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Effect of Unilateral Eyestalk Ablation on the Biochemical Composition of Commercially Important Juveniles of *Macrobrachium malcolmsonii* (H. Milne Edwards)

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Abstract: An investigation was made on the juveniles of a commercially important and cultivable species of second largest freshwater prawn *Macrobrachium malcolmsonii* for its responses to unilateral eyestalk ablation, different diets and some biochemical aspects. Protein content of the adult *Artemia* fed animals was higher than that of artificial feeds 1 and 2, oyster and earthworm fed eyestalk ablated prawns. In intact control animals, protein content was differ significantly when the animals were fed with different feeds except the artificial feed 1 and oyster. Eyestalk ablation had no significant effect on the body carbohydrate content of *M. malcolmsonii* fed with different feeds. The lipid content of the eyestalk ablated and intact control animals fed with adult *Artemia* showed significantly higher values when compared to other feeds. The content of free amino acids in eyestalk ablated animal fed with adult *Artemia* and earthworm was significantly higher from those fed with other feeds. Intact control animals offered with adult *Artemia* showed significantly higher contents of free amino acids as compared to those fed with other feeds. The eyestalk ablation had no effect on water content. The ash content of the eyestalk ablated animals fed with oyster and earthworm was significantly higher than those fed with other feeds. The intact control animals showed higher ash content when fed with earthworm, oyster and artificial feed 2 than those fed with other feeds. The energy content of the eyestalk ablated and intact control animals fed with adult *Artemia* was significantly higher than those fed with other feeds.

Key words: *Macrobrachium malcolmsonii*, eyestalk ablation, juveniles, *Artemia*, earthworm

INTRODUCTION

The freshwater prawns, *M. malcolmsonii* (H. Milne Edwards) and *M. rosenbergii* (De Man) are suitable for culture, as they grow to the marketable size reaching an average weight of 40-70 g in 6 months (Kannupandi, 1995). Of the two species, *M. malcolmsonii* is quite suitable for both mono and polyculture with carps in Tamil Nadu (Anonymous, 1985). Studies on the biochemical composition of edible organisms are important from the nutritional point of view. Only few reports in this aspect are available on penaeid shrimps and studies are very limited to palaemonid prawns with reference to eyestalk ablation. Hence, the present study was aimed to know the effect of unilateral eyestalk ablation on the body organic constituents of *M. malcolmsonii* juveniles when fed with different feeds.

MATERIALS AND METHODS

The feeding experiment was conducted in the juveniles of *M. malcolmsonii* for a period of 60 days. The ovigerous females of *M. malcolmsonii* were collected from the freshwater areas of

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Manampadi near Parangipettai (Lat. 11°29' N and Long 79°46' E) in Tamil Nadu. The larvae were reared in the laboratory up to juvenile stage. Healthy juveniles of *M. malcolmsonii* weighing 0.90 to 0.92 g and 3.5±0.5 cm lengths were selected. The experimental animals were divided into two groups. One group served as the control (intact control) and another group was used for eyestalk ablation experiment. Unilateral eyestalk ablation was performed by following the method of Caillouet (1973). Five juveniles were introduced in to each plastic trough (35 cm diameter × 13 cm height) filled with 10 L of freshwater. Each experiment had 8 replicates and the juvenile were fed once a day in the morning with live and artificial feeds at 10% of the body weight. The experimental set-up was kept under the following conditions: dissolved oxygen 6.2±1 mg L^{-1}, pH 8.2±0.5, light dark regime 12/12 and temperature 28±2°C. On termination of feeding trial the juveniles from each treatment were starved for one day to ensure complete evacuation of gut. They were dried at 60°C to estimate water content and biochemical constituents such as total protein, carbohydrate, lipid and free amino acids following standard methods.

Energy

The total energy content of the animal was calculated by using the following conversion factors 17.16 J mg^{-1} for carbohydrate, 23.65 J mg^{-1} for protein and 39.55 J mg^{-1} for lipid (Crisp, 1984).

Statistical Analysis

The data were analysed for statistical significance by ANOVA. Differences in treatment means were determinate by Duncan's multiple range test ($p<0.05$) using SPSS/PC$^+$ package

RESULTS

The results of biochemical constituents and their energy content of unilaterally eyestalk ablated and intact control juveniles of *M. malcolmsonii* after 60 days of experiment are displayed in Table 1 and 2.

