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Original Article

Comparative performance of Chlorella, yeast, and commercial feed for the culture of *Moina siamensis*

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Abstract: *Moina siamensis* is one of the most successful Cladocerans used for mass culture as live feed for the larvae of crustaceans and different fish. *Moina* is fed *Chlorella*; however, there is a risk of predation by larger zooplanktons, which appear in the tanks where the *Chlorella* are grown. As a solution, specialized commercial feeds have been produced. The main objective of this study was to explore an alternative to *Chlorella* for *Moina* culture. The present trial was conducted to compare the performance of *Chlorella*, yeast, and a commercial diet on the biography, population growth, and density. Seven adult *Moina* as replicates per treatment were placed in each plastic cup individually and monitored. They were fed three types of feed at a concentration of 6×10^6 mL^{-1}. The experiment continued until all the adult *Moina* died. The result showed that the average day of maturation, the number of newborns per day, the number of newborns per crop, and the number of crops from different diets were similar. The *Moina* fed *Chlorella* sp. showed the longest life span (9.00±0.8 days), and the shortest with the commercial feed (5.6±0.3 days). However, commercial feed and the *Chlorella* did not differ in terms of the final population of *Moina,* but they produced more than when fed the yeast. Therefore, the results demonstrated that the commercial feed was similar to that of *Chlorella* and can serve as an alternative diet.

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Introduction

Live feeds play a critical role in larval aquaculture, with high demand in the aquaculture industry, especially for some fish species and crustaceans. Livefeeds can be phytoplankton or zooplankton, which are the major foundations of any aquatic food web. They are either grown in culture systems or offered to other animals after they have grown separately. They are abundant throughout the day and night, allowing fish and crustaceans, as well as their larvae, to feed at any time. Another benefit of the live feeds is that they are small in size (200-1,500 µm), which can be preyed on and swallowed by the tiny larvae (Rodmongkoldee et al., 2020). The mouth of the newly hatched larvae is small and remains in a semi-developmental stage, requiring food of a suitable size to fit into their mouths.

Artemia has been one of the most widely used live feeds in the crustacean industry (New et al., 2010). Varying quality of *Artemia* cysts, timely hatching management, and high prices are its significant disadvantages (New et al., 2010). Therefore, for freshwater prawn, *Moina*, Daphnia, Copepods, and Rotifers are proposed as alternatives to *Artemia,* as Cladocerans are the most common components of natural freshwater ecosystems (Smirnov, 2013). Among them, *Moina*, also called a water flea, is the most common. It is a crustacean within the order Cladocera that belongs to the family Moinidae. *Moina* is parthenogenetic in reproductive behavior with a

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short lifespan. It shows fast reaction to environmental changes and has an important role in the food web as a primary consumer that consumes phytoplankton (Newcombe and Macdonald, 1991).

Moina represents a group of 138 zooplankton species known so far, which is widely known as an important live feed for fish or prawn larvae. *Moina siamensis* is one of them found in Thailand (Choedchim and Maiphae, 2023). According to Alonso et al. (2019), the size range of this species is 200-990 *μm*. It contains 61.04% protein and 7.96% lipids. The size and nutritional value are very suitable for many fish and crustacean larvae as natural food. The quantity and quality of *M. siamensis* affect the nutrition of the consuming larvae. At the same time, the quantity and quality of cultured *Moina* are affected by the food it consumes. Therefore, selecting a suitable diet for the *Moina* culture is important. Freshwater *Chlorella* sp., which is abundantly found in green water, has commonly been used to culture *Moina* sp., *Rotifer* sp., and *Daphnia* sp., etc. Most *Moina* farms utilize open-area or outdoor farms, either earthen or concrete ponds. However, seasonal variation and contamination of *Chlorrella* sp. by other zooplanktons are the major problems. To address these problems, many farmers in Thailand are exploring alternative methods to cultivate freshwater zooplankton, excluding *Chlorella* sp. Clear water, Baker's yeast (*Saccharomyces cerevisiae*), commercial powder feed, animal manures such as pig and cow, and others have been tried (Neri et al., 2020; Magouz et al., 2021; Hansan et al., 2023). Some companies have developed commercial feed, but it has yet to be widely adopted. Therefore, there is no clear solution for a standardized feeding protocol for *Moina* culture. Therefore, the present research was conducted in central Thailand to compare the performance of Chlorella, a commercial diet, and yeast in terms of maturation, population growth, lifespan, number of newly born individuals, number of crops, and final population of *M. siamensis*.

