Probiotic administration of *Litopenaeus vannamei*: Is there any negative effect on the fatty acid profile of meat?

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Abstract

It has been found that appropriate probiotic applications increased growth performance and disease resistance in shrimp. *Bacillus subtilis* has been suggested as a potent probiotic in improving growth performance and enhancing immune response in white shrimp, *Litopenaeus vannamei*. The aim of this work was to evaluate the possible effect of *B. subtilis* administration on the meat fatty acid profile of white shrimp, *L. vannamei*. Two groups of shrimps received *B. subtilis* strains L10 and G1 from the *B. subtilis*-supplemented feed (10^5 and 10^8 CFU g^{-1}) while two other groups received it from the rearing water (10^5 and 10^8 CFU ml^{-1}). One group received no *B. subtilis* and served as control. According to the results, there was no significant difference between the muscle fatty acid profiles of shrimps administrated by probiotic and control group. This study showed that *B. subtilis* administration, in either diets or water, did not have any negative effect on fatty acid profiles of *L. vannamei* meat.

Keywords: Probiotic, Fatty acid, *Litopenaeus vannamei*, Nutrition

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Introduction

Probiotics are defined as a live microbial supplement which beneficially affects the host animal by improving its microbial balance (Gram *et al.*, 1999). It has been found that appropriate probiotic application would increase growth performance and disease resistance in shrimp (Castex *et al*., 2008, van Hai and Fotedar, 2010). A variety of microbial species have been successfully applied as probiotics in shrimp including species of *Bacillus*(Balcázar and Rojas-Luna, 2007), *Lactobacillus*(Chiu *et al.*, 2007) and *Pseudomonas*(Alavandi *et al*., 2004).

Several mechanisms of action of probiotics have been found in human and terrestrial animals including prevention of overgrowth of potentially pathogenic micro-organisms, stimulation of the intestinal immune defense system, participation in the regulation of intestinal functions such as mucus utilization and nutrient absorption, production of nutrients and micronutrients of special importance such as fatty acids and vitamins (see the reviews by Bengmark, 1996 and Fuller, 1989). Some of the above mentioned functions have also been found in shrimp including production of inhibitory compounds in the intestine of the host as well as acting as an immune ~~e~~stimulant (see the review by Verschuere *et al*., 2000). However, there is no unambiguous experimental data on the production of nutrients, such as fatty acids in the intestine of aquatic animals administrated by probiotics.

It has been shown that administration of *Lactobacillus amylovorus* and *Enterococcus faecium*mixed culture increases monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in pig meat (Ross *et al*., 2012). On the other hand, there is evidence that supplementation of commercial probiotic containing *Saccharomyces cerevisiae* and *L.acidophilus* reduces the 18:3n-3 concentration in goat plasma (Paengkoum and Yong, 2009). Dietary supplementation of probiotics has been also found to play an important role in altering the lipid metabolism of chickens and to affect their egg fatty acid profile (Mikulski *et al*., 2012). As far as we know, there is currently no published information about the effect of probiotic administration on the meat fatty acid profile of fish and crustaceans.

*B.subtilis*has been suggested as a potent probiotic in improving growth performance and enhancing immune response in white shrimp, *L.vannamei* (Zokaeifar *et al.*, 2012b). Shrimp are regarded as the world's most popular shellfish and considered as a good source of long chain n-3 PUFA (Turan *et al.*, 2011). Therefore, any probiotic administration is needed to be monitored to prevent n-3 PUFA reduction in their meat. Any dietary manipulation and/or probiotic administration that change shrimp fatty acid profile may affect its nutritional value for final human consumption. The aim of the present study was to evaluate the possible effect of*B. subtilis* administration on the meat fatty acid profile of white shrimp, *L. vannamei*.

Materials and Methods

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Bacterial strains and Culture Conditions

B.subtilis strains L_{10} and G_1wereisolated and identified from fermented pickles (Zokaeifar *et al.*, 2012a), and used as probiotics. Probiotic strains were kept at -20 °C in Luria-Bertani broth (LB; Difco) containing 15% v/v sterile glycerol. Cultures were further activated by growing in LB broth at 30°C for 48h as explained by Zokaeifar *et al*. (2012b).

