The influence of dietary sources on the biological changes of a subterranean termite, *Coptotermes formosanus* Shiraki

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Abstract. To understand the effects of various dietary sources on the biological changes of a subterranean termite, *Coptotermes formosanus* Shiraki, the three lignocelluloses, i.e: softwood, hardwood, and grass with the different of samples shape/size (intact and powdered) were subjected to *C. formosanus*, and we analysed the biological changes of *C. formosanus* workers (survival, body mass and the presence of protists). The results showed that at the end of observation, the survival, body mass and presence of *Pseudotrichonympha grassii* and *Holomastigotoides hartmanni* in the guts when consumed wood diets were much higher than those of the termite workers when consumed rice culm straw diet, whereas the presence of *Spirotrichonympha leidyi* in the guts were similar for all the samples. We failed to detect significant difference between intact and powdered diets from each lignocellulose, suggesting that the difference of lignocellulose shape/size (intact and powdered) had no effect on the biological changes of a subterranean termite, *C. formosanus*.

1. Introduction

It has been well known that termites decompose lignocellulose as nutrient source [1-3]. They decompose lignocellulose by enzymes from host and microorganism in their guts [4-7]. Based on the presence of protists in the guts of termite workers, subterranean termites can be divided to be two classifications, i.e: higher termite and lower termite, in which higher termites do not have protists in their guts, whereas lower termites possess protists in their guts [8]. A subterranean termite, *Coptotermes formosanus* as important pest in the world is categorized as a lower termite [9-11]. Some earlier studies have been reported that three protists inhibit in the guts of *C. formosanus* workers, i.e: *Pseudotrichonympha grassii*, *Holomastigotoides hartmanni*, and *Spirotrichonympha leidyi* [12]. They have a specification role in degrading cellulose. Tanaka et al. [13] reported that *P. grassii* degrade high-molecular weight cellulose, whereas *H. hartmanni* and *S. leidyi* degrade low-molecular weight cellulose.

In nature, lignocellulose is divided to be three classifications, i.e: softwood, hardwood, and grass. Our previous study has well-documented regarding the effect of various lignocelluloses on the biological changes of a lower termite, *C. formosanus* [14,15]. However, such studies only
focused in the intact lignocelluloses, therefore in this present study we examined the effects of various diet sources from three lignocellulose, i.e.: softwood (Japanese cedar), hardwood (Japanese beech), and grass (rice culm straw) with difference of lignocellulose shape/size (intact and powdered samples) due to the difference of diets affected the biological changes of a subterranean termite, *C. formosanus* [13].

2. Materials and Methods

2.1. Samples preparation

In this study used woods (Japanese cedar and Japanese beech), and rice culm straw. Intact samples were prepared using a cutting machine wood and a scissor. Powdered samples were prepared using grinding machine and a sieve machine.

2.2. Bio-assay test

The bio-assay test and sample size referred to our previous study [14,15]. Acrylic cylinders and plastic cups were prepared for testing. 50 workers and 5 soldiers of *C. formosanus* and sample were then placed in an acrylic cylinder. Whereas, powdered sample was put in a plastic cup. The bio-assay test was conducted for 6 weeks in triplicate, and survival and body mass of termites were observed at every week.

2.3. Observation of protists profile in the guts of the termites

Various diet sources as described above and 130 workers of *C. formosanus* were put in acrylic cylinder for intact samples, and in a plastic cup for powdered sample. The observation of protists profile in the guts of termite workers referred to our previous study [14,15]. The protists were observed using a digital microscope (VHX-5000, Keyence, Osaka, Japan) in triplicate.

2.4. Statistical analysis

The biological changes parameters (survival, body mass, and the presence of protists in the guts) were analysed using SPSS ver. 23 software (IBM, Armonk, NY). Significance levels were set at *P* < 0.05 [14,15].

3. Results and discussion

3.1. Survival of *C. formosanus* workers

*Figure 1* shows the periodic changes in termite survival. At 1st-week observation, the present study observed no significant difference in termite survival (*F*=21, *P*=0.19) for the intact diets from wood diets, and rice culm straw diets at 95%, and 91%, respectively, or at 2nd-week observation (*F*=11, *P*=0.122) at 92-93%, and 89%, respectively. However, at 3rd-week observation, we observed significant difference for termite survival between woods and rice culm straw, in which the termite when consumed wood diets were much higher than those of the termites when consumed rice culm straw (*F*=81, *P*<0.05), but there was no significant difference in termite survival among wood diets (*F*=5, *P*=0.115) at 90%, and 75%, for wood diets, and rice culm straw, respectively. Similarly with results at 6th-week observation, we found the survival of the termites when consumed wood diets were much higher than those of the termites when consumed rice culm straw diet (*F*=98, *P*<0.05). In addition, the present study found that termite...
survival was similar among wood diets \((F=10, P=0.108)\) at 75-76%, and 26%, for woods, and rice culm straw, respectively. Our finding is consistent with an earlier study [14].

