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Influence of White Spot Disease on Muscle Lipid Content and Fatty Acid Profile of Cultured *Penaeus monodon*

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Abstract

Shrimp infected with white spot disease (WSD) are often released to Sri Lankan market as well as international markets for human consumption. Therefore, it is of interest to the consumer to know if this disease has any effect on the quality of the flesh of the shrimp. Using gas chromatography, we measured lipid levels and fatty acid profiles of muscle tissue from randomly collected, healthy and WSD cultured *Penaeus monodon* over a period of seven months from October 1999 to May 2000. The mean percentage dry weight lipid content of WSD shrimp was significantly higher (4.3 ± 0.1) than that for healthy shrimp (3.9 ± 0.2) ($p < 0.05$). The predominant fatty acids in muscle tissue for both were palmitic (16:0), stearic (18:0), oleic (18: $1n - 9$), linoleic (18:$2n - 6$), eicosapentaenoic (20: $5n - 3$) and docosahexaenoic (22: $6n - 3$). White spot disease (WSD) shrimp showed significantly lower levels ($p<0.05$) of linoleic acid (9.3 ± 2.0) than healthy shrimp (14.3 ± 3.1). However, significantly higher levels of stearic acid (14.1 ± 3.5) and palmitoleic acid (3.1 ± 1.15) were found in WSD shrimp than in healthy shrimp (11.7 ± 0.4. and 1.7 ± 0.2, respectively). The saturated fatty acid content was higher (41.0 ± 1.6) in WSD shrimp and polyunsaturated fatty acid content was higher in healthy shrimp (44.8 ± 1.4).

Introduction

The *P. monodon* shrimp aquaculture industry is one of the fastest growing export oriented industries in the South East Asian region. It seems that, shrimp production of the South East Asian reagion is 506,035 mt or 53.7% of total shrimp production. If all of Asia is considered together as one region then it contributes 737.380 mt or 78% of the world's farmed shrimp (Yap 1999). Shrimp exports, mainly *P. monodon*, have contributed 53% to 73% to the total foreign exchange earnings from the fisheries sector during the past two decades in Sri Lanka, and contributed Rs 5000 million during the year 2000 (NARA 2001). Crustaceans play an important role in human nutrition and health, because these serve as a rich source of energy and carriers of fat soluble vitamins such as vitamins A and D (Edirisinghe et al. 1998, Kinsella 1987). Crustacean lipids are a good source of both saturated and unsaturated fatty acids. Some of these such as linoleic acid (18:2*n*-6), arachidonic acid (20:4*n*-6), eicosapentaenoic acid (20:5*n*-3) and docosahexaenoic acid (22:6*n*-3) are essential fatty acids (Kinsella 1987). Humans need specific essential fatty acids, such as linoleic acid and its elongated, desaturated products, arachidonic acid, for membrane structure, fluidity, skin integrity and numerous tissue functions. The critical function of dietary linoleic acid is to provide arachidonic acid, which is also the precursor of the prostanoids and leukotrienes. These compounds modulate many vital physiological functions related to cardiovascular, renal, pulmonary, secretory, digestive, reproductive and immune functions (Kinsella 1987). It is claimed that eicosapentaenoic acid reduces the risk of heart attack by competing with arachidonic acid for the enzyme responsible for production of prostaglandins and thromboxanes (Kinsella 1986), and thus producing fewer of the platelet-aggrating eicosanoids (Johnston and Hunter 1987).Consequently, the frequency of coronary heart disease may be decreased by augmented fish consumption (Dyerberg 1986). Omega – 3 polyunsaturated fatty acids might also have an anti-inflammatory effect (Robinson et al. 1987).

