Methyl Farnesoate through Feed: a Growth Manipulator in Female Crab Oziothelphusa Senex Senex

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Abstract: Methyl farnesoate (MF), the predominant juvenile hormone-like compound found in crustaceans. In crustaceans, MF mediated growth was reported and succeeded in the laboratory, but was not done successfully at the field level. The present investigation is aimed to test the role of dietary MF on growth of crustaceans in the semi-controlled environment. To test this, MF was supplemented through commercial shrimp pellet diet to female freshwater crab Oziothelphusa senex senex (Oss) with a concentration of $10^{-9}$, $10^{-8}$ and $10^{-7}$ moles/crab in an every alternative day for about 40 days along with eyestalk ablated (ESX) and control groups. Dietary supplementation of MF enhanced the growth of female crab by inducing molt. The molt induction frequency found in this study as MF concentration $10^{-8}$ moles/crab > $10^{-9}$ moles/crab > $10^{-7}$ moles/crab ≤ ESX and recorded molt percentage of 25%, 12.5%, 10%, 7.5% respectively. Evidencing that the dietary MF supplementation induces growth in cultured crustaceans thereby increases the yield of the culture.

Keywords: Methyl farnesoate; Molting, Oss; Crustacea

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Introduction

Crustacean aquaculture plays an important role in producing quality protein, but is facing difficulties for quality protein production at the end. To attain more quality protein, methods are employed to induce growth. One of such popular conventional method to induce growth is eyestalk ablation (ESX), where unilateral or bilateral eyestalk ablation experiments were carried out (Venkitraman et al., 2010 and Amer et al., 2015). Removal of eyestalk causes increase in ecdysteroid secretion from Y-organ which induces precocious molting in many decapods (Techa et al., 2015, Allayie et al., 2011; Neelima et al., 2016). Though ESX induces growth, it promotes mortality due to loss of large amount of hemolymph. An effective alternative for ESX is hormonal manipulation with respect to molt regulation.

Endocrine manipulation is nothing but exogenous administration of modulators and screening or observing its effect on molting. Past to present, the manipulation of crustacean molt has led to use of several endocrine modulators including external and internal molecules (Aktas et al., 2005; Sainath et al., 2011). The molt regulatory hormones, especially positive regulators supplementation accelerates the molt and growth in crustaceans. Molting is accelerated by endogenous hormone methyl farnesoate (MF), secreted from mandibular organ (MO) and ecdysteroids synthesized and released from Y-organs. The mandibular organs located at the base of the mandibular tendon, secretes the sesquiterpene MF and farnesolic acid (FA) (Tiu et al., 2012) MF (methyl-(2E,6E,10E)-3,7,11-trimethyldodecatri-2,6,10-eneoate) is structurally similar to insect JHIII (methyl farnesoate) which induces precocious molting in many decapods. MF is capable of inducing synthesis and release of ecdysteroids (MOIH) produced by the eyestalk X-organ sinus gland (Nagaraju, 2007). The commercially available shrimp pellets were purchased from Echlon Biosciences, Salt Lake City, USA and purchased other chemicals from Merck, Mumbai, India and HiMedia Private Limited Laboratories, Mumbai, India used in the present study. MF was dissolved in 95% ethanol and diluted with crab ringer solution (6.5 g NaCl, 0.42 g KCl, 0.25 g CaCl2 and 0.2 g of sodium bicarbonate) so that the final concentration of ethanol was made into 10%. According to Reddy (Reddy, 1991) hemolymph was calculated as 27% of body mass in the fresh water crab sox and the volume of hemolymph and supplemented volume of MF is calculated according to Reddy (Reddy et al., 2004; Tamone et al., 1993). The commercially available shrimp pellets were purchased and are dried with 10², 10⁴ and 10⁷ moles of MF/each 100 mg pellets in three separate preparations.

Dosage of MF: The stock test chemical MF was purchased from Echlon Biosciences, Salt Lake City, USA and purchased other chemicals from Merck, Mumbai, India and HiMedia Private Limited Laboratories, Mumbai, India used in the present study. MF was dissolved in 95% ethanol and diluted with crab ringer solution (6.5 g NaCl, 0.42 g KCl, 0.25 g CaCl2 and 0.2 g of sodium bicarbonate) so that the final concentration of ethanol was made into 10%. According to Reddy (Reddy, 1991) hemolymph was calculated as 27% of body mass in the fresh water crab sox and the volume of hemolymph and supplemented volume of MF is calculated according to Reddy (Reddy et al., 2004; Tamone et al., 1993). The commercially available shrimp pellets were purchased and are dried with 10², 10⁴ and 10⁷ moles of MF/each 100 mg pellets in three separate preparations.

