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Growth performance and reprobiology of *Macrobrachium rosenbergii* feed with supplementation of *Indigofera longeracemosa* root extract

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Abstract

Macrobrachium rosenbergii de Man, 1879 is known as giant freshwater prawn. It is important to commercial species in the world wide. In the present study, observation of growth performance, reproductive biology and histological analysis of micro and macroscopical identification of development of ovary stages in *M. rosenbergii* fed with control diet containing 1%, 2% and 3 % of *Indigofera longeracemosa* roots extract for 60 days. The results of the present study, the all experimental diets fed with *M. rosenbergii* was a favorable growth performance of percentage of weight gain, specific growth rate and feed conversion ratio observed by $p<0.005\%$ significant, when compared with than the control diet (basal diet). The root extract was identified by active compounds were flavonoids, terpenoids, and phenolic compounds. The conclusion of the present study effects of *I. longeracemosa* roots supplementation in the control diet fed on *M. rosenbergii* reveal that improve growth, reduce the mortality rate, and enhances the reproductive performance of the in this species.

Keywords: Medicinal plants, Ovary, histology; stunted growth; *Macrobrachium rosenbergii*

Introduction

There are 150 species of *Macrobrachium* in the world wide which 49 are commercial. Twenty-seven of the commercial species are found in Asia and the Pacific region. The giant fresh water prawns *Macrobrachium rosenbergii* is cultured and commercial species in the worldwide. It is found in the tropical and sub-tropical region, Indo-Pacific regions, Malaysia, Thailand, Philippines, India, Sri Lanka, Bangladesh, Myanmar, Indonesia and Vietnam (New, 2002; New, 2005). India is the third largest producer of *Macrobrachium* species in the year 2007 and it is aquaculture production rise to 43,000 metric tonns (t) in 2005 from less than 500 tonns in the year 1995. The production of *Macrobrachium* species has been declining and in 2008-09 and it was 12,856 tonns, a reduction of more than 70% compared to the year 2005. Several factors of the affected production of Macrobrachium species is declining, such as slow growth rate, poor survival, disease outbreaks, increase in the cost of production, and availability of low risk alternative prawn species. The poor quality of seeds leading to unsatisfactory growth and survival rates in ponds. The development of good quality of seeds was improving growth rate and ensuring high survival rate. Hence, the study of reproduction is important for the life of the organism. The knowledge on gonad development and reproductive performance was important for culture systems to be improved. In the present study, evaluation of effects of *I. longeracemosa* root extract supplementation to control diet fed on *M. rosenbergii* observed in growth performance and reproductive analysis for 60 days experimental periods.

Materials and Methods

Plant Materials

The plant materials of *I. longeracemosa* roots were collected from the Courtallam forest region, Southern Western Ghats, Tirunelveli districts, Tamil Nadu, India.

Preparation of ferns extracts

The collected plants materials of *I. longeracemosa* roots were washed with sterile distilled water and shade dried for two weeks. The dried roots were powdered and extracted with alcohol in a Soxhlet apparatus for 5 hrs. The crude extract was obtained and dried in a vacuum desiccator. Final residue was collected and stored in refrigerator at 4°C for further use. The preliminary phytochemicals analysis for standardized method (Harborne,1984).

Preparation of experimental diets

The compositions of control diet and experimental diets were prepared by the table-1. The experimental diets were prepared to contain 0% of *I. longeracemosa* (Control diet), 1% *I. longeracemosa*, 2% *I. longeracemosa,*3% *I. longeracemosa* and 4% *I. longeracemosa* extract. The mixture of *I. longeracemosa* extract and distilled water was sprayed on the experimental diets, which were then dried in a dryer at 30°C

for 48hrs in order to volatilize remaining ethanol. All diets were stored in air-tight containers at -20°C until used.