Protein

Protein content of the adult *Artemia* fed animals (64.15%) was significantly higher than that of earthworm (59.15%), oyster (60.91%) and artificial feeds 1 (59.06%) and artificial feed 2 (62.19%).But the protein content did not show any significant difference among the animals fed with earthworm,

Table 1: Biochemical constituents (%) and their energy levels (J mg^{-1}) of unilaterally eyestalk ablated juveniles of *M. malcolmsonii* fed with different diets

Feed	Protein	Carbohydrate	Lipid	Free amino acids	Water	Ash	Energy
Adult *Artemia*	64.15±1.72^{c}	3.82±1.67^{a}	8.94±1.09^{c}	16.53±0.93^{b}	73.69±1.40^{a}	18.96±1.35^{a}	19.09±1.51^{b}
Earthworm	59.58±1.51^{ab}	3.94±1.26^{a}	7.93±0.99^{b}	15.41±0.76^{a}	73.81±1.49^{a}	20.40±1.06^{b}	18.54±0.75^{ab}
Oyster	60.91±4.08^{ab}	3.57±1.19^{a}	7.84±0.70^{b}	15.27±0.88^{a}	72.71±1.40^{a}	20.31±1.22^{b}	18.54±1.06^{ab}
Feed 1	59.06±3.92^{a}	3.83±1.03^{a}	6.56±0.53^{a}	15.46±0.74^{a}	72.84±1.48^{a}	19.54±0.92^{ab}	17.69±1.06^{a}
Feed 2	62.19±0.99	4.13±1.46^{a}	7.69±0.74^{b}	17.16±0.98^{b}	72.59±1.69^{a}	19.97±1.46^{ab}	18.53±1.07^{ab}

Means with different superscripts are statistically different ($p<0.05$; Duncan's multiple range test)

Table 2: Biochemical constituents (%) and their energy levels (J mg^{-1}) of intact control juveniles of *M. malcolmsonii* fed with different diets

Feed	Protein	Carbohydrate	Lipid	Free amino acids	Water	Ash	Energy
Adult *Artemia*	61.66±1.51^{d}	3.06±1.19^{a}	8.29±0.90^{c}	15.43±1.41^{b}	73.43±0.92^{a}	18.57±0.93^{a}	18.54±0.75^{c}
Earthworm	56.57±1.19^{a}	3.65±1.42^{a}	7.40±0.74^{b}	14.91±1.46^{ab}	73.06±0.50^{a}	20.16±0.98^{b}	17.41±1.06^{b}
Oyster	59.04±3.92^{b}	3.05±1.03^{a}	7.20±0.35^{b}	13.79±1.15^{a}	72.69±0.92^{a}	19.77±0.46^{b}	16.16±0.83^{a}
Feed 1	58.17±0.84^{b}	3.69±0.74^{a}	6.29±0.46^{a}	14.89±0.99^{ab}	72.56±1.07^{a}	19.41±0.74^{ab}	17.26±0.70^{b}
Feed 2	60.41±0.78^{c}	3.58±1.06^{a}	7.31±0.46^{b}	15.27±0.88^{ab}	72.43±1.03^{a}	19.69±1.06^{b}	17.59±0.53^{b}

Means with different superscripts are statistically different ($p<0.05$; Duncan's multiple range test)

oyster, artificial feeds 1 and 2 (Table 1). In the case of intact control animals, protein content showed a significant difference in all the feeds used except the artificial feed I and oyster fed animals (Table 2).

Carbohydrate

Eyestalk ablation had no significant effect on the body carbohydrate content of *M. malcolmsonii* fed with different feeds (Table 1 and 2).

Lipid

Duncan's multiple range tests showed that the lipid content in the eyestalk ablated animals fed with adult A*rtemia* (8.94%) was significantly higher when compared to other feeds. However earthworm (7.93%), oyster (7.84%) and artificial feed 2 (7.69%) fed animals did not show any significant difference between their lipid contents. Eyestalk ablated animals fed with artificial fed 1 (6.56%) showed the lowest lipid content (Table 1). Intact control prawns also showed a similar trend regarding lipid content (Table 2).

Free Amino Acids

Duncan's multiple range test indicated that eyestalk ablated animals fed with adult *Artemia* (17.16%) and earthworm (16.53%) were significantly higher from those fed with other feeds with respect to free amino acid content while adult *Artemia*, earthworm and also oyster, artificial feeds 1 and 2 did not differ significantly (Table 1). The intact control animals offered with adult *Artemia* showed significantly higher contents of free amino acids then those fed with other feeds. However, the animals fed with other feeds showed nonsignificant difference (Table 2).

