International Journal of Zoology and Applied Biosciences
Volume 3, Issue 1, pp: 41-47, 2018
https://doi.org/10.5281/zenodo.1313019
Research Article

ISSN: 2455-9571
http://www.ijzab.com

EFFECT OF RATION LEVELS OF *ARTEMIA* ON CHOSEN PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN CARP, *CIRRHINUS MRIGALA* (HAMILTON, 1822)

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Article History: Received 17th January 2018; Accepted 23rd January 2018; Published 25th January 2018

ABSTRACT

Effect of different ration levels of *Artemia* diet on growth, body compositions and respiratory metabolism were studied in *Cirrhinus mrigala* for 30 days. An increase in ration levels significantly enhanced the feeding and growth rates in *C. mrigala.* A significant and positive correlation ($P < 0.05$) was obtained between ration levels and feeding ($r = 0.983$) / conversion ($r = 0.921$) rates. The maintenance, optimum and maximum rations of *C. mrigala* were 5.33, 38 and 116 mg g^{-1} live fish day^{-1} respectively. The FCR value was low 2.16 – 1.79 in fish fed with 40 and 60% ration levels and they did not show significant differences ($t = 1.38$; $P > 0.05$) between them and other ration levels. The RNA: DNA ratio did not vary much in *C. mrigala* received the higher ration levels (60 – 100%) as compared to 40% ration. Hence, 40% ration is considered as optimum feeding to enhance feeding and growth in *C. mrigala.*

Keywords: Ration levels, *Artemia*, growth, RNA : DNA ratio, Optimum ration, *Cirrhinus mrigala.*

INTRODUCTION

Feeding rate is important for the growth, feed conversion, nutrient retention efficiency and chemical composition of fish (Hung and Lutes, 1987; Storebakken and Austreng, 1987). Determination of the nutrient requirement is also affected by feeding rate (Talbot, 1985). A restricted feeding rate will cause impaired health (Storebakken and Austreng, 1987) or slow growth (Hung and Lutes, 1987; Fontaine *et al.,* 1997). Conversely, over-feeding of fish will cause the overload of stomach and intestine, and decrease the efficiency of digestion and absorption, and thus reduces feed efficiency (Hung and Lutes, 1987; Storebakkkem and Austrong, 1987). An optimum feeding rate is helpful to minimize the feed loss, reduce pollution and decreases cost of aquaculture production. To our knowledge no study has yet been published on the effects of different ration levels of *Artemia* on growth in *Cirrhinus mrigala*. Hence, the present work was undertaken to study the effects of different ration levels of *Artemia* on growth and proximate composition in carp *C. mrigala.*

Previous authors have studied the requirement of feed in relation to body weight and feeding regimes in cultivable fishes (James and Sampath, 2004). However, there is paucity of information on the effect of ration levels of *Artemia* diet on growth, RNA:DNA ratio and energy content in cultivable fishes. The attractive movements and nutrionally rich prey organisms minimize the temporal and energy costs of feeding and maximize the growth in cultivable fish (James *et al.*, 1993). Hence, the present investigation was undertaken to study the effect of ration levels of *Artemia* diet on growth, nucleic acid and body compositions in a carp, *C. mrigala*.

MATERIALS AND METHODS

Maintenance of fish

C. mrigala was collected from Manimuthar Dam, Tirunelveli, Tamil Nadu, India and held for 30 days in laboratory conditions (DO: 4.38 ± 0.45 ml O_2 l^{-1}; temperature : 28.8 ± 0.5°C; pH : 7.8 ± 0.04; salinity : 0.19 ± 0.01 ppt and hardness ($CaCO_3$) : 215 + 5.7 mg l^{-1}). During acclimtization, water was changed daily and fish were fed *ad libitum* with pelletized diet containing 35% protein.

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Artemia collection

Artemia were collected once in two days from local salt pan and maintained in a cement tank containing sea water. Before feeding to fish, the *Artemia* were washed 3-5 times in freshwater to remove the salt. Traces of water content in the *Artemia* were removed by pressing the *Artemia* between folds of filter paper. The proximate compositions of *Artemia* are given in Table 1.

Table 1. Proximate composition (%) of experiment diet, *Artemia.*

Components	%
Crude protein	56.5 ± 3.45
Crude fat	7.9 ± 0.27
Ash	8.6 ± 0.34
Nitrogen free extract (NFE)	27.0 ± 2.16
Moisture	86.4 ± 5.16
Energy*	30.54 ± 1.6

* K J g^{-1} dry matter.

