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Effect of Various Animal Manures on the Growth of a Freshwater Green Algae (*Chlorella* spp.) and Rotifer (*Brachionus calyciflorus*)

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Abstract

The effects of chicken, duck, quail, buffalo and horse manure on the growth of *Chlorella* spp. and *Brachionus calyciflorus* were investigated. While the effects vary considerably, results indicated that all manures tested promote growth of *Chlorella* spp. The greatest growth with maximum density of 11 x 106 cells·ml^{-1} of *Chlorella* spp. was obtained in treatment with chicken manure. The density of *Chlorella* spp. decreased two days after inoculation of *B. calyciflorus*. No direct promotive effect of animal manure on the growth of rotifer was observed. However, animal manure promoted the growth of *Chlorella* spp. which in turn enhanced the growth of *B. calyciflorus*. Maximum density of *B. calyciflorus* was found under semi-continuous culture at 542 ind·ml^{-1}, when treated with chicken manure. Best growth of *Chlorella* and *B. calyciflorus* occurred in treatment with chicken manure, which was probably related to its high nitrogen concentration.

Introduction

The importance of fertilizers in pond production in modern fish culture is well established (Yusoff and McNabb 1989). However, in most tropical countries, suitable fertilization schemes (actual mixtures, quantity and application schedules) have not been widely practiced. Animal manure is the most common fertilizer used in fish culture in tropical countries such as Indonesia. It is applied as compost, liquid manure or in its fresh and untreated state. In China, the application of animal manure in all three forms is widely practiced (Yamada 1983).

Utilization of chicken and duck manure to enhance production in fish ponds has been well studied (Woynarovich 1980; Cruz and Shehadeh 1980; Wetcharagarun 1980; Turner *et al.* 1986; Bombeo *et al.* 1989; Green *et al.* 1989; Waichingsin 1989; Edirisinghe 1990; Schroeder *et al.* 1990). Various cultivated food organisms are used for rearing fish larvae, particularly *Brachionus* spp. and *Chlorella* spp., which are commonly used in mass culture. Utilization of various types of manure for Chlorella production as a diet for rotifer is also well documented. In this study, we evaluated the effect of five

types of manures (chicken, duck, quail, buffalo and horse) on the growth of both *Chlorella* spp. and freshwater rotifer *B. calyciflorus*.

Materials and Methods

Treatment I. Chlorella growth

Batch and semicontinuous culture of Chlorella were conducted inside and outside the laboratory. Five kinds of animal manure, from chicken, duck, quail, buffalo and horse, were obtained from a local breeder in Pekanbaru. Samples of each dried manure (3.15 g) were placed in a cooking-pot containing 300 ml of filtered wellwater and boiled for 20 minutes. The extract was then filtered through a membrane filter (1 μm pore size) and diluted by adding wellwater up to 900 ml, which was further divided into three equal replicates.

Freshwater Chlorella obtained from the Freshwater Aquaculture Research Center, Depok, West Java, was inoculated with an initial density of 105 cells·ml^{-1} to 300 cells·ml^{-1} culture medium in 500-ml flasks for each treatment. All growth studies were conducted in triplicate. Algae were cultured in a simple incubator with continuous aeration, illuminated by four daylight fluorescent lamps with a light intensity of 4000 lux and temperature between 23 to 28°C. A 12 hours light and 12 hours of dark (12 L:12 D) cycle was maintained. The algal density was enumerated daily for 16 d using a hemocytometer under a binocular microscope.

B. calyciflorus culture medium was also used to grow Chlorella with the same treatment and methods as described previously. On the sixth day after Chlorella inoculation, *B. calyciflorus* was inoculated at 1 ind·ml^{-1} and Chlorella growth was determined daily for 16 d before and after *B. calyciflorus* inoculation.