Table-1: The control and experimental diets composition of formulating diets

Ingredients (gms)	Experimental diets				
	Control	1% Extract	2% Extract	3% Extract	4% Extract
Fish meal	38	38	38	38	38
Groundnut oilcake	40	39	38	37	36
Coconut oilcake	3	3	3	3	3
Rice bran	3	3	3	3	3
Wheat bran	3	3	3	3	3
Maize bran	3	3	3	3	3
Tapioca flour	2	2	2	2	2
Egg albumin	7	7	7	7	7
Vitamin mix*	1	1	1	1	1
BI	100	100	100	100	100
Root extract of IL	0	1	2	3	4
Total feed ingredients	100	100	100	100	100

**B.I.- Basal Ingredients; IL: *I. longeracemosa*

Growth and reproductive performance

Collection of prawns

The freshwater prawn, *M. rosenbergii* were collected from the Tamiraparani River, Keela Eral, Tuticorin District, Tamil Nadu. The collected prawns of *M. rosenbergii* were brought to the laboratory in a plastic cover with aerated water and transferred into plastic tanks. Daily changed in the water and they were fed *ad libitum* with commercial pelletized food. They were maintained in the laboratory condition for 3 weeks for acclimatization periods. Five months old female prawn, weighing 13±2gms were selected. A total of 50 prawn was selected and divided into 5 groups. Each groups 10 prawn were introduced. Experiments were conducted in 3X2meters cement ponds. Diets at the level of 4% body weight/d were supplied three times daily at 08:00 h, 13:00 h and 18:00 h, respectively. After 180 days were observed growth performance of specific growth rate, weight gain and feed conversation rate. Parameters were calculated by using the following formulae so as to evaluate the efficacy of feeds prepared.

Survival Rate (SR) % = No. of live animals / No. of animals introduced X 100

Weight Gained (WG) = Final weight - Initial weight

Food Conversion Ratio (FCR) = Feed intake / Weight gained

Ovarian development of histological analysis

End of the experimental period, the dissection of the ovary was removed and weighed, fragments of their middle region were fixed for 24hrs in 4% formaldehyde and dehydrated with alcohol and xylene series and embedded in paraffin wax. A microtome section of ovary 3µm thickness and stained with eosin, hematoxylin and Malachite green were examined. Sections were micro photographed in a light microscope. The measurement oocytes diameter ranges at the identification of developmental stages in *M. rosenbergii*, the longer diameter range of minimum 20 cells per stage and section of ovaries were measured with ocular micrometer in eye piece. The sectioned of oocytes of *M. rosenbergii* were observed by nuclei in the approximately at the equatorial plane were measured. The frequency of oocyte size was estimated and duplicate counting of the total number of oocytes observed by according to Tan-Fermin and Pudadera, (1989) method. The development of ovarian stages was determined (Chaves and Magalhaes, 1993; Htun-Han, 1978; Martins *et al*., 2007; Okumura and Aida, 2000).

Results and Discussion

Phytochemicals

The results of the present study investigation of the phytochemicals of the crude extract of I. longeracemosa roots revealed the presence of alkaloids, flavonoids, terpenoids, steroids, phenolics, and quinone. The active constituents of alkaloid are used for antimalarial, analgesics and stimulant agents previously reported (Duke and Ayensu,1985).

Growth Performance

The results of the present study were observed by experimental diet development and supplementation of *I. longeracemosa* root extract highly exhibited in the significant growth performance represented in the Table-2. The growth performance of weight gain was observed by *I. longeracemosa* root extract supplemented with control diet fed on *M. rosenbergii* seen in table-1. The specific growth rate was highly significant in the all diets compared than the control. According to Prianka Paul and Anisur Rahman,