Experimental design

Healthy juveniles (360 nos.) of *C. mrigala* (0.90 ± 0.03 g) were selected from the acclimation tank and divided into 6 groups of 20 individuals each. Triplicates were maintained for each group. They were starved for 24 h prior to commencement of the experiment. Group 1 served as control and starved during the experiment. Test animals belonging to 2nd, 3rd, 4th, 5th and 6th groups were fed with 20, 40, 60, 80 and 100% rations of *Artemia* respectively. The experiment was conducted in cement tank (capacity: 110 *l*) containing 100 *l* of water. The clean unchlorinated well water was used and physico-chemical parameters were monitored biweekly. Dissolved oxygen averaged to 4.03±0.15 ml O_2 l^{-1}, temperature: 28.0±0.3^0 C, pH: 7.6 ± 0.1, salinity: 0.25 ± 0 2 and water hardness: 180 ± 4.48 mg $CaCO_3$ l^{-1}. The tanks were drained twice a week and replenished with freshwater to remove feces accumulated at the bottom of the tanks.

Feed consumption

During the experimental period, the chosen test groups were fed with different rations of *Artemia* diet. Test fish maintained at 20 and 40% ration levels were offered food once a day at 7.00 AM. To prevent food remaining in the water too long, test groups with 60, 80 and 100% rations were fed twice a day at 7 AM and 5 PM However, the first group was starved during the experiment. The water content of *Artemia* was estimated daily by drying a known weight of sample at 80°C. Unconsumed feed was removed after 1 hr of feeding and dried in hot air oven at 80°C for two days. Feed consumption (mg) was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of feed offered. Feeding rate was computed as:

$$\text{Feeding rate (mg g}^{-1}\text{ live fish day}^{-1}) = \frac{\text{Feed consumed (mg)}}{\text{Initial wet weight of fish (g)} \times \text{Duration (days)}}$$

Growth rate

Fish were weighed at the beginning and at the end of the experiment. Growth or weight gain was calculated as the difference between the wet weights at the beginning and end of the experiment. Rate and efficiency of conversion (growth) were computed as:

Conversion rate (mg g^{-1} live fish day^{-1}) =

$$\frac{\text{Weight gain (mg)}}{\text{Initial wet weight of fish (g)} \times \text{Duration (days)}}$$

Conversion efficiency (%) =

$$\frac{\text{Weight gain (mg)}}{\text{Feed consumption (mg)}} \times 100$$

Feed conversion ratio (FCR) was calculated by relating the feed consumption to weight gain of fish.

$$\text{Feed conversion ratio} = \frac{\text{Feed consumption (mg)}}{\text{Weight gain(mg)}}$$

The experiment lasted for 30 days. Fish / feed sample and unconsumed feed were weighed in an electrical monopan balance to an accuracy of 1 mg.

After the termination of the experiment on day 30, test groups were subjected for the estimation of body compositions, energy content and muscle nucleic acids content. Three fish were removed from each experimental group after 30 days. Oxygen consumption of test animal was separately estimated following Winkler's iodomatric method.

Estimations of nucleic acids and body compositions

Briefly, six fish were removed from each experimental groups for the estimation of nucleic acids (RNA and DNA), energy content and body compositions in muscle tissue. RNA and DNA were estimated in test animals following the method of Searchy and MacInnis (1970a and 1970b). The protein and lipid contents were estimated following the method of Lowry *et al.* (1951) and Bragdon (1951) respectively. The ash content was estimated following the method of Paine (1964). The nitrogen free extract was calculated by subtracting the protein, lipid and mineral contents (ash) from the weight of the feed / fish samples. For want of bomb calorimeter, the energy content of feed and fish samples was estimated by wet combustion method (Karzinkin and Tarkovskaya 1964).

Students 't' test was followed to detect the significance of mean values between experimental groups. Correlation was adopted following the method of least square (Zar 1984).

RESULTS

An increase in ration levels significantly enhanced the feeding and growth rates in *C. mrigala* (Table 2). A significant and positive correlation (P < 0.05) was obtained between ration levels and feeding (r = 0.983) / conversion (r = 0.921) rates. The geometric relationship between growth rate and ration levels is depicted in Figure 1. The point at which the curve cuts the x-axis represents the maintenance level at which weight equilibrium is attained in the animal without weight change. A tangent to the curve from the origin, the ration provides the maximum growth with the least feed intake-optimum ration. The point at which the curve flattens gives the ration which stimulates the maximum growth – maximum ration. The maintenance, optimum and maximum rations of *C. mrigala* were 5.33, 38 and 116 mg g^{-1} live fish day^{-1} respectively (Figure 1).

