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EFFECT OF MICROALGAL DIETS AND ITS BIOCHEMICAL COMPOSITION ON GROWTH AND SURVIVAL OF ASIATIC FRESHWATER CLAM

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ABSTRACT

Corbicula fluminea clam is one of the most popular food ingredients and nutritional supplements in Taiwan. Increasing the biomass of the clam by culturing it with a proper diet is necessary. For the potential of food availability of the *C. fluminea* clam, growth and survival were studied by rearing five species of live microalgal diets for eight weeks. The results revealed that the clams fed on *C. pyrenoidosa*, *O. multisporus* and *C. cryptica* showed outstanding results in shell growth and live weight gain. The maximum percentage of clam growth rate, which was measured by shell length, live weight gain and survival rate were found when fed on *C. pyrenoidosa*, *O. multisporus* and *C. cryptica*, respectively. However, the clams had a negative live weight gain when fed on *S. acutus* and *C. microporum*, due to the inappropriate size of the diets. The most significant protein content in clam tissue was shown when fed on *C. pyrenoidosa* (58.34%), and *C. cryptica* stimulated the highest lipid content in clam tissue (25.06%). Therefore, it suggested that the most suitable live microalgal foods are *C. pyrenoidosa*, *O. multisporus* and *C. cryptica*, for which useful algae and non-toxic species were selected to support *C. fluminea* growth.

Key words: Algal diet, asiatic clam, biochemical composition

INTRODUCTION

The bivalve culture industry was developed around the middle of the twentieth century (Quayle, 2012). The freshwater clam *Corbicula fluminea* is of Asiatic origin and has spread into many parts of the world, including Southern Asia, the Eastern Mediterranean, Australia, North America and Europe. It is cultivated for many popular foods in Taiwan and Japan and appears in Asian food markets. In Taiwan cuisine, *Corbicula* sp. is appreciated as a delicacy and is typically used as a health supplement and is the main ingredient of clam chowder, miso soup or hot pot (Jou *et al*., 2006). Moreover, freshwater clams are effective in reducing hypercholesterolemia and good for human liver functioning (Chijimatsu *et al*., 2008). Middle-aged or older people are interested in *Corbicula* extract as a nutritional food to improve the human liver function.

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Nowadays, one of the major problems in mollusc culturing to meet aquaculture targets is the production of appropriate diets for feeding. Production of microalgae is one of the main processes used in mollusc hatcheries for feeding clams. The importance of algal species as a food source for captive juvenile mussels is probably a compromise between their physical characteristics and their nutritional properties. Microalgae have a digestible cell wall to make nutrients available and specific on the size and shape of the cell. To determine how to select an appropriate microalgal diet for mollusc, feeding must depend on size, form, non-toxicity, the ability of the mollusc to trap and the biochemical composition of the microalga (Costa *et al*., 2012). There are many microalgal utilisation reports for bivalve culture. Growth rates and survival rates (>90%) of *Ostrea edulis* (the European flat oyster) achieved when fed on algal diets of *Isochrysis galbana* (Parke), *Phaeodactylum tricornutum* (Bohlin) and *Pavlova lutheri* (Ferreiro *et al*., 1990). Gale and Lowe (1971) found that

Sphaerium transversum bivalve chose to uptake green algae and diatoms. Growth increased in *Lyropecten subnodosus* (the lion-paw scallop) when fed on *Pavlova lutheri* and *Chaetoceros muelleri* (Ceron-Ortiz *et al*., 2009). Also, microalga is a promising source of high potential nutritional value, especially protein, carbohydrate and lipids, which are the main factors influencing mollusc growth and survival (Marshall *et al*., 2010). Avagyan (2008) reported that the biochemical characteristics of microalgae contain up to 50 to 70% protein and 30% lipids.

Therefore, it is crucial to increase *C. fluminea* biomass as it is one of the most aquaculture potentials of Taiwan clam for producing food ingredients and nutritional supplements by using clam extract. Five microalgal species were isolated for feeding to the Asiatic clam *C. fluminea*. In this study, the expected result was to explain the differing growth to both physiological (shell length and live weight gain), biochemical compositions and survival rate of the *C. fluminea* clam to select the most suitable microalgal species for rearing *C. fluminea* on farms.