Experimental Conditions

Pathogen-free *L. vannamei* juveniles of an initial weight of 0.70 ± 0.10 g (mean ± SD) were obtained from the Marine Science Research Station and Biology Field Station, Universiti Putra Malaysia, and the experiment was conducted at the same place. The shrimp were randomly distributed into 10 fiberglass tanks containing 500 l seawater with the stocking density of 100 shrimps per tank and acclimated for 3 days prior to start of the grow out experiment.

Two groups of shrimp received *B. subtilis* from the *B. subtilis*-supplemented feed while two other groups received this from the rearing water. For the feed groups, a mixture of *B. subtilis* strains L10 and G1 were sprayed over a commercial *vannamei* feed (Blanca, Charoen Pokphand Malaysia; crude protein 35%; crude fat 5%) to give a final concentration of approximately 10^5 CFU g^{-1} (L10, 5×10^4 and G1, 5×10^4 CFU g^{-1}), namedas FB5 and 10^8 CFU g^{-1} (L10, 5×10^7 and G1, 5×10^7 CFU g^{-1}), named asFB8. For the water groups, mixtures of *B. subtilis* strains L10 and G1 were added into the rearing water to give a final concentration of approximately 10^5 CFU ml^{-1} (L10, 5×10^4 and G1, 5×10^4 CFU ml^{-1}) name das WB5 and 10^8 CFU ml^{-1} (L10, 5×10^7 and G1, 5×10^7 CFU ml^{-1}) named asWB8. One group served as the control and received probiotics from neither food nor rearing water. Both water groups as well as control group were fed un-supplemented commercial feed. The experiment was conducted in duplicate for 8 weeks. Feed and rearing water supplementation procedures were done twice a week. Shrimps were fed three times a day at 5% of body weight. At the end of the experiment, 5 shrimp from each replicate were randomly collected and their meat tissue was collected, lyophilized for 48 h and stored at -20 °C for further fatty acid analysis.

Water Supply and Analysis

The water was supplied directly from the sea. After series of treatment and filtration process, the salinity was reduced to 20 ppt using freshwater. Continuous aeration was provided and kept at a dissolved oxygen level of 5.0±0.5 in each tank. Water quality was monitored every three days; pH was found between 7.4 and 8.6 and temperature ranged between 27 and 29°C. The nitrite-N, nitrate-N and ammonia-N were always within the acceptable ranges and found to be below 0.01, 4.0 and 1.1 mg l^{-1}, respectively.

Fatty Acid Analysis

Lipid from lyophilized meat of shrimp was extracted with a chloroform:methanol (2:1 v:v) mixture, saponated by KOH and transesterified with methanolic boron trifluoride(Kamarudin *et al.*, 2012). Fatty acid methyl esters (FAMEs) were then analyzed using a gas chromatograph (Agilent 7890A) equipped with a split/splitless injector (ratio 1:10), a fused

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silica capillary column (Supelco SP-2330: 30m × 0.25mm, 0.20 µm in film thickness) and a flame ionization detector (FID). High purity hydrogen was used as the carrier gas. Column temperature was set at 100°C for 2 minutes, increased to 170°C at a rate of 10°C/min, maintained for 2 minutes and increased again from 170 to 200°C at a rate of 7.5°C/min and maintained at 200°C for 20 minutes. Injector and detector temperature were 250 and 300°C, respectively. Fatty acids were identified by comparing the relative retention time with 37 component FAME mix standards (Supelco, Bellefonte PA, USA) and menhaden oil. The results were expressed as the area percentage of total identified fatty acids.

Statistical Analysis

Homogeneity of variances was tested using Levene's test, and data identified as nonhomogeneous were subjected to arcsine transformation before statistical analysis. All experimental data were analyzed by one-way analysis of variance (ANOVA). Significantly different means were then elucidated using Duncan's multiple range test. Statistical tests were conducted at 95 % confidence level using SPSS 19 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The muscle fatty acid profiles of shrimp administrated with probiotic as well as the control group are shown in Table 1. The carbon chain length of the identified fatty acids was between 14C to 22C with 0–6 double bonds. The major fatty acids identified in the muscle of shrimps were C14:0, C16:0, C18:0, C18:1n-9, C18:2n-6, C20:4n-6 (arachidonic acid; ARA), C20:5n-3 (eicosapentaenoic acid; EPA) and C22:6n-3 (docosahexaenoic acid; DHA) while the most abundant fatty acids were found to be C16:0, C18:2n-6, C18:0, C18:1n-9, EPA and DHA, ranging from 83.5% to 85.8% of the total fatty acids in the muscle of *L. vannamei*. The latter fatty acids except for C18:2n-6 were found as the major constituents of fatty acid profiles in muscle of *L. vannamei*(Montano and Navarro, 1996). The high amount of C18:2n-6 in the muscle of all examined groups in this study was most probably due to high ratio of this fatty acid in their commercial diet. It has been shown that high inclusion of C18:2n-6 in the diet of *L. vannamei* increases the percentage of this fatty acid in their body tissues (Zhou *et al*., 2007).

