ε−3	Eicosapentaenoic acid	1.19	1.91	0.13	1.11
C22:3ε−3	Docosa trienoic acid	–	1.51	–	1.39
C22:4ε−6	Docosa tetraenoic acid	2.19	–	2.13	–
C22:5ε−3	Docosa Pentaenoic acid	8.25	7.9	6.92	11.28
C22:6ε−3	Docosa Hexaenoic acid	0.11	–	–	0.17
Σ of PUFAs		36.12	35.41	33.72	35.8
C11:0 Iso		0.10	0.09	0.07	0.11
C11:0 Anteiso		–	0.12	–	0.12
C12:0 Iso		–	–	–	–
C12:0 Anteiso		0.13	–	0.17	–
C14:0 Iso		–	0.17	–	0.19
C15:0 Anteiso		0.19	–	0.11	0.31
C16:0 Iso		–	0.16	–	–
C17:0 Anteiso		0.12	–	–	0.13
C18:0 Anteiso		0.17	0.64	0.59	–
C19:0 Iso		–	–	0.14	–
C20:0 Iso		0.14	–	0.17	0.54
C20:0 Anteiso		–	0.19	0.73	0.13

Table 4
The presentage of fatty acid profile of different stages of infested *F. indicus* tissue (%).

Carbon Chain	Fatty acid	Low	Moderate	Severe	Control
C10:0	Capric acid	0.12	0.19	–	–
C11:0	Undecyclic acid	–	0.17	0.13	1.01
C12:0	Lauric acid	2.30	1.93	–	1.13
C13:0	Tri decyclic acid	–	2.19	2.93	1.27
C14:0	Myristic acid	2.87	–	1.95	2.91
C15:0	Penta decyclic acid	0.67	1.92	1.07	0.97
C16:0	Palmitic acid	19.19	20.13	20.57	19.02
C17:0	Margaric acic	2.59	2.67	2.77	2.97
C18:0	Stearic acid	4.10	3.91	3.57	3.41
C19:0	Nonadecyclic acid	1.93	2.01	2.63	1.59
C20:0	Arachidic acid	1.57	1.09	1.53	1.10
C21:0	Heneicosanoic acid	1.81	1.79	0.86	1.63
C22:0	Pehenic acid	1.93	1.90	1.59	2.19
C23:0	Tricosanic acid	–	1.91	0.96	1.07
C24:0	Lignoceric acid	2.59	2.90	2.91	2.03
Σ of SFAs	41.67	44.78	43.47	42.30	
C14:1ε−7	Myristolec acid	0.19	0.67	0.43	0.18
C16:1ε−7	Palmitoleic acid	1.43	1.79	1.84	1.56
C18:1ε−5	Cis−5−Octadecenoic acid	0.67	0.09	0.18	0.84
C18:1ε−7	Cis−7−Octadecenoic acid	0.54	0.06	0.63	0.79
C18:1ε−9	Oleic acid	2.19	2.06	2.17	2.53
C19:1ε−8	Nonadecenoic acid	1.07	1.01	1.91	1.09
C20:1ε−5	Cis−5− Eicosenoic acid	0.93	–	0.92	0.97
C20:1ε−7	Cis−7− Eicosenoic acid	0.51	0.49	0.63	–
C20:1ε−9	Cis−9− Eicosenoic acid	0.49	–	0.47	0.64
C20:1ε−11	Trans − 11− Eicosenoic	0.87	0.41	0.19	0.53
C22:1ε−7	Cis−7− Docosenoic acid	–	0.69	0.81	0.19
C24:1ε−3	Cis−3− Tetrasenoic acid	0.73	0.51	0.74	0.98
C24:1ε−6	Cis−6− Tetrasenoic acid	0.64	0.57	0.61	0.13
C24:1ε−9	Trans−9−Tetrasenoic acid	0.54	0.63	–	0.51
Σ of MUFAs		10.80	8.98	11.53	10.94
C18:2ε−6	Linoleic acid	3.09	3.17	3.09	3.11
C18:3ε−3	Alfa linolenic acid	1.17	1.51	1.12	1.13
C18:3ε−6	Gamma linolenic acid	2.61	2.79	2.89	2.11
C18:4ε−3	Stearidonic acid	6.19	6.01	6.57	6.18
C20:2ε−6	Eicosadienoic acid	3.97	3.67	4.01	4.91
C20:3ε−6	Dihomogamma linolenic	3.16	3.91	2.17	2.17
C20:4ε−6	Arachidonic acid	9.10	9.81	8.93	9.92
C20:5ε−3	Eicosapentaenoic acid	2.06	2.09	2.61	2.19
C22:3ε−3	Docosa trienoic acid	3.17	3.11	3.50	3.16
C22:4ε−6	Docosa tetraenoic acid	3.63	0.51	0.47	0.97
C22:5ε−3	Docosa Pentaenoic acid	1.31	1.93	1.97	1.21
C22:6ε−3	Docosa Hexaenoic acid	1.01	0.51	0.11	2.93
Σ of PUFAs		40.47	39.02	37.44	39.99
C11:0 Iso		0.63	0.61	0.59	0.57
C11:0 Anteiso		0.19	0.27	0.61	–
C12:0 Iso		0.17	0.23	0.62	0.61
C12:0 Anteiso		0.83	0.69	0.47	0.73
C14:0 Iso		0.71	0.61	–	0.18
C15:0 Anteiso		–	0.81	0.39	0.69
C16:0 Iso		0.59	0.73	0.47	0.91
C17:0 Anteiso		0.48	0.19	0.52	0.61
C18:0 Anteiso		0.19	–	0.61	0.72
C19:0 Iso		0.87	0.91	0.81	0.93
C20:0 Iso		0.81	0.83	0.73	0.11
C20:0 Anteiso		0.67	0.57	0.83	0.12