White spot syndrome virus (WSSV) causes WSD, a recurring regional problem specially in Asia in the culture industry of *P. monodon* (Mohan and Shankar 1998). The name WSD comes from prominent white spots which are about 0.5 to 0.2mm dia under the cuticle of infected shrimp (Sudha et al. 1998). The deposits of calcium phosphate minerals on inner side of the cephalothoraxes give this white spot appearance (Sudha et al. 1998). However, the presence of white spots does not always mean that the condition is terminal. For instance, under non-stressful conditions, infected shrimps that have white spots may survive indefinitely (Lo et. al 1997).

However, nothing can be done to save the shrimp that are severely affected with WSD. The only thing that can be done here is emergency harvest and pond disinfection (Mohan and Shankar 1998). These harvested WSD *P. monodon* are frequently released to markets for human consumption. Therefore, it is of interest for consumers to know whether WSD causes nutritional quality changes in shrimp flesh. This aspect has not been investigated much and very little literature is available on the importance of the disease on the nutritional quality of the flesh of shrimp. The present study reports on the muscle lipid composition and fatty acid profiles of healthy and WSD cultured *P. monodon*

Materials and Methods

Healthy and WSD *P. monodon* of approximately similar weight (between 30 to 40 g) were collected from shrimp culture facilities along the North Western coastline of Sri Lanka over a period of seven months from October 1999 to May 2000. Specimens with visible white spots under the cuticle were designated as WSD specimens and their infection status was later confirmed by two step PCR assay. *P. monodon* that showed no circular white spots and had negative results for 2 step PCR assay were considered healthy.

From the body mussel to be tested, PCR templates were prepared with proteinase K,N-cetyle N,N-trimethylammonium bromide (CTAB) treatments, phenol/chloroform extraction and ethanol precipitation (Lo et al. 1996, Lo et al. 1997). The quality of the DNA extract was checked for each sample by two step PCR with the decapod 18S rRNA gene specific primer pair (Lo et al. 1996). Subsequently each sample was subjected to the WSD diagnostic PCR test. In the present study, pms 146 F_1/R_1 and F_2/R_2 primer sets and the PCR reaction conditions described by Lo et al. (1996) were utilized for WSDV diagnostic PCR.

After collection, samples were immediately cooled in crushed ice, packed in labeled polythene bags and transported to the laboratory. The range of length, standard (from tip of rostrum to posterior edge of the last abdominal segment) total (from tip of rostrum to tip the telson) and weight were measured. Head, carapace and tail were removed 3 to 5th after capture. Only minced body muscles were used for further analysis of lipids and fatty acid.

Lipids were extracted and purified from the body muscle samples using a modification of the Bligh and Dyer method (Radin 1981). Briefly, samples of body muscles (2 g) were blended in a cold hexene: isopropanol mixture (3:2 v/v, 2 ml) and filtered (medium porosity scintered glass Buchner funnel), after which the filtrate was extracted once with 0.47 M Na_2SO_4 to remove the isopropanol. The hexane layer was then removed, concentrated by evaporation at 55°C under nitrogen, and vacuum-dried (0.58 bar, 50°C) for 2d. After weighing, samples were suspended in hexane (5 ml) and held at -20^0C (Hanson and Olly 1965).

Fatty acid methyl esters (FAME) of these lipids were prepared by base hydrolysis followed by trans-esterification (Berner and Berner 1994) and separated by capillary column gas chromatography using a Shimadzu GC–14A gas chromatograph with a supelco wax 10 fused silica capillary column (length 30 m, 0.32 mm ID, 0.25 mm film thickness). Statistical significance of percentage lipid and fatty acid (at 0.05 levels) were tested by student t-test (Mead and Curnow 1987).

Results

Body weight, length and moisture content of healthy and WSD shrimp were within the similar range (Table 1). The muscle lipid percentage of healthy *P. monodon* ranged between 3.7 to 4.1% dry weight while that of WSD shrimp was 4.2 to 4.5%. The mean lipid content in healthy shrimp (3.9% ± 0.2) was significantly lower than that in WSD shrimp (4.3% ± 0.1) ($P<0.05$) (Table 2). The most abundant fatty acids in both groups were palmitic (16:0), stearic (18:0), oleic (18: 1*n*–9), linoleic (18:2*n*–6), eicosapentaenoic (20: 5*n*–3) and docosahexaenoic (22: 6*n*–3), with palmitic (16:0) giving the highest content (20–25%).

