JARQ 42 (2), 131–136 (2008) http://www.jircas.affrc.go.jp

Preliminary Trials on the Effect of Lighting for the Population Growth of the Rotifer, *Brachionus plicatilis*

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Abstract

The effect of lighting to rotifer culture water was investigated preliminarily using freshwater *Chlorella* as food in terms of vitamin B_{12} (VB_{12}) production. The marine rotifer *Brachionus plicatilis* was batch-cultured for five days at 25°C in 25 psu seawater with or without lighting (*L*:*D* = 13:11 and *L*:*D* = 0:24). Rotifers were fed VB_{12}-enriched or VB_{12}-free *Chlorella vulgaris* with or without cobalt compound supplementation (cobalt (II) sulfate heptahydrate: $CoSO_4 \cdot 7H_2O$). When VB_{12}-enriched *Chlorella* was fed, rotifer with lighting showed better population growth than in complete dark. On the other hand, no difference was observed in the population growth between light and dark groups when VB_{12}-free *Chlorella* was used. Nevertheless if the cobalt compound was supplemented to VB_{12}-free *Chlorella*, the light group showed higher population growth than non-supplemented groups and dark groups, and a much higher amount of VB_{12} was detected from the tanks than those from other groups. In addition to that, even in the group fed VB_{12}-free *Chlorella* without Co supplementation a daily increase of a small amount of VB_{12} was observed when they were lit up. From these experimental results, we can conclude that lighting plays an important role for the population growth of rotifers and Co compound supplementation to rotifer culture water promotes the reproduction performance of rotifers due to the enhanced production of VB_{12} by lighting.

Discipline: Aquaculture
Additional key words: cobalt compound, culture, larviculture, light, vitamin B_{12}

Introduction

In recent years, the aquaculture production in the world has been showing a continuous increasing trend[2]. To produce fish and crustacean seedling fry, marine *Brachionus* rotifers are the most important live food and play an indispensable role as an initial food item in hatcheries. For that reason, all over the world many studies have been conducted so far to establish the stable production of rotifers[3,4,6,9,10,12].

Vitamin B_{12} (VB_{12}) is a water soluble vitamin containing cobalt as an important component element[1,5,7]. In aquaculture relevancy, the function of VB_{12} for enhancing population growth performance of rotifers was studied and reported previously[8,17,30,31]. Recently we found a cobalt compound (cobalt (II) sulfate heptahydrate: $CoSO_4 \cdot 7H_2O$) supplementation to rotifer feed also effectively enhanced the population growth of the S-type rotifer *B. rotundiformis*[23]. We also experienced lately that lighting of the rotifer culture tank practically stabilized the culture and improved its production performance. To elucidate this newly observed phenomenon we investigated preliminarily the lighting effect of the culture tank to enhance

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Received 27 July 2007; accepted 13 November 2007.

rotifer productivity correlating this with the supplementation effect of the Co compound in terms of VB_{12} synthesis.

Materials and methods

1. Rotifer culture

The so called L-type rotifer *Brachionus plicatilis* (Kinki Univ. strain, Noto-jima Stn, National Station for Stock Enhancement, Fisheries Research Agencies) was used for the experiment. The rotifer stock-cultured with commercial freshwater *Chlorella* was harvested and inoculated into 100 L polycarbonate tanks (Exp. I) and 800 mL glass bottles (Exp. II) at densities of 500 individuals (ind)/mL and 400 ind/mL, respectively. The rotifer was cultured under 25°C with high purity oxygen gas (> 95%, Exp. I) supplied from an electric oxygen-concentrator (Ozinator 600, Kinki-Sanso Co., Japan) or with a mixed gas of oxygen and air (Exp. II). Each treatment group was duplicated. Culture water was prepared after diluting sand-filtered seawater (32−34 psu) with freshwater to adjust its salinity at 25 psu.