FCR was inversely proportional to the increase in the ration levels of *Artemia* in *C. mrigala.* The FCR value was low 2.16 – 1.79 in fish fed with 40 and 60% ration levels and they did not show significant differences (t = 1.38; P > 0.05) between them and other ration levels.

RNA and DNA contents and RNA:DNA ratio of *C. mrigala* were increased with increasing ration levels of *Artemia* in the diet. *C. mrigala* consumed 100% *Artemia* diet enhanced the RNA content about 2 times as compared to fish received 20% ration. Similar trend was obtained in RNA : DNA ratio also. However, the RNA : DNA ratio did not vary much in *C. mrigala* received the higher ration levels (60 – 100%) as compared to 40% ration. Hence, 40% ration is considered as optimum feeding to enhance feeding and growth in *C. mrigala.* The concentration of DNA did not change in experimental groups whereas it was significantly (t = 34.35; P < 0.01) declined in fish subjected to starvation (Table 3).

The proximate compositions (protein and lipid) and energy of *C. mrigala* were increased with increasing the ration levels of *Artemia* in the diet (Table 4). Fish fed with 100% ration diet enhanced the maximum chemical components and energy than those fed on other ration levels. The rate of oxygen consumption of test animal was gradually declined with increasing the ration of *Artemia* diet. Obviously, the rate of oxygen consumption was inversely proportional relationship with increasing ration levels of *Artemia* (Figure 2).

Table 2. Effect of feeding different ration levels of *Artemia* feed on selected food utilization parameters in *Cirrhinus mrigala.* Each value is the mean (± SD) of three observations.

Parameters	Ration levels (%)					
	0	20	40	60	80	100
Feed intake (g wet weight)	Starvation	8.76±0.36	12.66±0.54	14.31±2.79	27.30±3.30	34.5±1.50
Feeding rate (mg g^{-1} live fish day^{-1})	0.00±0.00	20.0±0.2	39.99±0.19	60.0±2.52	78.4±1.6	116.66±16.66
Weight gain (g wet weight)	-3.68±0.25	2.69±0.41	5.90±0.2	7.98±0.03	8.97±0.38	9.56±1.19
Conversion rate (mg g^{-1} live fish day^{-1})	-0.68±0.04	6.11±0.68	18.73±1.35	27.32±2.82	26.04±2.55	32.57±3.24
Gross conversion efficiency (%)	-	30.71±2.81	46.60±3.65	55.77±3.90	32.86±2.71	27.71±1.98
FCR	-	3.31±0.37	2.16±0.17	1.79±0.34	3.03±0.24	3.65±0.31

Students 't' test

Conversion rate : 40 Vs 60% : t = 3.89; P < 0.05
Conversion efficiency : 40 Vs 60% : t = 2.43; P > 0.05

Table 3. Effect of ration levels of Artemia on nucleic acids (mg g-1 wet tissue) and RNA : DNA ratio in the muscle of *Cirrhinus mrigala.* Each value is the mean (± SD) of three observations.

Parameters	Ration levels of *Artemia* (%)					
	0	20	40	60	80	100
RNA	2.56±0.01	4.55±0.13	6.98±0.34	7.85±0.42	8.68±0.51	9.45±0.19
DNA	1.65±0.01	2.20±0.12	2.43±0.11	2.50±0.12	2.71±0.23	2.80±0.23
RNA : DNA ratio	1.55±0.01	2.25±0.01	2.87±0.22	3.14±0.13	3.20±0.11	3.38±0.22

Students 't' test

RNA : 40 Vs 60% : t = 2.28; P > 0.05
DNA : 40 Vs 60% : t = 0.61; P > 0.05
RNA : DNA ratio : 40 Vs 60% : t = 2.08; P >0.05
40 Vs 20% : t = 7.29; P < 0.01

Table 4. Effect of feeding different ration levels of Artemia feed on proximate composition (%) in *Cirrhinus mrigala*. Each value is the mean (± SD) of three estimations.