Table 1. Comparison of body weight and length of cultured healthy and WSD *P. monodon*

Sampling Date	Sample No[a]	No. of Shrimps	Moisture (%)	Lipid (%)	
				Wet weight	Dry weight
Cultured Healthy Shrimps					
8 Nov 99	5	67	35.6 ± 5.2	16.9 ± 1.2	18.4 ± 1.1
22 Nov 99	1	70	36.8 ± 4.7	17.2 ± 1.5	18.5 ± 1.1
20 Dec 99	2	68	35.9 ± 10.0	17.1 ± 1.3	18.6 ± 1.2
20 Dec 99	3	65	36.7 ± 9.0	17.4 ± 1.7	18.4 ± 1.5
23 Dec 99	4	64	37.3 ± 8.9	16.9 ± 1.6	18.4 ± 1.6
Average			36.7 ± 0.6	17.2 ± 0.2	18.5 ± 0.1
White Spot Diseased Shrimps					
8 Nov 99	5	67	35.6 ± 5.2	16.9 ± 1.2	18.4 ± 1.1
22 Nov 99	6	55	34.7 ± 8.1	15.7 ± 4.2	16.6 ± 1.0
16 Dec 99	7	65	33.1 ± 6.4	15.1 ± 1.4	15.4 ± 1.5
20 Dec 99	8	64	35.3 ± 5.4	16.4 ± 0.9	17.9 ± 0.9
Average			34.7 ± 1.1	16.0 ± 0.8	17.1 ± 1.4

[a]Each sample consist of five sub samples

Table 2. Percentage lipid content of cultured healthy and WSD *P. monodon*

Sampling Date	Sample No[a]	No. of Shrimps	Moisture (%)	Lipid (%)	
				Wet weight	Dry weight
Cultured Healthy Shrimps					
22 Nov 99	1	70	74.9 ± 0.7	1.0 ± 0.1	4.1 ± 0.4
20 Dec 99	2	68	75.0 ± 0.7	0.9 ± 0.2	3.9 ± 0.7
20 Dec 99	3	65	75.0 ± 0.5	0.9 ± 0.3	3.7 ± 0.1
23 Dec 99	4	64	74.4 ± 1.6	1.1 ± 0.1	4.0 ± 0.3
Average			74.8 ± 0.3	0.9 ± 0.1	3.9 ± 0.2
White Spot Diseased Shrimps					
8 Nov 99	5	67	77.9 ± 0.6	1.0 ± 0.1	4.5 ± 0.6
22 Nov 99	6	55	77.5 ± 1.3	0.9 ± 0.2	4.2 ± 0.8
16 Dec 99	7	65	77.5 ± 0.8	0.9 ± 0.1	4.3 ± 0.6
20 Dec 99	8	64	77.9 ± 0.8	0.9 ± 0.1	4.2 ± 0.6
Average			77.7 ± 0.2	0.9 ± 0.1	4.3 ± 0.1

[a]Each sample consist of five sub samples

There were differences in the percentage composition of saturated, monounsaturated and polyunsaturated fatty acids between the healthy and WSD shrimp (Tables 3, 4 and 5). WSD shrimp gave the highest percentage of saturated fatty acids (41.0% ± 1.6). Of these stearic acid (18:0) in the WSD shrimp was significantly higher (14.1% ± 3.5) than in the healthy shrimp (11.72% ± 0.38) ($P < 0.05$). Healthy and WSD shrimp had similar percentages of total monounsaturated fatty acids (14.6% ± 0.4 and 15.1 ± 0.8, respectively) although C16:1 in WSD shrimp was significantly higher (3.1% ± 1.2) than in healthy shrimp (1.7% ± 0.2) ($P < 0.05$).