(2016) observed that specific growth rate (% SGR per day) of prawns of fed for locally market available feed ranged from 1.89±1.59 to 2.19±1.80. Our study observed for high specific growth rate of the highest value 3.21 in 4% extract in *I. longeracemosa* root. Hossain, (2004) observed SGR of prawn ranged between 2.74 to 3.12 and reported that SGR (% per day) of *M. rosenbergii* were higher when fed supplementary diet (starter and grower) and diet containing 32% protein. Feed Conversion Ratio was observed by good results in all experimental diets fed on *M. rosenbergii* (Table-2). Earlier studies, Dandapat *et al*., (2003) reported that supplementation of vitamin-E improved growth, metabolism and survival of post larvae, *Macrobrachium rosenbergii*. Anne Rebecca and Saravana Bhavan, (2014) reported that feed conversion ratio (FCR) *Zingiber officinale* extract formulated with control diet fed with *M. rosenbergii* was observed by the higher level. Flavanoids are one among the natural active principle compounds that have been reported to promote various activities like anti-stress, growth promotion, appetite stimulation, tonic and immunostimulation and to have aphrodisiac and antimicrobial properties in finfish and shrimp larviculture (Citarasu, 2009; Sivaram *et al*., 2004).

Table -2: Growth performance and survival rate in *M. rosenbergii* fed with formulated feeds

Growth Parameters	Experimental Diets				
	Control	1%	2%	3%	4%
Survival rate (%)	75	100	100	100	100
Weight gain (%)	0.38±0.01	0.56±0.06	0.64±0.01	0.072±0.04	0.078±0.09
SGR	2.45±±0.03	2.46±0.08	2.78±0.03	2.98±0.01	3.21±0.07
FCR	0.93±0.02	0.78±0.01	0.67±0.07	0.47±0.02	0.39±0.05

Values are Triplicates (Mean ± SD)

The reproductive biology

The present study observed that reproductive biology of *M. rosenbergii* was studied by comparing the macroscopic and microscopic characteristics and the identification of gonadal developmental stages (Plate-1). We observed that *M. rosenbergii* was histological identified by five maturation stages of ovary recognized by the clear pattern and variations in colour, as well as development and arrangements of cells type. The identification of different stages was designated as undeveloped (translucent), developing (yellow), nearly ripe (light green), ripe (green) and spent (white/creamy) (Table-2). The results of the present study, the histological identification of the mature ovaries, nearly mature, matured and spent were exhibit light green, dark green or transparent color observed by experimental periods (Table-3). The present study showed that oogonia cells found throughout the ovarian development, though they were predominated in the undeveloped and spent stages (Plate-1). In the undeveloped stage, oogonia were found as clustered in a well-defined area of the ovary wall along the gonad, known as the "zone of proliferation" (plate-1).

Table-3:Characteristic features of the ovary of *M. rosenbergii* during different stages of reproductive cycle (Chengal Reddy *et al*.,2013)

States	Ovary color	Development stage
1	White and cream	Spawn
2	Yellow	Spent
3	Faint orange	Proliferative
4	Deep orange	Premature
5	Orange-Red	Mature

This study was conducted to explore the ovarian development of female *M. rosenbergii*, as an important foundation for the knowledge of its reproductive biology. Specimens were collected in four seasons throughout the year to investigate the ovarian cycle and germ cell development, by examining the external morphology and histological structure of the ovaries. Developing ovaries and germ cells were characterized throughout the entire ovarian cycle. Differentiating germ cells were classified mainly into oögonia, primary oöcytes, secondary oöcytes, and mature oöcytes. Among the four major germ cells, secondary oöcytes were further divided into five types (Photo-2). Previous researchers studied in ovarian histology of penaeid shrimp was identified by five ovarian developmental stages such as quiescent, developing, early maturity, ripe and spent (Tuma,1967; Crocos and Kerr,1983). Similarly, Amanat and Qureshi, (2011) observed by five developmental stages identified as undeveloped, developing, nearly ripe, ripe and spent in *Penaeus indicus*. Our result was five stages was clearly identified by based on colour, size and texture of the ovary in *M. rosenbergii* (Fig.1). According to the size and shape of cells, nuclei and nucleoli, and to the NP (ratio of nucleus to cytoplasm) and the vitelline accumulation, the oogenesis was divided into five stages in oogonium stage such as oocyte I stage, oocyte II stage, early mature oocyte stage and mature oocyte was identified in *M. rosenbergii*. Earlier studies, different stages of the developing ovary were classified based on the changes in external characteristics of the ovary, the timing of embryogenesis, the pre-mating, molt, oviposition, and histological observations of the ovary (Donovan *et al*., 1984; Chang and Shih,1995; Meeratana and Sobhon, 2007). Moreover, different germ cell types were identified according to their size, yolk accumulation, and follicular cell stage (Donovan *et al*., 1984; Meeratana and Sobhon, 2007).