Materials and Methods

Source of Moina and preparation: *Moina siamensis* cysts were purchased from a farm in Chanthaburi Province. They were sold in 0.1-gram packets, valued at 100-150 Baht (approximately US$3-5) per packet. The adult life and dried cysts can be cultured commercially and are available in local and online markets in Thailand. Cysts of Moina were kept in a transparent bottle with one liter of clean water per packet of cysts for hatching. They hatched after 24 hours. Newly hatched larvae were siphoned to a black plastic bowl (diameter of 30 cm and 13 cm high) with three litres of clean water, and larvae were fed with *Chlorella vulgaris* as a regular feed for 2 days.

Diet preparation: Three types of diets were used as three treatments. The first treatment (T1) was the *C. vulgaris* of a pure strain at high concentration (5×10^6 cell/mL) sourced from Chulalongkorn University, Bangkok. *Chlorella vulgaris* was cultured in six litres of plastic transparent bottles with four litres of clean water, one litre of *C. vulgaris* stock, and 5 mL of commercial fertilizers (NPK: 15-15-15, Urea: 46-0-0). Treatment two (T2) was the Baker's yeast (*S. cerevisiae*) diet, prepared from the yeast mixed with sugar and corn starch, 2.5 g each. All the ingredients were mixed with 500 mL of clean water and kept in a plastic bottle. The third treatment (T3) was the commercial feed (size 200-400) for shrimp produced by the INVE group. The standard composition of the feed was: 42% crude protein; 7% crude lipid; less than 3% crude fiber, and less than 10% moisture. Liptoma was used as a source of DHA from *Schizochytrium* spp. Shrimp feed and Liptoma, each at 5 grams, were mixed by adding 500 mL of clean water and blending with a kitchen mixer. After mixing well, the feed was screened to remove smaller particles by passing it through a fine net (50 μm). After grading, the feed was collected and kept in a plastic bottle. Yeast diet and commercial feed were kept in the refrigerator.

Feeding: Three different diets were used as treatments to culture the *M. siamensis* experiment. The T1 group was fed *Chlorella* as the control, the T2 group was fed with yeast, and the T3 group was fed the commercial feed. The density of *C. vulgaris*, yeast, and commercial shrimp feed cells was 6×10^6 mL^{-1}. The cells were counted using a hemocytometer. Feeding

Table 1. Average maturation day, newly born Moina per day, per crop, number of crops, and life span of *Moina siamensis* on different diets.

Biography		Diets		
		Chlorella	Yeast	Commercial feed
Maturation period (day)		3±0	3±0	3±0
Life span	Average	9±0.8[a]	6±0.9[ab]	5.6±0.3[b]
	Min-Max	6-11	6-9	5-7
Newly born *Moina*	Total	196	164	168
Number of crops	Average	4.1±0.6	3.0±0.6	2.9±0.4
	Min-Max	2-6	1-5	1-4
Number of newly born in the whole life span females	Average	28.0±6.4	23.4±5.1	24.0±3.4
	Min-Max	7-57	6-38	10-36
Number per crop	Average	6.3±0.8[b]	7.4±0.5[ab]	8.7±0.7[a]
	Min-Max	3.5-8.75	3-9.5	5.75-12
Average population		257±66[a]	33±8 [b]	341±22[a]
Population growth rate	(%)	53±3[a]	34±3[b]	58±1[a]

and water exchange were performed daily after counting the *Moina*.

Experimental design: Three different diets were used as treatments to culture *M. siamensis* in seven plastic cups for each feed group as a replication. The *Moina* were selected and sorted out from the stock kept in the black plastic bowls. Before the start of the experiment, the stock of the *Moina* was stopped feeding one day earlier. The experiment was maintained at the water temperature range of 27-30ºC, pH 8, and photoperiods of 12:12 (Light: Dark).

