MOULTING CYCLE RELATED CHANGES IN BIOCHEMICAL, ANTIOXIDANTS GENES AND FEEDING RATES IN COMMERCIAL PENAEIDAE SHRIMPS

METAPENAEUS AFFinis (H. MILNE EDWARDS, 1837)

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Abstract

There is an urgent need to understand the behaviors of important species in aquaculture, to facilitate their reproduction. Metapenaeus affinis is the most commercially important species. The hallmark of crustacean physiology is the periodic removal of its old exoskeleton, which was achieved by moulting. All processes such as crustacean metabolism, reproduction and behavior are affected by the periodic removal of the exoskeleton and the characteristics of the moulting cycle. The present study explained assess the components of biochemical structure and to estimate the antioxidant enzymes as well as to determine the feeding behavior during the different stages of moulting in M. affinis. The maximum level of total protein in muscle tissue (62.83%) mg/g in stage (postmoult) and the lowest level (62.05%) mg/g in stage (premoult).

While A sharp reduction in total lipid level (3.01%) mg/g at stage (postmoult) was observed compared with the high levels (6.08%) mg/g at stage (moult). It had the highest value of moisture 3.80% at the stage (ecdysis) while the lowest 1.86% at the stage (premoult), the highest level of ash (0.105%) in the (intermoult) then the lowest value (0.01%) at stage (moult). The contents of glutathione, catalase and reactive oxygen species were determined in muscle tissue in the studied species, the results obtained showed the types of parameters examined different ranges within the stages of the moulting cycle. This work represents the first study of its kind and has shown that these parameters are considered biological markers suitable for determining the state of cellular oxidation in different stages of moulting in commercial shrimp M. affinis. Relative important index IRI was adopted to evaluate the importance of different nutrients during different stages of moulting in the studied species. The results of the study showed different nutritional ratios in the Premoult, Intermoult and Postmoult stages, while the ecdysis stage, this stage was characterized by microscopic examination 243 animals that the foregut of the gastrointestinal tract was empty. This study documents and expands our understanding of the physiological and biochemical changes that occur in M. affinis shrimp during four different stages of moulting, through which useful criteria can be provided in the life cycle of this commercial shrimp.

Keyword: Ecdysis, biochemical constituents, glutathione, catalase, ROS, feeding, jinga shrimp.

Introduction

The shrimp is one of the largest groups of crustaceans, which contains more than 3000 species but only commercially exploits 40 species (Khodanazary, 2019). Shrimp has various benefits that are important in the economies of many East Asian countries. It is a nutritious source of meat protein and has a high economic importance for humans as an exportable commodity (Shokoohmand et al., 2017). Arthropods do not have an adaptive immune system, which relies on highly specific antibodies and receptors (Zhang et al., 2013), so that the biochemistry of crustaceans can benefit from the knowledge available about insects (Zhang et al., 2017).

The biochemical changes happen in crustaceans during molting, are an essential basis for determining suitable diets (Yan et al., 2017). Details on seasonal changes in biochemical structure is therefore important in the study of crustacean reproductive biology (Yan et al., 2017). Comparative biochemistry has evolved in crustaceans in recent years due to interest in aquaculture. Several preliminary hypotheses have been taken in the field of biochemistry of insects because of their evolutionary affinity with crustaceans specifically (Sugumar et al., 2013), so that the biochemistry of crustaceans can benefit from the knowledge available about insects (Zhang et al., 2017).

Most cells acquire the relevant antioxidant mechanisms to maintain the lowest possible level of ROS within the cell. Protection mechanisms include both enzyme oxidation systems such as glutathione and non-enzymatic antioxidant systems such as catalase (Jyoti et al., 2018). The importance of enzymes in the production of different types of chemical and biological products has become a well-established technique in the food and other industries, because processes containing enzymes usually reduce process time, waste quantity and shorten the reaction steps (Kaushal et al., 2018). Deprivation of nutrition and moulting can affect all behaviors, and contribute to individual variation in feeding behavior,
emphasizing the importance of knowledge of individual variation in aquaculture.