MATERIALS AND METHODS

Sample collection and microalgal cultivation

Freshwater clams *C. fluminea* were obtained by purchasing from farm in Taichung, located at the mid-western of Taiwan region. Samples of clam were cleaned to remove any fouling and were acclimated in aerated plastic tanks, containing water at 25±2°C. Five dominant species of microalgal (non-toxic strain), including *Chlorella pyrenoidosa*, *Ourococcus multisporus*, *Scenedesmus acutus*, *Coelastrum microporum* and *Cyclotella cryptica* were collected from clam pond which was the freshwater sources in Changhua, Taiwan. Four chlorophyte microalgae; *C. pyrenoidosa*, *O. multisporus*, *S. acutus* and *C. microporum* were grown in sterilised media of JM medium. F/2 medium was used for culturing *C. cryptica* diatom. All batch cultures were scaled-up from 3 to 20 litres of plastic carboys. The algal culture conditions were set up at 25±2°C, pH of 7.5 and photoperiod of 12:12 (light/dark cycle) which was provided by 40 W fluorescent tube. The microalgal cells were harvested at the exponential phase for rearing *C. fluminea* clam.

Experimental set-up

Three replicates of plastic container (fifteen litres of plastic container; 27×36.8×23 cm) were used for culturing the clams. Fifteen clams of the same size and weight (2.65 cm of shell length and 4.15 g of live weight) were placed in plastic container. Live microalgal density of 80×10^4 cell/mL was fed in each plastic container. Microalgal cell density in container was daily microscopically counted by hemocytometer. If microalgal density in container was less than 30×10^4 cell/mL, a stocking microalga was added as initial concentration (80×10^4 cell/mL). All containers were placed in walk-in incubator at temperature of 25±2°C with 12:12 of light/dark photoperiod and illumination was provided by 40W fluorescent lamp. The aeration was constantly set to each container. The treatment containers were cleaned and the water was changed every five days to eliminate the waste products, then the new stocking microalgal diet was added. The experiment was carried out for eight weeks.

Clam growth measurements

Live weight gain of the clams was weekly obtained by electronic balance in gram unit. Shell length of the clams was weekly measured by a vernier calliper in centimetre unit. Growth rate of clam was calculated to compare the effect of different microalgal diets with the following equation (Brinkhuis, 1985):

$$\text{Growth rate (\% day}^{-1}) = (\ln L_t - \ln L_0) / t \times 100\%$$

Where,
L_0: values of shell length (cm) or live weight (g) at the beginning
L_t: values of shell length (cm) or live weight (g) at the end
t: the duration of the experiment in day

Survival rate was determined as the final number of the clams in proportion to the initial number of the clams. The final number of the clams was obtained by counting all individuals in aliquot samples from each treatment. The survival rate of the clams was calculated as percentage of initial (n=15).

$$\text{Survival rate (\%)} = (\text{Final clam number} / \text{Initial clam number}) \times 100$$

Biochemical composition analysis

Biochemical composition was investigated in clam tissue and microalgal diets. Tissues of clam and microalgal diets were frozen until the weight was constant. The Kjeldahl method was used for protein analyses (AOAC, 2005). Crude lipid content was analysed using Soxhlet distillation system. Moisture content was investigated by difference of sample weight before and after drying the sample at temperature of 105°C for 3 to 5 min. The different weight was calculated as ash content. Total carbohydrate content was determined by different

values between 100 and the sum of lipid, protein, ash and moisture content (FAO, 2002). The analyses were repeated three times for each sample.

Statistical analysis

The results were analysed with the SPSS version 17.0 (SPSS Inc.). One-way ANOVA was used to calculate significant differences between groups. If ANOVA displayed significant differences among means, a multiple comparison of means was examined by Duncan's multiple range test. Differences were considered statistically significant when $p< 0.05$.

RESULTS

Clam growth measurements

Live weight gain of clam highly attained when fed on *C. pyrenoidosa* (4.501 g), which had a significant difference from the other treatments, followed by clam fed on *O. multisporus* (4.490 g) and *C. cryptica* (4.481 g) (Figure 1a). However, the clams fed on *S. acutus* and *C. microporum* diet showed a significant difference on live weight gain when compared to initial clam weight ($p< 0.05$). The greatest shell length increased when fed on diet of *C. pyrenoidosa* (2.83 cm), followed by *C. cryptica* (2.81 cm) and *O. multisporus* (2.79 cm) (Figure 1b). In contrast, shell length of clam slightly decreased when fed on *C. microporum* and *S. acutus* (2.71 & 2.69 cm), respectively. Nevertheless, these values were significantly different from the initial shell length at day 0 ($p< 0.05$).