Population dynamics: To investigate the population dynamics of *M. siamensis*, the animals were fed three different diets as treatments, with seven replications. One newborn (<6 hours old) was selected and separately cultured in a transparent cup with a diameter of 7.5 cm and a height of 8 cm, using 50 mL of clean water. The experiment was conducted until all the *Moina* had survived to record their lifespan.

Monitoring and assessing the *M. siamensis* population: All *M. siamensis* in each culture cup were monitored and counted every day in the morning. Water in the cup was drained into a petri dish. The cups used for the culture were washed, and new water was added at the same level (50 mL), and the *Moina* were cultured continuously. Newly born *Moina* were counted individually under a stereomicroscope in each treatment and then moved to a new culture cup. Maternal *Moina* was still cultured in the same culture cup to investigate the date of maturation, number of crops, number of newly born, and life span of *M. siamensis* fed different diets, which were recorded daily until the animal survived. At the end of the experiment, the newly born babies, which were separated into the new culture cup from each treatment, were counted individually to evaluate the number and biology. Population and vital rates were estimated following Kohyama et al. (2017), using the formula of the population growth rate (G%) = (Ln N_T- Ln N_0) / T x 100, where, Ln = the natural log, N_0 = initial count of individuals, N_T = final count, T = the number of days of the culture.

Data analysis: One-way analysis of variance (ANOVA) was used for each parameter to test the effects of the factor (different diets). Tukey's HSD test was used to compare the treatment groups. The significance level was set at $P<0.05$. All statistical analyses were performed using the IBM SPSS Statistics (Version 23).

Results

Biography of *M. siamensis*: The results showed that *Moina* matured in all the treatments and gave birth to the first newly born *Moina* on the third day of the experiment (Table 1). The average number of newborns per treatment was 28±6.38, 23.43±5.07, and 24±3.39 when fed *Chlorella*, yeast, and commercial feed, respectively, which showed no significant difference ($P>0.05$). An average number of newly born per spawn showed the highest value in the commercial feed (8.7±0.75), followed by the yeast diet (7.4±0.46) and the *Chlorella* diet (6.3±0.83).