Some studies have been conducted such as the study of both Abdul-Sahib and Sultan (1996) possible relation of the moulting of *M. affinis* with the tidal phase was investigated. Abdul-Sahib and Ajeel (2005) studied the biochemical structure (proteins, lipids, carbohydrates, ash) and caloric contents in the males and females of the commercial shrimp *M. affinis*. The study of (Saud et al., 2014) examined the nutritional value and physical condition of *M. affinis, Parapeneaeopsis stylifera* by studying the chemical composition of the two species. Al-Maliki and Al-Khafaji (2018) studied the human consumption of contaminated shrimps on human health, As well as studied the use of marine shrimp as a bio-indicator of heavy metal pollution in the environmental monitoring studies. Corgos et al. (2007) studied to quantify growth at moult of *Maja brachydactyla* in the Ría de A Coruña (NW Spain) using data from a mark-recapture experiment, and compare these data with two previous studies performed in the laboratory and extensive culture. Wang et al. (2012) illustrated the relative expression of Prx, CAT, and GPx in the cephalothorax of Chinsese shrimp. *Fenneropenaeus chinensis* was at studied transcription and translation level after one of them was silenced by double-strand RNA (dsRNA) injection. A study of jung et al. (2013) showed that muscle growth in crustaceans accompanied moulting and included periodic changes in size, muscle protein recovery and shrinkage characteristics during evolution. Xue et al. (2017) studied moulting in the crustaceans is controlled by ecdysteroids and the MIH hormon of x-organ/sinus gland and in turn prevents the moulting process. This species is the most important economic species in the decapods common in our territorial and inland waters, so it is necessary to understand the changes associated with the moulting cycle.

Hence, the main objective of this study is to assess some of the biochemical structures and feeding behaviour, As well as understand the relationship of antioxidant reactions in shrimp. During the different stages of moulting in this penaeid shrimp *M. affinis*.

**Materials and Methods**

**Collection of Shrimps**

Fresh shrimp *M. affinis* were obtained for this study from the River Al Masahab East South of Al Hammar marsh Basrah Governorate. It was stored in glass basins, their capacity ponds (60 x 60 x 30 cm) with recirculating system and two aerators for each bin.

Then category was approved for samples by length (5.5–8.5 cm) to monitor the phase-out moulting stages within 24 hours. During the experiment, salinity was maintained at 3.5% both in the bins and aquaria and temperature ranged from 28–30 °C. 50% of the water is replaced daily for one month. Experimental animals are fed with 45% protein, 4% raw fat, 6% raw fiber, 11% moisture) twice a day at 07:00 and 14:00. The waste was siphoned from the aquaria once every two days, the animals were weighed to the nearest 0.01 gm using an electric balance with an accuracy of 0.01 g after disposal of excess water with absorbent tissues. The total body length, were measured with a Caliper Vernier with an accuracy of 0.01 mm.

**Analysis of moulting stage**

Setal development of *M. affinis* was observed at the posterior median part of the uropods. Uropods were examined and photographed under a light microscope (Olympus BX40) connected to a digital camera (DCE-2). The criteria used for moult staging were used according to Drach (1939), modified by Warner (1977) and Lowery (1988).

**Estimation of total protein, total lipids, ash**

- Protein (%) was estimated by calculation the amount of nitrogen by micro-Kjedahle method to determine the amount of nitrogen, and the total amount of protein obtained N * 6.25 (A.O.A.C., 2000)
- Lipids (%) were determined by sexhlet extraction method for intermittent extraction to measure lipid ratio using solvent cyclohexane for 6 hours (Folch et al., 1957).
- Moisture (%) The moisture content was determined by drying a known weight of the sample using an oven at 105 °C until the weight was constant (A.O.A.C., 2000).
- Ash (%) Determination of ash content by combusion in the oven (550 °C) from 3-4 hours (Jafri et al., 1964).

**Estimation of glutathione, catalase, reactive oxygen species**

Reduced glutathione was measured following the method of Sedlak and Lindsay (1968). Estimation of total, protein-bound and nonprotein sulfhydryl groups in tissues with Ellmans reagent.

Catalase activity has been estimated following the method of Hadwan and Abed (2016). Data supporting the spectrophotometric method for the estimation of catalase activity.