The results from the powdered diets, somehow were similar with results from the intact diets. The results detected no significant difference for termite survival at 1st-week observation \((F=11, P=0.129)\) for the intact sample diets from wood diets, and rice culm straw at 97-98%, and 95%, respectively, or at 2nd-week \((F=9, P=0.112)\) at 94-95%, and 90%, respectively. But, at 3rd-week, the survival of the termites when consumed rice culm straw diet was much lower than that of the termites when consumed wood diets \((F=27, P<0.05)\), and there was no significant difference in termite survival among wood diets \((F=5, P=0.115)\) at 90%-91%, and 77%, for wood diets, and rice, respectively. Based on the statistical analyses, the present study failed to detect the significant difference between intact sample and powdered diets from each lignocellulose, even at 6th-week, suggesting that the difference of lignocellulose shape/size had no effect on the survival of the termites, but the survival of the termites was affected by lignocellulose type. These results support our previous finding that wood was preferably than rice culm straw [14,15].

### 3.2. Body mass of the workers

The difference in the body mass of the termite workers when consumed diet sources are presented in Figure 2. For intact diets, the results observed that values of the body mass of the termite workers were similar among all the intact diets until at 3th-week observation \((F=12, P=0.119)\). However at 4th-week observation, the present study detected the body mass of the termite workers were significantly decreased when consumed rice culm straw those of the termite workers when consumed wood diets \((F=26, P<0.05)\) at 87%, and 76% for wood diets, and rice culm straw diet, respectively. At the end of the observation, values of the body mass of the termite workers when consumed wood diets were also much higher than those of the termite workers when consumed rice culm straw diet \((F=22, P<0.05)\), but the body mass of the termite workers were similar for wood diets \((F=15, P=0.128)\) at 86%, and 75% for woods, and rice straw, respectively. These results are also consistent with our previous study [14].

As reported earlier by some studies [14,15], the silica components in rice have negative effect on the body mass of termite workers. In addition, the results in the body mass of the termite
workers from powdered diets were similar with results from intact diets. The body mass of the termite workers among the three lignocellulose diets were similar until at 3rd-week ($F=21$, $P=0.186$). Significant difference was found at 4th-week observation, the values of the body mass of the termite workers when consumed wood diets were much higher than those of the termite workers when consumed rice culm straw ($F=65$, $P<0.05$) at 87%, and 75% for woods and rice culm straw, respectively. Our previous studies suggested that nutrient in rice culm straw is inadequate for termites [14,15]. Earlier studies reported that content of mineral components in rice straws is much higher than that in woods [16,17]. Furthermore the statistical analyses recorded no significant difference between intact and powdered diets for each lignocellulose, suggesting that the lignocellulose shape/size had no effect on the body mass of the termites.

![Figure 2](image-url)

**Figure 2.** Periodic changes in the body mass of *C. formosanus* workers fed on diet sources. Jc: Japanese cedar diet (softwood); Jb: Japanese beech diet (hardwood). Rc: rice culm straw diet (grass).

### 3.3. Protists profile

The protists profile in the guts of termite workers when consumed diet sources are shown in **Figure 3**. We observed three protists in the guts of the termite workers (*P. grassii*, *H. hartmanni*, and *S. leidy*). The present study found 100% of *P. grassii*, *H. hartmanni*, and *S. leidy* in the guts. Interestingly, the presence of *P. grassii* in the guts when consumed woods was significantly higher than those in the guts when consumed rice culm straw ($F=37$, $P<0.05$) at 3rd-week observation (Figure 3a). We found significant difference for *H. hartmanni* at 4th-week observation (Figure 3b), in which *H. hartmanni* in the guts of the termite workers when consumed woods were much higher than those in the guts of termite workers when consumed rice culm straw ($F=54$, $P<0.05$). At the end of the observation, the present study observed no significant difference for *S. leidy* for all the intact diets, and 100% *S. leidy* in the guts of the termite workers when consumed woods (Figure 3c). The results for the powdered diets were also similar with the intact diets. The termite workers when consumed rice culm straw diet resulted in 10% of *P. grassii* lost from the guts at 2nd-week observation (Figure 3a), and 13% of *H. hartmanni* lost at 4th-week observation (Figure 3b). In addition, the results showed that presence of *S. leidy* in the guts of termite workers was similar when consumed all the powdered diets even at the end of
the observation (Figure 3c). These results suggest that the lignocellulose shape/size had no effect on the protists profile in the guts of *C. formosanus* workers.

![Graph](image)

**Figure 3.** Protists profile of *P. grassii* (a), *H. hartmanni* (b), and *S. leidy* (c) in the guts of *C. formosanus* workers when consumed diet samples.

4. Conclusion

Lignocellulose shape/size had no effect on the biological changes of a subterranean termite, *C. formosanus*. The present study supports the view that the biological changes of *C. formosanus* workers were affected by silica component in rice culm straw, and in the future silica component could be promoted as agent for termite control.
5. Acknowledgement

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