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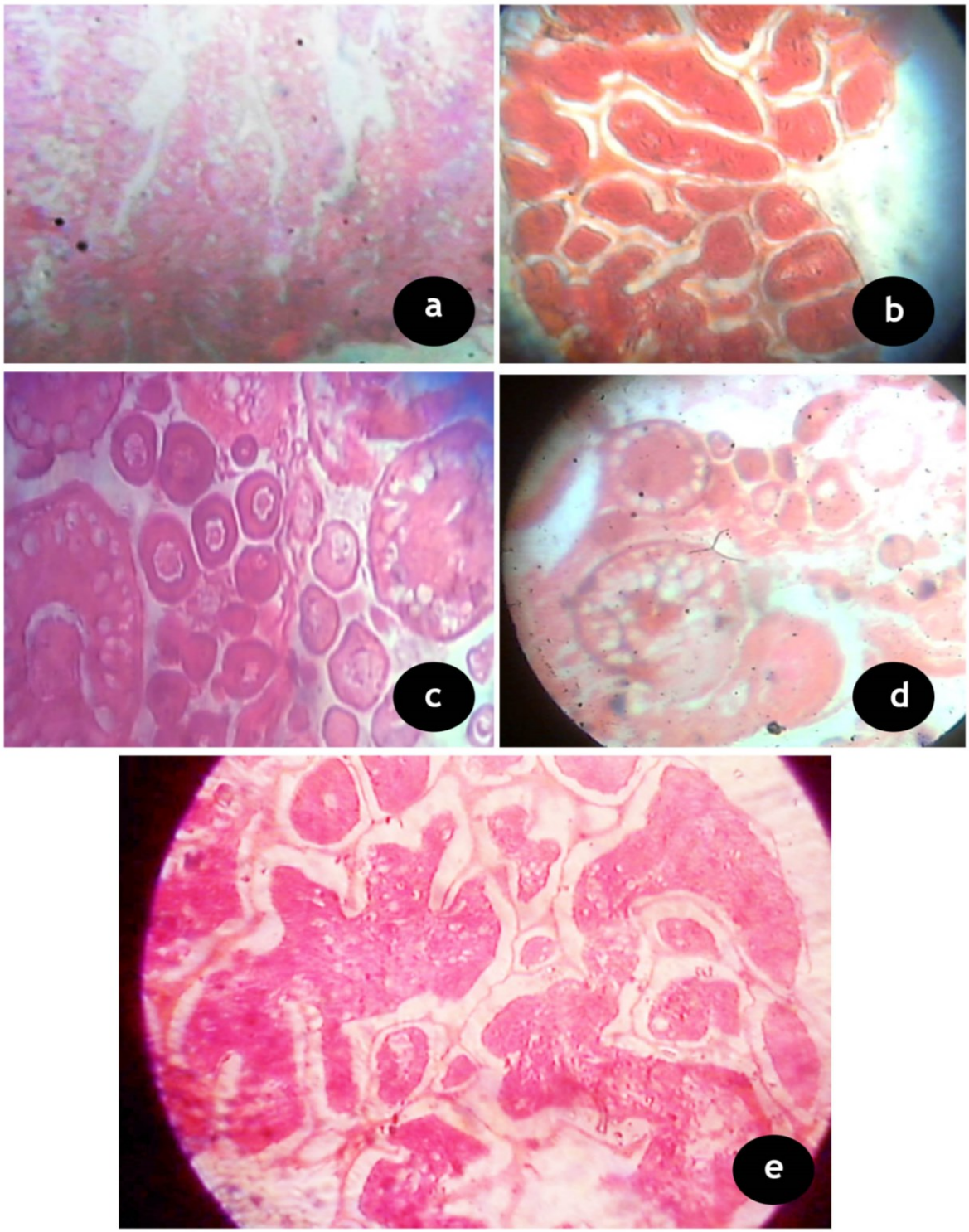
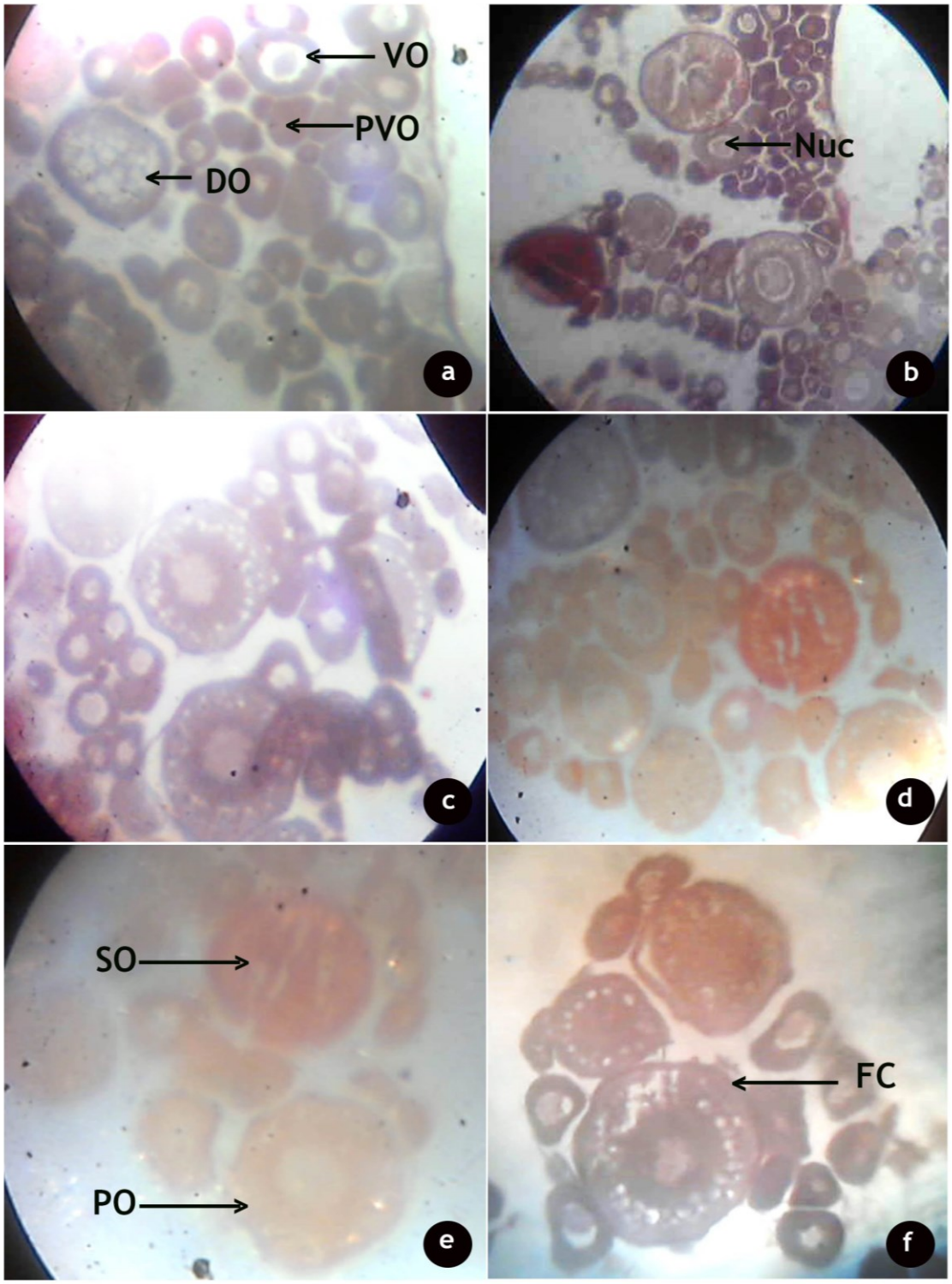