Reactive oxygen species (ROS) were estimated using a method by Erel (2005). Anew automated colorimetric method for measuring total oxidant status.

**Food**

The first third of the gastrointestinal tract was adopted for species that do not have distinct stomachs, in order to study their nutritional contents after measuring their lengths to the nearest millimeter using Vernia or ruler and weighing in grams using a laboratory scale. Sartorius of German origin. The digestive tract was extracted and the first part was adopted and its contents were placed in a petri dish and the sample was taken from the food after being mixed well with water and placed on a glass slide and examined by a microscope installed under a magnification of 10x and 40x. It was filled with 3/4 full, 1/2 full and 1/4 full and gave her points 0, 5,10,15,20 respectively. Points were distributed to different food ingredients depending on their suitable size, points were collected for each food component and the percentage of total points was calculated, Adopted in the classification of food ingredients as Edmondson (1959) was used to classify crustaceans and aquatic insects, while Hadi etal. (1984) and Wehr and Sheath (2003) were classified as phytoplankton. Two methods were used to determine the composition of food according to the nature of the food of the studied species, the point's method (p) and the method of...
repetition of presence (o) according to Hyslop (1980), Hansson (1998).

**Results**

**Describe the stages of the moulting cycle**

A detailed explanation of the moulting stages of *M. affinis*, moulting stages were characterized by morphological changes such as cuticle hardness or changes in the development of setae in pleopod and europod. On the basis of setogenesis, the moulting cycle was classified into four specific stages: postmoult, intermolt, premoult, Moult as shown in Table 1.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Diagnostic characters</th>
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<tbody>
<tr>
<td>Postmoult</td>
<td>Granular protoplasmic matrix, setae thin walled, carapace hard and rigid with development of cuticular nodes, pleopod soft</td>
</tr>
<tr>
<td>Intermoult</td>
<td>Setal cone formation, setal lumens narrow, exocuticle remains hard, setal wall translucent.</td>
</tr>
<tr>
<td>Premoult</td>
<td>Beginning of epidermal retraction (apoplosis), cuticle remains soft, retraction zone between old cuticle and epidermis widens, new setae clearly visible, epidermal retraction continues.</td>
</tr>
<tr>
<td>Ecdysis (moult)</td>
<td>The animal withdraws from the old cuticle and for a short time absorbs water rapidly.</td>
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Table 1: Characteristics of moulting cycle stages in *M. affinis*

Moult cycle stages based on (Drach, 1939)

**Fig. 1**: Morphological variation in different stages of moulting in uropods *M. affinis* shrimps were examined and photographed under a light microscope (Olympus BX40) connected to a digital camera (DCE-2). The criteria used for moult staging followed Drach staging. Scale Bar =50 µm. EE=epidermal edge, L= white layer, S= setae, SC= seta cone, Sn=setal node, Ns= new setae, E=epiderme.

= wavy edge of epidermis.
☆ = clear zone between cuticle and epidermis.

**Protein**

The results showed a high percentage of total protein in muscle tissue during different stages of the moulting cycle, the highest concentration (62.83%) mg/g in postmoult stage and the lowest level 62.05% mg/g in premoult stage were observed, differences in total protein levels in muscles were evident (Figure 2).
Moulting cycle related changes in biochemical, antioxidants genes and feeding rates in commercial penaeidae shrimps Metapenaeus affinis (H. Milne Edwards, 1837)

Lipid

The results showed differences in total lipid levels in muscle tissue during the different stages of moulting. A sharp reduction in total lipid level 3.01% mg/g at postmoult stage was observed compared with the high levels 6.08% mg/g at stage moult (Figure 3).

Moisture

The moisture ranged from 1.86% - 3.80% in muscle tissue during the different stages of moulting (Figure 4).

Ash

The ash percentage showed the highest value (0.105%) was in the intermoult stage then the lowest value 0.01% at moult stage (Figure 5). It shows the percentages of ash at different stages of the moulting cycle.

Antioxidant enzymes

Glutathione (µg/mg.protein)

The results showed significant differences in glutathione levels in muscle tissue during the different stages of moulting, the highest value 13.26 µg/mg protein was in the intermoult then the lowest value 3.28 µg/mg protein at premoult stage (Figure 6).