In an outdoor study, *Chlorella* spp. was cultured in 30-l polycarbonate tanks with water volume of 20 liters containing various kinds of animal manure. This *Chlorella* spp. was used as food for *B. calyciflorus*. Boiled water was mixed with exctract of animal manure to a concentration of 3.5 g·l^{-1}. After filtering through a membrane filter (1.0 um pore size), *Chlorella* spp. was inoculated to the culture media with an initial density of 105" cells·ml^{-1}. Cultures were maintained in outdoor tanks at 23 and 28°C with natural illumination. The number of Chlorella was counted daily for 16 d. On the sixth day, one liter of the culture medium was taken from each treatment for rotifer culture, and one liter of new medium was added to maintain constant volume. The culture media were changed every day for 10 d. The growth of *Chlorella* spp. in each treatment was observed daily for the 16-d culture period.

Treatment II. Rotifer Growth

Effect of chicken, duck, quail, buffalo and horse manure on the growth of *B. calyciflorus* was determined in 300 ml of culture media, containing 3.5 g·l^{-1} of respective manure type. *B. calyciflorus* in actively growing culture was inoculated into each culture medium with an initial density of

1 ind·ml^{-1}. All treatments were in triplicate with continuous aeration. The population density of rotifer was counted daily for 10 d.

The growth of *B. calyciflorus* in Chlorella culture media enriched with 3.5 g·l^{-1} of the five-type animal manure was studied under semi-continuous culture conditions.

In semi-continuous culture, chlorella was cultured in 30-l container with different manures type as described above. After the sixth day, 300 ml of this medium was placed into a 500-ml culture bottle and inoculated with *B. calyciflorus* at a density of 1 ind·ml^{-1} and continuously aerated. Each treatment was done three times. During the culture period, 2/3 of each culture medium was changed daily at 0900 h. Culture medium was filtered through a 40-μm plankton net to prevent the loss of rotifers. The same volume of the culture medium was added to the bottle from actively growing *Chlorella* spp. culture. The numbers of *Chlorella* spp. and rotifers were counted under a microscope daily before the culture medium was changed.

Analysis of variance was used to test for differences between all treatments with three replications. Significant differences between means were analyzed using Student-Newman Keuls multiple range test.

Growth of Chlorella spp.

It was revealed that chicken manure supported the best growth of Chlorella, with the density reaching 11.8 x 106" cells·ml^{-1} at day 12 and declining slightly at the end of the experiment (Fig. 1). A high density of *Chlorella* spp. was also found in the treatment with duck and quail manures, which had maximum cell densities of 5.4 x 106 cells·ml^{-1} and 5.5 x 106 on days 8 and 13, respectively. Rotifer feeding on chlorella is clearly shown in Fig. 2, where the density of chlorella in each culture decreased by about 50% on the fourth

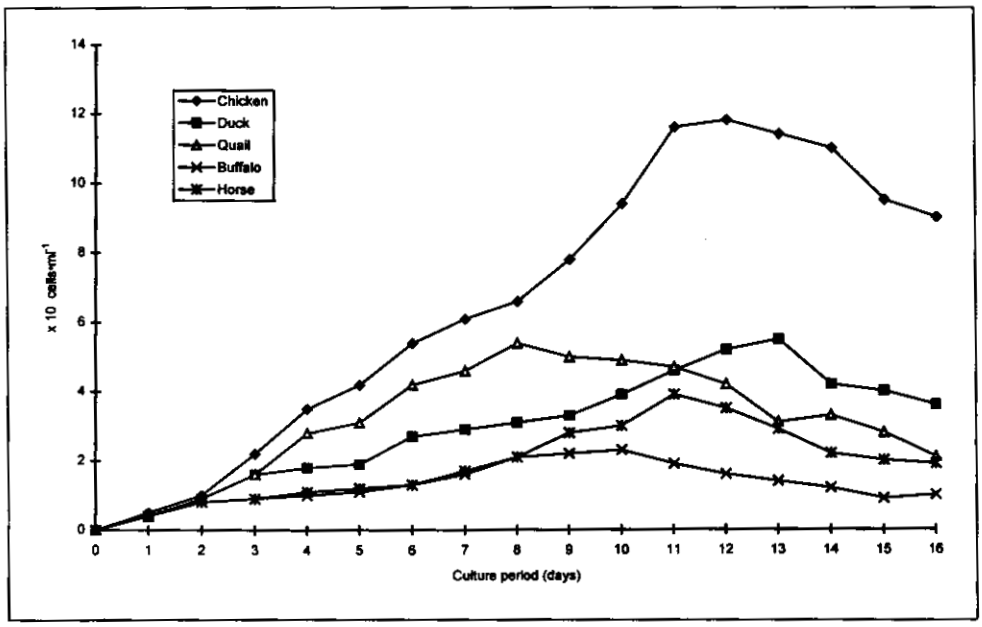

Fig. 1. Growth of *Chlorella* spp. with various animal manures.

