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Effects of Supplementation Substances on Survival Rate of *Lutraria Rhynchaena* During Seed Transportation

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ABSTRACT

This study was conducted to evaluate the effects of Chaetoceros algae and other substances (glucose, active char coal and Oxygen balls) on the survival rate of otter clam *Lutraria rhynchaena* during transportation and after 20 stocking days. Juvenile clams were packed in nylon bags (2 L of seawater 30‰) with the density of 300 ind./L and preserved during 24 hours at stable temperature of 25^{o}C. Results showed that total ammonium concentration increased abruptly after 24 hours in the treatments without supplements. Adding glucose and Oxygen tablets resulted in higher algal clearance rate of otter clams (70.4 – 75.0%), these levels were significantly higher than those from others ($p<0.05$). Survival rates of otter clams in active charcoal (83.3%) or Oxygen tablets (83.3%) were significantly higher than in only algae (74.4%) or glucose (71.7%). Although there was not significant difference but after 20 days of rearing otter clams which were preserved with glucose showing the higher results in survival rate (78.9%) and total weight (0.23g). Our findings indicated the ability to supply Chaetoceros algae together either glucose, active charcoal or Oxygen tablets during long transportation to improve the survival rate of otter clams.

Keywords: Juvenile, *Lutraria rhynchaena*, survival, transportation

INTRODUCTION

Otter clam (*Lutraria rhynchaena* Jonas, 1844) is a bivalve mollusk with high economic value. Otter clam meat is quite rich in nutrients, the meat contains 11.63% protein, 0,42% sugar, 1.22% mineral salts and especially with 18 types of amino acids, some of which are irreplaceable amino acids (Thuoc, 2006). In previous years, there have been a number of experimental studies on farming otter clams in Quang Ninh, Hai Phong and Khanh Hoa provinces of Vietnam (Xan *et al*., 2001; Thang and Thuy, 2004; Thanh and Huong, 2008). In Vietnam, otter clams are mainly produced in the Northern and Central regions. Transporting the seeds over a long period of time requires special techniques to ensure the animals have a high survival rate and good health conditions in order to improve production efficiency for the farmers.

Otter clam seeds are often transported in seawater and adding oxygen like shrimp and fish fingerlings because this bivalve species has a siphon that protrudes from the body and can never close the valves tightly. Prolonged transportation can cause otter clams to lose energy, reduce resistance and reduce survival rate. Practical observations show that small otter clams have the ability to digest food quickly, so they can lose more stored energy, and their wastes can also cause the water quality in the transportation bags to become worse. Research results by Welborn & Manahan (1990) showed that oyster larvae *Crassostreagigas* and abalone larvae *Haliotisrufescens* have the ability to directly absorb glucose, maltose, cellobiose and cellotriose in seawater. Uchida *et al*. (2010) added glucose to the nursery system of clam *Ruditapes Anadphilippinarum,* authors showed that they can absorb glucose and this substance contributes to growth as well as increases the content of organic acids in the clam tissues. The supplementation of a number of substances during the transportation and storage of blood cockles *Anadara granosa* has been done by Thao & Anh (2015), in which spraying 25 ‰ clean sea water combined with glucose from 50-100 mg/L can improve the survival rate of cockle seeds during storage and the newly stocked stage. Actually, there are not many research results on the addition of supporting substances in the transport of molluscs in

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Vietnam. This study was conducted to evaluate the effects of supplementation algae and some supporting substances during seed transportation on the survival rate and ability to survive in newly stocked stage of otter clams.

MATERIALS AND METHODS

Otter clam juveniles with shell length from 7-10 mm were used for seed transport experiments. Clams were packed with a density of 300/L in a plastic bag with a volume of 2 liters. The bags containing the otter clams were placed in air conditioner room that maintains a temperature of 25^{o}C. Each transportation method was repeated 5 times. The salinity in the transporting bag was maintained at 30‰ and the experiment duration lasted for 24 hours.

The experiment included 4 treatments with different types of supplements during seed transportation: 1). Control (only adding *Chaetoceros* algae); 2). Supplement *Chaetoceros* algae + glucose 50 µg/L; 3). Supplement *Chaetoceros* algae + activated charcoal (5 g/bag of seed); 4). Supplement *Chaetoceros* algae + Oxygen tablets (5 tablets/bag). *Chaetoceros* sp. algae were cultured to reach a density of 8-10 million cells/mL and then filtered through a 50 µm mesh to add to the otter clam seeds transfer bags. The monitoring criteria are the survival rate of otter clams at 0, 12 and 24 hours. Environmental factors such as total nitrogen (TAN), NO_2, pH and algal density were checked at the beginning of the experiment, after 12 and 24 hours of transportation to determine the algal filtration rate of clams.

