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Utilization of Organic Manure for Culture of Cladocerans, *Daphnia carinata*, *Ceriodaphina carnuta* and Copepod, *Thermocyclops decipiens* under laboratory conditions

Bilal Ahmad Paray*[1, 2] & Mohammad K. Al-Sadoon[1]

[1]Zoology Department, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia.

[2]Centre for Aquaculture Research and Extension (CARE), St. Xavier's College, Palayamkottai Tamilnadu- 627 002, India.

*[E mail: bparay@ksu.edu.sa].

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Mass culture of the cladocerans (*Daphnia carinata* and *Ceriodaphina carnuta*) and copepod (*Thermocyclops decipiens*) was carried out using chicken manure (500ppt) in the medium. The study lasted for twenty-one (21) days. Result showed that total number of individuals produced was highest in *C. carnuta* culture (10725 ± 846) on 18th day of inoculum using chicken manure. This was significantly different ($P<0.05$) from *D. carinata* and *T. decipiens* cultures. Maximum density of *D. carinata* (4660 ± 523) and *T. decipiens* (3706 ± 192) was recorded on 9th and 15th day respectively. pH ranged from 6.2-7.5, 6.5-7.8 and 6.0-7.5 in first, second and third treatments respectively. Optimum density was found when dissolved oxygen level ranged between 4.63-6.43mg/l, 4.08-5.9mg/l and 3.46-5.65mg/l in first, second and third treatments respectively. This study suggests that chicken manure is one of the ideal media for use in zooplankton culture to feed fish larvae.

[Keywords: Cladocerans, copepod, chicken manure, dissolved oxygen.]

Introduction

Mass culture of zooplankton has been the subject of many investigations during recent years because of the importance of live zooplankton in the rearing of fish larvae[1,2]. Several studies on shellfish and finfish larval rearing suggest that natural food is vital in the early stages of fish[3]. So fertilization of farm ponds has become a common practice to stimulate zooplankton production. However, unnecessary pond fertilization can lead to deterioration of water quality parameters and outbreaks of fish disease[4]. The chicken manure acts as a fertilizer to promote algal blooms on which the zooplankton feed. As fish larvae switch from yolk sacs to external feeding, zooplankton are the initial prey items. Stomach content analysis of the fingerlings of *Clarias gariepinus* and *Clarias anguilaris*, showed that, zooplankton was the predominant food item within the first few weeks of life[5].

Zooplankton are rich in proteins, amino acids, lipids, essential minerals and enzymes needed by fish larvae, for effective growth and survival[6]. The cladoceran genera such as *Miona* and *Daphnia* have been successfully used in fish larviculture, their biochemical profile with respect to organic and inorganic components are reported to be higher than the levels prescribed for fish larvae[7]. In hatchery seed production, provision of suitable live-feed is one of the important factors and if live-feed is developed from locally available planktonic organisms, seed production can be cost effective[8]. The cladocerans remove particulate organic matter from water by filtration and their ability to ingest food of a wider size range, and their higher filtering rates, give them a better competitive edge over the rotifers[9-11]. Copepods are of great ecological significance and serve as a major food for numerous organisms including fish and crustaceans[8]. The various developmental stages of copepods particularly the small nauplii of

about 50µm in size are important as feed for fish larvae.

The use of imported artemia cyst hatched into nauplii, has been anticipated as the most preferred food for fish hatchlings in the country. However, studies of Porticelli[12] have shown that artemia does not serve as the best feed for fresh water fish fry and larvae because artemia as a marine organism dies in fresh water within two hours of introduction, due to osmoregulatory problems. As a result of problems associated with the importation of artemia as well as the lack of technical know-how in its usage, by the local farmers, the present study was undertaken to assess the effect of chicken manure on zooplankton abundance and water quality profile and to optimize the dosage of chicken manure for fertilization to determine the best, easy and sustainable means of producing zooplankton for use in fish culture.

Materials and Methods

This study to compare the zooplankton abundance produced using chicken manure as sources of organic manure, was conducted at Unit of Reproductive Biology & Live Feed Culture, Department of Zoology, The New College, Chennai 600 014, Tamilnadu, India. The study lasted for twenty-one (21) days. Chicken manure collected from a local poultry farm was dried for 3 days to remove the moisture and stored in air tight plastic jars for further use. Chicken manure was micronized by grinding and required quantity was dissolved in clean water to get suspensions of required quantity to fertilize the culture medium. Micronisation of Chicken manure is necessary for an efficient filtration of the suspended particles. Nine glass tanks (50 L) capacity were respectively filled with 40 L of tap water previously aerated for 24 hrs to dechlorinate the water[8]. Three tanks were used for each treatment and labelled A1 – A3, B1 – B3 and C1 - C3. Dosage of 500 ptt of chicken manure was put into each of tanks A1 – A3. Tanks B1 – B3 and C1 – C3 also received the same proportion.

