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The Effects of Different Intensive Culture Systems on White Shrimp (*Litopenaeus vannamei*) Muscle Protein Pattern

Ni Nyoman Dian Martini*, Happy Nursyam, and Mohamad Fadjar
Fisheries and Marine Science Faculty, University of Brawijaya, Indonesia
*Corresponding author's e-mail: martininyoman@ymail.com

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ABSTRACT: Application of biofloc system, a heterotrophic culture with minimal water exchange, has gained interest particularly in relation to providing high productivity, low feed-conversion ratios, and a stable culture environment, especially in shrimp. In biofloc system, heterotrophic bacterial tends to form noticeable aggregates (biofloc), which can be consumed by shrimp as a natural protein source. In relation to this, the impact of different culture systems on white shrimp (*L. vannamei*) muscle protein patterns was investigated. In this research, an SDS-PAGE study was performed on white shrimp muscle tissue along the production cycle in intensive shrimp culture ponds with different culture systems (Phytoplankton, Semi-Biofloc, and Biofloc). The study was located in different areas of the District of Tuban, East Java. The aim of this study was to accomplish systematic characterization of the white shrimp muscle protein pattern, which derives from each of the ponds with different rearing systems. The result of the study showed that the biofloc system yields variability of protein pattern as it maintains good water quality throughout the culture and provides an alternative protein source (as biofloc) for the shrimp besides the pellet.
Key words: White shrimp (*L. vannamei*), biofloc system, muscle protein pattern, SDS-PAGE

INTRODUCTION

Shrimp is one of the most popular and healthy seafood as it is rich in protein and minerals and is very low in fat and calories and it is also the most valuable traded marine product in the world and is producing now well over four million tons [1]. White shrimp (*L. vannamei*) is a species of crustacean that can be reared in intensive ponds. There are a number of techniques that are generally used in intensive culture technology of rearing shrimp, namely, phytoplankton, semi-biofloc, and biofloc. These days, the closed 'biofloc' system has gained more interest among the shrimp farmers than the other techniques because this system supports the management of wastes and simultaneously recycles them to become dietary protein source so that it offers sustainability and compatibility of better environment for the shrimp farming community [2] as well as better growth of the shrimp in relation to the availability of an additional natural protein source in the culture pond [3].

Unlike the conventional (e.g. the open) culture system, the closed biofloc culture system uses two dietary sources for the reared biota, namely, pellet and biofloc. Dietary shift can affect shrimp metabolic and physiological responses to phyto-chemical components present in the diet at the molecular level. This describes on how specific nutrients and compounds produce specific changes at the molecular level, which in turn cause metabolic and physiological changes in shrimp [4]. In the field known as 'nutrigenomics', researchers will be able to understand how different components of the diet influence molecular mechanisms which in turn determine shrimp physiology, and in this way find strategies to optimize nutrient use and increase the quality of the final product [5].

Protein expression is influenced by dietary protein source [4], however little is known about protein patterns of muscle in shrimp reared under different cultivation conditions. The present study was therefore undertaken to investigate the effect of different cultivation systems in regards with input diet on the differentiation of muscle protein patterns in white shrimp.

MATERIAL AND METHODS

Sampling Technique

The sample investigated in this study was taken from white shrimp (*L. vannamei*) intensive rearing ponds with different culture systems involving phytoplankton system, semi-biofloc system, and biofloc system respectively in the District of Tuban, East Java. The shrimp sample was taken from culture system A (phytoplankton), culture system B (semi-biofloc), and culture system C (biofloc), each consisting of three rearing ponds, when the white shrimp was at the age of 0, 40 and 70 days. The white shrimp was then put in a plastic bag one by one, which was afterwards placed in a cool box before it went to a freezer with a -20^{o}C temperature.

Protein Extraction

Protein extraction was done by homogenating 0.5 gram of white shrimp muscle with 400 µl buffer extract (50 mMTris-Cl pH 7.5, 0.1 mM EDTA, 1mM merkaptoethanol or dithiothreitol, and 1 mM PMSF in DMSO). The homogenate was put in a 1.5 ml tube, which was then centrifugated at the speed of 10.000 rpm for 10 minutes under a temperature of 4^{o}C. The supernatant was then put in another 1.5 ml tube and kept in a temperature of -20^{o}C [6].