After transportation, otter clams from each treatment were placed in baskets containing fine sand and stocked in tanks with a volume of 1m^3, the salinity maintained at 30‰. The rearing process was conducted for 21 days, data were collected on shell length, total weight, and survival rate of clams. Every day, clams were fed with centrifuged *Chaetoceros* sp. algae at a density of 10,000 cells/mL. Water exchanges were performed weekly, the volume of water exchanged was 50% for each times.

RESULTS AND DISCUSSION

Variation of environmental factors during seed storage

During the storing and transporting seeds, the pH value in clam bags fluctuates slightly over time and between different types of preservative methods (Table 1). TAN and Nitrite concentrations varied greatly between control treatment and others. TAN concentration increased very high (from 1 to 5 mg/L) when no preservatives were added, while this value increased very little in the seed bags supplemented with activated charcoal (from 0.1 to 1.3 mg/L) or Oxygen tablets (from 0.1 to 0.8 mg/L). Nitrite also increased when no preservative was added (from 0 to 2.0 mg/L) while it increased less when adding glucose, activated charcoal or Oxygen tablets (Table 1).

Table 1. Variations of pH, TAN, NO_2^- concentrations during experiment and among treatments

Parameters	Period (h)		Supplementation substances		
		No adding	Glucose	Charcoal	Oxy tablet
pH	0	7.5 ± 0.0^{a}	7.5 ± 0.0^{a}	7.5 ± 0.0^{a}	7.5 ± 0.0^{a}
	12	7.5 ± 0.0^{a}	7.5 ± 0.0^{a}	8.0 ± 0.0^{a}	7.5 ± 0.0^{a}
	24	7.8 ± 0.3^{a}	7.3 ± 0.3^{a}	7.8 ± 0.3^{a}	7.5 ± 0.0^{a}
TAN (mg/L)	0	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
	12	5.0 ± 0.0^{c}	1.0 ± 0.0^{b}	0.1 ± 0.0^{a}	0.3 ± 0.0^{a}
	24	5.0 ± 0.0c	2.0 ± 1.7b	1.3 ±0.6^{ab}	0.8 ± 0.3^{a}

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NO_2^- (mg/L)	0	0	0	0	0
	12	2.0 ± 0.0[c]	0.7 ± 0.0[a]	1.0 ± 0.0[b]	0.8 ± 0.3[ab]
	24	2.0 ± 0.0[b]	0.8 ± 0.3[a]	0.8 ± 0.3[a]	0.7 ± 0.3[a]

Values in the same row with the same letter are not significant different (p>0.05).

Algae filtration efficiency of otter clams during the transportation period

Chaetoceros sp. algae were added to the seed bag at the beginning of the experiment at a density of 5 million cells/mL. Results of checking algae density after 12 and 24 hours in the transport bags showed that despite in closed bags and under cool conditions, otter clams in all treatments filtered out the algae after 12 hours (Table 2), filtration efficiency varied from 90.7% (in control) to 99.1% (oxygen tablets supplement). Algae were added a second time with the same amount and then the density was rechecked after the next 12 hours. The results showed that the algal filtration efficiency of otter clams in glucose supplementation (75%) or Oxygen tablets (70.4%) was higher than in bags supplemented with algae alone (43.7%) or Algae supplement + activated charcoal (34.6%). This result can be the basis for choosing a supplement such as glucose or Oxygen tablets during transport of otter clam seeds for more than 14 hours because it can better stabilize the environment and maintain the ability for filtering food that helps otter clams maintained the better conditions.

Table 2. Algae filtration efficiency of otter clams (%)

Period (h)	Supplementation substances			
	No adding	Glucose	Charcoal	Oxygen tablet
0	100	100	100	100
12	90.7 ± 3.9[a]	95.0 ± 0.0[a]	93.1 ± 9.3[a]	99.1 ± 0.6[a]
24	43.7 ± 12.3[a]	75.0 ± 2.0[b]	34.6 ± 7.7[a]	70.4 ± 14.0[b]

Values in the same row with the same letter are not significant different (p>0.05).

Survival rate of otter clams in different supplementation substances (%)

The survival rate of otter clams after 12 hours of transfer ranged from 86.7% (glucose supplementation) to 90% (in control or Oxygen supplementation), but there was no significant difference (p>0.05). After 24 hours, the survival rate of otter clams decreased in all treatments (Table 3). When supplementing glucose, the survival rate reached 71.7%, that was similar to supplementing algae alone (74.4%), and lower than adding activated charcoal or Oxygen tablets (83.3%).