For a successful monoculture a pure inoculum is essential. For preparing inoculum, zooplankton samples were collected from culture and examined under a binocular dissection microscope. The cladoceran and copepods were sorted out using a sub-sampler and then picked up with the help of a fine dropper. Commonly occurring species such as *Daphnia carinata, Ceriodaphnia cornuta and Thermocyclops decipiens* were identified by using the key provided by Altaff[13] and isolated for pure monoculture. Each species isolated were inoculated in 20 ml glass tube containing 10 ml of filtered water. Feeding was done with chicken manure fertilized medium (500 ppt). After 24hrs the animals were observed in the test tubes and counted. Each cladoceran yields 6 - 10 offspring's in 24 hrs. Test tube cultures were changed into 250 ml beakers and feed was given as in the test tube culture. After 24 hrs, these animals were transferred into 1 or 2 L jar and continued with same feed as given in test tube culture. After 2-3 days, these jar cultured animals were used as inoculum for the mass culture in tanks.

Culture of the cladocerans *D. carinata* and *C. cornuta* and the copepod *T. decipiens* were undertaken in the present study. Required densities of cladoceran and copepod were raised in the laboratory and they were inoculated at the rate of 45 - 50 No./l in the culture tanks. The inoculum consisted of neonates and mature animals.

Culture tanks were fertilized with Chicken manure as a function of population density every 3rd day using the formula of DePauw et al[14]:

$$Y = [(\log 10^N/10) - 0.2] \times V \times d.$$

Where,

Y = Quantity of Chicken manure

N = Population density (No./l)

V = Volume of culture (l)

d = number of days for which the food is to be given.

Water change was carried out after every 3rd day by removing 50% of the water. Atmospheric temperature, water temperature, pH and dissolved oxygen of the culture media were recorded weekly.

Zooplankton were harvested every third day by the horizontal trawl method, using a micro-filament plankton net of 50 μm^{-1} mesh size and collected in a 50 ml glass bottle. The samples collected were stored in 10% buffered formalin. 1 ml sub-samples were pipetted onto the Sedgwick

Rafter Cell and observed under binocular dissection microscope. Five such sub-samples were analyzed to determine the population of the cladocerans and copepods. Results were expressed as number of individuals per liter (No./l). Experiments were conducted in triplicate, mean and standard deviation of the planktons were calculated and data collected were analyzed, using one-way analysis of variance (ANOVA).

Results

At the dosage of 500 ppt of chicken manure the peak of *D. carinata* was observed on the 9th day of the culture (4660±523 /l) and gradually the number of plankton declined. Lowest value (490±20/l) was recorded on 3rd day. Population density ranged between 50±5/l and 4660±523/l during 21 days culture period (Fig. 1 a). Maximum Atmospheric and

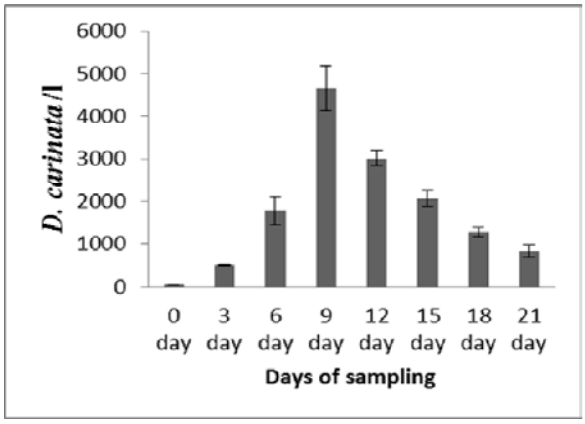

Fig. 1.**a** — Population density of *D. carinata* during different days of culture fertilized with 500 ppt chicken manure (Mean±SD)

water temperature during culture period were 29 ± 2°C and 27 ± 2°C respectively, while pH and DO ranged between 6.20 -7.5 and 4.63 – 6.43 mg/l respectively in the present experimental conditions.

During the culture period the *C. cornuta* population ranged between 50 ± 5 and 10725 ± 846/l at the dosage of 500 ppt of chicken manure. The culture peaked on the 18th day producing a maximum density (10725±846/l). The lowest value (765±83/l) was recorded on 3rd day (Fig. 1 b).