Measuring the Protein Concentration

The concentration of protein was measured using UV-Vis Quantification [7]. A mass of 10 µl protein sample with 990 µl PBS pH 7.4 was measured at a wave length of 280 nm and 260 nm. The protein concentration (mg/ml) was measured using the formula: Concentration (mg/ml) = (1.55 x A280) – (0.76 x A260).

Preparing the Protein Sample

The preparation of protein sample involves a treatment of 50 µl sample with 100 µl reducing buffer sample (0.1 M Tris-Cl pH 6.8, 20% gliserol, 4% SDS, 2 ml Merkaptoethanol, 0.001% bromophenol blue). The sample was heated at a temperature of 100^{o}C for 5 minutes and used for electrophoresis or kept under a temperature of -20^{o}C [6].

Electrophoresis and Measurement of Molecular Weight

The analysis of the protein bands of the white shrimp muscle used Laemmli's SDS PAGE method called "Denaturing (SDS) Discontinuous Gel Electrophoresis." The 12.5 % separating gel was made of a composition of 2480 µl polyacrylamide 30%, 1500 µl Tris-Cl pH 8.8, 1818 µl aquadest, 75 µl SDS 10%, 75 µl acid persulfate (APS) 10%, and 5 µl TEMED. A solution of 5% stacking gel was composed of 450 µl polyacrylamide 30%, aquadest 2100 µl, 380 µl Tris-Cl pH 6.8, 30 µl SDS 10%, 30 µl APS 10% and 5µl TEMED. An amount of 5µl sample was put in each well of the gel. The protein marker used ranges from 15 to 260 kDa molecular weight. Electrophoresis was done at a voltage of 100 volt for two hours. Finally the gel was stained overnight with Coormassie blue R-250. Measurement of the protein molecular weight was done by measuring the migration distance of protein bands [8].

RESULTS AND DISCUSSION

Protein Composition of White Shrimp Muscle

The result of the study showed that the dominant myofibrillar protein content in the muscle structure of *L. vannamei* treated under different culture systems using SDS-PAGE consisted of myosin and actin (Figures 1 and 2). The myosin consisted of myosin high chain (MHC) with molecular weight (MW) of about 200 kDa [9, 10], subfragment-2 heavy chain (S2) (about 66.3 kDa) [10], and myosin light chain (MLC) with MW of about 20 kDa [10] or about 12-22 kDa [11]. The actin consisted of alpha actinin (αA) (about 97 kDa) [12] or about 94-103 kDa [13], actin (about 45 kDa) [9], and beta actin (βA) (about 42 kDa) [14]. Besides, other types of myofibrillar protein were also detected, namely, paramyosin and isoform paramyosin with MW of about 105-110 kDa [15] or about 100-140 kDa [16], troponin T (TnT) (about 50-55 kDa) [17], tropomyosin (about 37 kDa) [17], and troponin I (TnI) (about 24-32 kDa) [17].

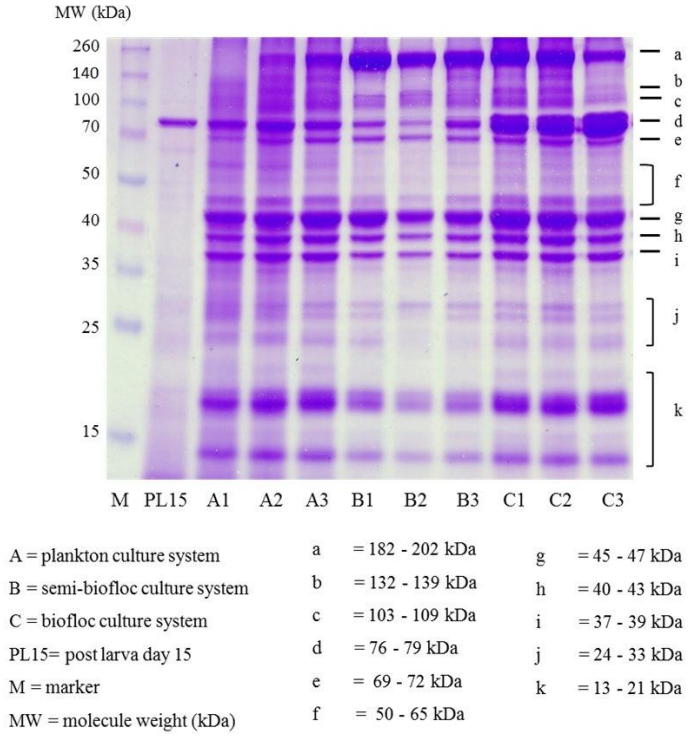

Figure 1. SDS-PAGE/Coomassie blue-staining analysis of muscle protein extracts from *L. vannamei* DOC 0 (PL15) and DOC 40

