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Article

# Urea as a Nitrogen Source in a Black Tiger Shrimp (*Penaeus monodon*) Closed Culture System

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## Abstract

Urea [$(NH_2)_2CO$] is an organic compound that serves an important role in the metabolism of nitrogen-containing compound by animals. Urea is widely used in aquaculture systems. This study investigated the effects of urea on growth of *Penaeus monodon*. Shrimp were reared in 500 l fiber tanks. There was no exchange of water throughout the experiment. Shrimp with an average body weight of 10.99 ± 0.19 g were stocked at a density of 32 shrimp/m$^2$ in 20 ppt diluted seawater and fed with 38 % protein diet for 9 weeks. Urea was added into the culture tanks at a concentration of 1.25 ppm once a week. The results show that urea slightly affects growth and survival of shrimp. Shrimp reared in the culture pond with added urea had a marginal better growth rate ($p > 0.05$) while the survival rate was significantly higher than the control group ($p < 0.05$). The urea in the closed culture tanks was shown to reduce the toxicity of ammonia in soil and promoted growth of plankton communities. Adding urea has no effect on water quality. This study concluded that urea is a potential nitrogen source in closed culture systems when the nitrogen input through the feeding regime is limited. It suggests that urea should be added at a concentration of 1.25 ppm once a week into culture systems with limiting nitrogen sources.

**Keywords:** Urea, nitrogen sources, *Penaeus monodon*, shrimp, phytoplankton, growth

## Introduction

*Penaeus monodon* is the most widely cultured shrimp species in Thailand. However, production of *P. monodon* has been decreasing rapidly for decades. The primary cause is waterborne diseases. In order to avoid such diseases, closed culture systems have been introduced. However, closed systems are prone to a laxity of nitrogen sources due to the limited amount of feed. Nitrogen is essential for the decomposing organisms in the culture systems. Nitrogen also promotes growth of food microorganisms such as phytoplankton, zooplankton, algae and benthos. These organisms play an important part in food ingested by shrimp. Indeed, Wasielesky *et al* [1] showed that natural food enhanced growth of shrimp when lower protein feeds were fed to the shrimp in closed culture systems. In fact, the supplement feed is only a part of the food consumed by shrimp in culture ponds. Focken *et al* [2] found that the major gut contents of *P. monodon* reared with supplementary feeds in earthen ponds were crustaceans and aquatic plants rather than the feed pellets.

Urea [$(NH_2)_2CO$] or carbamine is an organic compound that is widely used in fertilizers as a convenient source of nitrogen. Urea serves an important role in the metabolism of nitrogen-containing compounds by animals. Urea is also present naturally in many ecosystems in various forms and is used as an energy source by chemolithotrophic aerobic ammonia oxidizing bacteria (AOB) [3]. Urea is also used in aquaculture [4] to establish phytoplankton populations that are a primary food of zooplankton, larval shellfish and crustaceans [5]. Chakrabarty *et al* [6] have also reported the use of urea in pond soil preparation. Diammonium phosphate together with urea was also used as a nitrogen source in shrimp ponds during pond preparation and showed enhanced shrimp growth [7].

However, information regarding the use of urea as a nitrogen source in a closed system shrimp culture pond has not been reported. This study investigated the effect of urea on the growth of black tiger shrimp when added regularly to the water of a closed culture system.

## Materials and methods

### Experimental Protocol

Shrimp used were collected from a cultured pond in Trang Province, southern Thailand. The experimental animals were acclimatized to laboratory conditions for 7 days. Shrimp with an average body weight of 10.99 ± 0.19 g were transferred to fiberglass tanks at a stock density of 32 shrimp/$m^2$ (20 shrimp per tank). The tanks were laid with shrimp pond bottom soil (5 cm thick) and filled with 350 l of 20 ppt diluted seawater. Shrimp were fed with 38 % protein at a total amount of 6 % body weight per day 3 times a day (7.00, 15.00 and 23.00) for 9 weeks.

The experiments were divided into 2 groups - the control and urea adding - each of 4 replications (20 shrimps per replicate). In the latter group, urea was added into the culture tanks at a concentration of 1.25 ppm at the beginning of the experiment and continuously once a week throughout the experiment. The optimal concentrations and the application frequency applied in this study had previously been tested by Suwanpakdee *et al* [8].