As a rotifer feed, commercial freshwater *Chlorella vulgaris* (Chlorella V_{12}, Chlorella Industry Inc., Fukuoka, Japan) containing ca. 320 pg/mL VB_{12} (Maker web site HP information) was used for experiment I. In experiment II, VB_{12}-free *C. vulgaris* produced using a laboratory jar-fermenter was used. The cultured *Chlorella* was centrifuged to the concentration of 10^{10} algal cells/mL and stored at 5°C until use for the experiment. *Chlorella* feed was given once in the morning at an estimated optimum feeding ratio of 150,000 algal cells/rotifer/day calculated based upon two previous findings: i.e. the optimum feeding ratio for the S-type rotifer *Brachionus rotundiformis* (i.e. 50,000 algal cells/rotifer/day) reported previously[24-27] and its approximate three-fold difference of their individual fresh body weights (S-type rotifer: 1.37−1.87 μg, L-type rotifer: 3.85–4.44 μg)[3].

2. Lighting condition

Rotifer culture vessels of the *L* group were exposed under photo period of *L*:*D* = 13:11 using an auto-timer controlled halogen lamp (150 W, color temperature 3,000 K, 1,300 lux at water surface level, Sankyo Co. Ltd., Japan). The lamp was set about 60 cm above the water surface with the greatest care in order not to affect the temperature of the culture water. The dark conditioned vessels (*D* group, *L*:*D* = 0:24) were set in a dark room and covered with black vinyl-sheet completely. Other conditions were identical to that of the *L* group.

3. Cobalt compound supplementation

In experiment II, the supplemental effect of cobalt (II) sulfate heptahydrate was investigated on each light control group in a laboratory scale culture (4 treatments: light with Co supplementation, light without Co supplementation, dark with Co supplementation, and dark without Co supplementation). The concentration of cobalt compound was decided based on a previous experimental result: i.e. 0.1 mg/L culture water/day[23]. Cobalt compound was dissolved into freshwater in advance and trickled into culture water when feed was given in the morning.

4. Vitamin B_{12} determination

Total VB_{12} contents of each collected sample were detected from 100 mL culture water by bioassay using *Lactobacillus delbrueckii* subsp. *lactis*[1,16]. Firstly the culture water was filtered with a plankton net (opening size 55 μm) to remove rotifers and large suspended substances. As a next step the sample water was centrifuged and rinsed with clean seawater several times, and the sedimentary fraction containing bacteria/bacterial flocks and tiny suspended particles was measured up with clean seawater and provided for the bioassay for VB_{12} determination. The samples were collected every day before feeding *Chlorella*. In the results of VB_{12} analysis, no VB_{12} was detected from the VB_{12}-free *Chlorella* provided for experiment II and culture water used for both experiments.

5. Evaluation of culture trials

The daily change of rotifer density was monitored by a direct count method from three 1 mL samples taken from each culture vessel. After sampling, the rotifers were killed and stained with one drop of iodine-alcohol solution, and counted under a dissecting microscope with the naked eye and the average value from triplicated counts was used for evaluation. From the culture water samples on the final day, environmental parameters were measured in terms of pH, total NH_4^+-N and DO, and each value was expressed as the mean of the duplicated treatment. Population growth data were analyzed statistically using Student's *t*-test at 0.01 and 0.05 significance levels to evaluate the lighting effect to rotifer culture tanks.

