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Histological observations of hepatopancreas, anterior intestine and stomach of white leg shrimp *Litopenaeus vannamei* (Boone, 1931) reared in biofloc shrimp farming

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Abstract
Biofloc technology is regarded as an approach that produces microbial flocs by degrading organic waste by microorganisms. The present study was executed to evaluate the effects of partial substitute of every day feeding intake with biofloc, and the histology of *Litopenaeus vannamei* a prawn species. The species was collected from the culture ponds during the period of December to march from Thirunagari, Tamil Nadu, India and were brought to the laboratory in live condition using seed bags for carrying out histological examination. Histological appearance of Hepato-pancreas, anterior intestine and stomach were observed for both control and biofloc cultured prawn. The obtained results clearly indicated that there were no significant histological alterations found between the control and Biofloc technology shrimp farming prawn. Only little more differences were observed in all three organs of biofloc shrimp farming prawn compared to the prawn from normal shrimp farming and the reason for this is not clear. There were no pathological changes and neat arrangements of cells in these organs evidenced the key role in digestion, absorption and storage of food in *L.vannamei*.

Keywords: Biofloc, *Litopenaeus vannamei*, histology, hepato-pancreas, anterior intestine, stomach

Introduction

Biofloc technology (BFT) shrimp farming is contemplate to avoid the deposition of poisonous nitrogen which are metabolizes in to NO_2, NH_3 and so on by manipulating and stimulating the carbon/nitrogen ratio (C/N) and altering such metabolic intermediates to microbial flocs, since in a minimum water exchange system (Avnimelech *et al.* 1994) [7]. The microbial flocs are enhanced out of protozoa, zooplankton, phytoplankton and hetero-trophic bacteria (Avnimelech 2007) [5]. The floc protein levels might varying between from 24 to 40 %, and the lipid levels from 4.6 to 8.3 %, because of differences in biofloc components (Avnimelech 2009) [6]. Thus, BFT is a cost-effective substitute for use in reducing the commercial diets of aquatic livings of ponds, ultimately lowering the possible environmental issues (Bauer *et al.*, 2012) [8]. The satisfied outcome of BFT on the activity of digestive enzyme (Becerra-Do´rame *et al.*, 2012) [9], growth performance (Crab *et al.*, 2010; Ekasari *et al.*, 2015) [10, 13], immune response (Xu *et al.*, 2013) [36] and water quality parameters (Mega-hed 2010) were revealed in a number of aquatic species.

Histological studies are a remarkable and promising field to understand the structural organization that occurs in the organs of the aquatic organisms due to contamination in the environment and have been extensively used to describe the health status of these organisms. Histological changes in tissues of animals are powerful indicators of earlier exposure to environmental stresses and are the outcome of unfavourable biochemical and physiological changes in an organism.

Crustacean is no exception to the general rule that all living organisms are subject to diseases. Various workers have shown that the diseases that results in prawn populations are end results of synergistic action of stressors like variations in the environmental factors, viruses, bacteria, fungi, protozoan parasites, chemical pollutants, other toxic substances and nutritional factors which influence much in tilting the homoeostatic mechanisms to bring about distress to the animal health (Iffat Jahan *et al.*, 2017; Anjaini *et al.*,2018 ; Madhuri *et al.*, 2018 ; Thomas Caceci *et al.*, 2019 ; Ruiz *et al.*, 2020) [18, 4, 21, 33].

Hepatopancreas (HP) is one of the large gland which carries out all the works of a liver and pancreas and employ as an indicator in the shrimp body specifically used to find out the healthiness of shrimp. Its foremost function is to regulate and transportng ions and water (Sousa and Petriella, 2006) [31]. Histological examination of intestine is invaluable since they relate distinct morphological architecture with physiological functions including digestion of food, nutrient absorption and adapt to the changes in salinity also to know the nutritional distress and to assess the disorders (Sousa and Petriella, 2006) [31].

The stomach plays a significant role in grinding and masticating the food and also serves as the indicator which can be used to identify the health conditions of shrimp. The feeding pattern of decapod crustaceans can be generally known from morphology of the foregut (Albertoni *et al.* 2003) [3]. There is inadequate findings regarding the histology of stomach but only little information is available on morphology of the digestive tract, makes concern to the commercially important species (Abrunhosa and Melo 2008) [1].