### Monitoring of Water and Soil Qualities

Temperature, pH and dissolved oxygen (DO) in the water were measured daily. The ammonia, nitrite, nitrate and orthophosphate concentrations were measured every 3 days. Analyses of total ammonia were performed using indophenol blue [9], nitrite by diazotization [9], the toxic ammonia was the secondary data derived from the relation of pH and temperature values measured in this experiment, nitrate by cadmium reduction [9] and orthophosphate by ascorbic acid methods [9]. DO was measured using a dissolved oxygen meter (YSI model 58).

Soil samples were taken 30 and 63 days from the experiment tanks for orthophosphate, total ammonia, nitrite and nitrate analyses, using 2 N potassium chloride extract [9].

### Monitoring of Microorganism

Bacterial growth in the culture media was determined every 3 days. Numbers of *Vibrio* sp. and *Bacillus* sp. were determined using the spread plate method on BTB-Teepol agar and Nutrient Agar (NA), respectively. Numbers of Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) were determined every 7 days using the Microtechnique for Most-Probable-Number (MPN) [10].

Growth of the phytoplankton populations were measured in terms of chlorophyll a, using a spectrophotometer after acetone extraction [9]. The comparison of means was performed using the t-test method.

### Growth Performances of Shrimp

The body weight of the shrimps were individually measured at days 30 and 63. The growth rate was measured as a percentage of weight gain. The comparison of means was performed using a t-test.

## Results and discussion

### Shrimp Growth

The results show that adding urea to the culture tanks does not affect the growth of shrimp. However, the final growth rate of shrimp in urea treatment tanks (60.86 %) was apparently higher than that of the control group (56.81 %) ($p > 0.05$) (**Figure 1**). The final survival rate of shrimp in urea treatment tanks (61.25 %) was found higher than that of the control group (50 %) ($p < 0.05$) (**Figure 2**).

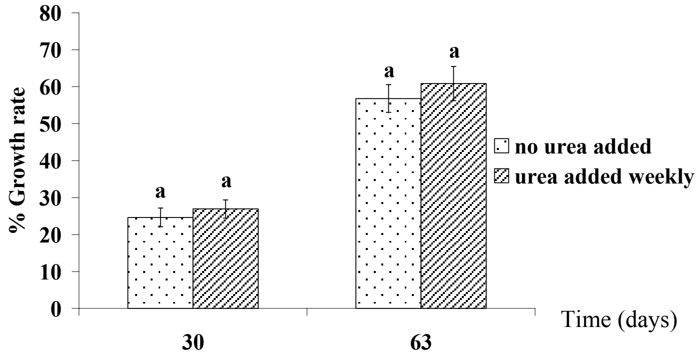


**Figure 1** Growth rates of shrimp reared under clay bottom conditions.

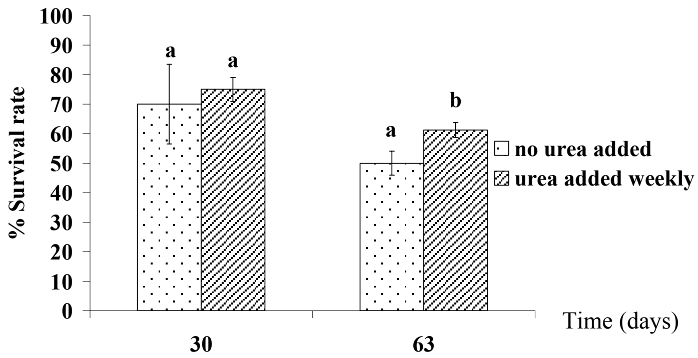


**Figure 2** Survival rates of shrimp reared under clay bottom conditions.

**Water and Soil Qualities**

The results show that adding urea into the culture tanks does not significantly change water quality in terms of ammonia, nitrite, nitrate and orthophosphate concentrations compared to the control group ($p > 0.05$) (**Table 1**). Temperature, pH and dissolved oxygen during the experiment ranged from 26 to 33 °C, 7.7 to 8.2 and 7 to 9 mg/l, respectively. Adding urea also does not affect soil quality in terms of nitrite, nitrate and orthophosphate concentrations ($p > 0.05$), except for the ammonia concentrations measured at day 30 which was low in the urea group ($p < 0.05$) (**Table 2**), however the difference was not observed at day 63 (**Figure 3**). The ammonium concentration in the control group was found to decrease after day 30; this may result from utilization of urea by bacteria in the processes of amino acid synthesis [11].