Table 3. Survival rate (%) of otter clams in different substances versus time

Period (h)	**Supplementation substances**			
	No adding	Glucose	Charcoal	Oxy tablets
0	100	100	100	100
12	90.0 ± 3.3[a]	86.7 ± 17.3[a]	88.9 ± 8.4[a]	90.0 ± 6.7[a]

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24	74.4 ± 8.4 [a]	71.7 ± 2.4[a]	83.3 ± 4.7[b]	83.3 ± 4.7[b]

Values in the same row with the same letter are not significant different (p>0.05).

Survival rate of otter clams in newly stocked days (%)

After transporting seeds with the addition of different substances, the otter clams was experimentally reared for 20 days to evaluate the survival rate and total weight. Results in Table 4 shows that although there was no statistical difference (p>0.05), clams in the glucose supplement treatment achieved higher weight (0.23 g) and survival rate (78.9 %) was also higher than other treatments.

Table 4. Total weight (g) and survival rate (%) of clams after stocking 20 days

Parameters	Days	**Supplementation substances**			
		No adding	Glucose	Charcoal	Oxy tablet
Total weight (g)	1	0.19 ± 0.01	0.19 ± 0.02	0.19 ± 0.02	0.19 ± 0.02
	20	0.20 ± 0.02	0.23 ± 0.02	0.20 ± 0.00	0.19 ± 0.02
Survival rate (%)	1	100	100	100	100
	20	77.8 ± 9.6	78.9 ± 1.9	73.3 ± 10.0	75.6 ±16.8

Data for the total weight or survival rate are not significant different (p>0.05).

Welborn & Manahan (1990) studied a method to directly determine sugar uptake by molluscan larvae from seawater. The authors confirmed that oyster larvae *Crassostrea gigas* have the ability to selectively absorb sugars, in which the simple sugar glucose is absorbed and L-rhamnose is not absorbed by oyster larvae. According to several previous studies, glucose is a common simple sugar in the seawater environment and may be the result of decomposition from complex polysaccharides (Ittekkot *et al.*, 1981; Sakugawa & Handa, 1983). Research results by Welborn & Manahan (1990) also showed that maltose is also absorbed by oyster larvae from seawater at the same rate as glucose. The ability to absorb a variety of sugars of varying complexity from seawater proves that mollusc larvae are capable of using most dissolved organic matter as a source of nutrition for body development.

Thao & Nha (2013) conducted experiments adding daily glucose with different doses (0, 35, 70 μg/L) to clam nursery tanks along with probiotic products containing *Bacillus* and *Lactobacillus* bacteria periodically added 1 times/week. Results after 70 days of rearing showed that clams in tanks supplemented with the highest dose of glucose had superior growth results in length and weight compared to other treatments (p<0.05). Uchida et al. (2010) added three different sugars: glucose, maltopentose and pullalan to the nursery system of clams (*Ruditapes philippinarum*) with concentrations of 10 mg/L and 100 mg/L, but the result was that only glucose was absorbed and contributes to clam growth. Glucose is absorbed directly by the body of bivalves through the mantle epithelium, so it can better replenish energy reserves during transportation and the first days of stocking. Results from previous studies and this study show the ability of larvae and molluscs to directly absorb glucose and the effects that this substance brings when used for seed transport as well as during the nursery process. According to Patterson *et al*. (1999), glycogen is the main energy reserve for bivalves. The conversion of glycogen to glucose allows immediate aerobic metabolism to promote organismal survival (Kelley, 2008). In studying the effect of ammonia on glucose utilization in the Indian freshwater mussel *Lamellidens borderis*, Chetty and Indira (1995) suggested that the increase in glucose concentration upon exposure to ammonia was higher in the shell, gills and foot. The cause is the rapid turnover of these energy fuels from the hepatopancreas or the ability to use less to conserve energy. Yusufzai *et al*. (2010) found significantly higher glucose concentrations in the membrane tissue of *L.*

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corrianus during water transport as well as moist transport compared to the gills and adductors initially and at 12 h during the transport. These results indicate that *L. corrianus* uses glucose as an energy source mainly from mantle tissues.

The content of TAN and NO_2 was clearly reduced in the water when adding glucose, activated carbon or oxygen pellets during the transportation of otter clam seeds. However, Lan *et al*. (2016) conducted the study by feeding biochar or charcoal to striped catfish (*Pangasius hypophthalmus*), the authors observed that levels of ammonia nitrogen (TAN), nitrite, phosphate and chemical oxygen demand (COD) in the tank water were reduced by adding biochar or charcoal to the feed, but not to the water. The authors suggested that biochar (and charcoal) facilitated the formation of biofilms as habitat for gut microbiota to improve growth rates when biochar and charcoal were added to the diet.