Growth rate and survival rate of *C. fluminea* clam

C. fluminea clam fed on *C. pyrenoidosa* grew faster than the other diets based on growth rate of live weight gain (1.012% day^{-1}) (Figure 2a) and shell length (0.819% day^{-1}) (Figure 2b). Conversely, the clams showed negative growth rate of live weight gain when fed on *S. acutus* and *C. microporum* diet. For survival rate, the clams fed on *C. pyrenoidosa* had the highest percentage of survival rate (97.78%), followed by *O. multisporus* (95.56%) and *C. cryptica* (93.33%) (Figure 3).

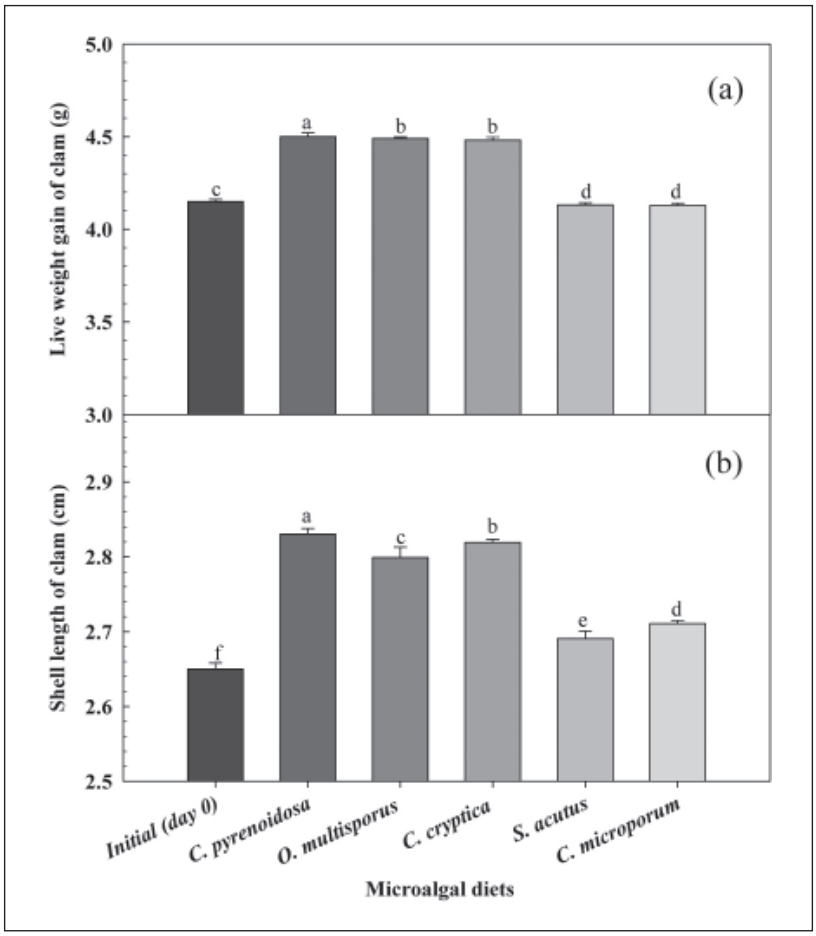

Fig. 1. Live weight gain (a) and shell length (b) of clam fed on five microalgal diets at the end of experiment (8 weeks). Different superscript letters represent significant differences ($p< 0.05$).