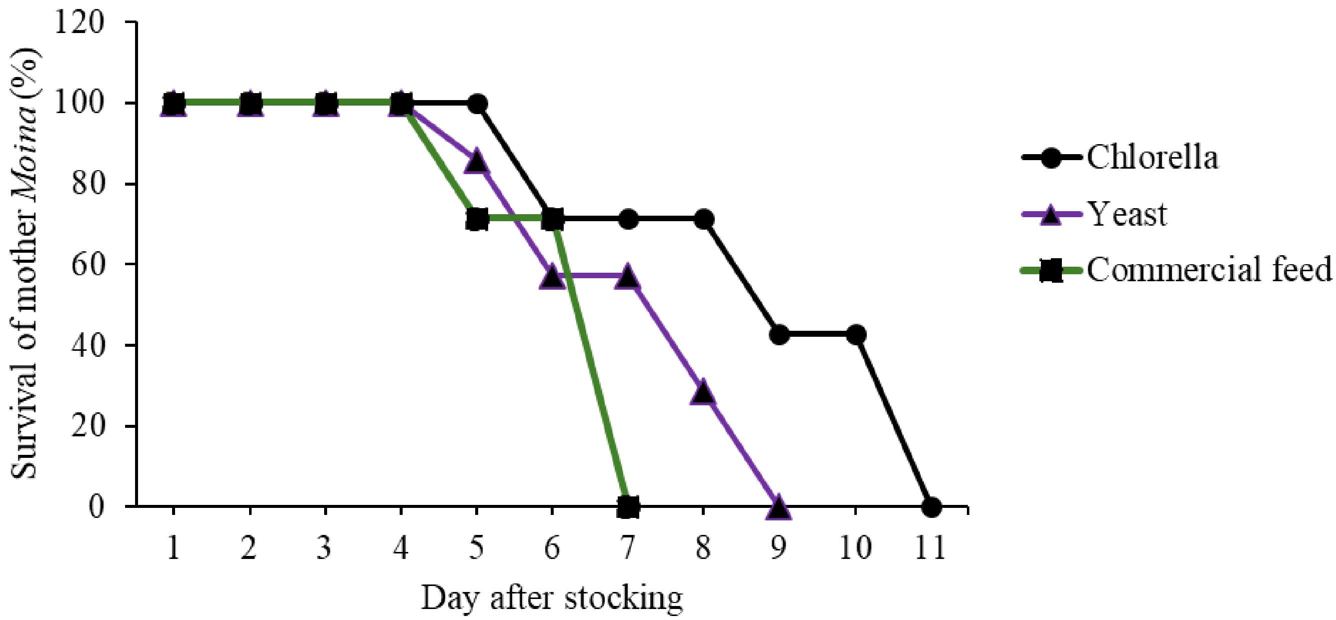

Figure 1. Survival of Moina fed with different diets by day.

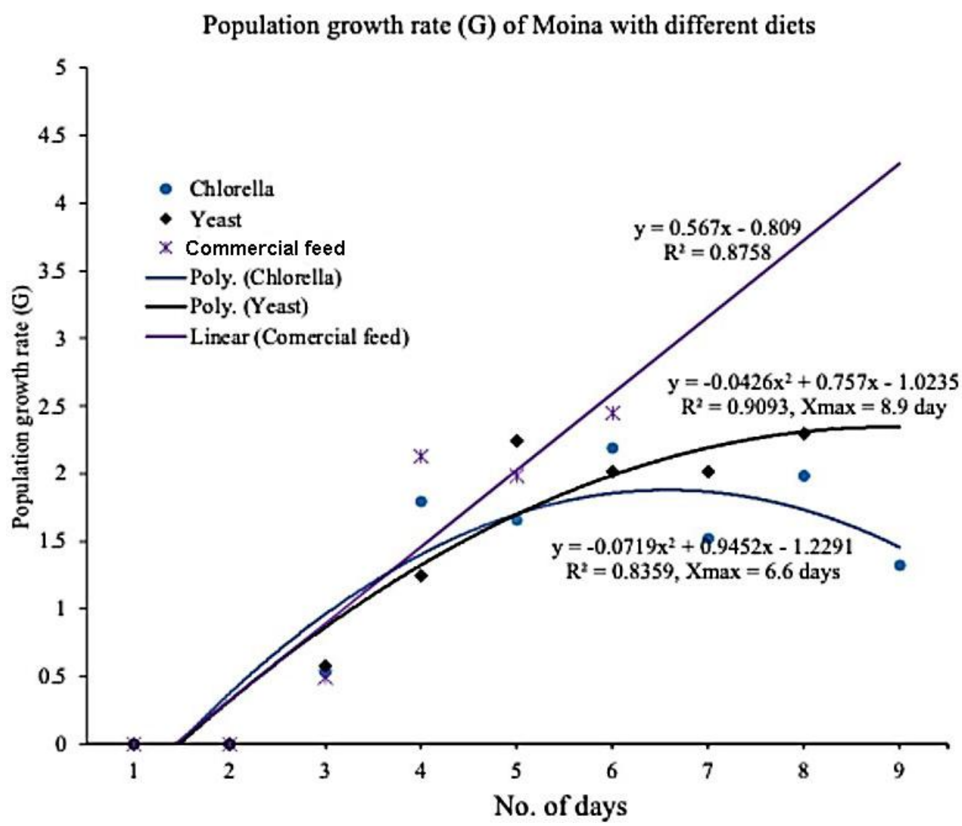

Figure 2. Population growth rate of *Moina siamensis* on different diets.

The average number of crops was similar among the treatments, i.e., 4.14±0.59, 3±0.58, and 2.86±0.4 in *Chlorella*, yeast, and commercial feed diets, respectively. The life span of *M. siamensis* was longest in the *Chlorella* diet (9±0.85 days) and shortest in the commercial feed diet (5.6±0.3 days). Maternal Moina's mortality started on day 5 in the two treatments, i.e., yeast and commercial diet.