Polyunsaturated fatty acids (PUFA) comprised 40 to 50% of total fatty acids for both groups. Amount of *n*-3 PUFAs recorded in healthy and WSD *P. monodon* was not significantly different ($P > 0.05$) while healthy *P. monodon* have significantly high percentage of *n*-6 PUFAs (17.51% ± 0.49) ($P<0.05$). The highest percentage of *n*-6 PUFAs of healthy *P. monodon* was obtained due to the significantly higher content of linoleic acid (14.3% ± 3.1) than the WSD shrimps (9.3% ± 2.0). Although the ratio *n*3/*n*6 for WSD shrimp (2.3% ± 0.1) was higher than that for healthy shrimp (1.6% ± 0.1), the difference was not significant ($P > 0.05$).

Discussion

It is known that the lipid content of shrimp varies with the species, season, location, physiological status, diet, body location and age (Ackman 1982, Kinsella 1988). Under farmed conditions, *P. monodon* is mainly fed with lipid rich formulated diets (approximately 20 to 25%). It has been reported that the high lipid content of cultured *P. monodon* is due to dietary imbalances (Ackman 1982, Kinsella 1988, Catacutan 1991). However previous studies (Jingzhi et al. 2000) have also indicated that viral load may influence the lipid composition on crustacean muscle. Also there are reports that tissue

Table 3. Percentage of saturated fatty acids of cultured healthy and WSD *P.monodon*

S[a]	14:0	15:0	16:0	17:0	18:0	Total
Cultured Healthy shrimps						
1	1.07 ± 0.42	0.35 ± 0.07	22.33 ± 1.65	2.21 ± 0.30	11.57 ± 3.26	37.64 ± 4.00
2	0.89 ± 0.72	0.33 ± 0.08	21.35 ± 0.32	2.12 ± 0.46	12.30 ± 1.98	37.01 ± 2.66
3	0.93 ± 0.20	0.39 ± 0.05	21.82 ± 0.42	2.05 ± 0.34	10.76 ± 2.97	35.97 ± 3.69
4	1.65 ± 1.14	0.43 ± 0.12	21.65 ± 1.21	2.55 ± 0.63	12.28 ± 4.89	38.57 ± 7.53
Average	1.13 ± 0.02	0.38 ± 0.02	21.38 ± 0.73	2.22 ± 0.05	11.72 ± 0.38	37.29 ± 1.09
White Spot Diseased shrimps						
5	2.63 ± 1.39	0.55 ± 0.31	21.32 ± 4.54	3.95 ± 2.83	14.6 ± 3.94	42.97 ± 8.03
6	2.58 ± 1.06	0.44 ± 0.10	21.34 ± 1.15	2.44 ± 1.11	13.18 ± 3.96	39.98 ± 3.97
7	2.39 ± 1.00	0.46 ± 0.07	21.95 ± 3.31	2.63 ± 1.14	14.21 ± 4.18	41.53 ± 4.91
8	2.22 ± 0.89	0.44 ± 0.11	20.33 ± 2.95	2.74 ± 1.37	14.30 ± 4.38	39.26 ± 5.06
Average	2.45 ± 1.03	0.47 ± 0.18	21.23 ± 1.75	2.95 ± 1.75	14.07 ± 3.50	40.97 ± 1.59

S: Sample number
[a]Each sample consist of five sub sample

Table 4. Percentage of monounsaturated fatty acids of cultured healthy and WSD *P.monodon*