Results

1. Experiment I

In experiment I, the effects of lighting to large scale rotifer culture tanks were investigated preliminarily using commercial freshwater *Chlorella*. The experimental results are shown in Fig. 1. In a complete dark condition, the rotifer population increased slowly and reached an average of 886.7 ind/mL from the initial inoculation den-

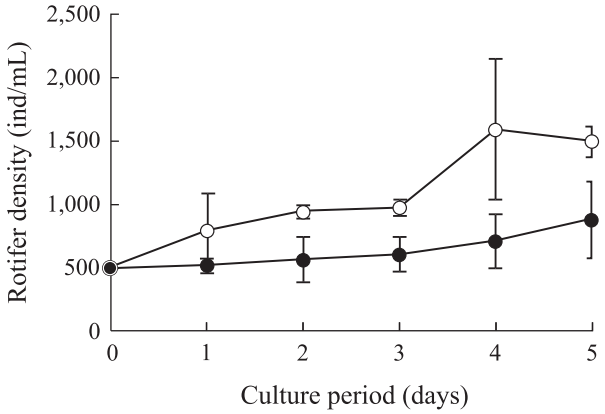

Fig. 1. Effect of lighting on the population growth of rotifers (Experiment I)
Symbols represent mean values; open circle: light group, solid circle: dark group. Vertical bars represent the range of values from duplicated treatment.
-○-: Light, -●-: Dark.

sity of 500 ind/mL in 5 days. On the other hand the population growth of the *L* group exceeded the *D* group after the second day, and the density eventually reached an average of 1,503.3 ind/mL on the final day. This mean final density in the *L* group was almost double that of the densities obtained in the *D* group, and a significant difference was noted at the $P < 0.01$ level between these two test groups.

The environmental parameters on the final day are summarized in Table 1. The DO of the culture water was always maintained at a high and hyper-saturated level (10–13 mg/L) in both *L* and *D* groups due to the use of a high purity oxygen gas supply. The pH and total NH_4^+-N levels were maintained around 8 and 60–70 mg/L, respectively, and all of these values were not at the critical levels that affect the reproduction of rotifers as reported previously[22,25,28,32].

2. Experiment II

In this experiment, the population growth of rotifers in each group was lower than those obtained in Exp. I, probably due to the poorer physiological conditions originating from the smaller culture scale and the absence of dietary VB_{12} in feed. Nevertheless the cobalt compound supplemented group with lighting (*L* + Co group) exceeded the other groups after the second day, and finally this group reached an average density of 898.0 ind/mL that was almost double of those obtained in other groups. On the 4th day after the onset of the experiment, there were significant differences ($P < 0.05$) between the *L* + Co group and other groups, but on the final day the difference had disappeared because growth stagnancy occurred in one of the duplicated culture vessels.

The environmental parameters on the final day of each group are summarized in Table 2. There were no differences in the values of DO, pH and total NH_4^+-N among them, and those values were all not at the critical levels that affect rotifer reproduction as well[22,25,28,32].

3. Vitamin B_{12} (VB_{12}) production

The VB_{12} production obtained in experiment II is shown in Table 3. Although no VB_{12} was detected in both the initial culture water and feed samples, 1.1–2.7 pg/mL of VB_{12} was detected in the initial culture water. Probably this trace VB_{12} originated and was taken over from rotifers used as inocula pre-cultured with VB_{12}-enriched commer-

Table 1. Environmental parameters on the final day (Experiment I)

Treatment	Light	Dark
DO (mg/L)	10.6*	12.6
pH	7.8	7.9
NH_4^+-N (mg/L)	70.3	59.3

*: Values are means from duplicated treatment.

Table 2. Environmental parameters on the final day (Experiment II)

Treatment	Light + Co*	Light	Dark + Co*	Dark
DO (mg/L)	5.1**	5.7	5.0	5.0
pH	8.4	8.4	8.5	8.5
NH_4^+-N (mg/L)	41.7	42.0	46.6	50.0

*: Cobalt (II) sulfate heptahydrate supplementation.
**: Values are means from duplicated treatment.