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Table 1: Fatty acid profile (% of total fatty acid) of meat of *Litopenaeusvannamei*cultured with probiotics *Bacillus subtilis*, strains L10 and G1 or fed with*B. Subtilis*-supplemented diet

Fatty acid	Treatments				
	Control	FB5	FB8	WB5	WB8
14:0	2.67 ± 0.43	3.12 ± 1.13	3.05 ± 1.28	1.51 ± 0.31	2.66 ± 0.94
15:0	1.19 ± 0.09	1.19 ± 0.05	0.88 ± 0.20	1.13 ± 0.04	1.00 ± 0.13
15:1	0.67 ± 0.08	0.86 ± 0.39	1.00 ± 0.39	0.68 ± 0.12	0.83 ± 0.10
16:0	20.09 ± 0.55	20.12 ± 0.61	21.17 ± 0.63	21.36 ± 0.27	20.04 ± 0.83
16:1n-7	1.46 ± 0.13	1.02 ± 0.12	1.07 ± 0.11	1.13 ± 0.08	1.47 ± 0.32
17:0	0.89 ± 0.16	0.71 ± 0.36	0.75 ± 0.17	1.03 ± 0.05	1.04 ± 0.10
17:1n-7	1.36 ± 0.10	1.57 ± 0.04	1.80 ± 0.23	1.30 ± 0.08	1.44 ± 0.07
18:0	14.11 ± 0.43	13.90 ± 0.58	14.17 ± 0.23	14.27 ± 0.76	13.65 ± 1.02
18:1n-9	13.63 ± 1.45	12.18 ± 0.46	13.80 ± 1.28	11.96 ± 1.29	14.39 ± 1.72
18:2n-6	19.06 ± 0.17	20.10 ± 0.64	20.15 ± 0.77	19.93 ± 0.69	19.71 ± 1.12
18:3n-3	1.56 ± 0.04	2.04 ± 0.23	2.11 ± 0.83	1.38 ± 0.26	1.88 ± 0.48
21:0	1.09 ± 0.08	1.82 ± 0.40	1.18 ± 0.25	1.27 ± 0.03	1.33 ± 0.09
20:4n-6	4.10 ± 0.11	3.39 ± 0.36	2.90 ± 0.22	3.22 ± 0.09	3.27 ± 0.32
20:5n-3	10.14 ± 0.47	9.88 ± 0.54	8.26 ± 0.41	10.32 ± 0.56	8.92 ± 0.66
22:5n-3	0.79 ± 0.14	0.75 ± 0.29	1.35 ± 0.48	1.59 ± 0.79	1.07 ± 0.27
22:6n-3	7.19 ± 0.07	7.35 ± 0.27	6.35 ± 0.79	7.91 ± 0.47	7.30 ± 0.62

Values reported are mean ± SE (n=10).

The distribution pattern of the fatty acids sum in total fatty acids within shrimp muscle was: PUFA > saturated fatty acid (SFA) > MUFA (Table 2). A similar distribution pattern has been found for *Macrobrachium rosenbergii* (PUFA: 43% > SFA: 35% > MUFA: 22% of total fatty acid) (Bragagnolo and Rodriguez-Amaya, 2001), *Penaeus monodon*(PUFA: 44.3% > SFA: 35.4% > MUFA: 20.3% of total fatty acid) and *L. vannamei*(PUFA: 42.2% > SFA: 35.8% > MUFA: 22% of total fatty acid)(Sriket*et al.*, 2007). However, this pattern can be extremely influenced by diet, season and geographical zone (Soriguer *et al.*, 1997, Tziouveli and Smith, 2012, Yanar, 2005).