4. Discussion

The results showed significance differences in the

biochemical profiles of healthy and WSSV infected shrimp of both the species. In the case of *L. vannamei*, there was a sharp increase in the carbohydrate (6.25%), calcium (277.05 mg/100 g), iron 4.32 (mg/100 g) and calorific value 66.04 (kcal/g) in the tissue which might owe to increase of viral load in the circulatory system. The presence of abundant viral protein in the hemolymph of *Manduca sexta* larvae infected with polydnavirus has been reported[24]. Generally, the carbohydrate level increases in infected or stressed animals. The possibility of high levels of total carbohydrate in hemolymph might be due to the transport of glucose and carbohydrate from hepatopancreas and muscles to hemolymph. A study of Hall and van Ham showed a significant elevation of blood glucose (carbohydrate) in *P. monodon* during stress condition[25]. Blood glucose, strongly and rapidly increased in response to repeated blood-sampling stress in juveniles of *L. vannamei*. In contrast, the proximate composition of wild and farm reared *L. vannamei* and the result confirmed that the wild caught shrimp had higher level of proximate composition than the cultured shrimp[26]. The proximate chemical composition of *Fenneropenaeus penicillatus* was determined in midgut gland, ovary and muscle during different stages of ovarian development that was found to be varied during maturation among the different tissues examined. The lipid content was the highest in the midgut gland, and protein content was found higher in the ovary. The carbohydrate was found to be higher in the mid gut gland. Different body parts need different compounds for their development and function. Protein was used for the embryo development, so it is higher in the ovary. The study of Nisa and Sultana showed that lipid was higher in the midgut and it was due to the intake of other diet[27]. A study was undertaken to determine the amount of protein content in different tissues of marine shrimps *P. monodon* and *L. vannamei*. The result shows that the protein concentration was higher in *P. monodon* than that of in *L. vannamei*[28].

In the present investigation, *F. indicus* challenged to WSSV, showed difference between severely infected (73.91%) and healthy shrimps (72.09%). The decrease in biochemical constituents of infected shrimps was probably caused by hemocytic accumulation at the site of injection for wound healing and phagocytosis of foreign bodies[29]. Another possibility is that the total haemocyte count decline would be due to cell burst resulting from budding of the virus, or by virus induced apoptosis, since this type of cell "suicide" may be induced or repressed during some viral infections[30]. Proximate composition and the amino acid profile of the female and male prawn *Macrobrachium rosenbergii*, which is commercially available, shows that the protein, lipid, carbohydrates, amino acids and fatty acids levels were comparatively higher than the male prawn according to Bhavan *et al*[31]. This may be due to the reproductive behaviour in the female prawn. This process needs more energy levels than the normal level. Male does not need this so that it has lower level than female. The PUFA and SFA were also found to be higher in female than male prawns. Protein, lipid and carbohydrates of both the species *Fenneropenaeus merguiensis* and *F. penicillatus* increases as the ovary matured. This indicates that the maturity of the animal sexually[32]. This is due to the need of excess energy to the animal for the maturity. Protein, lipid and carbohydrates were uniform in all the parts of the body till it gets sexually matured. When the maturity starts, the size and weight of the animal stops, and gonads start to get matured.

