academicJournals

Vol. 14(1), pp. 10-16, 7 January, 2015
DOI: 10.5897/AJB09.30
Article Number: 597B9AD49425
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Comparison of fatty acid profile of wild and farm reared freshwater prawn *Macrobrachium rosenbergii* (De Man) brooders for broodstock diet formulation

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Received 5 March, 2009; Accepted 12 December, 2014

Fatty acid profiles of midgut gland (MG), ovary and eggs of *Macrobrachium rosenbergii* of wild and farm reared brooders indicate a significant variation of their components during the sexual maturation and spawning. In both groups, major fatty acids found in the chosen tissues were 14:0, 16:0, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 and 22:6n-3. Of these, saturated fatty acids dominate over the mono-unsaturated (MUFA), polyunsaturated (PUFA) and highly unsaturated fatty acids (HUFA). Though all the four groups of fatty acids are found in both groups except n-3 HUFA (P>0.05) other categories are found in higher wild brooders (P<0.05). There was a significant difference in total weight, total length and clutch weight (P<0.05) between farm and wild brooders, but the gonadosomatic index (GSI) and midgut gland somatic index (MSI) did not vary significantly (P>0.05).

Key words: Fatty acids profile, morphometry, *Macrobrachium rosenbergii*, wild and farm brooders.

INTRODUCTION

A thorough knowledge on the physiology, metabolism and biochemistry of commercially important species during maturation is essential for a complete understanding of its reproductive processes for hatchery operation (Mourente et al., 1994); indeed reproductive control of a cultured species is importance only next to its consumer demand (Bardach et al., 1972). Reproduction in crustacean entails maternal mobilization, biosynthesis and bioaccumulation of materials for export as self sufficient capsules; the unfertilized eggs (Harrison, 1990). The eggs are rich in yolk substances that are used as nutrient for embryonic development processes. Usually protein, one of the main components of yolk, plays an important role in both morphogenesis and energy supply in embryos (Holland, 1978; Luo et al., 2004). In decapod eggs, lipid content is relatively high and constitutes one of the major energy sources. Lipids play an important role in embryonic metabolism as they are the most important energy source and provide at least 60% of the total energy expended by the developing crustacean embryo; during the stages of

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embryonic development, lipids also serve as the components of biological membranes and pigments of compound eyes (Wehrtmann and Graeve, 1998).

In the early 1990s, commercial stocks of giant prawn have experienced productivity decline due to inbreeding depression; this is believed to have resulted from brood stock sourced from grow-out ponds (New, 2000). The fecundity and brood quality of wild *M. rosenbergii* are far better than the farm reared females (Wilder et al., 1999). But wild stocks of *M. rosenbergii* have also declined in recent years, as a result of over-harvesting, habitat loss and increased pollution; this is further compounded by the seasonal availability of wild brooders (Hien et al., 1998). Decline in quality and quantity of wild population has been reported in Bangladesh, India, Indonesia, Malaysia, the Philippines and Thailand (New et al., 2000). Hence *M. rosenbergii* hatcheries are constrained to depend on brooders procured from farms (Hien et al. 1998).

Understanding the interaction between nutrition and reproduction, quantification of the nutrient requirement is essential to promote successful maturation and spawning which in turn enable year round hatchery seed production (Harrison, 1997). In this regard, fatty acids of n-6 and n-3 series play a vital role in the reproductive performance of crustaceans. Hence a through evaluation of fatty acid profile during maturation and spawning is essential for sustainable aquaculture.

In India, freshwater prawn culture industry declines in terms of growth and production due to poor seed quality, random selection of brooders by hatchery, improper management practices in culture and imbalanced feeds. In this study area also for the past one decade a vicious cycle of supply of brooders from farms to hatcheries and the resultant juveniles from hatcheries to farms (unpublished data). In this scenario, the present study aims to find out the variations in the chosen morphometry and biochemical profiles of brooders collected from two different farms are the main suppliers for hatcheries with the wild brooders to find out the differences in brood quality between wild and farm reared *M. rosenbergii* as a measure of brood quality assessment.