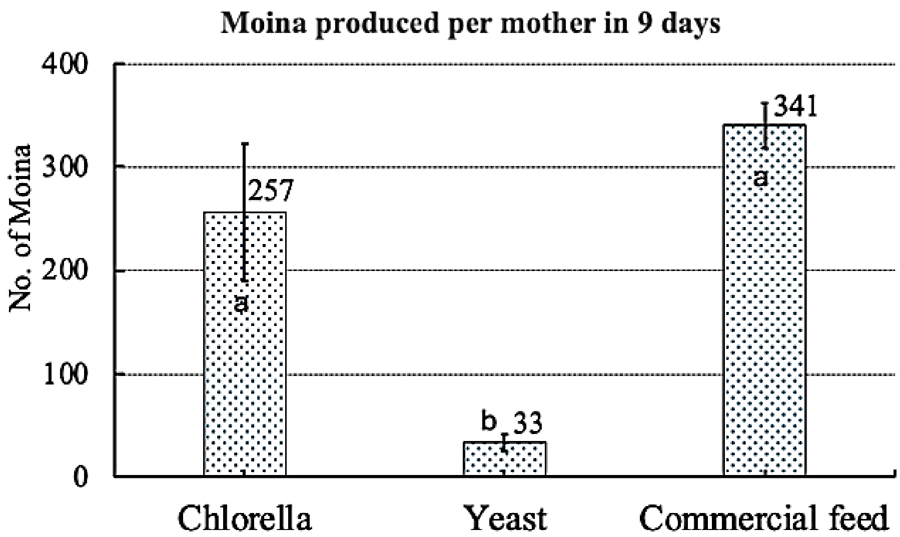

Figure 3. The average final population of Moina fed different diets on the ninth day.

Meanwhile, mortality started on day 6 of the *Chlorella* diet. The last mother *Moina* survived until the 7th, 9th, and 11th days when fed commercial feed, yeast, and Chlorella, respectively (Fig. 1), indicating that Moina nourished with commercial feed died all earlier, i.e., by the 7th day. Those with *Chlorella* survived longer, 70% *Moina* survived until the 8th day, and the last died on the 11th day.

Population growth: All *M. siamensis* cultures fed different diets showed a significant increase in population; the highest population growth rate was obtained from the commercial feed diet (58±1%), compared to yeast (34±3%) and *Chlorella* (53±3%) diets. The population growth was linear until the mother *Moina* died, i.e., on the fifth day of commercial feed. Whereas the growth trends were quadratic in the cases of the Chlorella and yeast groups, which peaked at days 6 and 8, respectively, and then declined (Fig. 2). At the end of the experiment, the population growth rate for each diet was assessed, showing significant differences ($P<0.05$). It was highest with the commercial feed and lowest with the yeast diet (Fig. 3).

Discussions

Monia siamensis is the most promising species among the live feed organisms for mass production and supply to hatcheries. The cost and standardization of a protocol for mass-scale culture require more work to develop, especially in terms of feeding management. In the present study, different diets were compared in terms of their performance to select a suitable diet for maximizing the quantity. *Chlorella*, yeast, and a commercial feed have affected reproduction and the population. In all the diets, the first maturation of *Moina* took two days. Release of newborns occurred on the third day of the experiment, which is almost similar to what was reported in *Moina siamensis, M. micrura,* and *M. macrocopa* by Rodmongkoldee et al. (2020), and in *M. mongolica,* i.e., 3.4 days, whereas it is double compared to *M. macrocopa,* i.e., 1-2.5 days (He et al., 2001; Benider et al., 2002). The first maturation and reproduction are important in the population ecosystem (Roff, 2001). The life span of some Cladoceran can be up to several months, which has been reported by several authors, such as 9 days (Sushchenya et al., 1990; Voronin and Makrushin, 2006; Makrushin, 2011), 11 days (Razlutskii, 1992), 12.5 days (Chuah et al., 2007) in *M. macrocopa* species*,* and up to 15 days (Garcia et al., 2004). Longer life spans have also been reported, e.g., 17 days in *M. weismanni* (Venkataraman, 1990) and 28 days in *M. rectirostris* (Razlutskii, 1992).