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Table 2: Sums of SFA, MUFA and PUFA (% of total fatty acid) in the meat of *Litopenaeusvannamei* cultured with probiotics *Bacillus subtilis*, strains L10 and G1 or fed with*B. subtilis*-supplemented diet

Fatty acid	Treatments				
	Control	FB5	FB8	WB5	WB8
∑ SFA	40.04 ± 0.96	40.86 ± 0.70	41.21 ± 1.10	40.57 ± 1.23	39.72 ± 2.55
∑ MUFA	17.12 ± 1.34	15.63 ± 0.71	17.68 ± 1.51	15.06 ± 1.10	18.13 ± 1.88
∑ PUFAn-3	19.68 ± 0.45	20.01 ± 0.44	18.06 ± 0.23	21.21 ± 0.56	19.18 ± 1.59
∑ PUFAn-6	23.16 ± 0.21	23.50 ± 0.97	23.05 ± 0.82	23.16 ± 0.78	22.97 ± 1.31
n-3/n-6	0.85 ± 0.02	0.86 ± 0.02	0.79 ± 0.04	0.92 ± 0.02	0.83 ± 0.03
EPA/DHA	1.41 ± 0.06	1.34 ± 0.05	1.34 ± 0.14	1.31 ± 0.02	1.23 ± 0.08
PUFA/SFA	1.07 ± 0.02	1.07 ± 0.05	1.00 ± 0.02	1.10 ± 0.06	1.08 ± 0.13

Values reported are mean ± SE (n=10).

Discussion

In the current experiment, there was no effect of probiotics, added either to feed or rearing water, on the individual fatty acid or sum of the fatty acids in the muscle of *L. vannamei*, compared to the control group. Recently, extensive consideration has been given to the potential of probiotics in changing lipid metabolism in terrestrial animals. However, most studies have focused primarily on serum fatty acid alteration imposed by probiotic administration (Ashayerizadeh *et al*., 2009, Paengkoum and Yong, 2009); and information about the effect of probiotics in altering meat fatty acid profile is still scarce. Ross *et al*., 2012 reported that inclusion of *Lactobacillus amylovorus* and *Enterococcus faecium* mixed culture increases total MUFA, C18:2n-6 and C18:3n-3 in pig meatsignificantly. This could be due to the ability of some bacteria to produce conjugated linoleic acid and affect host metabolism and fat composition in liver and adipose tissue in different animal species (Wall *et al.*, 2012). According to our results, *B. subtilis* strains L10 and G1 should not be able to produce fatty acid in shrimp intestine in a way that affects tissue fatty acid composition. On the other hand, probiotic administration has a potential to facilitate biohydrogenation of unsaturated fatty acids to more saturated ones and reduce the unsaturated FA to saturated FA ratio in the body of ruminants (Paengkoum and Yong, 2009), which is not a desirable alteration for human health. Such a negative effect of probiotic has not been observed in broiler chicken. Král *et al*. (2013) reported that there is no significant difference between SFA, MUFA and PUFA amounts in the meat of broiler chicken fed with *B.subtilis*-supplemented diet and those fed diet with no probiotic supplementation. Our results also showed that supplementation of shrimp feed with *B. subtilis* did not increase SFA amount in their muscle. The PUFA/SFA ratios in the muscle of all shrimps ranged from 1.00 to

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1.10 without any significant difference. Saturated and trans fatty acids increase cardiovascular risk, while both n-6 and n-3 PUFAs have been associated with lower cardiovascular risk (Erkkila *et al.*, 2008). Meanwhile, the n-3/n-6 ratio is known as an important factor in human diet and many health disorders are linked with high intake of n-6 fatty acids through diet (Steffens, 1997). The n-3/n-6 ratios in our study were found to be between 0.79 and 0.92 and there was no significant difference between different treatments.

Results of this study revealed that *B. subtilis* strains L10 and G1 administration, either through the rearing water or by feed supplementation, did not change the fatty acid profiles of total lipid content of muscle of *L. vannamei*. Therefore, *B. subtilis* should not be able to produce fatty acids in shrimp intestine in a way that affects their tissue fatty acid composition. Moreover, *B. subtilis* administration does not have any negative effect on fatty acid profiles of *L. vannamei* meat.

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growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol,*33**,** 683-689.

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