MATERIALS AND METHODS

Brooder collection

M. rosenbergii females captured from Vembanad lake (Lat 9º 28' & 10º 10' N and long 76º 13' & 31' E) Arookkutty Tool, Cochin, Kerala, India were transported to the laboratory in polyethylene bag filled with aerated freshwater with least disturbance. Farm reared brooders purchased from Kottaipatinum (9º 57' N, 79º 30' E, Puddukotai District, Farm I) and Srikandapuram, (10º 59' N, 79º 35'E, Nagapatinum District, Farm II) Tamilnadu, India were also transported under above condition to the laboratory. The prawns were measured individually for wet weight (± 0.1 g) and total length (±0.1 cm) (from tip of rostrum to the end of the telson). The prawns were then sorted into two groups: a) females with ripe ovaries extending beneath the third rostral spine of the cephalothorax and b) incubating females with bright yellow colour newly spawned eggs (Pandian and Balasundaram, 1982; Chang and Shih, 1995). In the first group (n=3) individuals were sacrificed and tissues were sampled from midgut gland and ovary. The gonado-somatic index (GSI) and midgut gland somatic index (MSI) were calculated as the percentage weight of the gonad and midgut gland (MG) to the total body weight respectively. Clutch weight was determined separately after removing excess water by blotting with filter paper; care was taken to sample eggs of the same stage since there is a significant variation in biochemical parameters during different developmental stages of the egg (Cavalli et al., 2001). All the samples were stored at -70°C until further analysis.

Biochemical analysis

Total lipid (Folch et al., 1957) and fatty acid methyl esters (FAME) (Miller and Berger, 1985) content of midgut gland, ovary and eggs were quantified adopting standard analytical procedures. FAME was analyzed using a Hewlett-Packard 5890 gas liquid chromatograph equipped with Diethylene Glycol Succinate (DEGS) column and flame ionization detector (FID) using nitrogen as the carrier gas. The column temperature was 180°C and injection temperature was 200°C. The detector response was recorded. The FAME were identified and quantified by comparison of peak area and retention time of standard fatty acids.

Statistical analysis

The biometric variables such as total weight, total length, gonadosomatic index (GSI), midgut gland somatic index (MSI), clutch weight and selected tissue biochemical composition of *M. rosenbergii* brooders of wild and farms are analyzed through one-way analysis of variance (ANOVA). Subsequently the significant means are ranked through multiple mean comparison tests with LSD.

RESULTS

Morphometry

The biometric variables of *M. rosenbergii* females are summarized in Table 1. There were no significant differences found in GSI and MSI of the brooders between wild and farms but total wet weight, total length and clutch weight varied significantly ($P<0.05$) between wild and farm brooders.

Fatty acid profile

The fatty acid profile of midgut gland, ovary and eggs of brooders collected from wild and farms are compared. The MG of wild brooder have maximum total lipid (34.3%) content than the farm reared brooders (Table 2) and the difference is statistically significant ($P<0.05$). The total lipid content of farm I (29.5%) and farm II (28.5%) also differed significantly ($P<0.05$). Fatty acids found predominantly in all these organs are saturated fatty acids like myristic (14:0), palmitic (16:0), stearic (18:0) and monounsaturated fatty acid such as oleic (18:1n-9) acid.

Table 1. Total weight, total length, GSI, MSI and clutch weight of wild and farm brooders of *M. rosenbergii*. Superscript letters within rows indicate significant differences (P<0.05). Values are mean ± SD (n=10).

