

# **Growth Performance of Asian Clam** *Corbicula fluminea* **(Müller, 1774) Fed with Different Feeds in Laboratory Scale Culture System**

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# **Abstract**

A sixty days feeding trial was conducted on the growth performance of Asian clam *Corbicula fluminea* (Müller, 1774), restricted to pedal-feeding in response to different diets; 1) fermented soy pulp (FSP), 2) treated quail dung (TQD), 3) chemical fertiliser (NPK) and 4) control group; based on the growth rate of dry body weight and shell length, lengthweight relationship, condition factor and daily consumption of organic matter. The findings revealed that NPK exhibited the highest growth for shell length with the increase of 21.0  $\pm$  3.00 µm.day<sup>-1</sup>, followed by FSP 17.0  $\pm$  3.00 µm.day-1 , which was significantly higher (*P* ≤ 0.05) than TQD 6.0 ± 1.80µm.day-1 and the control group 3.0 ± 0.1 µm.day-1 . Fermented soy pulp indicated the highest growth performance based on dry weight of *C. fluminea* with 10.0 ± 0.40 μg.day<sup>-1</sup> followed by NPK at 8.0 ± 0.30 μg.day<sup>-1</sup>, that was significantly different than TQD at 3.0 ± 0.10 μg.day<sup>-1</sup> and the control at  $-5.0 \pm 0.5$  µg.day<sup>-1</sup>. The length-weight relationship demonstrated negative allometric growth for all treatments except for the control, while low Fulton's body condition factor (K) was observed in all treatments. The daily organic matter consumption was the highest for TQD (1.20 µg.clam<sup>-1</sup>.day<sup>-1</sup>), followed by NPK (0.67 µg.clam<sup>-1</sup>.day<sup>-1</sup>), FSP (0.28 µg.clam<sup>-1</sup>.day<sup>-1</sup>) and control treatment (-042 µg.clam<sup>-1</sup>.day<sup>-1</sup>). The recorded water parameters were similar to the ambient condition of *C. fluminea* habitat, which excludes TSS and turbidity. These findings suggest that both NPK and FSP could be utilised to promote higher growth performance of *C. fluminea*.

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**Keywords:** growth trial, length-weight relationship, Asian freshwater clam, *Corbicula fluminea*, pedal-feeding

# **Introduction**

*Corbicula fluminea* (Müller, 1774) is a native freshwater clam in the South East Asia region including Malaysia. In Malaysia, this clam has been served as a protein source of the local's diet. However, the exploitation of *C. fluminea* due to its high market demand has unfortunately led to the decline of this clam's population in the wild. *Corbicula fluminea* is commonly sold at 3 US\$.kg-1 (Lee et al., 2018) in the local market and there is limited information on the culture of *C. fluminea* (Karatayev et al., 2007).

Based on the literature survey, *C. fluminea* possess two feeding methods; filter-feeding and pedal-feeding (Sousa et al., 2008). Besides phytoplankton, organic matter is an important diet for *C. fluminea* by ingesting it through pedal feeding (Kasai and Nakata, 2005;

Marroni et al., 2013). The first study on the use of organic matter from terrestrial sources as feed showed that there are potentials to improve the growth performance of *C. fluminea* (Lee et al., 2018). Organic and inorganic matters are applied in the semi-culture pond to feed the aquaculture species directly and to increase their natural food sources (Rahman and Yakupitiyage, 2006). For example, leguminous plant, animal manure, plant residue and artificial feed (ninegrain cereal, rice flour and rye bran) are among the potential feeds for *C. fluminea* (Mamat and Alfaro, 2014). Rapid growth rate, ease of spawning, shorter turnover times, high fecundity (Sousa et al., 2008) and customer's acceptance (Lee et al., 2018) are the other characteristics that can further promote *C. fluminea* as a suitable freshwater clam for aquaculture. Thus, this study aims to determine the growth performance of *C. fluminea* restricted to pedal-feeding by using three

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different types of feed, namely treated quail dung (TQD), fermented soy pulp (FSP), and chemical fertiliser (NPK).