S[a]	16:1	17:1	18:1n-9	18:1n-7	20:1n-9	Total
Cultured Healthy shrimps						
1	1.53±0.42	0.35±0.03	8.87±2.63	2.53±0.59	1.20±0.32	15.06±1.97
2	1.72±0.71	0.31±0.02	7.73±1.43	2.58±0.46	1.21±0.26	14.09±0.89
3	1.55±0.20	0.22±0.36	9.12±2.35	2.67±0.09	1.01±0.04	14.70±1.64
4	2.15±1.52	0.46±0.11	8.13±2.77	2.57±0.18	0.98±0.14	14.55±1.30
Average	1.74±0.17	0.36±0.02	8.46±2.51	2.59±0.04	1.10±0.26	14.60±0.40
White Spot Diseased shrimps						
5	3.60±1.74	0.63±0.29	6.29±3.12	3.16±0.70	1.06±0.23	15.22±3.04
6	3.02±1.55	0.51±0.24	5.95±3.18	2.73±0.61	1.17±0.45	13.97±3.57
7	3.31±1.11	0.48±0.19	7.01±3.67	3.25±1.01	0.93±0.25	15.32±3.79
8	2.64±0.56	0.68±0.19	8.31±2.81	2.95±0.46	1.22±0.24	15.92±1.96
Average	3.14±1.15	0.57±0.25	6.89±3.09	3.02±0.67	1.09±0.31	15.11±0.83

S: Sample number
[a]Each sample consist of five sub sample

Table 5. Percentage of polyunsaturated fatty acids of cultured healthy and WSD *P.monodon*

S[a]	*16:2n-4*	*16:4n-3*	*18:2n-6*	*18:3n-3*	*18:4n-3*	*20:4n-6*	*20:5n-3*	*22:4n-6*	*22:5n-3*	*22:6n-3*	*Total*	*n-3 PUFA*	*n-6 PUFA*	*n-3/n-6*
Cultured Healthy shrimps														
1	0.56 ±0.11	0.93 ±0.55	14.64 ±2.69	0.47 ±0.37	0.47 ±0.35	3.15 ±0.47	10.11 ±0.66	0.42 ±0.12	0.74 ±0.06	13.22 ±0.87	44.34 ±3.41	25.57 ±1.82	17.79 ±3.10	1.48 ±0.35
2	0.61 ±0.06	0.77 ±0.51	14.50 ±3.25	0.57 ±0.38	0.56 ±0.24	3.28 ±0.50	10.52 ±0.80	0.40 ±0.05	0.89 ±0.14	14.16 ±1.16	45.93 ±2.51	27.15 ±2.14	17.78 ±3.74	1.61 ±0.57
3	0.53 ±0.03	1.34 ±0.51	14.54 ±2.32	0.71 ±0.66	0.69 ±0.20	3.30 ±0.35	10.54 ±0.89	0.42 ±0.09	0.72 ±0.24	13.92 ±0.41	45.99 ±2.29	27.22 ±1.17	17.85 ±2.67	1.55 ±0.33
4	0.68 ±0.26	1.29 ±1.08	13.46 ±3.37	0.32 ±0.09	0.67 ±0.19	3.16 ±0.57	9.78 ±0.60	0.35 ±0.07	0.73 ±0.10	12.84 ±1.28	43.03 ±5.89	25.43 ±1.83	16.82 ±4.09	1.57 ±0.37
Ave	0.59 ±0.16	1.08 ±0.78	14.32 ±3.10	0.52 ±0.38	0.62 ±0.25	3.22 ±0.51	10.24 ±0.86	0.40 ±0.08	0.77 ±0.18	13.35 ±1.16	44.82 ±1.41	26.34 ±0.97	17.56 ±0.49	1.55 ±0.05
White Spot Diseased shrimps														
5	5.52 ±0.23	0.98 ±0.27	7.90 ±3.30	0.81 ±0.88	0.42 ±0.10	3.23 ±2.89	10.91 ±2.55	0.27 ±0.07	0.94 ±0.50	11.23 ±3.94	36.27 ±8.52	25.05 ±6.34	11.13 ±2.89	2.28 ±0.36
6	0.41 ±0.12	1.31 ±0.26	8.57 ±1.30	0.69 ±0.09	0.48 ±0.08	3.58 ±0.18	11.73 ±0.98	0.27 ±0.04	0.91 ±0.56	13.07 ±0.92	40.43 ±4.29	27.68 ±4.05	12.15 ±0.82	2.27 ±0.28
7	0.27 ±0.08	1.02 ±0.25	9.63 ±1.50	0.52 ±0.25	0.51 ±0.09	2.11 ±0.45	11.08 ±2.11	0.34 ±0.06	0.78 ±0.30	13.29 ±3.62	39.03 ±6.52	26.82 ±5.12	11.73 ±1.85	2.29 ±0.33
8	0.37 ±0.06	1.50 ±0.41	9.42 ±1.00	0.46 ±0.17	0.45 ±0.26	2.33 ±0.60	9.81 ±1.53	0.29 ±0.07	1.10 ±0.47	14.08 ±2.21	40.24 ±4.59	28.06 ±3.90	11.75 ±1.05	2.38 ±0.20
Ave	0.28 ±0.06	1.15 ±0.13	9.29 ±2.0	0.51 ±0.42	0.46 ±0.13	2.50 ±1.49	11.20 ±1.76	0.30 ±0.06	0.82 ±0.37	13.20 ±3.28	38.93 ±1.92	26.89 ±1.33	11.69 ±0.41	2.30 ±0.05