Variable	Wild	Farm I	Farm II
Total weight (g)	44.5±13.4^{b}	42.4±12.7^{ab}	40.3±12.0^{a}
Total length (cm)	17.4±2.9^{c}	15.5±2.5^{a}	16.8±2.7^{b}
GSI (%)	5.1±0.9	4.9±0.8	4.8±0.8
MSI (%)	4.0±0.6	3.8±0.6	3.9±0.5
Clutch weight(g)	4.1±0.8^{b}	3.1±0.6^{a}	3.2±0.1^{a}

Table 2. Selected principal fatty acid content (mg g^{-1} dry weight) of the MG of wild and farm broodersof *M. rosenbergii*. Each value is a mean ± S.D (n=3). Different superscripts within rows represent significant differences (P<0.05).

Fatty acid	Wild	Farm I	Farm II
Total lipids	34.3±2.4 ^{c}	28.5±1.9^{a}	29.5±2.1^{b}
14:0	38.04±2.3^{c}	11.61±1.2^{b}	5.50±0.68^{a}
16:0	32.03±2.2^{c}	5.53±0.61^{a}	7.96±0.95^{b}
18:0	28.83±1.7^{c}	5.89±0.64^{a}	6.44±0.77^{b}
18:1n-9	33.82±2.4^{c}	4.54±0.59^{a}	6.76±0.87^{b}
18:2n-6	28.73±2.0^{c}	10.71±1.07^{a}	11.89±1.30^{b}
18:3n-3	7.46±0.52^{c}	4.47±0.63^{a}	5.57±0.72^{b}
20:4n-6	5.51±0.44^{c}	2.04±0.76^{a}	3.29±0.46^{b}
20:5n-3	5.02±0.40^{a}	5.82±0.75^{a}	6.96±0.90^{b}
22:6n-3	4.59±0.41^{a}	5.31±0.48^{a}	6.59±0.72^{b}
Σ Saturates*	98.89±4.94^{b}	23.04±2.3^{a}	20.89±2.29^{a}
Σ Mono-unsaturated**	33.82±2.4^{c}	4.54±0.59^{a}	6.76±0.87^{b}
Σ n-6 PUFA	34.24±2.74^{c}	12.75±1.40^{a}	15.19±1.36^{b}
Σ n-3 HUFA	17.06±1.36^{a}	15.59±1.24^{a}	19.12±1.72^{b}
DHA/EPA ratio	0.913	0.912	0.947
n-6/n-3 ratio	2.01	0.817	0.794

*Saturates: 14:0; 16:0; 18:0. **Monounsaturates: 18:1n-9, n-6 PUFA: 18:2n-6; 20:4n-6. n-3 HUFA: 18:3n-3; 20:5n-3; 22:6n-6.

The n-6 polyunsaturated fatty acids (PUFA) such as linoleic (18:2n-6) and arachidonic acid (20:4n-6) and n-3 highly unsaturated fatty acids such as eicosapentaenoic (20:5n-3) and docosahexaenoic acid (22:6n-3) are present at an intermediate levels in all the tissues studied.

The comparison of fatty acids of MG indicate that the wild brooders (98.8 mg g^{-1}) have maximum saturated fatty acids which is significantly (P<0.01) different from farm reared brooders. But there was no such significant difference (P>0.05) between farm I (23.0 mg g^{-1}) and farm II (20.8 mg g^{-1}) brooders. There was significant difference in the amount of monounsaturated fatty acid (18:1n-9) (P<0.01) in the wild brooders (33.8 mg g^{-1}) when compared with Farm II (6.8 mg g^{-1}) and Farm I (4.5 mg g^{-1}) brooders. The content of n-6 PUFA were also significantly higher (P<0.05) in wild (34.2 mg g^{-1}) than the farm reared brooders. Interestingly in MG, the n-3 HUFA level showed a significant variation between farm and wild brooders.