The Effect of Day Culture on Protein Pattern of White Shrimp Muscle

There was a difference in protein band pattern between the muscle structure of *L. vannamei* at the age of 40 days and that at the age of 70 days, that is, at the age of 40 days and 70 days there was a mean total of successively 22 and 15 protein bands as detected using SDS-PAGE. At the age of 70 days there was some amount of myofibrillar protein of *L. vannamei* with a molecular mass lower than that of *L. vannamei* at the age of 40 days. That is, as the study showed, the paramyosin and paramyosin isoform of *L. vannamei* at the age of 40 days and 70 days were respectively found to be about 123-139 kDa and 97-107 kDa; alpha actinin was respectively found to be about 109-115 kDa and 92-96 kDa; troponin T was respectively found to be about 50-59 kDa and 50-52 kDa; and MLC was respectively found to be about 13-21 kDa and 13-17 kDa. This difference was caused by the transition or adaptation toward the developmental stages of the shrimp muscle structure at the age of 40 days and that at the age of 70 days. The occurring transitional protein development may correlate with the rise in the maximum shortening speed of the fast muscle fibers during the life span of the shrimp. The different successions of protein isoforms indicate the independent regulation of different syntheses of myosin sub-units. The molecular change in the muscle contractile characteristic or the phenotype of the muscle structure causes a change in the gene expression pattern of crustacean muscle protein [17]. The main function of muscular system is served in eating and moving. The muscle molecular structure will continuously develop in accordance with the function of certain protein in the muscle as the effect of the expression variation of the myofibrillar protein isoforms in the muscle as such [18]. The variation of muscle development of *L. vannamei* in DOC 40 and DOC 70 in this study may relate to the change of expression in the muscle protein in which there is a molecular correlation toward kinetic and behavioral change. Myofibrillar protein is found in some isoform types which originate from multigene family (isogene). Additional isoform, including the product of tropomyosin, MLC, and troponin, can result from the same gene through an alternative promoter. The isogene expression patterns vary along the muscle development, which is related to the origin of myogenic cell and different primary/secondary fiber generations and is influenced by hormonal and nervous conditions [18].

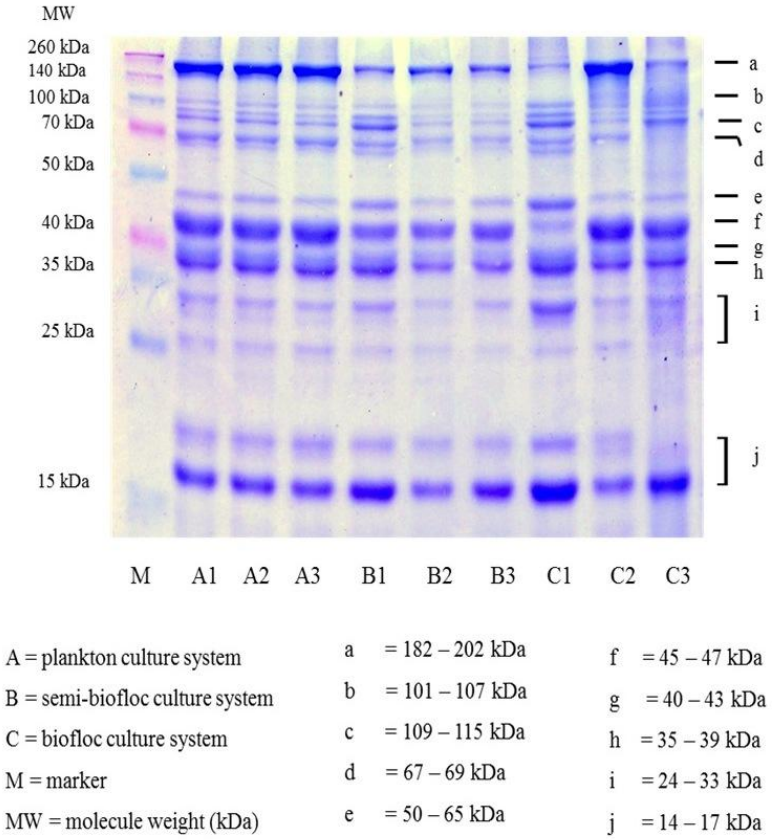

Figure 2. SDS-PAGE/Coomassie blue-staining analysis of muscle protein extracts from *L. vannamei* DOC 70