# **Materials and Methods**

# *Feed preparation*

Different feeds were employed to observe the growth performance of *C. fluminea,* which included treated quail dung (TQD), fermented soy pulp (FSP), and chemical fertiliser (NPK). Fresh quail dung was treated with hydrogen peroxide, and sulphuric acid as the feed used for the experiment was from waste products, and it contained strong odour and high ammonia emission (Lee et al., 2018). The dung mixture was then sun-dried (O'Neill et al., 1976). Meantime, the fermented soy pulp was prepared by mixing raw soy pulp, molasses and yeast solution with the proportion of 1:0.1:0.01, respectively before being fermented for 2 weeks in a closed container. The mixture was sundried afterwards. As for positive treatment, chemical fertiliser (10N:10P:10K) (AgroBridge, Malaysia) was used. Finally, each of the feeds was grounded into fine particles using the mortar and pestle and was then passed through the sieve of ≤34 µm (Mesh size = No. 400) as the adult clams can ingest food particles of 1 µm to 64 µm size (Cheng, 2015).

### *Description of experimental aquarium tanks*

The research was carried out at the Aquatic Laboratory in Universiti Malaysia Kelantan (UMK). The experimental design is as follow. A total of 12 aquarium tanks, each measuring 60 cm × 120 cm × 40 cm provided with aeration were used in the present study. The water flow rate into each tank was fixed at 70 mL.s-1 . Each feed treatment was set-up in three replicates. Meanwhile, sand (grain size <0.1 mm) collected from the clam habitat was autoclaved, and oven-dried to remove the organic residues and sieved with mesh size ≤0.1 mm. The sand was then used as a substrate for each treatment to mimic the clam culture system; while the remaining sand was used to determine the initial organic matter percentage in the substrate by loss on ignition (LOI) method. For the treatments, 18 g of each feed was mixed with the sand in the tray (50 cm  $\times$  35 cm) in order to homogenise the mixture and the sand was added until it reached 2.5 cm depth. Then, dechlorinated water was added into the aquarium tanks until 20 cm depth. As for the control treatment, no feed was added in the sediment. After the feed and sand were mixed, each tank was operated with water flowing into the aquarium for 3 weeks to stimulate the accumulation of organic matter in the sediment that acted as feed for the clam. The culture system for the clam *C. fluminea* is illustrated in Figure 1.



Fig. 1. *Corbicula fluminea* culture system. Arrow shows the direction of water flow.

# *Experimental design*

*Corbicula fluminea* was collected from a river in Tumpat, Kelantan. The clams were labelled with numbers using white paint, followed by the process of measuring the initial weight and length linear distance from posterior to anterior end using a digital calliper (0.01 mm precision) and analytical balance (0.1 mg precision). Clams of 15.5 mm average size were selected for the experiment. A total of 360 clams were selected and divided into four treatment groups. Each treatment set-up in triplicate and conditioned 3 weeks earlier with TQD, FSP, NPK and the control were each stocked with  $131.25$  cm<sup>3</sup>.clam<sup>-1</sup>. This feeding trial was conducted for 60 days. The water was changed every 10 days to restrict the clam to only consume the trial feed and prevent other variables from influencing the pedal-feeding method (Nichols et al., 2005).

To obtain the actual weight of *C. fluminea* with water trapped in it, a fresh-dry-weight regression line was established (Basen et al., 2011) to estimate and use to calculate their growth performance of the clam. The method involved 90 live samples of *C. fluminea* taken at the beginning of the study to represent a similar number of clams used in each treatment. All *C. fluminea* were weighed and dried at 100 °C in the oven until a constant measurement was achieved. The initial dry weight of clams used in the growth experiment was estimated from fresh-dry-weight regression equation as follows:

#### $Dry weight = (0.667 \times Fresh weight) + 0.0179$

 $(R^2 = 0.9218, P \le 0.05)$ 

Meanwhile, the final dry weight of *C. fluminea* was recorded after performing similar method as above to the clams used for growth test.

