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Nucleic Acid and Protein Concentrations in the Muscle of *Macrobrachium rosenbergii* Juveniles at Different Periods of Growth

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Abstract

Variations in muscle protein and nucleic acid content in juveniles of *Macrobrachium rosenbergii* fed on diets with varying protein levels at different periods of growth have been reported. The changes in RNA-DNA ratio and the protein content in the muscle were correlated to growth rates of the experimental animals fed on varying dietary protein levels such as 10%, 20%, 30%, 35% and 45% at 15,30,60 and 90 days of feeding. A positive relationship between the amount of dietary protein level and the RNA-DNA ratio was observed .The RNA-DNA ratio showed an increase during the period of feeding trials up to 30 days while prolonged feeding showed no significant changes. Similar changes were observed with RNA and protein content of the muscle, but not as significant as RNA-DNA ratio. The concentration of DNA content declined progressively with an increase in dietary protein level up to 35% and showed an increase with further increase in dietary protein levels. The RNA-DNA ratio and muscle protein contents were correlated with the dietary protein levels and growth rate of juvenile *M. rosenbergii* suggesting the usefulness of RNA-DNA ratio as a sensitive tool in monitoring the growth of the fresh water prawn.

Introduction

In crustacea, growth is an increase in the dry weight of the body which generally occurs in the periods between molts, when the absorbed water is

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gradually replaced by protein and DNA controls the development of the organism by controlling the formation of RNA (Thomas 1993). Growth in terms of accumulation of protein is always accompanied by high turnover rate of RNA concentration, which is a prime factor of protein synthetic machinery. Since the DNA content per cell is generally constant, the RNA-DNA ratio reflects the growth (Buckley 1984). It has proven a useful indicator of nutritional condition as has been shown in several larval fish studies (Bulow et al. 1981; Martin et al. 1985).

The giant fresh water prawn *M. rosenbergii* is widely distributed all over the country and is observed to be an ideal species for culture in fresh water ponds and reservoirs. An appropriate feeding is a major factor in the success of aquaculture ventures. Protein is the most important nutrient for growth, which constitutes the major component of the diet and usually the most expensive ingredient in artificial feed. The quality and quantity of dietary protein strongly influence growth rate in fishes (Love 1980; Wilson and Halver 1986).

In the present study an attempt has been made to study the changes in DNA, RNA, and the protein content in the muscle during the development of *M. rosenbergii* juveniles fed on variable dietary protein levels in order to investigate the usefulness of RNA: DNA ratio as a growth assessing parameter.

Materials and Methods

Preparation of experimental diets and feeding trial

To prepare a growth differential test of the RNA-DNA technique, two controlled laboratory-feeding experiments were conducted. In both experiments, 3 month old *M. rosenbergii* - were fed on the experimental diet on an *ad libitum* basis at approximately 3% of the total wet body weight of prawn per day until they got acclimatized to the laboratory conditions. Five test diets with a crude protein range of 10%, 20%, 30%, 35% and 45% were formulated using purified dried clam meat powder as protein source. The composition of the formulated diets and the proximate analysis of the clam meat used as the protein source are given in table 1. The proximate composition such as moisture, fat, ash and crude protein were estimated using the method of AOAC (1984). Diets were made isocaloric by adjusting with potato starch and alpha cellulose. The vitamin and mineral premixes were added and egg albumin was used as the coating material. The feed was dried in an oven for 12 hours at 60 ± 2°C. After drying, the feeds were stored in airtight containers. The preparation of diets were as described by the method of Halver (1976).

The experiment was started with 50 animals each in 15 fiberglass tanks of 100 liter capacity. Aeration was provided throughout the experiment. Each diet was fed to triplicate groups and the prawns were fed with the test diet for one week to acclimatize them to the feed. The animals were then

given the particulated feeds every day at a daily rate of 3% of their live weights. After every fortnight, the quantity of the feed was readjusted based on the growth of the animals. The sampling of the animals was done on 15,30,60 and 90 days of feeding trial. Water quality parameters in the tanks were monitored routinely. They included temp (28°C), dissolved oxygen (7 to 8ppm), pH (7), nitrite and ammonia N (<0.02 ppm). Five animals each were selected in triplicate from each treatment for the analysis of tissue DNA, RNA and the protein content.