The total lipid and fatty acid profile of ovary of wild and farm reared brooders are presented in Table 3. The total ovarian lipid content of wild brooders was 37.4%, which is differed significantly (P<0.05) when compared to farm I (33.5%) and Farm II (33.7%). Though there is a variation in farm and wild among the farm reared brooders there is no such difference. Saturated fatty acids are found higher in the ovary of wild brooder (52.8 mg g^{-1}) than the farm reared brooders (P<0.05). Monounsaturated fatty acids 18:1n-9 was found rich in wild (35.3 mg g^{-1}; P<0.01) followed by farm II (7.6 mg g^{-1}) and farm I (6.3 mg g^{-1}) (P<0.05) reared brooders. The n-6 series of polyunsaturated fatty acids of wild brooder (35.5 mg g^{-1}) was significantly higher (P<0.05) than Farm I (16.4 mg g^{-1}) and Farm II brooders (15.4 mg g^{-1}). There was no significant difference in n-3HUFA level among brooders

Table 3. Selected principal fatty acid content (mg g^{-1} dry weight) of the ovary of wild and farm brooders of *M. rosenbergii*. Each value is a mean ± S.D (n=3). Different superscripts within rows represent significant differences (P<0.05).

Fatty acid	Wild	Farm I	Farm II
Total lipids	37.4±2.99^{b}	33.5±3.02^{a}	33.7±3.03^{a}
14:0	14.59±1.60^{c}	4.53±0.41^{a}	5.97±0.55^{b}
16:0	23.89±2.62^{b}	9.76±0.87^{a}	9.68±0.77^{a}
18:0	14.41±1.58^{c}	9.93±0.89^{b}	8.03±0.72^{a}
18:1 n-9	35.39±3.89^{c}	6.39±0.61^{a}	7.67±0.69^{b}
18:2 n-6	30.57±3.36^{c}	14.45±1.58^{b}	13.03±1.43^{a}
18:3 n-3	4.53±0.45^{a}	6.26±0.55^{b}	5.59±0.63^{ab}
20:4 n-6	4.95±0.44^{b}	1.98±0.39^{a}	2.43±0.52^{a}
20:5 n-3	6.59±0.59^{a}	6.98±0.62^{a}	7.78±0.70^{b}
22:6 n-3	6.77±0.61^{a}	5.28±0.61^{a}	6.05±0.54^{a}
Σ Saturates	52.89±5.82^{b}	24.23±2.66^{a}	23.68±2.84^{a}
Σ Mono-unsaturated	35.39±3.89^{c}	6.39±0.61^{a}	7.67±0.69^{b}
Σ n-6 PUFA	35.51±3.91^{b}	16.43±1.81^{a}	15.46±1.70^{a}
Σ n-3 HUFA	17.89±1.96^{a}	19.53±2.14^{a}	19.44±2.13^{a}
DHA/EPA ratio	1.050	0.848	0.788
n-6/n-3 ratio	2.053	0.846	0.804

Table 4. The selected principal fatty acid content (mg g^{-1} dry weight) of eggs of wild and farm brooders of *M. rosenbergii*. Each values is a mean ± S.D (n=3). Different superscripts within rows represent significant differences (P<0.05).

Fatty acid	Wild	Farm I	Farm II
Total lipids	34.7±3.81^{b}	33.4±4.00^{a}	33.6±4.03^{a}
14:0	10.58±1.37^{c}	7.19±0.64^{b}	3.53±0.63^{a}
16:0	14.66±1.61^{c}	4.98±0.44^{a}	6.95±0.62^{b}
18:0	13.09±1.44^{b}	6.68±0.59^{a}	5.55±0.75^{a}
18:1n-9	30.59±3.36^{b}	5.74±0.47^{a}	6.15±0.52^{a}
18:2n-6	36.33±3.99^{c}	13.09±1.57^{b}	9.79±0.87^{a}
18:3n-3	3.91±0.35^{a}	4.41±0.62^{ab}	5.16±0.46^{b}
20:4n-6	3.83±0.66^{b}	1.57±0.63^{a}	2.03±0.24^{a}
20:5n-3	5.36±0.63^{ab}	4.71±0.53^{a}	6.06±0.54^{b}
22:6n-3	4.66±0.73^{a}	4.83±0.53^{a}	5.11±0.45^{a}
Σ Saturates	38.33±4.98^{b}	18.85±2.26^{a}	16.54±2.12^{a}
Σ Mono-unsaturated	30.59±3.36^{b}	5.74±0.47^{a}	6.15±0.52^{a}
Σ n-6 PUFA	40.16±4.82^{c}	14.66±1.76^{b}	11.82±1.42^{a}
Σ n-3 HUFA	13.94±1.67^{a}	13.95±1.68^{a}	16.33±1.95^{a}
DHA/EPA ratio	0.848	1.026	0.833
n-6/n-3 ratio	3.05	1.081	0.723