# *Percentage of total organic matter*

The percentage of organic matter in the substrate was analysed using loss on ignition method. The total organic matter was obtained by comparing the weight

of the substrate before and after the substrate was ignited (Hakenkamp and Palmer, 1999; Marroni et al., 2013). This method was conducted three times, i.e. before and after adding feed and at the end of the culture period. The total organic matter (OM) before and after the substrate was ignited, and daily consumption of organic matter of a clam per day was calculated using the following equations:

Total organic matter  $(\%)$  =

*Before ashing*  $(g) - After asking (g) \times 100 \%$ Before ashing  $(g)$ 

Consumption of organic matter ( $\mu$ g. clam<sup>-1</sup>. day<sup>-1</sup>) = Total organic matter  $(\%) \times$  Feed weight

#### Total clam  $\times$  Total rearing days

### *Clam growth performance*

The initial and final shell length and dry body weight of *C. fluminea* was recorded individually. The relative growth rate was calculated by using the formula stated by Basen et al. (2011). Furthermore, the lengthweight relationship was estimated using a non-linear regression equation, as described by Recker (1975). The condition factor was calculated according to the equation developed by Bervoets and Blust (2003). Therefore, the relative growth rate in terms of shell length and dry body weight, length-weight relationship and Fulton's condition factor were calculated using the following equations:

*Relative growth rate* (
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\mu
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m. $day^{-1}$ ) =  $\frac{\ln Lt - \ln L^{\circ}}{t}$ 

L<sup>o</sup> refers to the initial shell length or dry body weight, *L<sup>t</sup>* refers to shell length or dry body weight at time t and t represents the total culturing time.

#### Length – Weight relationship,  $W = aL^b$

W refers to the weight of freshwater clam, whereas L is the shell length of clam; *a* is the intercept and *b*  represents the slope. Hence, the length-weight relationship can be expressed in the logarithmic form below:

 $\log W = \log a + b \log L$ Condition factor = Weight  $\div$  Length $^b \times 100$ 

Weight is represented in mg and length is described in mm. Meanwhile, the exponent *b* is derived from the length-weight relationship at each aquarium tank which is described as W = *a*L b .

#### *Water quality measurement*

Water samples were collected weekly from each tank and analysed at the Aquatic Laboratory, UMK. Several water parameters which including temperature, dissolved oxygen (DO), conductivity, pH, and total

dissolved solids (TDS) were measured in-situ with a multi-parameter (YSI, UK). In addition, total suspended solids (TSS) and ammonia level were measured using a spectrophotometer (Hach, Germany) following the methods stated by Hach Company (2007). The turbidity levels were determined using the turbidity meter (Thomas Scientific, USA) as suggested by Suganthi et al. (2018).

#### *Ethics statement*

The clams were sampled, handled and sacrificed according to the methods approved by the Institutional Animal Care and Use Committee, UMK.

#### *Statistical analysis*

The data were reported as mean ± standard deviation (SD). The growth performance in terms of shell length and body dry weight and water parameters were analysed using a one-way analysis of variance (ANOVA) by applying treatment as a factor. Moreover, the differences between the treatments were evaluated using the Tukey test. The statistical significance was set at *P* ≤ 0.05, which was carried out using IBM SPSS Version 21 software.

# **Results**

### *Growth performance*

Table 1 indicates the results of the growth rate of shell length and dry body weight of freshwater *C. fluminea* that received different feeding treatments. The shell growth rate was observed to be higher in NPK with 21.0  $\pm$  3.00  $\mu$ m.day<sup>-1</sup>, followed by FSP 17.0  $\pm$  3.00  $\mu$ m.day<sup>-1</sup>, TQD 6.0  $\pm$  1.80  $\mu$ m.day<sup>-1</sup> and control treatment  $3.0 \pm 0.10 \mu m$ . The growth rate in terms of dry body weight was higher in FSP 10.0  $\pm$ 0.40  $\mu$ g.day<sup>-1</sup> followed by NPK 8.0  $\pm$  0.30  $\mu$ g.day<sup>-1</sup>, TQD  $3.0 \pm 0.10$  µg.day<sup>-1</sup> and control treatment -5.0  $\pm$  0.5 µg.day-1 . Statistical analysis indicates that NPK and FSP were not significantly different from each other but significantly different to TQD and control treatment for both shell length and dry body weight.

Table 1. Daily growth performance of *Corbicula fluminea* using three different feeding treatments, namely fermented soy pulp (FSP), treated quail dung (TQD), chemical fertiliser (NPK) and no feed (Control).



Values are presented as mean  $\pm$  SD. Means followed by the same letters (a, b) are not significantly different (*P* ≤ 0.05).