Table 3. Daily Vitamin B_{12} production (pg/mL) of each treatment group (Experiment II)

Treatment	Ini.	2nd	3rd	4th	Final
Light + Co*	1.1	1.1	5.0	16.0	75.6
Light	2.0	2.1	8.5	10.5	26.0
Dark + Co	2.7	1.4	1.1	4.3	2.4
Dark	2.0	0.6	0.7	6.2	3.4
Culture water**	ND***				
Chlorella**	ND				

*: Cobalt (II) sulfate heptahydrate supplementation.
**: Vitamin B_{12} contents of the culture water and *Chlorella* used.
***: Not detected.

cial *Chlorella*. During the culture no change in the value was observed on the second day, but the VB_{12} contents in the *L* + Co group increased day by day, and eventually 75.6 pg/mL of VB_{12} was detected. This average value was approximately three times higher than that of the *L* group without Co and several tens of times higher than those from *D* groups. The *D* groups produced only 0.6–6.2 pg/mL VB_{12}, regardless of Co compound supplementation or not. The *L* group without Co supplementation also produced 26.0 pg/mL VB_{12} that was 7.5–11 times higher than those of *D* groups, but only one third of the content obtained in *L* + Co groups; i.e., 75.6 pg/mL.

Discussion

Vitamin B_{12} is an important nutritional element to maintain good health for mammals and other animals. This particular vitamin is a member of the vitamin B complex, a group of water soluble vitamins, and is found primarily in animal foods, like meat, eggs and many dairy products. VB_{12} is necessary for the synthesis of red blood cells, the maintenance of the nervous system, and normal growth and development in human infants[1,5]. In fish, poor appetite, poor growth and poor food conversion were found as VB_{12} deficiency symptoms[7]. The physiological function of VB_{12} for zooplankton has not yet been studied, but the supplementation of this vitamin to culture water was reported previously to be effective for enhancing reproduction performance of rotifers[8,17,30,31]. From these experimental findings, feed companies supplement it and enrich the contents of this vitamin to enhance the dietary value of their products, in spite of its expensiveness[13-15].

VB_{12} contains a cobalt atom (Co) as its component element, so it is also called cyanocobalamin ($C_{63}H_{88}N_{14}O_{14}CoP$). In nature this cobalt-containing vitamin is produced by bacteria and fortified to the surrounding ecosystem[1,5,7]. Many bacteria commonly found in aquaculture grounds, including some kinds of phototrophic bacteria, are reported to have high productivity of VB_{12}[11,18,19,30,31]. As for rotifer culture, we previously reported its bacterial abundance especially when the rotifer was cultured in a high density condition[21]. Under this unique culture environment with high temperature (ca. 30°C) and a very eutrophic condition of rich metabolites from rotifers, many kinds and huge numbers of bacteria exist and form special flora in the tanks[20,21,29], and probably some of them could be deeply involved with the synthesis of VB_{12}.

Recently we reported the dietary supplementation of a water-soluble cobalt compound (cobalt (II) sulfate heptahydrate: $CoSO_4 \cdot 7H_2O$) also effectively enhanced the population growth of the S-type rotifer *B. rotundiformis*[23]. Cobalt (II) sulfate heptahydrate is a cheap compound easy to obtain in the market and used as a common and safe trace mineral food additive for many livestock and cultured fish in many countries[7]. As mentioned above, Co is a key element for synthesizing VB_{12}, and in a previous paper we implied that the enhanced dietary value obtained in our culture results were probably due to the increased VB_{12} content that occurred by the supplementation of dietary Co. Hence in this study, we conducted culture trials of rotifers and analyzed how much VB_{12} had been actually produced after Co supplementation under various light conditions.

In the present experiments, when VB_{12}-enriched commercial feed was given, the rotifer population increased smoothly with light (*L*-group in experiment I) as shown in Fig. 1, although poorer growth was obtained if there was no light notwithstanding the use of VB_{12}-enriched *Chlorella* as feed (*D* group in experiment I). This experimental result meant that the dietary value of *Chlorella* fluctuated according to the light conditions. This finding about the necessity of light for rotifer culture is novel and there have been no similar reports on it so far. This indicates that lighting to culture water plays a significant role in population growth of rotifers and under a com-

plete dark condition rotifers exhibit reduced reproduction performance. On the other hand, there appeared no significant differences in population growths between *L* and *D* groups when VB_{12}-free *Chlorella* was fed (Fig. 2). This result shows that VB_{12}-free *Chlorella* had poorer dietary value in comparison with VB_{12}-enriched *Chlorella* as in previous findings[30,31]. Nevertheless if a small amount of Co compound was supplemented to the tanks with VB_{12}-free diet, a clear positive lighting effect was observed and a better population growth was obtained in the lighting tanks (Fig. 2).