day after rotifer inoculation. The concentration of Chlorella in all treatments were below 106" cells·ml^{-1} after the fifth day. This suggests that the grazing rate of rotifer was higher than the growth rate of *Chlorella* spp. In outdoor culture, the best growth of *Chlorella* spp. occurred in chicken manure (9.8 x 10 6" cells·ml^{-1}), followed by duck (4.9 x 106" cells·ml^{-1}), quail (4.7 x 106" cells·ml^{-1}), buffalo (2.2 x 106" cells·ml^{-1}) and horse manure (1.9 x 106" cells·ml^{-1}) (Fig. 3).

Rotifer growth

No appreciable rotifer growth was observed in culture media with animal manure alone (Fig. 4). All rotifers died on the fourth day after inoculation. The

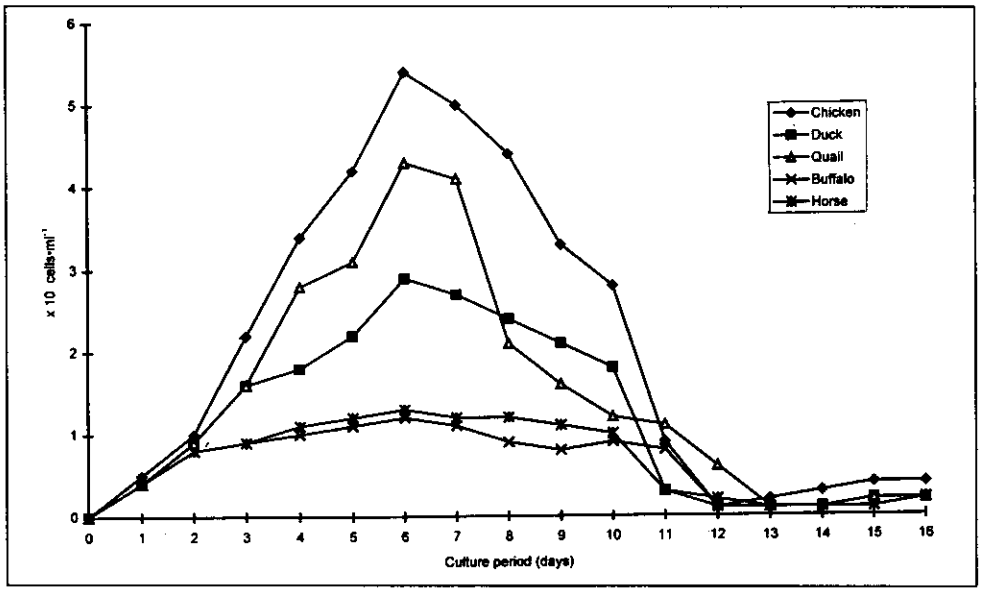

Fig. 2. Growth of *Chlorella* spp. before and after inoculation of *B. calciflorus*.