**Table 1** Water quality in shrimp rearing tanks (mean (SD)*).

| Parameter | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | No urea added | | | Urea added weekly | | |
| | day 0 | day 1 - 30 | day 31 - 63 | day 0 | day 1 - 30 | day 31 - 63 |
| **Water qualities** | | | | | | |
| Total ammonia (mg-N/L) | 0.41 (0.07) [a] | 2.18 (1.90) [a] | 0.37 (0.09) [a] | 0.38 (0.08) [a] | 2.46 (2.16) [a] | 0.40 (0.09) [a] |
| Toxic ammonia (mg-N/L) | 0.03 (0.01) [a] | 0.12 (0.10) [a] | 0.03 (0.01) [a] | 0.02 (0.01) [a] | 0.14 (0.12) [a] | 0.02 (0.01) [a] |
| Nitrite (mg-N/L) | 0.05 (0.01) [a] | 0.15 (0.02) [a] | 0.12 (0.03) [a] | 0.05 (0.01) [a] | 0.15 (0.02) [a] | 0.11 (0.04) [a] |
| Nitrate (mg-N/L) | 0.11 (0.02) [a] | 0.01 (0.02) [a] | 0.02 (0.02) [a] | 0.13 (0.02) [a] | 0.01 (0.02) [a] | 0.03 (0.02) [a] |
| Orthophosphate (mg-N/L) | 0.15 (0.03) [a] | 0.25 (0.12) [a] | 0.14 (0.07) [a] | 0.17 (0.02) [a] | 0.25 (0.14) [a] | 0.14 (0.09) [a] |

* Comparisons were made between the two groups (e.g. week 0 with week 0 in that order).
Values within the same row sharing a common superscript are not significantly different ($p > 0.05$).

**Table 2** Soil quality in shrimp rearing tanks (mean (SD)*).

| Parameter | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | No urea added | | | Urea added weekly | | |
| | day 0 | day 1 - 30 | day 31 - 63 | day 0 | day 1 - 30 | day 31 - 63 |
| **Soil qualities** | | | | | | |
| Total ammonia (mg-N/kg. dry soil) | 11.08 (2.10) [a] | 49.55 (5.90) [b] | 34.95 (7.09) [a] | 11.08 (2.08) [a] | 34.07 (4.03) [a] | 35.36 (4.04) [a] |
| Toxic ammonia (mg-N/kg. dry soil) | 0.63 (0.18) [a] | 3.91 (0.32) [b] | 2.92 (0.62) [a] | 0.63 (0.15) [a] | 2.98 (0.35) [a] | 3.10 (0.35) [a] |
| Nitrite (mg-N/kg. dry soil) | 2.36 (0.34) [a] | 1.23 (0.27) [a] | 1.24 (0.11) [a] | 2.36 (0.36) [a] | 1.38 (0.29) [a] | 1.22 (0.26) [a] |
| Nitrate (mg-N/kg. dry soil) | 3.21 (0.58) [a] | 4.52 (0.77) [a] | 3.62 (0.78) [a] | 3.21 (0.61) [a] | 4.90 (0.26) [a] | 5.88 (1.06) [a] |
| Orthophosphate (mg-N/kg. dry soil) | 8.39 (2.24) [a] | 4.12 (1.51) [a] | 4.47 (1.53) [a] | 8.39 (2.18) [a] | 3.91 (1.10) [a] | 5.61 (2.11) [a] |

* Comparisons were made between the two groups (e.g. week 0 with week 0 in that order).
Values within the same row sharing a common superscript are not significantly different ($p > 0.05$) and a < b.

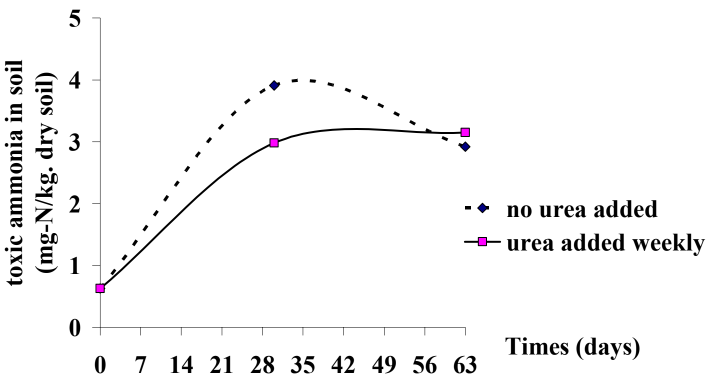


**Figure 3** Toxic ammonia concentrations in the bottom soil of the shrimp rearing tanks.

**Bacteria and Chlorophyll A**

The bacterial counts in the culture media were similar in the two groups. The AOB counts were obtained at 5.42 to $6.70\times10^4$ m/l and NOB counts were 2.75 to $4.49\times10^4$ m/l (**Table 3**). In both groups, the AOB populations were found to increase in week 1 - 5 and then dropped off in week 6 - 9, while the NOB populations increased throughout the period of the experiment. In combining the water quality parameters previous described and the growth characteristics of AOB and NOB, it suggests that adding urea within the experimental dose (1.25 ppm once a week) does not adversely affect the normal nitrifying cycle according to the AOB and NOB activities. The urea also has been reported to increase *Nitrosomonas*, an AOB, in rice field soil [12].