The Effect of Culture System on Protein Pattern of White Shrimp Muscle

The protein bands structure pattern of *L. vannamei* at the age of 40 days (DOC 40/day of culture 40) (Figure 1) did not show any difference among the three culture systems, namely, plankton, semi-biofloc, and biofloc culture systems. It was observed that in the protein band with MW of about 76 kDa the muscle structure of *L. vannamei* from the biofloc culture system has the highest thickness intensity as compared with those from the other culture systems, like the case observed when the shrimp was at DOC 0 (PL 15). The thickness of protein band indicates the concentration of the protein, in which protein with thicker intensity has higher concentration [19]. Protein with MW 76 kDa is the type of glycoprotein that is found in crustaceans which is called *prophenoloxidase.* Another study also found this glycoprotein in the form of enzyme called *prophenoloxidase* (proPO) in *Penaeus monodon* using a cloning technique at MW 78.7 kDa [20]. Further, it is reported that proPO constitutes an important defense component of invertebrates, including crustaceans, in combating pathogen. They also found this proPO protein at MW 76 kDa through purification of the blood cells of fresh water lobsters (*Pacifastacus leniusculus*) [21].

It was further found that at the age of 70 days (Figure 2) the protein bands pattern of the muscle structure of *L. vannamei* did not show any difference between the plankton culture system and the semi-biofloc system, but showed a difference in the biofloc culture system. It was found that in C1, C2, and C3 wells, which contained the muscle structures of *L. vannamei* taken respectively from ponds 1, 2, and 3, in which the biofloc culture system was applied, there was an addition of protein band at MW of about 35 kDa. In C2 well, there was an addition of protein band MW of about 18 kDa and 110 kDa as well as a deletion of protein band at MW 63 kDa. An addition of protein band was also found in C3 well, which was at MW of about 57 kDa and 110 kDa, besides a deletion of protein band at MW 63 kDa and 66 kDa. The C1 well showed higher protein bands density than that of C2 and C3 wells, that is, in alpha actinin, troponin T, beta actinin, tropomyosin, troponin I, and MLC. The difference in this protein bands pattern may be caused by differentiation of the protein gene expression of the shrimp's (*L. vannamei*) muscle structure in responding to the change or combination of nutrition obtained during the rearing.

Unlike the phytoplankton and the semi-biofloc system, the biofloc culture system uses two dietary sources for the reared biota, namely, pellet and biofloc. The differentiation in the dietary protein sources during the rearing activities influences the protein nutrition value, that is, its amino-acid content, which in turn influences the protein quality or protein profile of the flesh [22]. Shrimp has the habit of eating a small bit of food but doing it often [23]. White shrimp (*L. vannamei*) which is reared in ponds using biofloc culture system does not have to wait for the pellet feeding as there is food enough for it to eat in the ponds for 24 hours a day [24]. It has been proven that *L. vannamei* has the ability to utilize and keep the nitrogen contained in the biofloc and about 29% of its daily dietary intake may come from that floc [25]. In this study, differentiation of gene expression (at DOC 70) was not observed in the specimens that did not undergo a combination or a shift in diet during the time of the research, namely, the shrimp flesh from the plankton and semi-floc rearing ponds. This result was in line with other studies, which maintains that the interaction of nutrient with the sensory system in the cell causes a change in gene, protein expression, and the metabolite production in accordance with the level of nutrient signal captured so that different diets will result in different gene patterns, protein expressions, and metabolite productions [26].

CONCLUSION

The result of the study showed that the biofloc system yields variability of protein pattern as it maintains good water quality throughout the culture and provides an alternative protein source (as biofloc) for the shrimp besides the pellet. With the addition of nutrition in the form of biofloc in the *L. vannamei* diet, increased differentiation of specific gene expression of muscle structure can be obtained.

Recommendation

For future researches, we suggest further studies in the evaluation of the differentiation muscle proteins expression in other aquatic species with biofloc system used for the rearing.

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