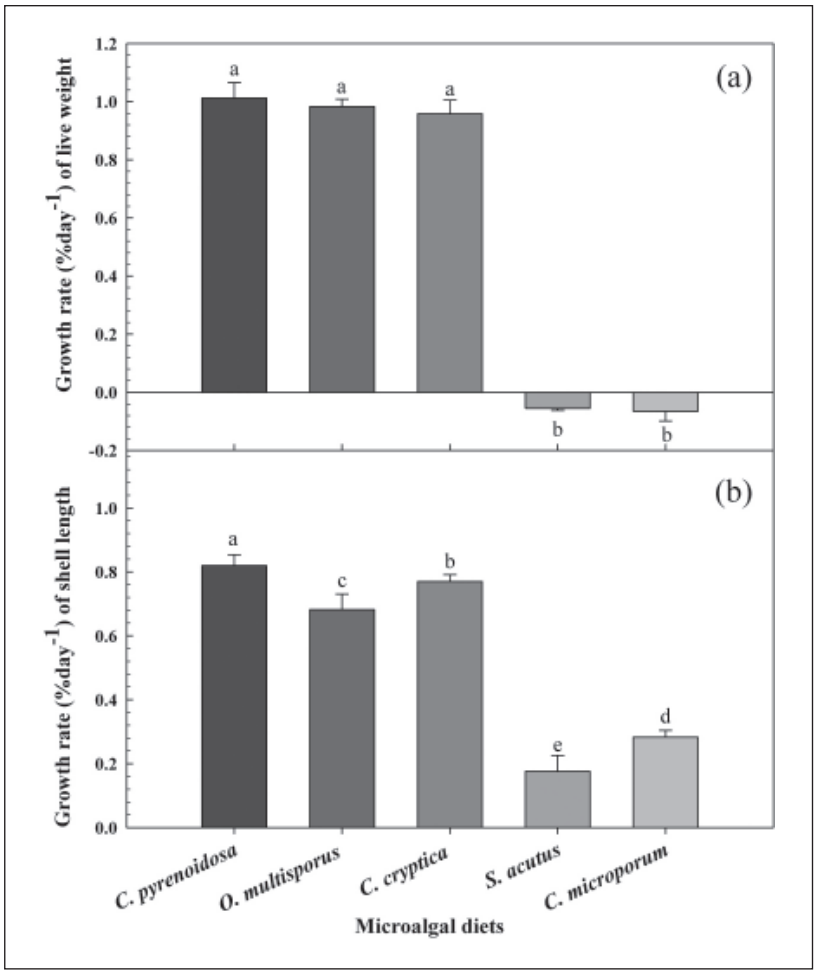

Fig. 2. Growth rate (% day^{-1}) of live weight gain (a) and shell length (b) of clam fed on five microalgal diets. Different superscript letters represent significant differences ($p < 0.05$).

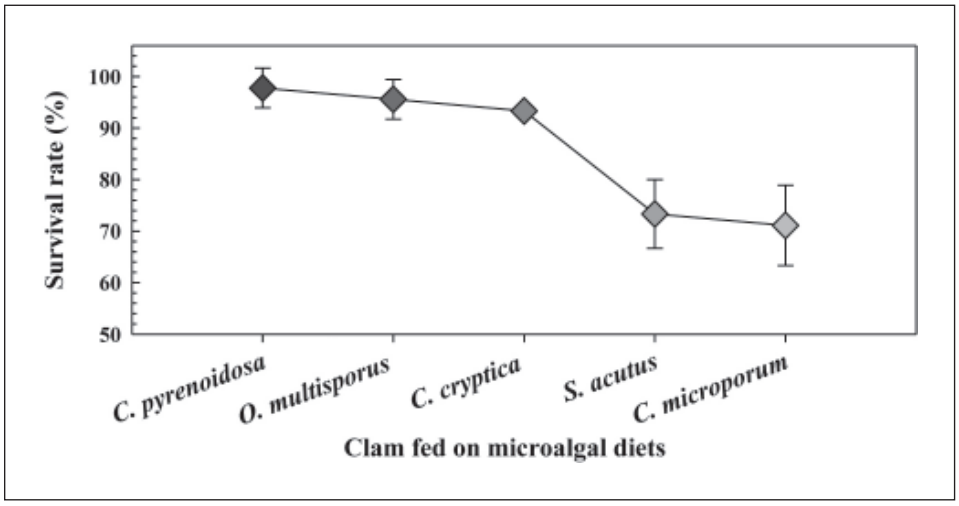

Fig. 3. Survival rate (%) of clam fed on five microalgal diets at the end of experiment.