Results from this study show that the survival rate of otter clams is high variable during transportation and in the early stocking period when supplemented with charcoal or Oxygen tablets. In contrast, these results were more stable when supplemented with glucose.

CONCLUSION AND RECOMMENDATIONS

Using glucose, activated charcoal and Oxygen tablets along with *Chaetoceros* algae added to the transport bag can improve the survival rate of otter clams after 24 hours, however, adding glucose gives stable and better results.

The transport time for otter clams should not exceed 12 hours because it will be significantly affects the survival rate.

REFERENCES

1. Chetty, A.N. Indira K. 1995. Adaptive changes in the glucose metabolism of a bivalve to ambient ammonia stress. Bulletin of Environmental Contamination Toxicoly, 54: 83–89.
2. Ittekkot V., Brockmann U., Michaelis W. and Degens E. 1981. Dissolved free and combined carbohydrates during a phytoplankton bloom in the North Sea. Marine Ecology Progress Series. Vol. 4: 299-305.
3. Kelley, L.C. 2008. Upper thermal limits in Mytilus trossulus and Mytilus californianus: glucose as major fuel for heat-stress response. OIMB Technical Report. Oregon Institute of Marine Biology, University of Oregon, Eugene.
4. Lan, T.T., Preston, T.R. and Leng, R.A. 2016**:** Feeding biochar or charcoal increased the growth rate of striped catfish (Pangasius hypophthalmus) and improved water quality. Livestock Research for Rural Development. Volume 28, Article #84. Retrieved September 29, 2023, from http://www.lrrd.org/lrrd28/5/lan28084.html
5. Le Xan, Son, H.N., and Hoang Hai. 2001. Initial investigation of the biological characteristics, reproduction, and production of artificial varieties of geoduck (*Lutraria philippinarum Deshayes*) in the waters of Cat Ba – Hai Phong. Collection of marine fisheries research projects. Marine Research Institute, Hai Phong. Vol. 2: 428 – 442.
6. Patterson, M.A., Parker, B.C. and Neves, R.J. 1999. Glycogen concentration in the mantle tissue of freshwater mussels (Bivalvia: Unionidae) during starvation and controlled feeding. American Malacology Bulletine, 15: 47–50.
7. Pham Thuoc. 2006. Report on investigation of the current situation, proposing some solutions to protect and develop otter clam resources in the waters of Hai Phong and Quang Ninh 2004-2005. Marine Research Institute, Hai Phong, Vietnam.
8. Sakugawa, H. and Handa, N. 1983. Chemical studies of dissolved carbohydrates. Journal of Oceanography Society of Japan, 39: 279-288.

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume X Issue X October 2023

9. Thang, H. D and Thuy, H. Đ. 2004. Initial research results on artificial seed production of (*Lutraria philippinarum* Reeve, 1854). Fisheries Journal (6): 19-23. (In Vietnamese with English abstract)
10. Thanh, T. T and Hương, L. T. T. 2008. Experimental production of artificial seeds of otter clam (*Lutraria rhynchaena* Jonas, 1844) in Khanh Hoa. Collection of scientific reports from the National Molluscan Workshop. Agricultural publishing house: 328 – 345 (In Vietnamese with English abstract)
11. Thao, N. T. T. and Nha, L.Q. 2013. Effects of glucose and probiotic supplementation in nursing juvenile clam Meretrix lyrata. European Aquaculture Society, Special Publication No. XX, Oostende, Belgium, 2013. Page 304-307.
12. Thao, N. T. T. and Anh, L. T. T. 2015. Effect of glucose on the storage and transportation of juvenile cockle (*Anadara granosa*). Can Tho University Journal of Sciences. Part B: Agriculture, Fisheries and Biotechnology, 36: 81-87. (In Vietnamese with English abstract)
13. Uchida, M., Kanematsu, M. and Miyoshi, T. 2010. Growth promotion of the juvenile clam, Ruditapes philippinarum, on sugars supplemented the rearing water. Aquaculture, 302 (3-4): 243-247.
14. Welborn, J.W. and Manahan, D.T. 1990. Direct measurements of sugar uptake from seawater into mollusc larvae. Marine Ecology Progress Series, 65: 233-239.
15. Yusufzai, S.I., Singh, H. and Shirdhankar, M.M. 2010. An evaluation of different methods for transportation of the freshwater mussel Lamellidens corrianus (Lea 1834). Aquaculture International June 2010. https://www.researchgate.net/publication/225430552. DOI 10.1007/s10499-009-9290-2.