### *Length-weight relationship and condition factor*

The results of length-weight relationship of parameters *a* and *b* and condition factor of clams in each treatment are presented in Table 2. Mean values of *a* and *b* are shown to be in the range of -0.009 to -0.97 and 2.5 to 3.2, respectively for three different treatments and control. The condition factor of the clam ranged from 0.03 to 0.16. The results obtained for *b* values were less than 3 in each treatment except for the control group; indicating a negative allometric growth in clams for all treatments and positive allometric growth for the control treatment.

## *Total organic matter in substrate*

Table 3 demonstrates the amount of total organic matter in the substrate before fertilisation  $(T_0)$ . The amount of total organic matter was similar in all treatments initially, but it increased after the substrate fertilisation period  $(T_1)$ . NPK showed the highest increment of total organic matter by 8.02 %, followed by TQD (3.35 %) and FSP (3.08 %). Meanwhile, the total organic matter in the control treatment was reduced by 0.28 %. At the end of the culture period  $(T_{60})$ , the highest reduction was shown by TQD with 10.02 %, followed by NPK (5.64 %) and FSP (2.33 %). The control treatment portrayed a negative reduction (-4.95 %), whereas the total organic matter increased at the end of the rearing period  $(T_{60})$ . While TQD recorded the highest consumption of organic matter (1.20 µg.clam-1 .day-1 ) followed by NPK (0.67 µg.clam- $1$ .day<sup>-1</sup>) and FSP (0.28  $\mu$ g.clam<sup>-1</sup>.day<sup>-1</sup>) whereas control treatment showed a negative value of organic matter consumption, indicating that organic matter increased in the treatment.

## *Water parameters*

The different mean ranges of environmental variables are shown in Table 4. The temperature (25.87 °C-26.64 °C) for all treatments showed minimal variations over the study period. Meanwhile, the dissolved oxygen (DO) was in the range of  $6.39$  mg. L<sup>-1</sup> to  $6.81$ mg.L<sup>-1</sup>. The total dissolved solids ranged between 14.06 mg.  $L^{-1}$  to 24.83 mg.  $L^{-1}$  in three treatments and control group, while salinity was 0.024 ppt to 0.04 ppt and pH remained alkaline (7.47 to 7.74). The turbidity was in the range of 1.00 NTU to 1.69 NTU whereas total ammonia levels remained below 1 mg. $L^{-1}$ . However, the result of statistical analysis showed that TSS and turbidity were significantly higher (*P* < 0.05) in the treatment of FSP and TQD as compared to the treatment of NPK and control.

# **Discussion**

The utilisation of different feeds supplements showed a significant influence on the growth of clam in an artificial culture system. The present study indicates that the growth performances in terms of shell length and dry weight of the clam were significantly higher in the FSP and NPK treatments as compared to TQD and the control treatment. NPK demonstrated a significant influence on the growth of clams in terms of shell length compared to FSP, TQD and the control treatment. Meanwhile, FSP portrayed a higher growth performance in terms of dry weight compared to other feed applications. The significantly higher growth of shell length seen in the clams in the NPK treatment could be due to the higher calcium content in NPK that is essential in inducing high growth performance of shell length, shell formation and muscle contraction (Ferreira-Rodŕiguez et al., 2017). Similarly, hard clam *Meretrix lusoria* showed a high rate of filtration and pseudofaeces production when fed soybean meal (Chien and Hsu, 2006).

The major part of soybean waste is linoleic acid (Shuhong et al., 2013) which can give a positive correlation growth in *C. fluminea* (De Sousa et al., 2011). Conversely, feeding low lipid content to *C. fluminea* could contribute to its low growth performance (Mamat and Alfaro, 2014) due to the failure to store important nutrient supply energy that eventually leads to mortality (Fernández et al., 2011). Since *C. fluminea* in this study was restricted to pedal feeding in the form of organic and inorganic matter, the growth result is also compared with *C. fluminea* in cold temperature habitat and artificial feeding testing.