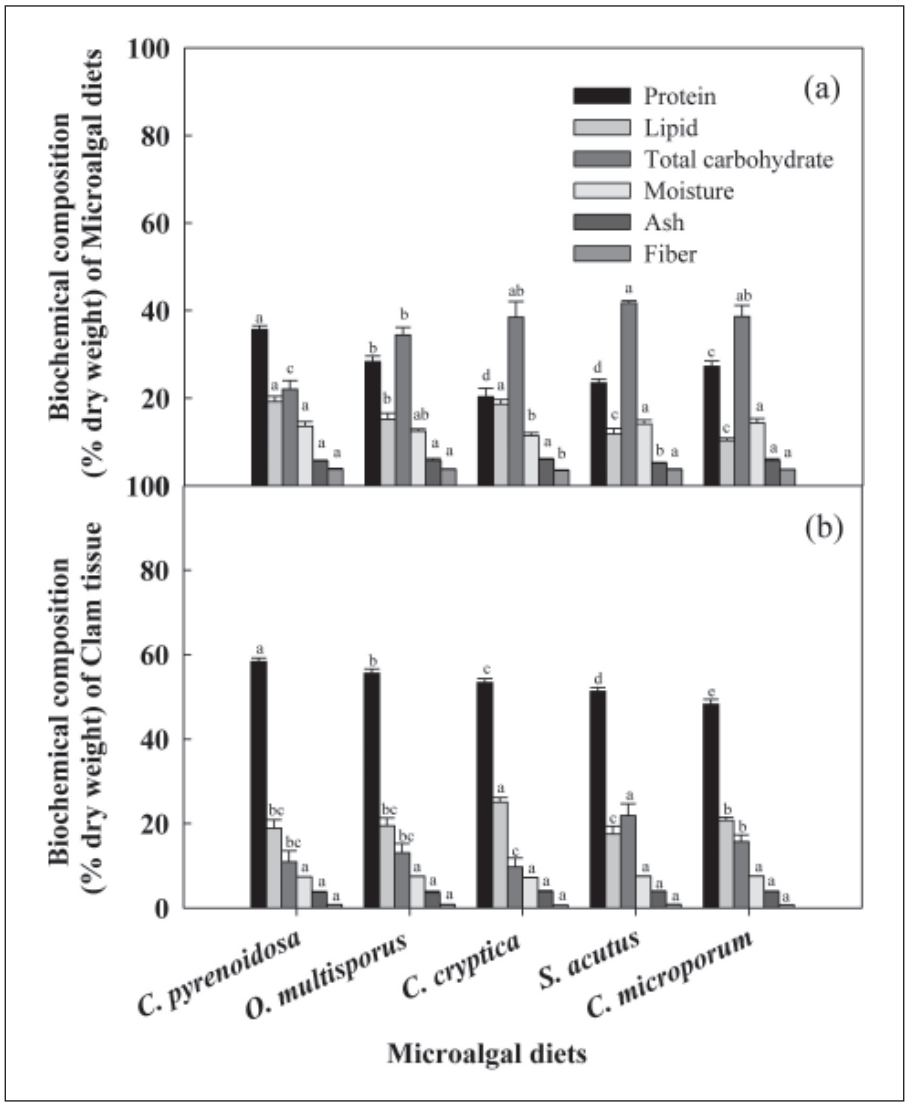

Fig. 4. Biochemical composition (% of dry weight) of microalgal diets (a) and in clam tissue (b). Different superscript letters represent significant differences ($p < 0.05$) of the biochemical composition between treatments.

Biochemical composition of diet and clam tissue

C. pyrenoidosa contained the highest amount of protein (35.67% of dry weight), followed by *O. multisporus* (28.33% of dry weight). However, lipid content highly accumulated in *C. pyrenoidosa* and *C. cryptica* at 19.23% and 18.60% of dry weight, respectively. Total carbohydrate content was highly observed in *S. acutus* diet (41.70% of dry weight) (Figure 4a). The greatest content of protein was found in clam tissue when fed on *C. pyrenoidosa* (58.34% of dry weight). Clams fed on *C. cryptica* contained high lipid content at 25.06% of dry weight. In this study, total carbohydrate content was highly found in clam fed on *S. acutus* (21.90% of dry weight). The values of moisture, ash and fiber content did not differ among treatments ($p < 0.05$) (Figure 4b).