a: un developed ovary; b: undevaloped ovary;
c: near ripe ovary;d: mature ovary; e: spend ovary

Plate-1: Histological section of different stages of ovarian maturation of *M.rosenbergii*



PO= pre-vitellogenic oocyte, VO= vitellogenic oocyte, N= nucleus, FC= follicular cells, PVO = post-vitellogenic oocyte

Photo-2: Histological section of ovary stages of *M. rosenbergii*

The histological results revealed that germ cells in the organ stage are mainly situated in the central region of the ovary, and subsequently migrate to the periphery during development. They observed that well developed sites are distributed in the periphery of the ovary (Plate-2). Charniaux-Cotton, (1985) who observed by well-developed sites are distributed in the periphery of the ovary of Crustacean species. The histological study revealed that different maturation stages of the ovary of M. Rosenbergii was identified (Plate-2). The spawn ovary of *M. rosenbergii* was enlarged in size more than the spent ovary (Plate-1e). The stromal and connective tissues from collapsed, and empty ovarian pouches become loosened and flaccid, while those of the spent ovary are firm and start to have more oogonia and previtellogenic oocytes (Plate-1e). The ovary was observed in accumulation of previtellogenic oocytes and significant numbers of distinguishing characteristic feature of proliferative ovary. The increase in size of premature and mature ovaries was identified and due to the increased number of vitellogenic oocytes were observed. Increase number of mature ovary sizes are enlarged due to the uptake of yolk protein from exogenous sources significantly. According to Luis and Ponte (1993) reported that the presence of polychaetes in the experimental diet may be important to induce ovarian maturation in *P. keruthurus* because they contribute essential fatty acids (Luis and Ponte, 1993; Luis and Passos, 1995). Earlier studies, ovary development of penaeid shrimps was identified by five different stages, named immature, developing, incipient, ripe and spent (Vogt *et al*., 1989; Castille and Lawrence, 1991; Tan-Fermin, 1991; Medina *et al*., 1996; Quintero and Garcia, 1998; Palacios *et al*., 1999). The present results showed that experimental fed with *M. rosenbergii* observed by an increase in oocyte diameter from stage I to IV in *M. rosenbergii* coinciding with developing ovary. The maturation process of oocytes development of M. Rosenbergii was comparable to that reported in other crustaceans (Van Herp and Soyez,1997), which is characterized by the accumulation of ribosomes, glycogen, lipid, yolk and marinas (Kleckner *et al*., 1994). Chang and Shih (1995) and O, Donovan et al., (1984) have reported that follicle cells (FC), surrounding pre maturing oocytes, possibly reflect their biosynthetic ability (Chang and Shih, 1995; Yano and Chinzei, 1987). These cells are involved in the process of vitellogenesis in crustacean species, which may contribute to the vitellogenin synthesis during the early vitellogenesis was observed (Tsutsui *et al*., 2000; Van Herp and Payen, 1991). Earlier studies, Tan-Fermin and Pudadera, (1989) reported that frequency of acute size and cut diameter, have proved to be good indicators of the ovarian developmental stage in *Penaeus monodon*. The site size, frequency was estimated for all experimental diet fed for *M. rosenbergii* was observed by the counting of the total number of oocytes appeared. The conclusion of the present results suggested that dietary *I. longerecemosa* root extract could be improved the reproductive growth and feed utilization of giant freshwater prawn *M. rosenbergii*. The identification of active compounds from the roots of *I. longerecemosa* root extract may be acted as involvement in maturation of ovary. The presence of vitellogenin (vitelline) in the ovary of M. Rosenbergii suggests its involvement in the maturation of ovary. High level of vitellogenin present in the ovary may stimulate in the oocyte increment as well as maturation. It is also concluded that vitellogenin transport nutrients to the ovary of the M. Rosenbergii. This study is an ideal model for studying the growth performance and reproductive biology of crustacean species and also, this species may be a potential aquaculture species.

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