Estimation of muscle protein and nucleic acid

A weighed quantity of muscle tissue was homogenized with distilled water and was treated with 10% cold trichloroacetic acid to precipitate the proteins. The residue was washed twice with 10% TCA to remove all acid soluble compounds. The resultant residue was treated with 95% ethanol twice and with solvent ether twice. The residual dried fat free sample was used for estimating protein and nucleic acid content. Protein was estimated following the method of Lowry et al. (1951) using bovine serum albumin as the standard. RNA was extracted and estimated by the method of Schneider (1957). A calibration curve was prepared using purified yeast RNA as the standard. DNA was extracted by the method of Webb and Levy (1955) and estimated using the technique of Ashwell (1957). The calibration curve was prepared using calf thymus DNA as the standard. RNA-DNA ratio was calculated by dividing RNA to DNA value.

Table 1. Diet formulation and proximate composition of clam meat

Ingredients	g/100g dry diet				
Protein % Constituent	10 Diet A	20 Diet B	30 Diet C	35 Diet D	45 Diet E
Clam meat	19.59	48.13	76.48	91	91
Casein	-	-	-	-	10
Sun flower oil	7.86	4.72	1.60	-	-
Mineral mix*	7.86	5.72	3.59	2.5	2.5
Starch	26.42	15.86	5.37	-	-
Cellulose	32.27	19.57	6.96	0.5	0.5
Vitamin mix*	3	3	3	3	3
Cholesterol	1	1	1	1	1
Egg albumin	2	2	2	2	2
E/Kcal/100 g	287	327	367	387	427

Proximate composition of clam meat used as the protein source

Components	Crude protein	Crude fat	Ash	Fiber	Moisture	NFE**
Composition (%)	38.5	11	7.5	0.88	8.62	33.5

Vitamin mix*- mg/kg dry diet (Supplivite M). Thiamine monochloride 45; Riboflavin 30; nicotinamide 100; pyridoxine hydrochloride 20; calcium-d-pantothenate 50; biotin 0.1; cyanocobalamine 0.01; choline chloride 400; Mineral mix* g/kg dry diet (USP Salt mixture). **Nitrogen free extract (determined by difference)

Specific growth rate

Growth was determined by the method described by Halver (1976). The initial and final weights of the animals under different treatments were monitored at different days of sampling. Specific growth rate was calculated using the formula.

Specific growth rate = [{ln(final mean weight)-ln(initial weight)}/number of culture days] x 100

Statistical analysis

The feeding experiments were designed on the basis of completely randomized design. The results obtained from the experiments, were subjected to analysis of variance ANOVA as per the method of Snedecor and Cochran (1968). To study the significance of the difference between days and treatments for various parameters, the experimental data was statistically analyzed using two-factor ANOVA.

Results

In this experiment the results of RNA and DNA content, protein in the muscle tissue and growth pattern in weight of prawns fed on diets of different dietary protein levels for a period of 90 days are presented. The relation of RNA-DNA ratio to specific growth rate and muscle protein content are also presented.

Table 2 shows the DNA content in relation to different protein concentration. From this, it appears that a 35% protein level shows the minimum value of DNA at all the sampling days followed by 30% protein level. The maximum value for DNA was obtained at 90 days for all the corresponding protein levels except for 35% level. Both periods and treatments are statistically significant at 5% level ($p<0.05$). From this it is clear that the DNA values show a low profile at 35% and 30% protein levels compared to other dietary protein concentrations.

Table 2. Changes in DNA concentration at different dietary protein levels (mg/100 g dry wt.)

Dietary Protein (%)	Days					F Value
	0	15	30	60	90	
10	227.90	327.90	294.70	202.64	262.83	4.8700*(treatments)
20	230.30	276.65	201.53	197.95	330.84	4.6926**(periods)
30	112.92	126.93	175.21	170.69	310.45	
35	104.729	130.06	155.59	174.74	135.64	
45	128.72	178.45	199.32	251.27	259.37	

*Significant at $p<0.05$
**Significant at $p<0.01$

Table 3 shows the RNA content in relation to different protein concentrations. The values show that there is a linear increase in the RNA content with an increase in the dietary protein concentration up to 35% and a decline on further increase of dietary protein levels, this being most pronounced at 30 days of feeding trial. Towards 90 days of feeding the RNA content of the muscle protein remains at a low level irrespective of the different protein levels.