In this study, the *Vibrio* counts obtained were 4.36 to 5.92×10$^5$ m/l and decreased in weeks 6 - 9. The *Bacillus* populations were relatively low (34 to 83 m/l) compared to other bacterial populations (**Table 3**).

The chlorophyll a concentrations were observed to increase throughout the culture period in both groups, but were significantly different between the two groups. The chlorophyll a concentrations increased from 0.16 mg/l at the start to 1.68 and 2.23 mg/l at the end of the experiment, in the control and urea groups, respectively (**Table 3**). This suggests enhancement of the phytoplankton populations in the culture tanks by urea. Subosa and Bautista [7] also reported that urea promoted growth of phytoplankton in shrimp ponds.

This study declares that adding urea into closed culture shrimp systems promotes growth of phytoplankton populations. In turn, phytoplankton promotes the growth of zooplankton that eventually serves as a natural food for the cultured shrimp. Saha and Chatterjee [13] also found the promotion of zooplankton populations when urea was added into the culture ponds. As a bottom devourer, shrimp may not consume phytoplankton and zooplankton directly. However, the dead zooplankton and algae cells in combination with some microorganisms such as bacteria, protozoan, marine yeast etc. makes the detritus masses large enough to be picked up and consumed by shrimp. This detritus is full of nutritive nutrients for shrimp growth [14].

**Table 3** Cell numbers (mean (SD))* of bacteria and chlorophyll a in shrimp rearing tanks (9 weeks).

| Parameter | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | No urea added | | | Urea added weekly | | |
| | week 0 | week 1 - 5 | week 6 - 9 | week 0 | week 1 - 5 | week 6 - 9 |
| **Bacteria** | | | | | | |
| Ammonia Oxidizing Bacteria (AOB × 10$^4$ m/l) | 5.90 (0.05)$^a$ | 6.55 (0.71)$^a$ | 5.42 (0.43)$^a$ | 5.93 (0.08)$^a$ | 6.70 (0.22)$^a$ | 5.52 (0.39)$^a$ |
| Nitrite Oxidizing Bacteria (NOB × 10$^4$ m/l) | 2.75 (0.08)$^a$ | 4.03 (0.39)$^a$ | 4.43 (0.08)$^a$ | 2.76 (0.11)$^a$ | 4.05 (0.46)$^a$ | 4.49 (0.37)$^a$ |
| *Vibrio* sp. (×10$^5$ m/l) | 4.57 (0.62)$^a$ | 5.92 (1.36)$^a$ | 4.36 (1.16)$^a$ | 4.37 (0.76)$^a$ | 5.63 (1.26)$^a$ | 4.42 (0.69)$^a$ |
| *Bacillus* sp. (m/l) | 80 (55)$^a$ | 55 (49)$^a$ | 55 (52)$^a$ | 83 (57)$^a$ | 53 (52)$^a$ | 34 (31)$^a$ |
| **Phytoplankton** | | | | | | |
| Chlorophyll a (mg/l) | 0.16 (0.04)$^a$ | 0.89 (0.32)$^a$ | 1.68 (0.47)$^a$ | 0.16 (0.05)$^a$ | 1.62 (0.35)$^b$ | 2.23 (0.41)$^b$ |

* Comparisons were made between the two groups (e.g. week 0 with week 0 in that order).

Values within the same row sharing a common superscript are not significantly different ($p > 0.05$) and a < b.

## Conclusions

This study shows the potential of urea as a nitrogen source in shrimp culture ponds especially when the nitrogen input through the feeding regime is limited. The limited nitrogen input may be caused by low protein content in the diet and also by restricted amounts of food given to the cultured shrimp. Adding urea at a concentration of 1.25 ppm once a week is a safe dose in closed culture systems regarding the concentrations of ammonia and nitrite compared to the control ponds. Adding urea once a week also helps to control the stability of toxic ammonia in the bottom soil. Finally, urea slightly promotes growth of shrimp by enhancing growth of phytoplankton that contributes to the availability of natural foods in the culture systems.

## Acknowledgements

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