Parameters	Ration levels (%)					
	0	20	40	60	80	100
Protein	12.3±0.11	27.53±0.47	30.38±0.43	33.44±0.43	35.60±0.55	37.12±0.37
Lipid	1.59±0.14	1.63±0.01	1.90±0.02	2.32±0.06	2.77±0.15	3.09±0.24
Ash	1.49±0.08	1.24±0.06	1.69±0.09	1.94±0.15	2.08±0.17	2.17±0.20
Nitrogen free extract	85.62±3.64	69.60	66.04	62.32	59.56	57.72
Energy	0.13±0.03	3.11±0.04	6.32±0.02	7.36±0.45	8.39±0.81	9.68±0.66

Students 't' test

Protein : 40 Vs 60% : $t = 7.12$; $P < 0.01$
80 Vs 60% : $t = 4.40$; $P < 0.01$
80 Vs 100% : $t = 3.30$; $P < 0.05$

Lipid : 40 Vs 60% : $t = 9.41$; $P < 0.01$
80 Vs 60% : $t = 4.01$; $P < 0.05$
80 Vs 100% : $t = 1.62$; $P > 0.05$

Energy : 40 Vs 60% : $t = 3.26$; $P < 0.05$

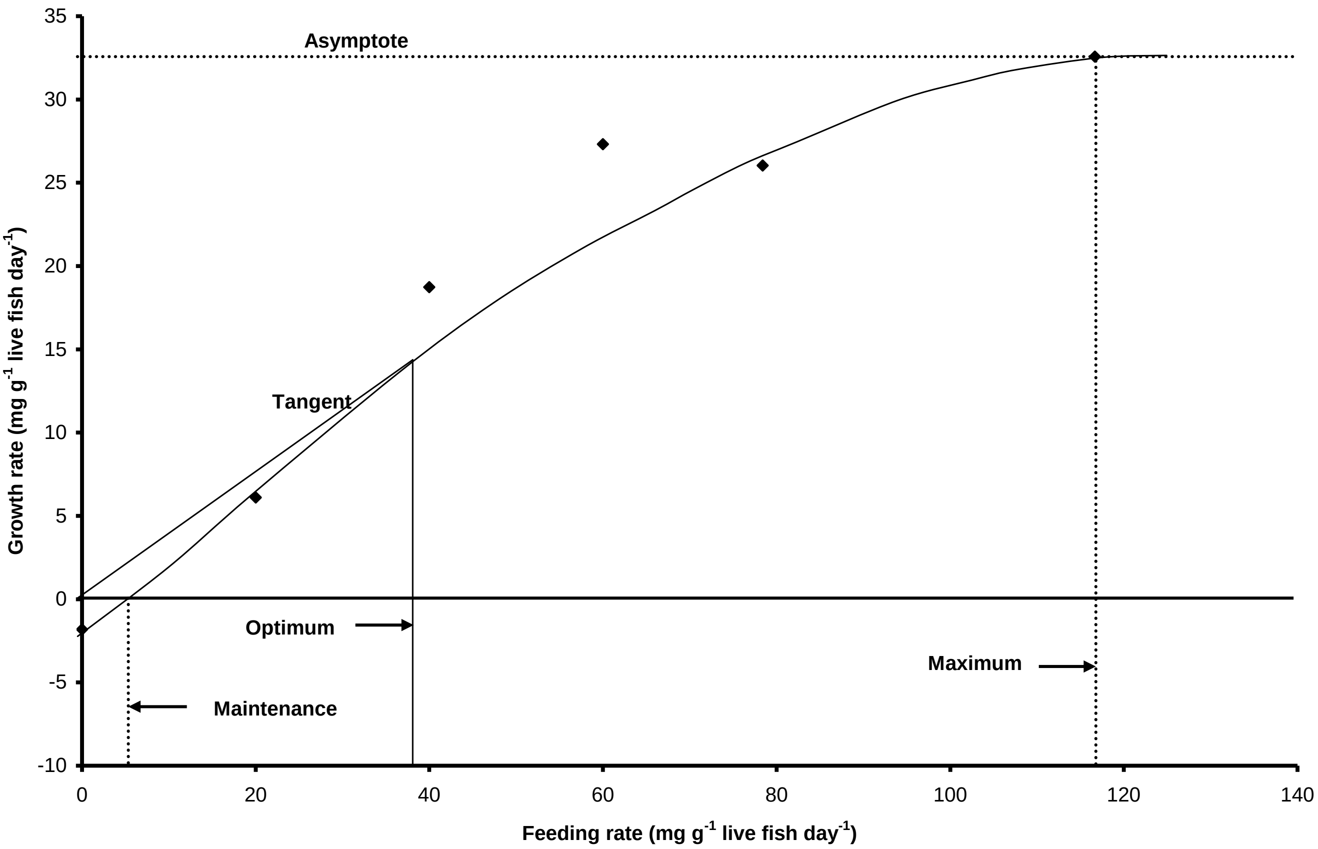

Figure 1. The geometric deviation of maintenance, optimum and maximum rationin *Cirrhinus mrigala* fed on *Artemia* diet for 30 days.