Catalase activity KU/L

Figure 7 shows the changes in the level of catalase activity during the different stages of moulting, as the maximum 154.04 ku/L in the intermoult stage and the lowest value 46.21 ku/L in postmoult stage.
ROS (Micromol/gm)

The results showed differences in reactive oxygen species levels in muscle tissue during the different stages of moulting, the highest value (52.95) micromole/gm in the premoult stage and the lowest value (32.68) micromole/gm in the moult stage (Figure 8).

Food preference

Chavanich et al. (2015) when analyzing the components of the stomach of penaeid shrimp species, diets including phytoplankton, crustacean's appendages, zooplankton, organic matter were found. Some studies have indicated that penaeid shrimp are considered carnivores, and may show cannibal behavior (Zhang et al., 2010).

Food composition

Food items examined on the basis of abundance observed in shrimp foregut of gastrointestinal tract, zooplanktons were, ranked first among the foodstuffs that present in the foregut throughout the study period, while organic matter ranked second among foodstuffs and phytoplankton constituted the third nutrient, and finally green algae were found in the final rank among contents of the foregut.

Figure (9) shows the percentages of relative importance index (IRI) of the food ingredients in foregut of gastrointestinal tract of *M. affinis* in the Premoult stage. The diet consists of five food ingredients, with zooplankton ranked first with 44.95%, organic matter came second with 28.88%, and algae ranked third with 18.27%. Diatoms ranked fourth with 4.17%, and phytoplankton ranked fifth with 3.74%.

Results showed *M. affinis* in the ecdysis stage, this stage was characterized by microscopic examination 243 animals that the foregut of the gastrointestinal tract was empty.

Discussion

Changes in the exoskeleton, reflect the regular cyclical growth that occurs in crustaceans during the molting cycle (El-sayed et al., 2015). Although setogenesis has been used as a criterion for molting for many years (Drach, 1939), these criteria include discernment in the internal cones and setal organs, setal lumen. Similar criteria have been used to determine stages for the penaeidal shrimp (Paray et al., 2014).

Differences in species related to setae morphology and development lead to crustaceans variation in both the gradient criteria and in the sub-divisions defined in the molting stages. The results of the present study showed apparent phenotypic changes in the uropod setae of the *M.
affinis during the moulting cycle, especially during the sub phases of pre-moult stage. In the Intermoult stage, the setae cones were arranged in one row on the bases of the setae without the withdrawal of pigments, and the epidermal tissue appeared to be attached to the setal cones. These characteristics were consistent with a study by Promwikorn, et al. (2004) on the black tiger shrimp (Penaeus monodon). On the other hand, Zilli et al., 2003 pointed out that the pigment seemed a thick line at the bases of the setal nodes in some shrimp species. Withdrawal of epidermal pigments during the D1-D2 sub phases within the Premoult stage, where the undulating edge of the epidermal tissue and the white area between the epidermis and the old setae bases have been agreed (Stevenson 1971; Skinner 1985; Zilli et al., 2003; Promwikorn et al., 2004; El sayed et al., 2015) On the other hand, the level of matrices in the setal lumens varies within the sub-stages during the pre-mouling cycle. These are agree with that demonstrated by (El sayed et al., 2015; Chansiu, et al., 1988). Europods was used to determine the stages of moulting because removal of other appendages leads to shock or death (Paray et al., 2014).

The process of moulting results in periodic changes in the chemical composition (Suod et al., 2014; Khodanazary, 2019), so we need more chemical analyzes of commercial aquatic organisms, because it gives importance to know the nutritional value and understanding metabolism, to obtain a healthy balanced food (Abdul-Sahib and Ajeel 2005), as well as it reflects the physiological status of the organism. On the other hand to show the negative effects of pollution in the environment and the extent of its impact on the components of the chemical composition, to see whether this sea food is still suitable for human consumption or not (Khodanazary, 2019).