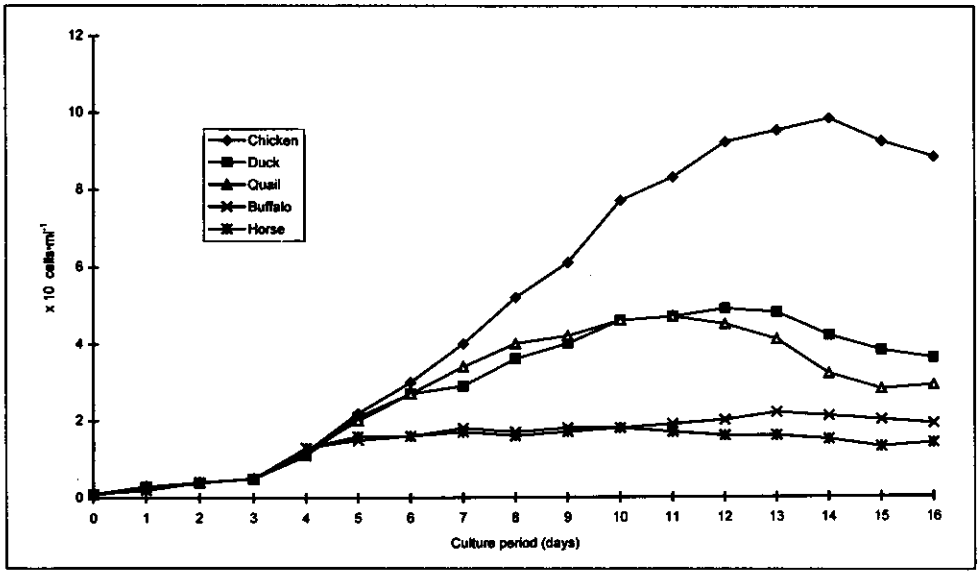

Fig. 3. Growth of *Chlorella* spp. with various animal manure in 20 l cultures.

existence of bacteria in the culture medium did not support the population growth of *B. calyciflorus*.

However, existence of *Chlorella* spp. in each manure treatment promoted the growth of *B. calyciflorus* (Fig. 5). The highest growth of *B. calyciflorus* was observed in the medium with chicken manure, where Chlorella density was also high. The maximum density of rotifer in chicken, duck, quail, horse and buffalo was 103, 82, 51, 39 and 20 ind·ml^{-1}, respectively. After the sixth day of culture period, rotifer growth in each treatment decreased. This may be related to the low concentration of *Chlorella* spp. as a food source for rotifer.

The growth of rotifer, however, increased with the daily exchange of 2/3 of the culture medium with Chlorella solution as shown in Fig. 6. Best growth

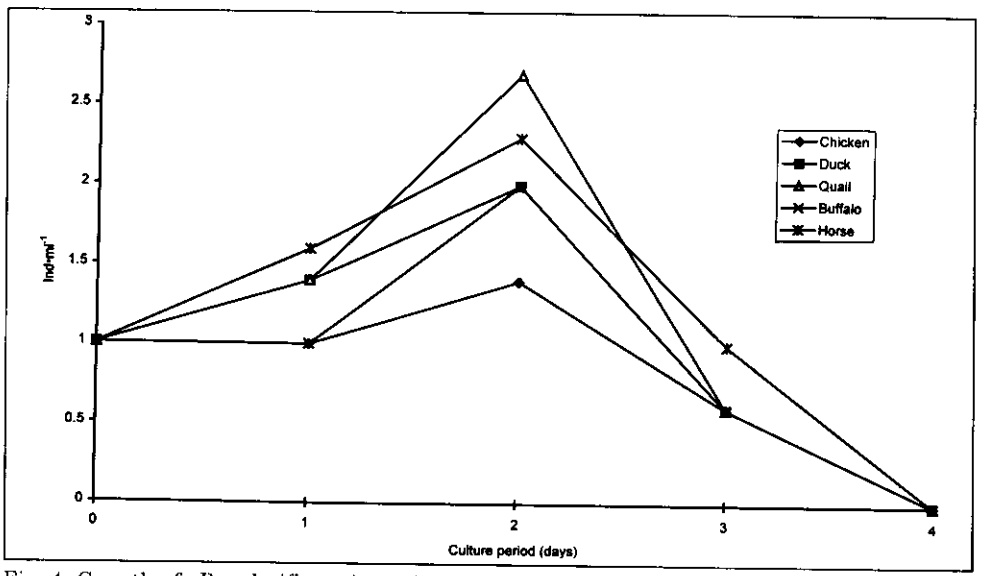

Fig. 4. Growth of *B. calyciflorus* in various animal manures without *Chlorella* spp.