Table 4 shows the variation in RNA: DNA ratio at different dietary protein levels.

In figure 1a to d, the variations in RNA: DNA ratio with muscle protein content at different periods of feeding is presented. Uniform increases in the RNA-DNA ratio and muscle protein content are observed with an increase in the dietary protein levels, maximum being at 35% level. At 45% level both these parameters show a decreasing trend. These changes are distinct at 15 to 30 days of feeding while at 90 days a stagnant level is reached. Two factor ANOVA analysis shows that the variations in RNA-DNA ratio and tissue protein content with dietary protein are highly significant ($p<0.001$).

The initial and final weight of the animals fed on different protein levels were monitored at different stages of growth. From this the SGR% and average weight gain were calculated. Figures 2a to d describe the variations of RNA-DNA ratio and SGR%. Among dietary protein levels, 35% level shows significantly higher values in both the parameters. There is a significant difference in SGR% between protein levels ($p<0.001$) and feeding days ($p<0.001$).

Table 3. Changes in RNA concentration at different dietary protein levels (mg/100 g dry wt.)

Levels (%)	Days					F Value
	0	15	30	60	90	
10	248.49	230.73	164.65	194.35	222.95	7.5545**(treatments)
20	268.78	262.15	189.59	227.18	193.26	1.4135 (periods)
30	280.12	270.56	284.45	298.34	225.69	
35	325.72	316.74	353.10	265.18	253.06	
45	230.48	212.27	263.07	257.20	228.57	

**Significant at $p<0.01$

Table 4. Changes in RNA-DNA ratio at different dietary protein levels

Levels (%)	Days					F Value
	0	15	30	60	90	
10	1.090	0.704	0.559	0.959	0.848	6.2764*(treatments)
20	1.168	0.948	0.941	1.148	0.584	11.1223**(periods)
30	2.481	2.132	1.623	1.748	0.727	
35	3.110	2.435	2.269	1.518	1.074	
45	1.791	1.189	1.132	1.024	0.881	

*Significant at $p<0.05$
**Significant at $p<0.01$

The growth pattern of juveniles of *M. rosenbergii* fed with varying dietary protein concentration is given in figure 3. It is found that there is a uniform increase in body weight with an increase in dietary protein levels. The group fed with 35% protein shows maximum growth throughout the feeding days closely followed by 45% protein group.

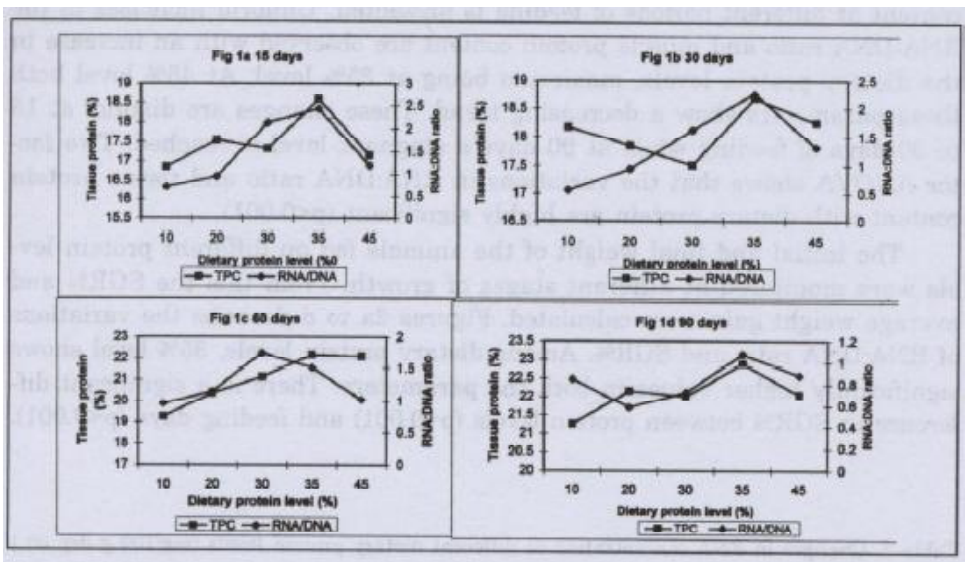

Fig. 1. Variations in RNA:DNA ratio with tissue protein content at different dietary protein levels and at different periods of feeding