Water

The water content was slightly higher in unilateral eyestalk ablated animals, but the difference was not statistically significant (Table 1 and 2).

Ash

Eyestalk ablation had no significant effect on ash content of the animals. However, Duncan's multiple range tests showed that the eyestalk ablated animals fed with earthworm (20.40%) and oyster (20.31%) was significantly higher then those offered with other feeds. The feeds among oyster (20.31%), earthworm (20.41%) and also adult *Artemia* (18.96%), artificial feeds 1 (19.54%) and 2 (19.97%) did not show significant difference (Table 1). The intact control animals when fed with earthworm (20.16%), oyster (19.77%) and artificial feed 2 (19.69%) showed significantly higher ash content than other feeds. But ash content between these three feeds and also between artificial 1 (19.41%) and adult *Artemia* (18.57%) did not show significant difference (Table 2).

Energy

The energy content of the eyestalk ablated animals fed with adult *Artemia* (19.09 J mg^{-1}) was significantly higher. However, animals fed with other feeds showed non significantly difference (Table 1). The intact control animals fed with adult *Artemia* (18.54 J mg^{-1}) showed significantly higher energy content than those fed with other feeds, while animals fed with earthworm (17.41 J mg^{-1}) artificial feeds 1 (17.26 J mg^{-1}) and feed 2 (17.59 J mg^{-1}) did not show significant difference. Intact control animals fed with oyster showed the lowest energy content (Table 2).

DISCUSSION

Biochemical studies are very important from the nutritional point of view. The biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity,

temperature and availability of food etc. The eyestalk hormones have regulative (positive) influence on carbohydrate, nitrogen and lipid metabolism in crustaceans (Highnam and Hill, 1979) which were found to vary with times and species (Vernberg and Vernberg, 1974; Madyasthan and Rengnekhar, 1976). In the present study, the levels of protein, lipid, free amino acids and energy content of the fresh water prawn *M. malcolmsonii* were altered significantly by unilateral eyestalk ablation. However, the eyestalk ablation had no effect on carbohydrate, water and ash content of the whole animal. Koshio *et al*. (1992) reported that neither unilateral eyestalk ablation nor feeding frequency affected the contents of the protein, lipid or the composition of the lipid class and fatty acids of *M. rosenbergii*. Surendranath *et al*. (1992) reported eyestalk ablation caused marked changes in haemolymph glucose, glycogen, proteins, free amino acids, total lipids, free fatty acids and glycerol in tissues of *M. monoceros*.

Sriraman (1978) found that biochemical constituents showed increasing trend from juvenile to adult in *Penaeus indicus* and *P. monodon*. Chen and Lin (1992) reported slightly increased level of lipid, protein and ash in *P. monodon* fed with arginine supplemented diet. However, Fox (1993) reported no significant effect of dietary chitin level on carcass protein or lipid.

In the present study, protein content was significantly higher in eyestalk ablated animals than those in intact control ones fed with different diets. Not only eyestalk ablation, but also feed, dietary proteins in particular, play important roles in the proximate composition of the animals. The protein content in the eyestalk ablated animals showed significant difference when fed with adult *Artemia,* earthworm and artificial feed 2. Protein is undoubtly, the most dominant biochemical constituent of penaeid prawns (Shaikhmahmud and Magar, 1957; Dabrowski *et al*., 1969; Achuthankutty and Parulekar, 1984) and palaemonid prawns (Pounuchamy *et al*., 1983; Ajith Kumar, 1990; Joseph *et al*., 1991; Sherief *et al*., 1992). Values of protein in the present study agree with other studies (Hilton *et al*., 1984; Ashmore *et al*., 1985; Nuwayhid and Young, 1985; Sheen and D'Abramo, 1991; Shah, 1995).

The average body protein of the *M. malcolmsonii* was directly related to the levels of protein in the diet up to 56%. Higher protein content was noticed in adult *Artemia* fed animals and lowest in artificial feed 1 fed animals. Similar pattern of protein changes are reported by Alava and Lim (1983) in *P. monodon*. Some ingredients in the feed also have some negative effect on the body biochemical composition. Lim and Dominy (1990) reported that there was no significant difference in the whole body composition of lipid, ash, calcium or potassium of *P. vannamei* fed with different diets incorporated with soyabean meal as a major ingredient. In the present study also, soya flour was used as one of the ingredient in artificial feed 1. This may be the reason for low body protein content in the prawn *M. malcolmsonii* fed with artificial feed 1.