DISCUSSION

In this study, a live microalgal diet was chosen to feed the bivalve. Pernet *et al*. (2003) stated that live microalgae are generally used as an algal diet by mollusc hatcheries. Some previous bivalve studies reported that *Mytilus edulis* mussel lost their weight gain when fed on dried algal diet (Williams, 1981; Winter, 1974). *Argopecten irradians* (the Atlantic bay scallop) grew better when fed on algae than when fed on detritus (Castagna, 1975). The nutritional value of microalgae can be an essential factor influencing mollusc growth. Food value is demonstrated by biochemical composition, especially lipid, protein and carbohydrate (Marshall *et al*., 2010). Biochemical compositions and survival rates of clam are often used as indicators of success and

growth rate is considered the key factor for clam. It was found that the *C. fluminea* clam fed on *C. pyrenoidosa* showed the maximum shell length growth and live weight gain corresponding to *C. pyrenoidosa* which had the highest protein content. Moreover, the *C. fluminea* clam had the highest lipid content when fed on diatom *C. cryptica*. According to Kheder *et al*. (2010), the better performances and high lipid accumulation were shown in Japanese oyster *Crassostrea gigas* when the diet was the diatom species. Mamat and Alfaro (2014) reported that protein and lipid were regularly metabolised to support energy usage of mollusc. Meanwhile, carbohydrate in tissue was rapidly catabolized for energy requirements in the reproduction process. The result was in line with previous studies; Uriarte *et al*. (2004) found that a high protein diet was linked to improve larval growth and survival in both *Crassostrea gigas* and *Argopecten purpuratus*. Gallager and Mann (1981) revealed that the high protein content of a microalgal diet stimulates the growth in many clams such as in *Tapes japonica* (the short-neck clam), *Crasosstrea virginica* (the pacific oyster) (Webb & Chu, 1983) and *Mytilus galloprovincialis* (the Mediterranean mussel) (Langdon & Onal, 1999). Conversely, Aranda-Burgos *et al*. (2014) demonstrated that high lipid and carbohydrate had a negative effect on growth and survival of the larvae *Ruditapes decussatus* (the grooved carpet shell).

In this study, the preference of small cell size (approximately 1 to 2 μm) of *C. pyrenoidosa*, *O. multisporus* and *C. cryptica* diets could be considered appropriate food for the *C. fluminea* clam, as clams attained their maximum growth and survival rate with these diets. Lora-Vilchis & Maeda-Martinez (2008) stated that the essential factors in bivalve nutrition are the size and shape of the microalgal cells. The maximum cell size that can be ingested is related to the bivalve size. This feeding behaviour may also be related to the essential nutrients and the different forms of the algae as the cell of *C. pyrenoidosa* was round and smaller in size than the other microalgal diets. In contrast, *S. acutus* and *C. microporum* showed the lowest of percentages of growth rate and survival rate because the cell size of these two algae (approximately 10 to 15 μm) was bigger than *C. pyrenoidosa*, *O. multisporus* and *C. cryptica*. The result of this study was agreed by the research of Beck and Neves (2002), which demonstrated that *Villosa iris* Mussel rejected large particle size of *S. quadricauda* (approximately 22.8 to 44.5 μm) but preferred *Nannochloropsis oculata* and *Selenastrum capricornutum*, based on the size range (approximately 2.8 to 8.5 μm). A freshwater bivalve *Anodonta calipygos* chose green microalgae in the range of 5 to 10 μm (Miura & Yamashiro, 1990). This complied with Ferreiro *et al*. (1990) that the lowest growth rate of *Ostrea edulis* oyster was found when fed with *Skeletonema costatum* (Greville) Cleve because of the algal spines and long cell chains. Nevertheless, each species of bivalve preferred different kinds of microalgae. A study by Gireesh and Gopinathan (2008) claimed that when fed *Isochrysis zhanjiangensis* to *Pinctada martensii* (the pearl oyster), they grew more rapidly than those fed on *Chlorella* spp.

CONCLUSION

In conclusion, microalgae are an important source of nutrition for the *C. fluminea* clam. It is suggested that *C. pyrenoidosa*, *O. multisporus* and *C. cryptica* should be considered as an appropriate size in the formulation of diets as their high protein and lipid content that stimulates clam growth. Moreover, those microalgae are safe to use as non-toxic algae hence having no impact on clam culturing or other aquatic animals on natural farms.

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