The present result also showed that the life span varies with individuals within the same species, which is influenced by the type of food and affects the production of offspring or newborns. Maternal age in *M. siemensis* showed that the shortest life span was found to be 5 days, and the longest life span was 11 days when fed with *Chlorella* sp., which is similar to the results of 8.7 days reported by Rodmongkoldee et al. (2020). Offspring and newly born declined when the age of the maternal Moina got older, which is known as the "Lansing Effect" (Bock et al., 2019; Liguori et al., 2023). The shortest lifespan observed was with commercial feed compared with other tested diets. However, it had the highest average number of newly born per day and may have some effect on brooders, which showed a negative correlation between maternal age and offspring (Rocca et al., 1992; Farrer et al., 1992; Barclay and Myrskyla, 2016). The quality and quantity of offspring depend on the age at which the parents breed. The maternal age influences the offspring's reaction to calorie restriction (Bock et al., 2019). Therefore, added protein and lipid could improve the offspring and maximize the breeding and population density.

Commercial feed is made from shrimp feed and mixed with DHA from *Schizochytrium* spp. Protein and lipid are essential nutrients for broodstock reproduction. *Schizochytrium* spp, rich in DHA, could affect the reproductive performance in female broodstock, such as egg fecundity. Moreover, the egg quality of the females fed *Schizochytrium* sp. showed good quality in the rainbow trout (Cardona et al., 2022). Li et al. (2009) reported that *Ictalurus punctatus* catfish fed *Schizochytrium* sp. supplements can enhance weight gain and improve the feed efficiency ratio (FER). However, broodstock nutrition is one of the most important factors influencing the survival of the newborn and reproductive efficiency (RE) (Lin et al. 2007; Murugan et al., 2009).

In addition, final population growth rate and final population density showed highest in commercial feed mixed with *Schizochytrium* sp. supplementation in diet on lipid metabolism due to high protein and DHA content is important role of fatty acid in reproduction system and effect on the reproductive performance and progeny of other factors such as protein content, amino acid profile additives, etc. (Cardona et al., 2022). Moreover, commercial feed, which is characterized by high protein, blended with water and mixed with a kitchen mixer, should have made appealing palatability by smell and having a small particle size, which could remain as a suspension, which might have been well fitted for the mouth of the small animal, i.e., *M. siemensis*. On the other hand, the average number of newly born per female in the whole life span in each diet was similar to that of other feeds, which could be because *Chlorella*, yeast, and commercial feed did not show a negative effect on the water quality, as in copepod culture (El-khodary et al., 2020).

The study indicated that *M. siemensis* fed on commercial feed had as high an average final population (341±22) as from the group fed *Chlorella* diet (257±66), but more than 10 times higher than from the yeast (33±8; respectively). Carli et al. (1995) reported that the type of diets strongly affects the fecundity and survival of Cladocerans such as *Harpacticoida Copepoda*. Previous studies have confirmed that the fecundity of copepods can probably be linked to the content of fatty acids in their diets (Støttrup and Jensen, 1990; Pan et al., 2016). The dietary fatty acids might have affected the production of newly born *M. siemensis,* which was mixed in the experimental diets. However, the life span, number of newborns, and population density of *M. siemensis* may depend on the environment, water quality, and quality of the food or diet during the culture period. More interestingly, the *Moina* fed commercial diets died earlier after giving birth to more offspring, whereas *Moina* reared in *Chlorella* gave birth to fewer but survived longer. This inverse relationship of survival and reproduction indicated that loss of energy for reproduction or energy spent on giving birth might have caused exhaustion, leading to early death. Implications for the commercial culture of *Moina* are that commercial feed is the most suitable diet as it helps boost Moina production, whereas *Chlorella* produces less but sustains longer.

Conclusions and recommendations: The present study indicated that commercial feed showed the highest final population density due to the higher production of newborns per spawn. However, *Moina* had a shorter life span in this treatment. *Moina* fed *Chlorella* survives longer, resulting in a smaller number per spawn and similar population density compared with *Chlorella*. For commercial mass-scale production of *Moina*, commercial food is a good alternative source of food for *M. siamensis*. Moreover, it is more hygienic and easier to handle during the culture and safe from contamination compared to Chlorella sp., which is easily contaminated by other organisms, especially during the rainy season. However, *Chlorella* could still be the cheapest source to use, provided it can be done without contamination and infection from other species on a commercial scale, with further studies. Yeast alone is not a good alternative; however, more research should be done using various combinations of *Chlorella,* Yeast, commercial feed, probiotics, and others.

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