S: Sample number
[a]Each sample consist of five sub sample

extracts from crustaceans have antiviral activities and that this activity significantly inactivated the tissue through lipid extraction (Jingzhi et al. 2000).

A significantly higher percentage of lipid in WSD shrimp has not been previously discussed in the literature. It is possible that the hepatopancreas of diseased *P. monodon* may be affected and this is reflected in lipid profile. But Mohan and Shankar (1998) have reported that WSD does not infect tissues of endodermal origin such as hepatopancreatic tubule epithelia and mid gut epithelia. But since the intracellular virus replicates in the nucleus of all cells of ectodermal and mesodermal origin including muscle tissue (Lo et al. 1997a) it is possible that this may interfere with the biochemical pathways which are responsible for lipid metabolism and finally would affect the muscle lipid levels. Thus, it is possible that it may interfere with lipid metabolism and affect lipid levels in those cells. The lethargic behavior of WSD shrimp may be related to interference in lipid catabolism, resulting in elevated lipid levels. However more studies are needed to confirm this assumption.

According to Changhu et al. (1993) cultured *P. monodon* contains 17:3 ± 1.6% of C18:2*n*-6 fatty acid. The comparison of fatty acids of cultured healthy and WSD *P. monodon* shows that healthy and diseased *P. monodon* differ from each other in the content of C18:0, and C18:2n-6 fatty acid levels. Infected *P. monodon* have higher percentages of mono-saturated fatty acids than the healthy P. *monodon*. This is mainly due to the significantly high amount of C18:0 fatty acid in WSD shrimp. This study shows a remarkable decrease in the levels of 18:2*n*-6 in WSD *P. monodon* (9.3% ± 2.0). The C16:1 fatty acids of infected *P. monodon* show significantly elevated levels (3.14% ± 1.15) when compared with the healthy condition (1.74% ± 0.17). These observations are rarely discussed in literature.

Among the *n*-3 polyunsaturated fatty acids (PUFA s) 20:5*n*-3 and 22:6*n*-3 contributed very high proportion to the total n-3 PUFAs (Edirisinghe 1999). The present investigation also shows that the body flesh of *P. monodon* is a rich source of both 20:5*n*-3 and 22:6*n*-3.

The present study indicates that the low level of 18:2*n*-6 and high level of C16:1 may be an indicator of infection by WSD. Though linoleic acid (18:2*n*-6) is very important in human nutrition and health specially for membrane structure fluidity, skin integrity and numerous tissue functions, the expected nutritional values cannot be gained by consuming WSD shrimps. Importance of 16:1 fatty acid on human health and nutrition are rarely discussed in literature. So elevated levels of 16:1 fatty acid in WSD shrimp may not have any remarkable effect on human health.

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