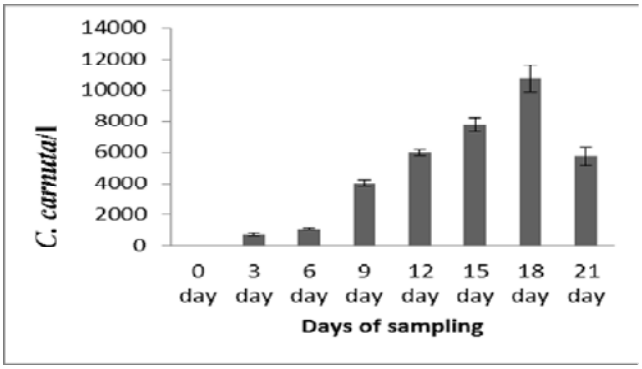

Fig. 1.b — Population density of *C. carnuta* during different days of culture fertilized with 500 ppt chicken manure (Mean±SD)

Atmospheric and water temperature during culture period were 29 ± 2°C and 27 ± 2°C respectively, while pH and DO ranged between 6.5 -7.8 and 4.08 – 5.90 mg/l respectively in the present 21 days experiment.

In the present study *T. decipiens* was cultured at a maximum density of (3706± 192/l) using chicken manure fertilized medium at a concentration of 500 ppt. Higher density of *T. decipiens* adult, copepodid and nauplii was obtained on 15th day (1273±47/l), 21st day (2333±120/l) and 15th day (1420±79/l) respectively. Lowest density of *T. decipiens* adult, copepodid and nauplii (36±2/l), (12±2/l) and (135±5/l) was obtained on 3rd day respectively (Fig. 1 c). Atmospheric temperature and water temperature during culture period were 27 ± 2˚C and 26 ± 1.5˚C respectively and pH and DO ranged from 6 - 7.5 and 3.46 – 5.65 mg/l respectively.

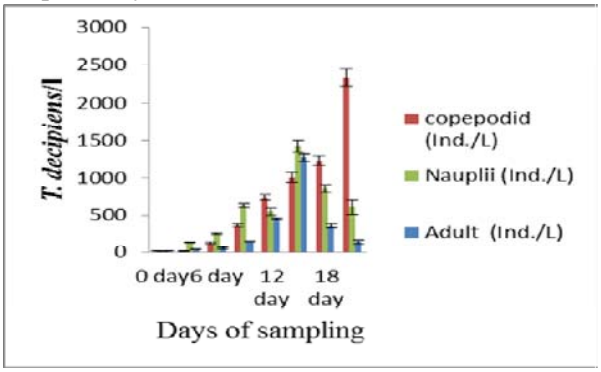

Fig. 1.**c** — Population density of *T. decipiens* during different days of culture fertilized with 500 ppt chicken manure (Mean±SD)

The peak populations densities obtained during the culture of *D. carinata*, *C. cornuta* and *T. decipiens* were significantly different ($P < 0.05$) from each other.

Discussion

The need for large quantities of live food organisms in aquaculture and the increasing need for valorizing organic wastes, such as animal manures and agro-industrial residues, have been the major initiatives for research on the culture of live feed organisms[11]. Chicken manure is a waste produced in poultry farms in large quantities and is cheap in price. Chicken manure can be stored for longer periods after drying and is easy to dose and it has none of the problems involved in maintenance of algal stocks and culture.

In the present study microorganisms produced with chicken manure promote higher density of *C. cornuta* population than *D. carinnata*. Such influence of food on the population density of Cladocera was also reported by Hebert[15] and Boersma and Vijverberg[16]. Density of *D. carinata* increased steadily and peaked (4660 ± 523 No./l) on the 9th day of culture, and thereafter the production declined. Such population dynamics was also reported by Pagano *et al.*[17] were maximum density was reported on 7th day. Advantage of culturing *D. carinata* by frequently applying low doses of poultry waste as a function of population density might provide sufficient food to the animals as well as maintain favourable water quality, which led to fast growth, early maturation and relatively long life span. Compared to the previous reports, higher population density (7764 ± 473/l) of *C. cornuta* was recorded in the present study in medium fertilized with 500 ppt concentration of chicken manure. Suresh Kumar[18] and Sivakumar[19] reported 5817/l and 6247/l respectively, in chicken manure and mixed algae mixture. Using a mixture of organic manures (cattle manure, poultry droppings and mustard oil cake) Shrivastava *et al.*[11] cultured *C. cornuta* at a maximum density of 1930/l.