Histological changes have been characterized in many crustacean species (Iffat Jahan *et al.*, 2017; Anjaini *et al.*,2018; Madhuri *et al.*, 2018 ; Thomas Caceci *et al.*, 2019; Ruiz *et al.*, 2020) [18, 4, 21, 33, 27]. Until now, no literatures are available related to the histology of shrimp cultured in biofloc farming. Hence, an attempt was made to find the histological appearance of hepatopancreas, anterior intestine and stomach of white shrimp *Litopenaeus vannamei* reared in both normal and biofloc shrimp farming.

Materials and method

Litopenaeus vannamei prawn species was collected from the normal as well as biofloc shrimp forming during the period of December to march from Thirunagari, Tamil Nadu, and India and were brought to the laboratory in live condition using seed bags to carrying out histological examination. The *L. Vannamei* from normal shrimp farming is considered as control. The fields were suitable for both seasonal and perennial prawn culture system.

Histological studies

The animals were dissected and selected tissues (hepatopancreas, anterior intestine and stomach) were pooled out for histological studies. The tissues were fixed in 5% formalin for 72 hrs. The fixed tissues were washed in running tap water over night and then dehydrated in ascending grades of alcoholic series. For block making, paraffin wax of melting point 58-60º C was used. Sections cut at 5 micrometer in thickness were finished and stained in Delafield's haematoxylin with eosin as counter strain. The photomicrographies of various sections of the tissues were taken for microscopic observations (Gurr, 1962) [15].

Results

General morphology of the hepatopancreas

Hepatopancreas is essentially composed of branched tubule and different type of epithelial cell lining the tubules. M cells, also known as basal cells, were triangular in shape and stayed in contact with the basal lamina, but their apex did not reach the hepatopancreatic tubule lumen and lacked microvilli. The nucleus is spherical and has many nucleoli, with extensive basophilia in the cytoplasm.

The F cells, also known as fibril cells were big in size with slightly elongated nucleus. The cells were prismatic and/or triangular in shape, with strong basophilia in the cytoplasm. This cell type penetrates the tubule lumen and contains microvilli.

Vesicular cells, also known as B cells, had a globular shape with a circular basal nucleus. They were the most numerous of the cell kinds discovered. The cytoplasm had many pinocytic apical vacuoles, one of which was always larger than the others and occupied a considerable portion of the cytoplasm. The B cell reached the hepatopancreas tubule lumen, the cytoplasm was acidophilic, and the apical region of the cytoplasm had microvilli.

R cells, also referred to as reabsorption cells, have a rounded nucleus in the basal region, a prismatic form, and multivacuolar and granular cytoplasm. They are not basophils like M, E, or F cells, but they are also not acidophilic like B cells. They reach the tubule's lumen and contain microvilli.

General morphology of the anterior intestine

The intestine joins the hindgut and flows caudally between the hepatopancreatic lobes. A thin layer of connective tissue surrounds the organ, which is lined by a simple columnar epithelium supported on an uneven PAS-positive basal membrane and displaying distinct hemolymph gaps. One cell type can be found in the intestinal lining epithelium, and it has two separate acidophilic properties.

A modest brush border, cytoplasm with small subapical vesicles, and a central nucleus are present in all epithelial cells. A layer of circular visceral muscle is covered by a thick layer of loose connective tissue on the outside. Small arteries, hemocytes, and an uneven system of hemolymph gaps can all be found in the connective tissue.

General morphology of stomach

Stomach extends posteriorly approximately to the midpoint of the hepatopancreas. Stomach consists of anterior and posterior chambers know as cardiac stomach and pyloric stomach respectively. Stomach wall consists of tooth like protections known as ossicles where the cuticle is calcified and serves as the grinding surface with the help of supra-lateral teeth.

Histological Sections of Hepatopancreas, Anterior intestine and Stomach of *Litopenaeus vannamei* cultured in normal and Biofloc (BF) shrimp farming

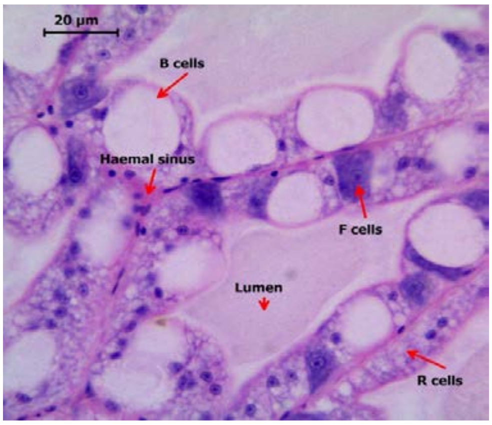

Fig A: Hepatopancreas (Pond)