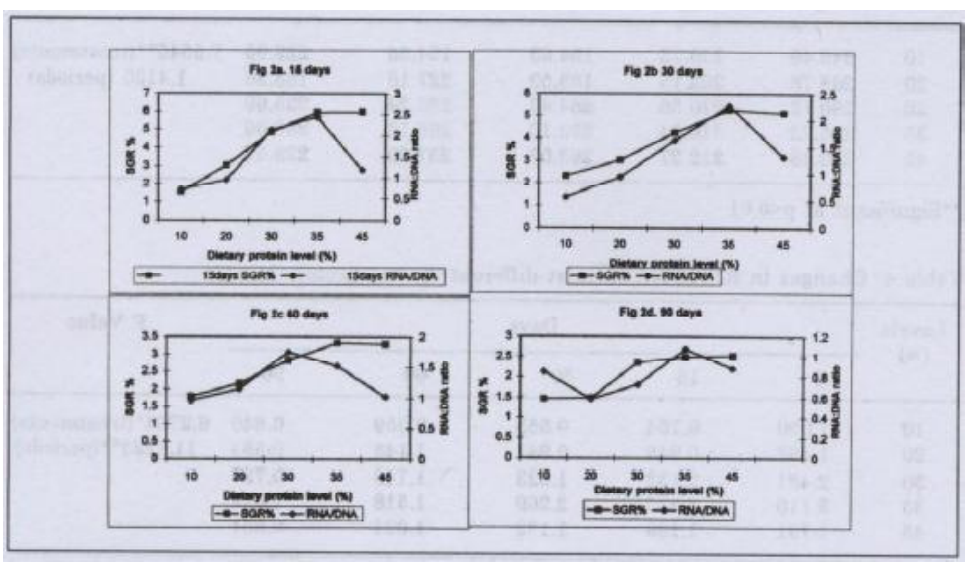

Fig. 2. Variations in RNA:DNA ratio with specific growth rate (%) at different dietary protein levels and at different periods of feeding

Discussion

The increase in RNA concentration appears to be the result of a more efficient utilization up to 35% of crude protein intake leading subsequently to an increased protein synthesis (Khan and Jafri 1991). A fall in muscle protein and RNA concentration beyond 35% dietary protein intake strengthens the already established fact that there are limits to the amount of protein that fish can convert to its body material (Love 1980). Sutcliffs (1965) and Dagg and Page (1972) found increased amount of RNA content during the exponential growth phase of the *Artemia salina*, but they did not find any significant change thereafter. The present findings of RNA concentration agree with the above observations. An increase in the level of RNA is necessary for an active protein synthesis and that was found to be mainly responsible for the growth of fish (Bulow 1970, Mustafa and Jafri 1977). The extent of the growth increase measured in terms of specific growth rate of the fish declined beyond the 35% dietary protein level. Any factor that prevents or slows down growth is known to be reflected by a reduced RNA concentration (Buckley 1979, 1980, 1982, 1984; Martin et al. 1985).

Bulow (1970) while estimating RNA-DNA ratio in relation to the growth rate of fish found that there was a slight decrease in the DNA content with an increased growth and a slight increase in the DNA content with an increased weight loss. He further explained that this change was probably due to the changes in the cytoplasmic volume. With food deprivation other cellular constituents are metabolized and DNA is preserved (Leslie 1955).