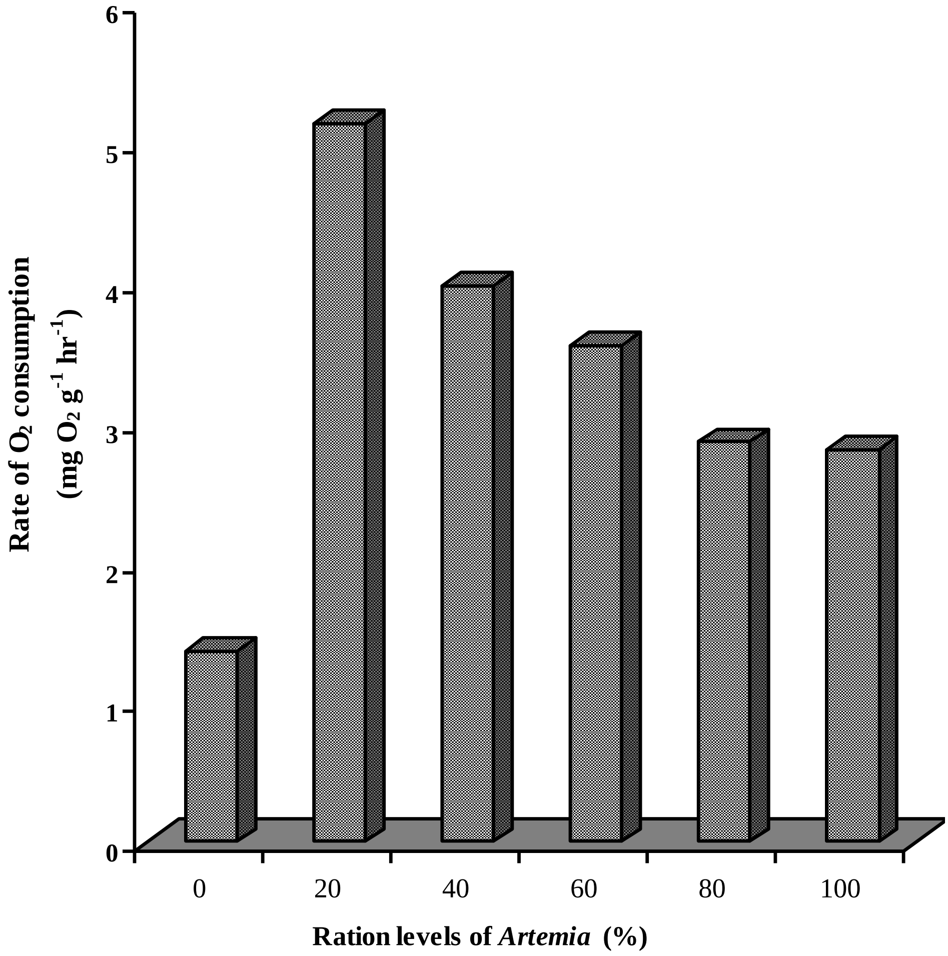

Figure 2. Effect of feeding different ration levels of *Artemia* on rate of oxygen consumption in *Cirrhinus mrigala*.

DISCUSSION

The present study reveals that, ration was positively related to feed intake, weight gain and growth rate in *C. mrigala* when consumed *Artemia* as diet. Working on *Mystus vittatus,* Arunachalam and Ravichandra Reddy (1981) observed enhanced consumption of *Tubifer tubifex* with increase in ration, supports the present study. Consumption rate determines the conversion rate (Raghuraman, 1973). A positive, linear relationship was observed between growth rate and ration levels in cat fish, *Mystus keletius* (Sampath and Lily Pramila, 1995). An increase in ration levels, conversion rate was enhanced to 3 times in 40% ration and 5.4 times in 60% ration. The present study also revealed that *Artemia* diet reduced the allocation of feed energy for maintenance, which was confirmed from the results obtained for oxygen consumption rate in *C. mrigala.* In aquaculture practices offering optimum ration to fishes would help to reduce the production cost by avoiding feed wastage and enhance their growth rate.