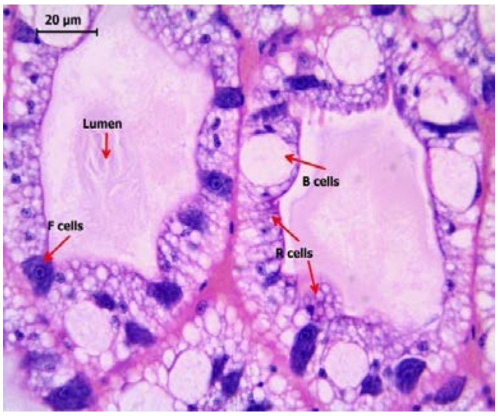

Fig B: Hepatopancreas (BF)

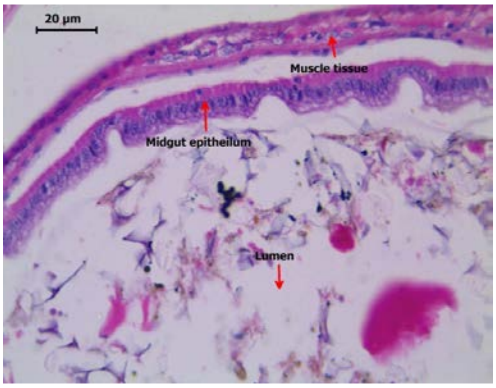

Fig C: Anterior intestine (Pond)

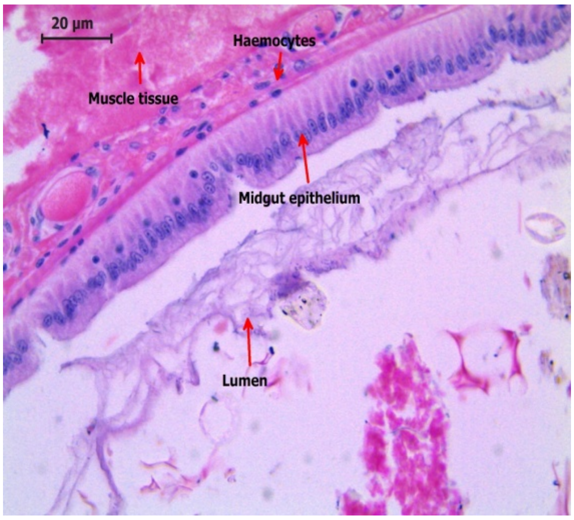

Fig D: Anterior intestine (BF)

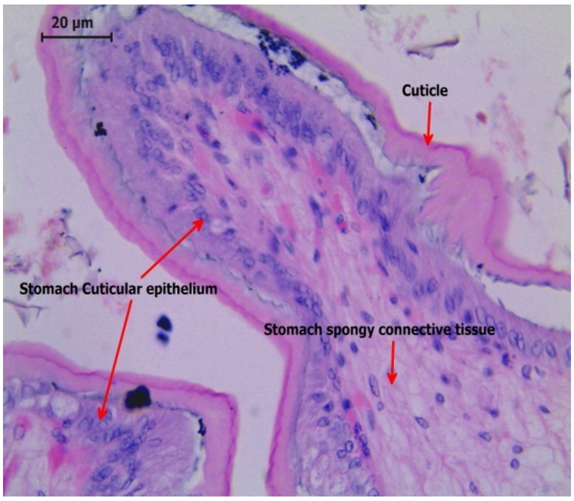

Fig E: Stomach (pond)

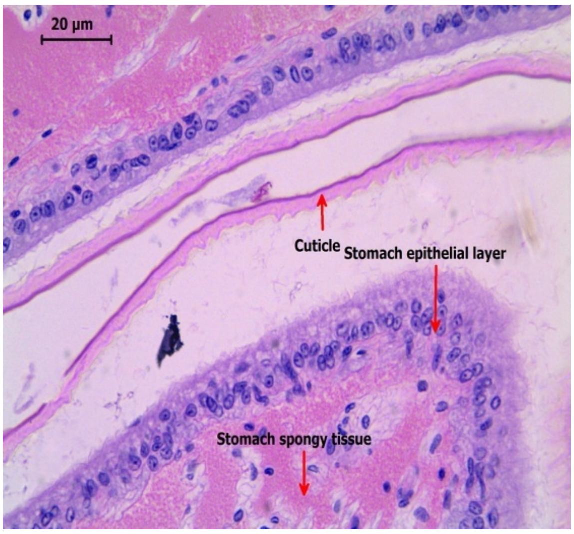

Fig F: Stomach (BF)