T. decipiens was cultured at a maximum density of 3706 ± 193/l on 15th day using chicken manure fertilized medium. The results of *T. decipiens* are highly encouraging. Higher density culture of copepods such as *Tisbe holothuriae, Amphiscalla subdebilis, Nitochra spinipes* and *Microstris elatensis*[20], *M. aspericornis*[21] and *C. bicolor*[22] was also reported.

Physico-chemical parameters appear to play an important role in the successful culture of Cladocera and copepods. Tay *et al.*[23] reported that there is no report to suggest the relationship between the physico-chemical parameters and physiological process of zooplankton and found that 50% mortalities of cladocerans occur when temperature is close to 40ºC. Shrigur and Indulkar[24] proposed the range of water temperature between 27 - 31ºC for optimum growth of *M. micrura*. In the present study temperature range of 29 ± 2 ºC and 27± 2 ºC appeared to produce optimum density of cladocerans and copepods respectively. Dissolved oxygen content of the culture medium is another important factor for the growth of zooplankton population. Dissolved oxygen between 4.6 and 8.7 mg/l and pH 6.5 to 8.9 was reported to be optimum for mass culture[25]. In the present study in all culture media dissolved oxygen level of 3.46 – 6.43 mg/l was recorded.

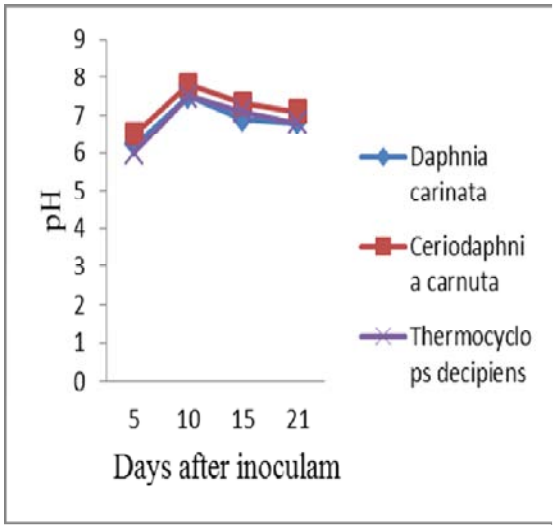

Fig. 2.**a** — Dissolved oxygen levels of water in three different experiments of zooplankton during 21 days of culture period. Each point represents the mean of values derived from three replicates.

DePauw *et al.*[14] also reported similar results, where dissolved oxygen level of culture medium for *D. magna* was above 5 mg/l with aeration. Tay *et al.*[23] had stressed that aeration is an important culturing parameter and correlation studies showed that dissolved oxygen decrease with organic loading of the media. Further, they stated that for the production of *Moina*, pH did not appear to be a critical factor. In the present study, pH of the culture medium ranged between 6.0 - 7.8, similar results were reported by Lee[26] and Pangano *et al.*[17].

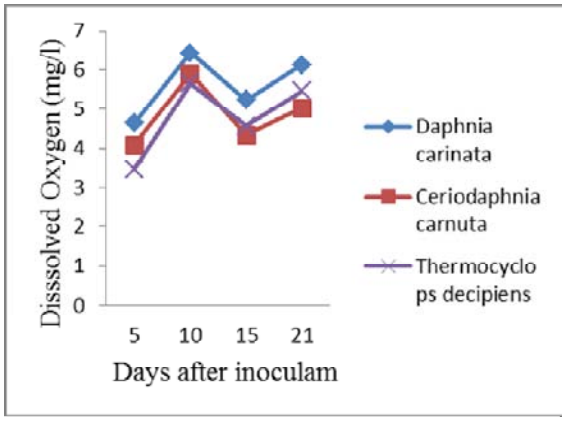

Fig. 2.**b** — pH levels of water in three different experiments of zooplankton during 21 days of culture period. Each point represents the mean of values derived from three replicates.

An absolute prerequisite to maintain cultures is to renew part of the culture water at regular intervals, to ensure a permanent good water quality. It is known from the literature that a wide range of live and inert feeds can be successfully used in culturing live feed organisms[19,27], a cheap food, available worldwide, will be more helpful and will reduce the cost of expenditures on live feed culture. It is evident from the results that with proper standardization using chicken manure fertilized medium mass culture of zooplankton is possible which will make it cost effective; thereby reduce the expenditures met on the seed production in aquaculture hatcheries. The search for suitable alternatives to salt water *Artemia* nauplii in rearing freshwater fishes remains paramount in aquaculture production. Freshwater zooplankton *D. carinata, C. cornuta and T. decipiens* could be suitable alternatives and consequently the need to mass produce it on a large scale becomes beneficial. The use of chicken manure, which is surplus in country like India, in raising these unique zooplankton is the trust of this research.

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