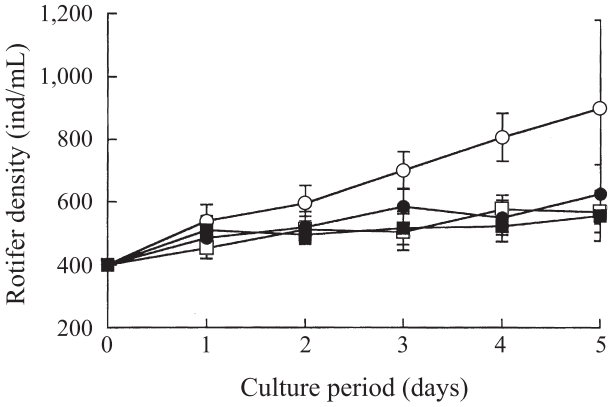

Fig. 2. Effect of lighting on the population growth of rotifers with Co compound supplementation (Experiment II)

Symbols represent mean values; open circle: light group with Co, solid circle: dark group with Co, open square: light group without Co, solid square: dark group without Co. Vertical bars represent the range of values from duplicated treatment.
-○- : Light + Co, -□- : Light, -●- : Dark + Co, -■- : Dark.

Under the present preliminary experimental conditions it would be difficult to estimate and predict how much higher the rotifer production would be improved by the interaction of these two factors: supplementation of Co compound and lighting. Nevertheless it is certain that Co compound supplementation to rotifer culture water promotes the reproduction performance of rotifers, and the lighting condition is indispensable for it. In this respect, a rational explanation about the better growth performance that occurred in the *L* + Co group became possible from the view point of VB_{12} production obtained as a result in this study. As shown in Table 3, the *L* + Co group produced and accumulated a much higher amount of VB_{12} (75.6 pg/mL) than other groups in 5 days culture. Probably this newly synthesized VB_{12} caused the better growth performance of the *L* + Co group rotifers in the experiment. In addition to that, even in the group fed VB_{12}-free *Chlorella* without Co supplementation a slight daily increase of VB_{12} contents (26.0 pg/mL on the final day) was noted when they were exposed to light. This increase of VB_{12} obtained in the tanks fed VB_{12}-free *Chlorella* without Co supplementation had possibly originated from newly synthesized VB_{12} in the tanks with light. Probably through a synergy effect of both dietary VB_{12} and newly synthesized VB_{12}, good and stable cultures can be maintained in rotifer culture tanks. Therefore from these experimental findings obtained in the present study, we can conclude that from the view point of VB_{12} production a certain level of lighting is essential for maintaining a stable culture with high reproduction performance. Furthermore attention should be paid to the lighting condition of rotifer culture tanks because rotifers are the most important initial food item for larviculture of marine fin-fish and crustaceans.

Still we do not know the mechanism of how VB_{12} was synthesized utilizing cobalt compound as a material and what promoted this phenomenon in the rotifer culture tanks. Also we have very limited information on the light required for rotifer culture: i.e. intensity, wave length, color, etc. Further intensive research would be necessary to elucidate the whole picture on the necessity of light for rotifer culture from now.

Acknowledgments

We thank Prof. Masahiro Hayashi of Miyazaki University for providing cultured vitamin B_{12}-free freshwater *Chlorella* for our experiment and Prof. Zhang Dong Ming of Jilin Agricultural University in China for his cooperation.

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