The result of growth performance in this study was higher than cold temperature habitat (Welch and Joy, 1984; Serdar, 2018) and artificial feed testing (Welch and Joy, 1984). Cold temperature habitat restricted the growth of natural food, thus limiting *C. fluminea* only to burrow organic matter in the substrate for food while suspended artificial feed allows *C. fluminea* to apply filter-feeding only. In contrast, the growth performance of *C. fluminea* in warm temperature habitat (Welch and Joy, 1984; Serdar, 2018) are higher than the result from this study. Thus it can be concluded that *C. fluminea* requires food from both sources of feeding method, i.e. pedal-feeding and filter-feeding for high growth performance of *C. fluminea*.

The results also indicate that NPK and FSP contain more nutrients to be absorbed by *C. fluminea,* which resulted in higher dry weight and shell length compared to TQD and control treatment. NPK and FSP are suitable feeds for *C. fluminea* growth in an artificial culture system. However, proper control must be taken to ensure that there is no excessive use of NPK and FSP, which can cause water pollution and a threat to *C. fluminea.*

The current findings indicate that the *b*-value of LWR for *C. fluminea* in all treatments were below 3.0, which shows a negative allometric growth pattern of the

Table 2. Length-dry weight relationship and condition factor of *Corbicula fluminea* in different feeding treatments, namely fermented soy pulp (FSP), treated quail dung (TQD), chemical fertiliser (NPK) and no feed (Control). Length-dry weight relationship is expressed in the equation of W = *a*L *b* .

Treatment	$\sigma$	b	Length-weight equation $(P \le 0.05)$	Correlation coefficient (R <sup>2</sup> )	Falcon's condition factor
<b>FSP</b>	$-0.75$	2.6	$W = -0.751^{2.6}$	0.819	$0.13(0.102 - 0.191)$
TOD	$-0.67$	2.5	$W = -0.671^{2.49}$	0.824	$0.16(0.138 - 0.194)$
<b>NPK</b>	$-0.01$	2.8	$W = -0.011^{2.83}$	0.882	$0.06(0.05 - 0.086)$
Control	$-0.97$	3.2	$W = -0.971335$	0.739	$0.03(0.02 - 0.033)$

*a*: a-value; *b*: b-value; W: average dry weight; L: average shell length. The values in parenthesis represent the range of condition factor.

Table 3. Percentage of total organic matter and daily organic matter consumption of *Corbicula fluminea* in different feeding treatments namely fermented soy pulp (FSP), treated quail dung (TQD), chemical fertiliser (NPK) and no feed (Control).



T<sub>0</sub>: Before fertilisation / Day 0 of clam rearing; T<sub>1</sub>: After fertilisation / Day 1 of clam rearing; T<sub>60</sub>: Day 60 of clam rearing. Values are presented as mean ± SD.

Table 4. Water quality parameters sampled in different feeding treatments, namely, fermented soy pulp (FSP), treated quail dung (TQD), chemical fertiliser (NPK) and no feed (Control).



Values are presented as mean ± SD. Means that are followed by the same letters (a, b) are not significantly different (*P* ≤ 0.05).

clams except for the control treatment with positive allometric growth. According to Froese (2006), bvalue, which is larger or smaller than 3.0 indicates the specimen is not in a proportional direction or having an allometric growth pattern. It also shows that although *C. fluminea* can grow faster in length, it will not affect its weight because faster length growth will cause a slower dry weight gain. Furthermore, the reverse effect is also inevitable whereby faster dry weight growth will produce a more gradual length development of C*. fluminea*. It should be noted that the *b*-values may vary due to several factors such as the growth phase and geographic situation (Muzzalifah et al., 2015). In this case, *C. fluminea* is believed to undergo a stress condition due to lack of food sources. Sharma et al. (2005) stated that the *b*value should remain between 2.5 to 4.0 in order for the mollusc to exhibit isometric growth. The

**Asian Fisheries Science** 33 (2020):50-57

statement is supported by Jaiswar and Kulkarni (2002) as they claimed that the mollusc has an isometric growth pattern although the *b*-value is not exactly 3.0. Therefore, it can be concluded that the *b*values of freshwater clams from all treatments in this study were within the range and adhered to the cube law. Whereas, the condition factor calculated for each treatment can be used to detect the variation in the condition of freshwater clams that may vary based on the abundance of food (King, 2007). The present study proved that the condition of freshwater clams in each treatment was less than 1.0, which, according to Narejo (2006) is an indication of poor growth of the aquaculture species. However, the finding is similar to the study conducted by Sharma et al., (2005) on green mussel *Perna viridis* (Linnaeus, 1758) with the condition factor ranging from 0.12 to 0.87. In this case, it can be inferred that condition factor in this study is within the acceptable range, but there was a stress condition resulting from scarce of food source (Gupta et al., 2011) or poor water quality parameters (Martina et al., 2010) that should be taken into consideration.