Eyestalk ablation (ESX): Both the eyestalks were removed from the crabs by cautering the eyestalk at the base without prior ligation but which cautery of the wound after operation. Eyestalk ablation (ESX) deprives the crab from eyestalk hormones as they regulate the major physiological processes like molting in crustaceans.

Identification of molting stages: Molt stages were determined based on morphological changes in the decapods during the molting cycle(Hosamani et al., 2016). At stage $D_{1}-D_{2}$ (early premolt) the pigment retracts from the bases of the setal nodes, leaving the old cuticle and during $D_{2}-D_{3}$ (late premolt) the new developing setae are observed. For molting stages determination live crabs were checked daily and the selected animals were placed on ice for determining the molt stages.

Dosage of MF: The stock test chemical MF was purchased from Echlon Biosciences, Salt Lake City, USA and purchased other chemicals from Merck, Mumbai, India and HiMedia Private Limited Laboratories, Mumbai, India used in the present study. MF was dissolved in 95% ethanol and diluted with crab ringer solution (6.5 g NaCl, 0.42 g KCl, 0.25 g CaCl2 and 0.2 g of sodium bicarbonate) so that the final concentration of ethanol was made into 10%. According to Reddy (Reddy, 1991) hemolymph was calculated as 27% of body mass in the fresh water crab sox and the volume of hemolymph and supplemented volume of MF is calculated according to Reddy (Reddy et al., 2004; Tamone et al., 1993). The commercially available shrimp pellets were purchased and are dried with 10², 10⁴ and 10⁷ moles of MF/each 100 mg pellets in three separate preparations.

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Experimental Design

About five small ponds were maintained with labelled 40
female crabs in each. Pond one and two served as control and ESX groups and other three for 10⁷, 10⁸ and 10⁹ moles of MF/each 100 mg pellets supplemented to each animal every alternative day. The control and ESX animals were fed with normal pellet diet. The crabs from each group are sacrificed on 10th, 20th, 30th and 40th day of experiment to check the molt stages except control group where some of them sacrificed on 0th day.

Molt stages were determined based on the setal development in the mastigobranch of 3rd maxilliped. The changes in the setal development are observed under phase contrast microscope (Olympus, Model BX41TF, Japan) and molt frequency was examined.

Result

The molt frequency was determined on 10th, 20th, 30th and 40th day of experiment in all experimental groups along with ESX and controls. No mortalities were recorded in experimental groups except in ESX animals. Throughout the experimental period the controls and ESX animals were fed with normal pellet diet. The groups and other three for 10th day.

Mortalities were recorded in experimental groups except in ESX animals. Throughout the experimental period the controls and ESX animals were fed with normal pellet diet. The animals supplemented MF 10⁷ moles/crab were in premolt stages D₃ (70%) and D₄ (30%) on day 10 of experiment. The molt stages C₃ (50%) and D₃ (50%) were observed in crabs supplemented with 10⁷ moles/crab of MF on day 10 of experiment (Table 1).

ESX crabs on day 20, were observed in early premolt D₃ (50%) and middle premolt D₄ (30%) stages. About 20% mortality was recorded on the same day in ESX group. In case of MF 10⁹ moles/crab supplemented animals, they were in middle premolt D₃ (60%) and late premolt D₄ (40%) stages. The group MF 10⁹ moles/crab supplemented were found in premolt D₃ (30%) and D₄ (50%) stages and 20% crabs were molted on day 20. Premolt stages D₃ (60%), D₄ (20%) and D₅ (20%) were observed in crabs supplemented 10⁷ moles/crab of MF on this day.

On day 30 of experiment ESX crabs were in premolt stages D₄ (22.22%), D₅ (33.33%), D₆ (11.11%) and some were molted in D₅ (early premolt stage) (23.08%) stage. About 38.46% mortality observed on 10th day of experiment in ESX group. Observed most of MF 10⁹ moles/crab supplemented females were in early premolt stage D₄ (80%) and only 20% in C₃ stage on 10th day of experiment. The animals supplemented MF 10⁸ moles/crab were in premolt stages D₃ (70%) and D₄ (30%) on day 10 of experiment. The molt stages C₃ (50%) and D₃ (50%) were observed in crabs supplemented with 10⁷ moles/crab of MF on day 10 of experiment (Table 1).