The body fat content of *M. malcolmsonii* juveniles increased when the protein content of the diet was high. Luquet and Sabaut (1973) and Cowey *et al*. (1972) noted that the protein contents in gilthead bream (*Chrysophrys aurata*) and plaice (*Pleuronectes platessa)* increased, while fat contents decreased with increasing percentage of protein in the diet. The protein content of the diets in the present study, ranged from 35.15 to 56.00% and the body protein which slightly high ranged from 59.06 to 64.15% in eyestalk ablated animals and from 56.57 to 61.66% in intact control animals. Brown trout fed with the lowest dietary levels of protein (16.0%) had the highest body protein, while the fish fed with intermediate levels (20.5, 40.0, 41.01 and 71.0%) contained the same levels of body protein (Poston, 1975). Dabrowski (1977) also observed that the body fat increased with increasing protein contents in the diets, while other components remained at similar levels in grass carp. As the levels of protein in the diet increased beyond the maximum requirement, the excess was stored as fat or as carbohydrate Cowey and Sargent (1979).

Carbohydrates constitute only a minor percentage of total biochemical composition. In the present study, neither unilateral eyestalk ablation nor diet had any effect on the content of carbohydrate in body of *M. malcolmsonii*. However, the results of Alava and Pascual (1987) and Diaz

and Nakagawa (1990) indicated that different types of dietary carbohydrates could influence the proximate composition of prawn. In *P. monodon*, carcass fat decreased while faecal fat increased with increasing dietary carbohydrate (Catacutan, 1991). This indicates that higher levels of carbohydrate depressed lipid deposition as noted by Alava and Pascual (1987) in *P. monodon* juveniles fed with 30% trehalose or 20% sucrose. On the contrary, the lipid content of *M. rosenbergii* was observed to increase with increasing non protein energy in barley diets (Ashmore *et al*., 1985).

In the present study, lipid content of the unilaterally eyestalk ablated animals increased significantly and showed significant difference only with the animals fed with oyster. However, Koshio *et al*. (1992) reported that the unilateral eyestalk ablation did not change the composition of lipid class and fatty acids in *M. rosenbergii* juveniles. This suggests that remaining factors and/or hormones in the unilateral eyestalk ablated, which was not removed, continue to control lipid metabolism to a certain extent. The contents of lipid in body of M. *malcolmsonii* juveniles were higher than those reported by Chanmugam *et al*. (1983) and Sheen and D'Abramo (1991), but were very similar to those of Ashmore *et al*. (1985), Koshio *et al*. (1992) and Shah (1995).

Fat served as food reserves along with protein. It is generally known that fat content in animal is subjected to periodic fluctuation. Temperature of external medium influences the fat content in some fishes (Johnstene, 1997). The wide fluctuations in the lipid composition have been reported to occur both in hepatopancreas and gonads of prawns during gonadal development (George and Patel, 1956; Pillay and Nair, 1973; Gopakumar and Nair, 1975). But this does not affect the lipid composition of muscle tissue to any great extent.

The level of free amino acids in eyestalk ablated animals was higher than that of intact control prawns. It showed significant increase when animals were fed with adult *Artemia*, earthworm and oyster except artificial feeds 1 and 2 which showed non significant difference. Surendranath *et al*. (1992) reported a similar pattern of changes when *M. monoceros* was performed with eyestalk ablation.

In the present study, the water content slightly increased in unilateral eyestalk ablated animals, but the difference was not statistically significant. Koshio *et al*. (1992) also observed similar result in *M. rosenbergii* after unilateral eyestalk ablation. Similar values of the water content were also observed by Ajith Kumar (1990), Joseph *et al*. (1991) and Sherief *et al*. (1992) in *M. idella* and *M. rosenbergii*, respectively.

The body ash content of the animals was affected by neither eyestalk ablation nor diet in the present study. A slight increase in the ash content with increasing size has been reported in juveniles of *P. monodon* (Sriraman and Reddy, 1977), *Parapanaeopsis stylifera* and *Metapenaeus affinis* (Achuthankutty and Parulekar, 1984).

In the present study, unilateral eyestalk ablation affected the energy content of the animals. The difference was significant only when oyster and artificial feed 2 were offered to the animals. The energy content was calculated from the body organic constituent's viz. protein, carbohydrate and lipid. Likewise, the diet energy was also calcnlated from the organic constituents of the diet. The diet energy content influenced the body energy content of the animals due to high accumulation of organic constituents in the body of prawn.

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