Histological observations

Hepatopancreas

The hepatopancreas (HP) is a main organ for the absorption and storage of nutrients, and acts as primary digestive organ of shrimp. It has the dual role of secreting digestive enzymes and absorbing nutrients. It's comprised of four types of epithelial cells respectively, R cells, B cells, F cells& E cells and each cell play a different role in hepatopancreatic function. Little more number of B cells was observed in biofloc shrimp farming prawn compared with control. No significant pathological changes were observed in (HP) of both cultured shrimps (Figs. A and B).

Anterior intestine

It is narrow and elongated part of the midgut and extended up to sixth abdominal segments. The anterior intestinal lumen made with epithelial or secretory cells, produced mucus layer with wrapped undigested particle to form the peritrophic membrane. Slight differences were noticed in the epithelial layers of biofloc cultured prawn than the control group. This study showed no pathological changes in anterior intestinal epithelial layer (Figs. C and D).

Stomach

The stomach is longest part of foregut, which divided into two parts, Cardiac chamber and pyloric chamber. The stomach epithelial layers were covered with thin cuticular layer. No significant differences were noticed between the organs of biofloc and control (Figs. E and F). In the present study, both culture system shrimp stomach showed normal architecture.

Discussion

To the greatest of our understanding, until this day, no research has reviewed the effects of BFT on histology in aquatic animals. On the account, this may be possibly the first study that has been aimed to evaluate the effects of Biofloc Technique on histology of hepatopancreas, anterior intestine and stomach in Prawn *L. vannamei*. As far as we realize, hepatopancreas, anterior intestine and stomach are the vital organs in nutrient metabolism, and histological alterations are informed to be a trusted indicator in the interpretation of nutritional condition (Ruiz, *et al*. 2020) [27].

The digestive system of Decapod crustaceans, according to their embryological origin, represents an internal tube subdivided into three parts: the midgut (hepatopancreas and intestine) arising from the endodermal layer the foregut (esophagus and stomach) and the hindgut are formed from the ectodermal layer (McLaughlin, 1983) [22].

The structure of the hepatopancreas is the brace for studies on nutrient requisite to evolve sustainable cultivation techniques, in addition the comprehension of the biology of the species (Icely and Nott, 1992; Diaz *et al*., 2010; Ruiz *et al*., 2019) [17, 27] and in general, reflects external environmental factors and varies greatly among species (Sousa and Petriella, 2007) [24]. The morphology of the midgut (Hepatopancreas and anterior intestine) in our present investigation of *L. Vannamei* shows considerable resemblances to other Decapoda taxa (Thomas Caceci *et al*., 2019. Ruiz *et al*. 2020) [33, 27] (Figs. A, B, C and D) However, some authors have described various dissimilarities in anatomy and histology, mainly within freshwater prawns (Felgenhauer, 1992, Rőszer, 2014) [14, 26].

Our present study revealed that, the Hepatopancreas of *L.vannamei* is mainly formed with branched tubule with distinct epithelial cell lining the tubules. This observation is strongly coincide with the reports of other workers (Diaz *et al*., 2010; Wu and Yang, 2011; Longo and Diaz, 2015; Ruiz *et al*., 2019) [28].

In our present study four types of cells namely B cells, R cells, E cells and F cells were located in hepatopancreas

(Figs. A and B) as reported by Iffat Jahan *et al*. (2017) [18], Anjaini *et al*. (2018) [4], Madhuri *et al*. (2018) [21], Thomas Caceci *et al*. (2019) [33] and Ruiz *et al*. (2020) [27]. They have also noticed these cells in the hepatopancreas of the species *L. Vannamei*, *Penaeus vannamei* and *Macrobrachium carcinus.*

Iffat Jahan *et al*. (2017) [18] also have noticed systematic glandular tubular structure as commonly perceived in other shrimp and are formed with numerous tubules dominated with four kinds of cells *viz*. B cells, R cells, E cells and F cells even after fed with feed having low levels of Potassium (K^+) and Magnesium (Mg^{2+}) for a period of 60 days. At the same time structural damages in hepatopancreas and gut were also noticed when fed with high contants of Potassium (K+) and Magnesium (Mg2+).