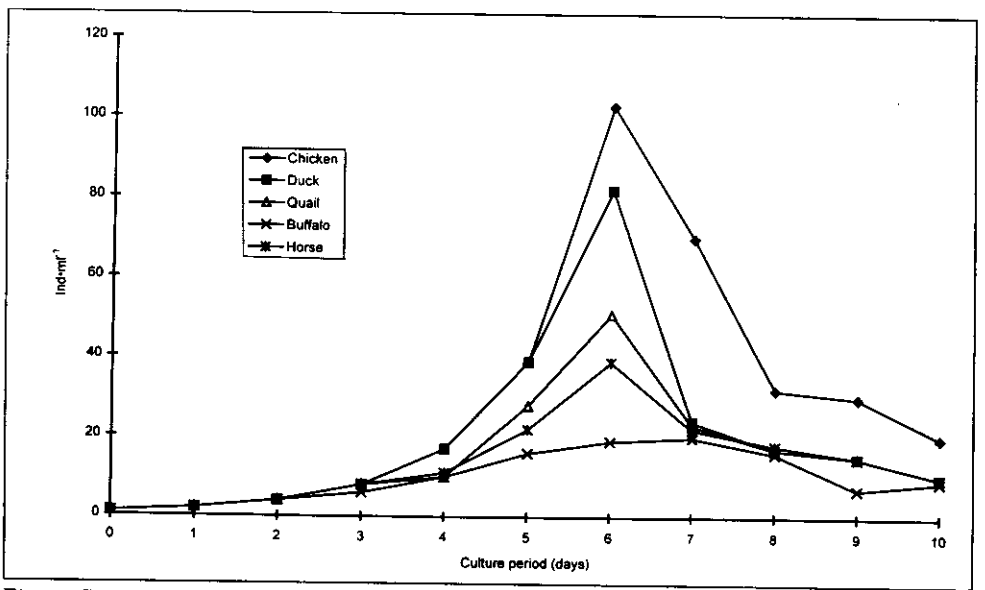

Fig. 5. Growth of *B. calyciflorus* in *Chlorella* cultures with various animal manures.

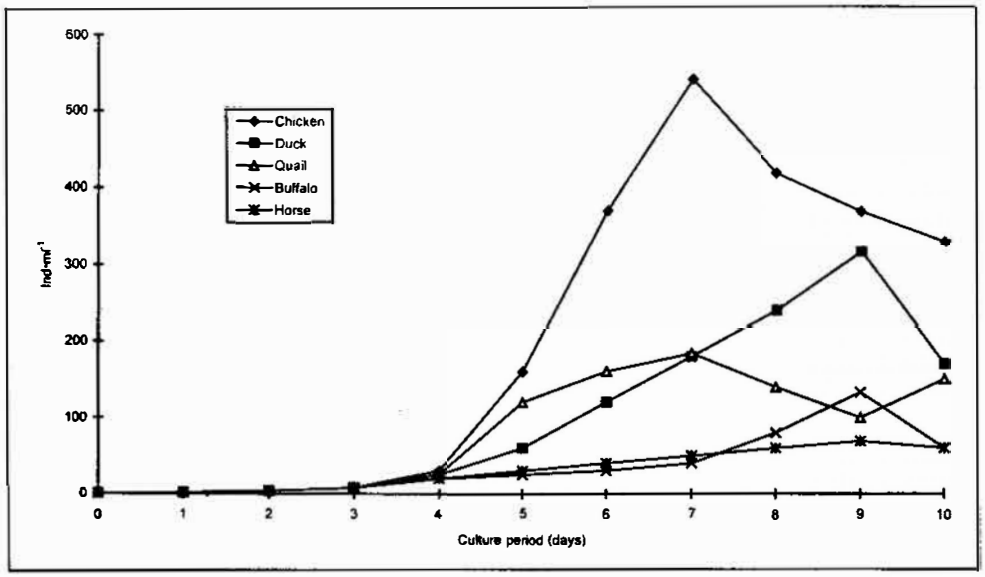

Fig. 6. Growth of *B. calyciflorus* in semi-continuous culture with *Chlorella* spp. as food and various animal manures as fertilizer.

was observed in chicken manure where the density reached 542 ind•ml^{-1} on the seventh day after inoculation. This was followed by duck (317 ind•ml^{-1}), and quail (184 ind•ml^{-1}), buffalo (133 ind•ml^{-1}) and horse manure (69 ind•ml^{-1}). Statistical analysis indicated a significant different between the use of animal manures on the growth of *Chlorella* spp. and *B. calyciflorus*.