The results were obtained for total protein levels in muscle tissue collected during the different stages of moulting. The maximum concentration in the postmoult stage was attributed to the formation of kitten in the new epidermis. While the total protein level was observed to be lower in the premoult stage, it may be attributed to the re-adsorption of kitten protein in the epidermis in the old exoskeleton and the degradation of proteins in energy production (Paray et al., 2014). Protein levels ranged from 62.83% -60.05% a study but higher than that recorded (Abdul-Sahib and Ajeel, 2005; Al-Maliki and Al-Khafaji, 2018). The high percentages of total proteins in the present study are attributed to different sampling time or sampling location. Total lipids levels in muscle tissue showed a sharp decrease in postmoult and premoult, compared to high levels in ecdisys and Intermolt stages. This is agree with the observed in penaeid shrimp species (Paray et al., 2014). High ash levels in the Intermoult stage, may be attributed to kitten compared to the low percentage in ecdisys.

The results of the study show the percentages of moisture in jinga shrimp Metapeneus affinis 3.80%, 2.82%, 2.53%, 1.86 in ecdisy, postmoult, intermoult and premoult respectively, that the increase in moisture content in the moulting stages can be explained that the increase in volume when crustacean moulting is, due to water absorption (Oconnor and Gilbert, 1969; NorFaadila et al., 2013) and this is consistent with a study (Travis, 1957) during the moulting cycle involved the water-to-mass ratio is greater in the moulting stage and early postmoult stage.

The role of the antioxidant defense system in invertebrates can be more important than in vertebrates, because during the phagocytosis process involving the production of (ROS) this is a major defense mechanism against invading microorganisms, due to their lack of antibodies and acquired immunity (Johansson et al., 1999; Wang et al., 2012). On the other hand, the radicals peroxide produced after the stress of the cell are converted to hydrogen peroxide and oxygen by superoxide dismutase (SOD), and the excess of hydrogen peroxide is stimulated into harmless water and oxygen by catalase and glutathione (Holmblad and Soderhall, 1999; Wang et al., 2012). illustrate Yeh et al. (2010) Increased respiratory activity in the giant freshwater shrimp Macrobrachium rosenbergii to eliminate invasive pathogens, so antioxidant enzyme activity and RNA transcription were regulated in order to convert ( ROS) excess from pathogenic infections. Confirmed Liu et al. (2010) in the black tiger shrimp Penaeus monodon associated with increased respiratory bursts significantly with Photobacterium damselae and WSSV-injected shrimp. Finally therefore, antioxidant enzymes may have key roles in the innate immunity of shrimp. The highest concentration of the antioxidant enzyme glutathione during the intramoulting stage to protect cells from damage, while decreasing during the ecdisys, this applies to the enzyme Catalase. This is due to the biological and chemical processes active in the intermoult stage, while the ROS level is high during the premoult and postmoult stages to prepare for any microbial attack. It is noted that shrimp are not fed during the moulting stage. A clear understanding of food preference and behavior is important in aquaculture and for efficient nutrition management, which in turn helps reduce food wastage and thus protect the ecosystem. During the moulting stage, the exoskeleton is so thin that it does not allow the activity of searching for food. On the other hand, shortly after the moulting stage, shrimp consumes exuvia (Philpen et al., 2000), which is rich in chitin, which in turn will compensate for the loss of minerals due to moulting. The natural food of the animal during the moulting stage includesditoms, algae, phytoplankton, zooplankton and organic matter, but the lack of nutrition during the moulting stage is noted, because the animal is busy with moulting. The postmoult and premoult phases observe the similarity of food types at almost the same proportions, this is agree with (Nguyen et al., 2014)

**Conclusion**

The developmental stage in setae provides a quick and accurate indication of the moulting stage in M. affinis, which would be without sacrificing of the animal, when it is intended the repetition of measurements taken from the same animal in order to monitor the rate of development. This study provides a detailed explanation of moulting cycle such as epidermal retraction and related setal formation in the epidermis, this present study confirms differences in the components of biochemical composition as well as the measurement of three antioxidant enzymes in muscle tissue during different stages of moulting cycle, which contribute to our understanding of physiology during the ecdisys of M. affinis. On the other hand, the reduction of feeding rates before and after moulting compared to excessive rates during the intermoult stage.
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