The results indicate the presence of higher percentage of biochemical constituents in the post challenged WSSV-infected tissues of *L. vannamei* than *F. indicus*. Changes in the level of SFA (%) in *L. vannamei* tissues appeared in the following order: severely infected (48.72%) > moderately infected (46.63%) > healthy (45.53%) > low infected (45.26%); and in *F. indicus*, the identical levels appeared as moderately infected (44.78%) > severely infected (43.47%) > healthy (45.53%) > low infected (41.67%). In addition, in *L. vannamei* the level of MUFA encountered as low infected (16.87%) > healthy (16.46%) > WSSV moderately infected (15.62%) > WSSV severely infected (15.42%); correspondingly in *F. indicus* which was recorded as severely infected (11.53%) > healthy (10.94%) > WSSV low infected (10.80%) > WSSV moderately infected (8.98%). Based on the results of the present investigation, a high amount of saturates and MUFA are found in the WSSV infected tissues of *L. vannamei* than *F. indicus*.

The present investigation also shows that the tissues of both the species, post challenge to WSSV are rich source of both 20:5 n-3 and 22:6 n-3. These levels were encountered high in low phase of WSSV infection. Results of present study showed the presence of high level of 18:2 n-6 with the amount of 3.55% in the *L. Vannamei* shrimps infected with low sign of WSSV. Contrarily, a maximum level of 4.59% was noticed in the healthy *L. vannamei* maintained in control tanks. While in the case of *F. indicus*, a uniform level (3.09%) of 18:2 n-6 found in the tissues of low and severe phases of infection. However, in the healthy *F. indicus*, linoleic acid was found more than that of in WSSV infected shrimps. Though, linoleic acid (18:2 n-6) is very important in human nutrition and health especially for

membrane structure fluidity, skin integrity and numerous tissue functions, the expected nutritional values can not be gained by consuming WSSV infected shrimps. Considerably of 16:1 fatty acid on human health and nutrition are rarely discussed in literature. So an elevated level of 16:1 fatty acid in WSSV infected shrimp may not have any remarkable effect on human health.

It can be concluded that, *L. vannamei* and *F. indicus*, the results of the proximate composition express higher value in shrimps maintained as control than the three stages of infection. Our study showed that the healthy shrimps are nutritionally rich than the WSSV affected shrimps. The level carbohydrate alone was little high in *L. vannamei* infected with low range of WSSV. Linoleic acid is present in the control shrimp in higher level when compared with the other three infected one. Finally the shrimp which is kept as a control is better for consuming than the shrimps infected with WSSV.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Seafoods are important source of nutrients in the human diet. Crustaceans such as shrimps, crabs and lobsters, are good sources of amino acids, protein and other nutrients. Aquaculture emerged worldwide in the last two decades. World shrimp aquaculture is producing now well over four million mt. Shrimp muscle is an excellent source of protein.

Related reports

Several related reports have been studied. A study of Hall and van Ham showed a significant elevation of blood glucose (carbohydrate) in *P. monodon* during stress condition. Nisa and Sultana showed that lipid was higher in the midgut and it was due to the intake of other diet.

Innovations and breakthroughs

Authors scientifically tried to prove the effect of WSSV on the biochemical changes in selected shrimps, and the study comes to a conclusion that the healthy shrimps are nutritionally rich than the WSSV affected shrimps.

Applications

Overall the research is application oriented. To check the quality of shrimps that are being marketed and to design biosensors to check the product quality, these sort of studies are highly essential.

Peer review

This is a written study in which the authors studied the difference in the proximate composition and fatty acid profile of *L. vannamei* and *F. indicus* infected with different stages of WSSV. The results are interesting that the healthy shrimps are nutritionally rich than the WSSV affected shrimps.

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