The components of nitrogen and phosphorus in each treatment were observed (Table 1). It was evident that the concentration of nitrogen was high in chicken, quail and horse manure while the concentration of phosphorus was high in duck and buffalo manure.

Discussion

Inorganic nutrients are one of the major factors that influence the growth and production of phytoplankton in a pond. Taiganides (1978) reported that animal manure contains all of the major inorganic nutrient components (N,P,K). Trace elements such as Ca, Cu, Zn, Fe and Mg are also found in animal manures. As much as 72 to 79% N, 61 to 78% P and 82 to 92% K are recovered from animal excreta.

Nitrogen and phosphorus are the two main nutrients that influence phytoplankton growth (Finenko and Akinina 1974; Fabregas *et al.* 1986). Therefore, the high concentration of nitrogen and phosphorus in a culture medium, especially in chicken manure, promoted the rapid growth of *Chlorella* spp. As Chlorella is an important live food for rotifers (Hirayama *et al.* 1989), adding rotifers to a *Chlorella* spp. culture effectively reduced the population density of the latter (Fig. 3). Hirayama and Ogawa (1972) reported that the ingestion rate of rotifer with empty guts was 200 cells•min^{-1}•ind.$^{-1}$.

The population growth of rotifer mass culture depends not only on the quality of the food given but also on its density (Dahril 1989). Although *B. calyciflorus* is capable of collecting and ingesting bacteria such as *Aerobacter*

Table 1. Nitrogen and phospore concentration in the different manures and mean water quality parameter in rotifer culture with and without *Chlorella* spp. and in semi-continuous culture.

Manure/ Treatment			Without *Chlorella* spp.				With *Chlorella* spp.				Semi-continuous culture			
	N (%)	P (%)	°C	pH	NO3-N	NO2-N	°C	pH	NO3-N	NO2-N	°C	pH	NO3-N	NO2-N
Chicken	3.19	0.17	30.5	7.5	0.41	0.46	32.5	8.0	0.73	1.21	32.0	8.5	2.20	1.17
Duck	0.67	1.37	29.0	7.0	0.04	1.25	32.0	6.5	0.12	0.38	31.5	8.0	0.04	0.81
Quail	2.61	0.02	30.0	7.5	0.91	0.76	32.0	6.5	0.12	0.38	31.5	8.0	0.99	1.85
Buffalo	0.88	1.81	30.5	7.0	0.59	0.59	32.0	7.0	0.97	1.06	31.5	7.5	0.79	1.20
Horse	2.76	0.47	30.5	6.5	0.61	1.58	32.5	6.5	0.91	1.79	32.5	7.5	0.73	1.79

aurogenes (Starkweather *et al.* 1979), no growth of *B. calyciflorus* was recorded in the medium without *Chlorella* spp. Hirayama *et al.* (1973) reported that the high rate of population growth of *B. plicatilis* was found in marine Chlorella at a density of 150 X 104" cells·ml^{-1}.

High concentration of ammonia and nitrite can also affect the growth and reproduction of rotifers in mass culture (Schluter and Groeneweg 1985; Fu and Hirayama 1986). Laboratory analysis of ammonia and nitrite concentration in each treatment at the end of this experiment show that the concentrations were too low to affect the growth and reproduction rate of rotifer (Table 1). Therefore, it is probable that food concentration was the main factor that influenced the growth of rotifer in the present study.

The results of this study show the effectiveness of various animal manures as nutrient sources for *Chlorella* spp., which in turn is available as a food source for *B. calyciflorus*.

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References

Bombeo T.I, R.F. Agbayani and P.F. Subosa. 1989. Evaluation of organic and inorganic fertilizers in brackishwater milkfish ponds. Aquaculture 76:227-235.

Cruz, E.M. and Z.H. Shehadeh. 1980. Preliminary results of integrated pig-fish and duck-fish production tests. In: Integratead Agriculture-Aquaculture Farming Systems (ed. R.S.V. Pullin and Z. H. Shehadeh). pp. 225-238. ICLARM, Manila.