Table 1: Methyl farnesoate pellet diet induced molt in female crab Oziothelphusa senex senex.

| Group            | Days of experiment | 0th          | 10th         | 20th         | 30th         | 40th         |
|------------------|--------------------|--------------|--------------|--------------|--------------|--------------|
| Control (n=40)   |                    | C₄ (8.0; 100)| C₄ (8.0; 100)| C₄ (8.0; 100)| C₄ (8.0; 100)| C₄ (8.0; 100)|
| ESX (n=40)       |                    | C₃ (5.0; 38.46)| C₄ (3.0; 23.08)| Died          | Died (2.0)   | Died (5.0; 38.46)|
|                  |                    |              |              |              |              |              |
| MF 10⁷ moles/crab|                    |              |              |              |              |              | Died (2.0; 22.22) |
| (n=40)           |                    |              |              |              |              |              |              |
| MF 10⁹ moles/crab|                    |              |              |              |              |              |              | Molted (1.0; 11.11) |
| (n=40)           |                    |              |              |              |              |              |              | Molted (2.0; 25) |
| MF 10⁸ moles/crab|                    |              |              |              |              |              |              |  |
| (n=40)           |                    |              |              |              |              |              |              |              |

The values in the parenthesis represent the number of animals in followed by percentage of each stage or sub-stage or molt and died animals on the respective day of experiment.

C₄: Intermolt; D₁, D₂, D₃ and D₄ premolt sub-stages; M: molted; ESX: eyestalk ablated
In the present study in crab Oss maintained in semi-controlled environment. Out of three concentrations of MF tested, 10^4 moles/crab dietary supplemented is found more effective molt inducer in female crab Oss. Moreover, the dietary MF at 10^4 and 10^7 moles/crab are also inducing molt in females. A number of studies were proved the molt induction capacity of MF injection in many crustacean species. In Oss molt induced studies were done with MF injections in laboratory (controlled) conditions (Neelima et al., 2016). It is reported in the same study that MF reduces the molt interval to 14 days i.e., within 2 weeks in both in males and females crabs. Besides Oss MF induced molt was studied in crustaceans like Cherax quadricarinatus (Abdu et al., 2001), Cancer magister (Tamone et al., 1993) Litopenaeus vannamei (Tariq et al., 2014) L. emarginata (Hosamani et al., 2019), S. serrata (Girish et al., 2015), Portunus trituberculatus (Xie et al., 2015), Neocaridina denticulata (Sin et al., 2015), Metapenaeus ensis (Gunawardene et al., 2002), Homarus americanus (Homola et al., 1997), Peneaus monodon (Suncetha et al., 2010), Chionecetes opolii (Marilyn et al., 2014), and Scylla olivacea (Akbar et al., 2016). On the other handecdysteroids from Y-organs are also released by MF thereby in molt induction. However, it is proved from the present study that dietary MF supplementation induced the molt in female crab. But the clear mechanistic action of MF on molt induction is not clear. It is predicted to be by direct induction or by releasing the ecdysteroids or both.

In the present experiment it is clear that the molt induction frequency of MF at 10^4 moles/crab supplemented through diet is showing high than other MF treatment groups in female crabs. The function of MF is to promote the molt cycle through induction of MH synthesis and release thereby growth in crustaceans. It is clear that dietary supplementation of MF induces molt effectively with reduced molt cycle durations than positive control ESX group in crab Oss. Moreover the MF 10^4 moles/crab supplemented crabs also showing reasonably good numbers with molt induction. This report is a base for implementing growth of crustaceans at the farm level. However, no recorded studies are available on dietary supplementation of MF for testing growth at semi-natural aquatic environment (Hosamani et al., 2017).

The present investigation is giving a base to increase the yield of crustacean protein at the pond level by supplementing MF through pellet diet. Since the dietary MF supplementation induces the molt by reducing the length of natural molt cycle in the crab Oss grown in semi-controlled environment, it may directed to the semi-controlled crustacean cultures and gradually to the open pond system. This study provides a base to initiate and test the dietary supplementation of other endocrine manipulators for growth in cultured crustacean species at the pond level. Moreover,
the molecular mechanistic action MF on molt induction is open for researchers to find out and to proceed further. However, MF dietary supplementation can serve for increasing the crustacean protein by reduced crop periods thereby reduced utilization of feed and pond management.

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