of wild and farms (P>0.05). Total lipid and fatty acids of eggs in wild and farm brooders show a similar trend like ovary (Table 4).

DISCUSSION

In India, commercial farming of *M. rosenbergii* culture face productivity declined in recent years. Poor quality of seeds and low feed conversion efficiency affect the growing industry. Previous studies on reproductive performance explained inbreeding depression as a major cause which is as a result of brood stock sourced from the grow-out ponds rather than from the wild (New, 2000; Mather and Bruyn, 2003). In *Macrobrachium* culture, brooders are chosen for seed production not on the basis

of size, but on the availability and ready to spawn. This results in selection of smallest brooders that in turn lead to a substantial reduction of mean size across generations and loss of performance (New, 1995).

In the wild, *M. rosenbergii* female first mature at a size of 20-40 g; eggs obtained from these females are of good quality and their larvae show high percent of survival. However, females of hatchery origin, which are cultured as broodstock often, mature at a size of 7-10 g. Selection of such a precociously mature females for seed production results in eggs and larvae of poor quality; offspring of these females may mature even more precociously (Wilder et al., 1999).

Among the various factors govern to the broodstock quality the prime factors is the nutritional status of the brooder (Harrison, 1990). The total lipid content of the tissues of wild brooders is reflected by the habit of the organism, that is omnivorous food habit in a natural ecosystem where it has ready access to variety of organisms such as worms, insect larvae, small mollusks, aquatic insects, fish and other crustaceans (Ling, 1969). This enhances the constant growth under natural conditions and suggests the organism's ability to meet out the seasonal food shortage (Sahavacharin and Pongsuwan, 1974).

Studies in crustacean brooders, indicate that there is an accumulation of lipid content in ovary when compare to the eggs and midgut gland. Such an increased in lipid content suggests that the dietary lipids stored in the MG are transferred to the ovary for the maturation (Harrison, 1990; Cavalli et al., 1999). As indicated above, the nutrition status of the organism determines the accumulation of nutrient reserves. In this study also there is a significant difference in total lipid content of wild brooders when compare to the farm reared ones.

The fatty acid profiles of MG, ovary and eggs of wild and farm brooders in this study showed that the proportion of saturated and monounsaturated fatty acids was higher than that of n-6PUFA and n-3HUFA series. Previous studies also support the present findings in *M. rosenbergii* eggs (Tidwell et al., 1998; Cavalli et al., 2000), newly hatched larvae (Roustaian et al., 1999), Juveniles (Chanmugam et al., 1983) and midgut gland and ovary of mature females (Cavalli et al., 2000). The wild brooder possesses highest level of saturated and mono-unsaturated fatty acids than the farm brooders in all the organs. The highest level of saturated and mono-unsaturated fatty acids accumulation in the tissue shows these organs are the major energy source for embryonic (Clarke et al., 1990) and early larval development (Roustaian et al., 1999).

The comparison of n-6PUFA and n-3HUFA content in the tissues show much difference in wild and farm brooders. The n-6PUFA fatty acids are found higher in all the organs compare to n-3HUFA. For *Penaeids* n-3HUFA, particularly EPA and DHA are considered as essential fatty acids (Kanazawa et al., 1979a; 1979). The maturation performance and offspring quality of *Penaeids* are linked to dietary n-3HUFA level (Middleditch et al., 1980; Teshima et al., 1998; Teshima et al., 1989; Xu et al., 1994). In the case of freshwater prawn, n-6PUFA series of fatty acids predominate over n-3HUFA fatty acids (Chanmugam et al., 1983). The essential role of n-6PUFA especially linoleic acid (18:2n-6) has been demonstrated for juveniles (Reigh and Stickney, 1989; D'Abramo and Sheen, 1993).