The current study, Biofloc and pond cultured shrimp hepatopancreas showed massive number of R cells indicated that capable of absorptive of lipid, glycogen storage and deposition of minerals such as calcium, magnesium, phosphorus, sulfur, and others. In crustaceans, hepatopancreatic R-cells plays the main part in metabolism and assimilation of nutrients, and also storing reserves (Hu and Leung, 2007). Consequently, hepatopancreatic ducts may be chiefly in digestive functions in *L.vannamei*, because they have the pattern of structures that rises the consumption activity and can also reserve vital nutrients in the hepatopancreas.

In our present study, little more B cells and vacuolization were also noticed in hepatopancreas of biofloc prawn than the control. It is correlated with the rate of secretion and digestion in HP as proposed by Ribeiro *et al.* (2014) [25], Longo and Diaz (2015), Vogt (2019) [34] and Ruiz *et al.* (2019) [28]. Vogt (2019) [34] proposed that the B-cells formed in the middle region feasibly linked to the process of luminal absorption for intracellular ingestion, while those established in the proximal region may be linked to the digestion and nutrient consumption processes. Ribeiro *et al.* (2014) [25] have reported that vacuolization in B-cells may indicate the functional stage of these cells. Longo and Diaz (2015) and Ruiz *et al.* (2019) [28] suggested that the various small vacuoles showing scarce acid glycoproteins in the B-cell cytoplasm, would indicate the inception of the digestion process.

The middle region of the hepatopancreatic tubules in *L. vannamei* in our present investigation is largely formed with F- and B-cells, as also reported for *Neocaridina heteropoda* and *Macrobrachium carcinus* (Sonakowska *et al.,* 2015, Ruiz *et al*., 2020) [30, 27], and can be measured as a region of profound secretion for extracellular digestion and the inception of intracellular digestion.

The midgut, besides the hepatopancreas, also shows the intestine with particular characteristics. In our present observation, the anterior intestinal lumen made with epithelial cells surrounded by connective tissues and the epithelial cells are neatly packed in both biofloc and normal pond prawn (Figs. C and D). The intestine in most crustaceans is lined by an epithelium made by a single cell type, which may apparent as a distinct stages in cell morphology (Icely and Nott, 1992) [17]. The intestinal epithelial cells of *M. carcinus* showed different acidophilia that could constitute two main stages in maturation, according to the development of organelles (Icely and Nott, 1992) [17]. The intestinal surface displays intestinal cells with a strong brush border. These particular characteristics of the intestinal epithelium of *L. vannamei* in our present study suggested that this anterior intestine may assist in absorption, as suggested for other crustaceans (Ahearn, 1987; Lovett and Felder, 1990) [2, 20].

According to Eckmann (1985) [12], whitefish (*Coregonus sp*) larvae were fed on excess supply of zooplankton led to histological changes of the intestinal epithelium in the foregut and mid-gut and the intestinal micro villi provided a vast absorptive surface area and was also observed that the rise in micro villi density can raise the nutrient absorptive ability (Sang and Fotedar, 2010) [29].

On the contrary to our present reports, apparent histological alterations in hepatopancreas were observed in pacific white shrimp, L. *vannamei*, reared in various concentrations of salinity of inland saline ground water (Madhuri S. Pathak *et al*., 2018) [21]. Anjaini *et al.* (2018) [4] have also noticed histological changes in Hepatopancreas and Gut of White Shrimp samples collected from 3 farms infected with white feces disease.

The stomach epithelial layers were covered with thin cuticular layer and are calcified with ossicles which serve as the grinding surface (Figs E and F). Little information is available related with the morphology of stomach (Albertoni *et al*. 2003**,** Suthers and Anderson 2006**,** Abrunhosa and Melo, 2008) [3, 1] and no literature is accessible about the stomach histology of prawns.

In our present study, there was no significant histological changes noticed in the white shrimp *L. Vannamei* cultured employing biofloc technology with heterotypic microbial flocs compared to the prawn from normal pond. Only meagre differences were noticed in all the three organs of biofloc cultured prawn than the normal pond prawn. The reason for this is not clear and yet to be investigated.

Conclusion

The results of the present study also established the constructive reactions of biofloc on histology in prawn. It was also noticed that in all cases there were no notable dissimilarities and no pathological problems observed. The results of the present work specified that the fortification of Biofloc is extremely advantageous to keep up the regular structure of hepatopancreas, anterior intestine and stomach of Prawn associated with standard growth especially when reared in raw inland ground water with stimulated heterotrophic microbial growth.

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