Current findings indicate that total organic matter in the substrate for treatment with NPK, FSP and TQD should be increased at different rates after the fertilisation phase. NPK showed the highest organic matter increment than the other treatment. According to Kumar et al., (2005) NPK is faster in nutrient releasing rate and decomposition process compared to the organic matter before turning into edible organic matter consumed by cultured species (Weil and Nyle, 2016). Moreover, organic matter such as FSP and TQD needs a longer time for the decomposition process than the inorganic matter (Soderberg et al., 2012).

After 60 days of *C. fluminea* culture, the highest final percentage of total organic matter and daily organic matter consumption per clam was shown by TQD, followed by NPK and FSP. This result is similar to the finding by Lee et al. (2018) as the majority of *C. fluminea* in the test responded positively by moving towards the direction of TQD. Moreover, the organic matter reduction and total consumption of organic matter for all treatments in this study were higher than the study by Hakenkamp and Palmer (1999) as they used feed originated from the clams' habitat. However, the high organic matter consumption may be contributed by two possible factors that occurred during the experiment. The organic matter presented in the substrate might include faeces and pseudofaeces that were possibly consumed by the clams (Nichols et al., 2005) or dissolved in the water before decomposing. Furthermore, the total organic matter in control treatment increased after 60 days of rearing. The organic matter increment can also be caused by the production of pseudo-faeces and faeces of the clams (Sousa et al., 2008) as it can produce a high amount of total organic matter in a

starvation state due to the excretion process (Zhang et al., 2011). The best reading of *C. fluminea* growth performance in terms of dry weight and shell length was shown by NPK and FSP. Similarly, both treatments also presented the least consumed organic matter, which is below 1.0 µg.clam<sup>-1</sup>.day<sup>-1</sup>. This indicates that NPK and FSP are a good choice as feed for *C. fluminea* due to least consumption by the clams but resulted to high growth performance.

There was little variation of water quality among all treatments in the present study, considering that all were provided with equal feed which prevent any detrimental effects to the freshwater clams. However, the results of TSS and turbidity were significantly higher in treatment FSP and TQD but still tolerable by the clams (Bucci et al., 2008) and within the acceptable minimum level (Tuttle-Raycraft et al., 2017). The long-term exposure to the high level of TSS can obstruct the gill function that leads to stress and eventually cause death of the clams (Yang et al., 2017). This statement could be related to the high clam mortality rate in the TOD and FSP treatments. Dissolved oxygen (DO) level and pH values in this study remained relatively stable and not deemed stressful to the clams (Erdoğan and Erdoğan, 2015; Nobles and Zhang, 2015). However, if the DO level is lower than 5.0 mg.L-1 , then the condition could cause the clams to die (Hua and Neves 2007). The temperature was also in the acceptable range, thus representing a similar finding obtained by O'Beirn et al. (1998). Finally, other water parameters in the present study were within the acceptable range for the culture of freshwater clams and conducive for growth. With the high reading of TSS and turbidity, it can be concluded that the use of each treatment was higher than that required by *C. fluminea*. Meanwhile, other parameters were suitable for *C. fluminea* breeding and growth especially ammonia, which was lower than the limit stated by Ferretti and Calesso (2011) of 2.35 mg. L<sup>-1</sup> for EC (effective concentration) 50 and 10.6 mg.  $L^{-1}$  for LC (lethal concentration) 50.

# **Conclusion**

In this study, chemical fertiliser (NPK) and fermented soy pulp (FSP) showed a significantly higher growth of *C. fluminea* compared to TQD and control treatment. However, the growth of the clams was not satisfactory because a low condition factor value found during the 60 days experiment. Therefore, more study related to artificial feed for *C. fluminea* is encouraged especially in terms of its nutritional value and micronutrient in organic matter form as well as its viability for use in a large scale of *C. fluminea* farming.

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