Increase in the amount of ovarian linoleic acid during maturation, indicates its importance in metabolism or its response. This increase influences the fecundity of *M. rosenbergii* (Cavalli et al., 1999). In the present study n-6PUFA fatty acids were abundantly present in wild brooders compare to farms; this may be true for the wild caught brooders of Vembanad lake where they have recorded a high fecundity of 227,161 eggs per female with a total length of 258 mm and weight of 208 g; the smallest female measuring a total length of 158 mm and a total weight of 33.7 g had a clutch size of 30,666 eggs (Sureshkumar and Kurup, 1998). The small sized farm reared brooder measured a total length of 155 mm and weight of 42.4 g had a clutch size 34,020 (Balamurugan, 2006). This gives a strong support on the role of richness in n-6PUFA in the wild when compared to farm brooders. Moreover, the high level of n-6 fatty acids, in wild brooders is not surprising because of its natural diets (Chanmugam et al., 1983).

Hence the evaluation of broodstock prawns captured at different times and different habitat is essential for quality seed production. Variation between these individuals and their nutritional condition play a vital role on brood quality (Marsden et al., 1997). The n-6/n-3 ratio of the farm brooder is higher than the wild brooders is due to the diet available in their environment. Increase in n-3 HUFA series of farm reared brooders of *M. rosenbergii* is as a result of formulated grow out diet which are rich in animal proteins of marine origin that are rich source of n-3 series of HUFA. In wild, shrimp maturation and reproduction are greatly influenced by environmental factors (Bray and Lawrence, 1992) and the type of autothonous food which includes a variety of food items (Ling, 1969). Changes in the abundance and distribution of these food determine the preference of n-3 and n-6 series of fatty acids and perform the pattern of reproductive activity (Crocos and Coman, 1997).

The n-3 HUFA content of ovary and eggs of farm I and farm II brooders are more than in the wild brooders. For instance the amount of docosahexaenoic and eicosapentanoic acid are more and these acids play an important role in hatching, larval survival and structural component of cell membranes and formation of central nervous system in embryo (Cavalli et al., 1999). In *M. rosenbergii* bioconversion ability of EPA to DHA is lacking (Teshima et al., 1992). A well-balanced feed enriched by the required fatty acids will enhance the synthesis of EPA to DHA (D' Abramo and Sheen, 1993).

Comparatively low level of n-3 HUFA series especially docosahexaenoic acid in tissues of wild brooders would adversely affect the hatchability which is evident from the brood stock collected from the wild (unpublished data). Hence while selecting the brood stock from the wild, the hatcheries should take care of the nutritional level especially with reference to the eicosapentanoic and docosahexaenoic acid levels since they determine the hatchability and larval survival.

Conclusion

The present study clearly explained the importance of n-6 PUFA and n-3 HUFA content in the brooders for fecundity, hatchability and larval survival of *M. rosenbergii* for sustainable culture. The wild brooders lipid and fatty acids analysis show more amount of n-6 PUFA content which increased their fecundity and their low level of n-3 HUFA content reduced the hatchability and larval survival. In such a way that the farm reared brooders lipid and fatty acid analysis show less amount of n-6 PUFA and high level n-3 HUFA content reflects reduced fecundity and better larval survive supplied to culture. So, the present study strongly recommend that maturation diet fortified with ingredients should have a rich sources of n-6PUFA and n-3 HUFA for enhanced fecundity, hatchability and larval survival for sustainable *M. rosenbergii* culture.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to UGC-New Delhi for the financial assistance in the form of Major Research Project to CB. The authors are indebted to Mr. Igbal, for liberal supply of brooders for this research. A grant awarded to the department thorough FIST, Department of Science and Technology is profoundly acknowledged.

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