The results clearly showed that, fish fed with *Artemia* exhibited significant ($P < 0.05$) effects on the rates of consumption and conversion. It may be due to the soft nature and palatability of *Artemia* which contains growth promoting components (57% protein). Feed intake of fish depends on size of the prey and predator, quality, density, physical attractiveness and mode of presentation of food (James *et al.,* 1993). The large size active movement and high protein (57%) content of adult *Artemia* sp. could have stimulatory effect on the predatory responses of experimental fish which enhanced the growth rate. James *et al.* (1993) reported that the wriggling movements of large and nutritionally rich prey organisms such as *Chironomus* and *Culex pipiens* larvae minimize the temporal and energy costs of feeding and maximise growth in *Cyprinus carpio* which supports the present findings. Feeding with adult *Artemia* provides more protein and most essential aminoacids (*Artemia* nauplii is deficient in histidine, methionine, phenylalanine and threonine) to fish (Claus *et al.,* 1979). Also, the live *Artemia* has some specific enzymes (Bengtson *et al.,* 1991) which help in initial digestion by juvenile fish enhancing appetite, feed intake and growth.

The concentration of DNA did not change in experimental groups where as the levels of RNA and protein were found to be increased with increasing of ration levels. The increase in RNA suggested that it is involved in protein synthesis without changing the DNA amount in the experimental fish which supports the findings of previous workers. The amount of DNA in each cell nucleus is constant for a species and it is considered as an index of cell number contributing to unit weight of tissues, while the concentration of RNA in cell is related to protein synthesis and metabolic activities of a tissue (Bulow, 1970). Therefore, RNA : DNA ratio indicates the amount of protein synthesis and could be a more sensitive tool for measuring the growth rate of fish (Fauconneau, 1985; Khan and Jafri, 1991). In the present study, RNA : DNA ratio has gradually increased with increasing of ration levels and it was 50% higher in fish received 100% ration than those received 20% ration. Fish received 40% ration of *Artemia* diet did not exhibit the significant ($P > 0.05$) increment of RNA : DNA ratio with higher rations while it showed significant ($t=7.29$; $P < 0.01$) increment with fish received 20% ration. Blue green alga, *Spirulina* has higher protein like *Artemia* diet also showed that, the elevated RNA: DNA ratio concomitant with improvement of growth in red sea bream supports the present study. Nandeesha *et al.* (1998) reported that, the muscle RNA : DNA ratio of *C. mrigala* fed with *Spirulina* diets was higher than that of fish fed with *Spirulina* free diet.

The results showed that the proximate compositions were increased with increasing the rations levels of *Artemia* diet. However, fish received 40% ration diet significantly extracted more quantum of protein ($t = 7.12$; $P < 0.01$) and energy ($t= 3.26$; $P < 0.05$) from *Artemia* and chanallized for growth than those received higher rations (60-100%) of *Artemia* diet. Working on *Cyprinus carpio,* James *et al.* (1993) found that, *Chironomus* larvae fed fish extracted the more nitrogen and energy, and diverted for muscle production than those fed on *Culex pipiens* and *Daphnia.* Also, lipid level was deposited in fish body in relation to rations of *Artemia* diet.

The rate of oxygen consumption was inversely proportional to the increase in the ration of *Artemia* levels in *C. mrigala.* It indicates that, higher feeding levels of *Artemia* did not influence the spontaneous metabolic activities which inturn reduced the energy loss on respiratory metabilism and hence more growth rate was registered in fish received higher rations. According to Kerr (1971) at higher feeding levels, the spontaneous activity increases, resulting in high metabolic energy needs which increase at increased amount of available feed. Therefore, a large fraction of energy is dissipated on metabolism and hence the growth rate of fish does not increase at high feeding levels.

CONCLUSION

In aquaculture practices offering optimum ration to fishes would help to reduce the production cost by avoiding feed wastage and enhance their growth rate. The present study concludes that, 40 to 60% ration was positively related to feed intake, weight gain and growth rate in *C. mrigala* when consumed *Artemia* as diet. Hence, 40% ration is considered as optimum feeding to enhance feeding and growth in *C. mrigala.* The present study also revealed that *Artemia* diet reduced the allocation of feed energy for maintenance, which was confirmed from the results obtained for oxygen consumption rate in *C. mrigala.*

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