Dahril, T. 1989. A review on rotifers study and its mass culture problems. Faculty of Fisheries, University of Riau, Pekanbaru. 57 p.

Edirisinghe, U. 1989. Effect of 'Veluwe' ducks on *Oreochromis niloticus* recruitment under extensive fish culture condition. Aquacultural research in Asia: Management techniques and nutrition. Proceedings of the Asian Seminar on Aquaculture, Malang, Indonesia, pp. 116-120.

Fabregas, J., C. Herrero, B. Cabezas and J. Abalde. 1986. Biomass production and biochemical composition in mass cultures of the marine microalga *Isochrysis galbana* Parke at varying nutrient concentration. Aquaculture 53:101-113.

Finenko, Z.Z. and D.K.E. Akinina. 1974. Effect of inorganic phosphorus on the growth rate of diatom. Marine Biology 26:193-201.

Fu, J.P. and K. Hirayama. 1986. The effect of un-ionized ammonia on the population growth of the rotifer in mass culture. Bulletin. Japanese Society of Scientific Fisheries 52:1509-1513.

Green, B.W., R.P. Phelps, and H.R. Alvarenga. 1989. The effect of manure and chemical fertilizers on the production of *Oreochromis niloticus* in earthen ponds. Aquaculture 76:37-42

Hirayama, K. and S. Ogawa. 1972. Fundamental studies on physiology of rotifer for its mass culture-I. Filter feeding of rotifer. Bulletin. Japanese Society of Scientific Fisheries 38:1207-1214.

Hirayama, K., K. Watanabe and T. Kusuno. 1973. Fundamental studies on physiology of rotifer for its mass culture-III. Influence of phytoplankton density on population growth. Bulletin. Japanese Society of Scientific Fisheries. 39:1123-1127.

Hirayama, K., I. Maruyama and T. Maeda. 1989. Nutritional effect of freshwater Chlorella on the growth of the rotifer *Brachionus plicatilis*. Hydrobiologia 186/187:39-42.

Schluter, M. and J. Groeneweg. 1985. The inhibition by ammonia of population growth of the rotifer, *Brachionus rubens* in continuous culture. Aquaculture 46:215-220.

Schroeder, G.L., G. Wohlfarth, A. Alkon, A. Halevy and H. Krueger. 1990. The dominance of algal-based food webs in fish ponds receiving chemical fertilizers plus organic manures. Aquaculture 86:219-229.

Starkweather, P.L., J.J. Gilbert and T.M. Frost. 1979. Bacterial feeding by rotifer *Brachionus calyciflorus*: Clearance and ingestion rates, behavior and population dynamics. Occologia 44:26-30.

Taiganides, E.P. 1978. Principles and techniques of animal waste management and utilization. FAO Soils Bulletin 36:341-362.

Turner, J.W.D., R.R. Sibbald and J. Hemens. 1986. Chlorinated secondary domestic sewage effluent as a fertilizer for marine aquaculture I. Tilapia culture. Aquaculture 53:133-143.

Waichingsin, A. 1980. Integrated animal-fish husbandry systems in Hong Kong, with case studies on duck-fish and goose-fish systems. In: Integrated agriculture-aquaculture farming systems (ed. R.S.V. Pullin and Z. H. Shehadeh), pp. 113-124. ICLARM, Manila.

Wetcharagarun, K. 1980. Integrated agriculture-aquaculture farming studies in Thailand, with case studies on chicken-fish farming. In: Integrated agriculture-aquaculture farming systems (ed. R.S.V. Pullin and Z. H. Shehadeh), pp. 243-250. ICLARM, Manila.

Woynarovich, E. 1980. Utilization of manure in fishfarming. In: Report of the Proceeding Fishfarming and Wastes (ed. C.M.R. Pastakia). pp. 78-95. Institute of Fisheries Management and Society of Chemical Industry, London.

Yamada, R. 1983. Pond production systems:fertilization practices in warmwater fish ponds.

Yusoff, F.M. and C.D. McNabb. 1989. Effects of nutrient availability on primary productivity and fish production in fertilized tropical ponds. Aquaculture 78:303-319.

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