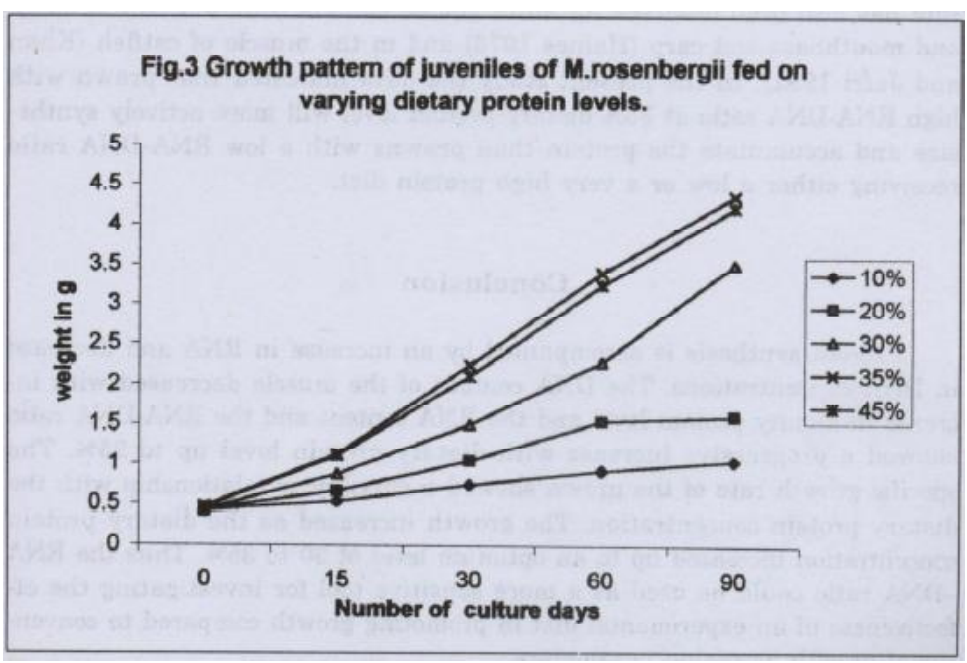

Fig. 3. Growth pattern of juveniles of *M. rosenbergii* fed on varying dietary protein levels

DNA carries the genetic material in each cell and is present in the nucleus in fixed quantities (Love 1980). It is considered as an index of cell numbers contributing to the unit weight of the tissue. In a weight losing fish, the size of the cells decreases. Thus the number of cells contributing to the unit weight of the tissue enhances the number of nuclei and this will contribute to an increase in the DNA content. In a weight gaining fish on the other hand the DNA content becomes diluted with a larger volume of cells per unit weight. The variation in the DNA content on diets containing low, optimum and high dietary protein can be explained on the basis of the above findings.

The RNA-DNA ratio is considered as a sensitive measure for the growth rate of fish (Love 1980, Buckley 1979). This concept is based on the fact that the quantity of RNA varies directly with the activity of the protein synthesis in tissues undergoing a faster growth while the amount of DNA per cell remains constant within the species. Thus the ratio of RNA to DNA, which is indicative of the amount of RNA per cell, is usually a more accurate index of protein synthetic activity than the RNA concentration alone because the ratio is not affected by the differences in the cell numbers.

There are some indications that size may affect the relation between the RNA-DNA ratio and the temperature and the growth rate in older fish (Buckley 1982). Large individuals with the same RNA-DNA ratio may grow at a slower rate than the smaller individuals. Also due to the increasing energy reserves, the macromolecular composition of the larger individuals may require a longer time to change in response to the changes in the food availability. RNA-DNA ratio analysis will continue to provide information on the growth rate and condition of the larval fish and shellfish in their active growth phase.

The direct positive relationship between the RNA-DNA ratio and growth rate has also been observed for adult golden shiners (Bulow 1970) in small and mouthbass and carp (Haines 1973) and in the muscle of catfish (Khan and Jafri 1991). In the present study the data indicated that prawn with high RNA-DNA ratio at 35% dietary protein level will more actively synthesize and accumulate the protein than prawns with a low RNA-DNA ratio receiving either a low or a very high protein diet.

Conclusion

Protein synthesis is accompanied by an increase in RNA and decrease in DNA concentrations. The DNA content of the muscle decreased with increase in dietary protein level and the RNA content and the RNA-DNA ratio showed a progressive increase with dietary protein level up to 35%. The specific growth rate of the prawn showed a curvilinear relationship with the dietary protein concentration. The growth increased as the dietary protein concentration increased up to an optimum level of 30 to 35%. Thus the RNA –DNA ratio could be used as a more sensitive tool for investigating the effectiveness of an experimental diet in promoting growth compared to conventional growth assessing parameters.

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