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Authors: Arquette, Timothy J., Mallette, Eldon J., and Rodriguez, Jose M.

Source: Florida Entomologist, 95(1) : 218-220

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.095.0138>

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## URIC ACID AND SOLUBLE PROTEIN CONTENT OF FECES FROM THREE SPECIES OF SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

TIMOTHY J. ARQUETTE<sup>1,2</sup>, ELDON J. MALLETT<sup>2</sup> AND JOSE M. RODRIGUEZ<sup>3</sup>

<sup>1</sup>Department of Entomology, Mississippi State University, Mississippi State, MS 39762

<sup>2</sup>Mississippi State University South Branch Experiment Station, Poplarville, MS 39470

<sup>3</sup>State Chemical Laboratory, Mississippi State, MS 39762

Like all animals, termites must dispose of excess nitrogen from protein and purine digestion and metabolism. Nitrogenous wastes in insect feces are primarily ammonia or uric acid (Cochran 1985, Chapman 1998). Ammonia is often the primary nitrogenous waste product of insects that live in aquatic or very moist environments. For insects that live in dry habitats, or need to conserve body weight for flight, or have a dry diet, uric acid is typically their main nitrogenous waste product (Chapman 1998, Cochran 1985). Although termites are uricotelic, feces of a subterranean termite, *Reticulitermes flavipes* Kollar, were reported to have only trace amounts of uric acid (Potrikus & Breznak 1980a).

Termites are coprophagous (Waller & LaFage 1987), but experimental data is lacking regarding the nutritional benefit termites receive from ingestion of their feces. Nitrogen in the feces would be of nutritional value to the xylophagous subterranean termite since wood is very low in nitrogen. Fecal protein has been suggested as a source of dietary nitrogen for termites (Nation 2002), but amounts of fecal protein have not been measured. The current study aimed to determine levels of uric acid and soluble proteins in the feces of 3 subterranean termite species, and in turn consider how these biomolecules are involved in termite digestion and nutrition.

*Reticulitermes virginicus* Banks and *R. flavipes* were collected from forested areas of Pearl River County, Mississippi, and either stored in the laboratory in original nest sapwood, or separated from wood for assay within 24 h after field collection. *Coptotermes formosanus* Shiraki were collected

in Pearl River County from a bucket trap. Soluble protein levels of whole termite workers and fecal samples were determined using Bradford reagent with a bovine serum albumin standard (Sigma-Aldrich, St. Louis, Missouri). For whole termite samples, 3 groups of 10 live workers were weighed, added to separate 1.5 ml centrifuge tubes, and ground with a micropestle. After 1 mL deionized water was added, tubes were vortexed and centrifuged 5 min at 20,600 g. Soluble protein levels in adult termites were determined spectrophotometrically at 595 nm following the standard Bradford assay procedure provided with the reagent. For determination of soluble protein levels in feces, the micro Bradford assay procedure was used. Small piles of fecal material were collected as described in Arquette & Rodriguez (2011) from laboratory arenas without food. After determination of fresh weight, feces were prepared for protein assay as for whole termite samples. Uric acid levels from feces and 3 groups of 10 whole termites were determined using a procedure detailed in Arquette & Forschler (2006). Data for uric acid and soluble protein levels are presented using the same units reported in earlier papers (Potrikus & Breznak 1980a; Arquette & Forschler 2006; Arquette et al. 2006).

Uric acid was not detected in feces of 7 of 9 populations assayed (Table 1). The 2 populations measuring trace amounts of uric acid in the feces had been kept in laboratory culture for approximately 1 year, whereas other groups were assayed soon after field collection. Fecal soluble protein levels ranged from about 1 to 5 percent fresh weight (Table 2).

TABLE 1. URIC ACID CONTENTS OF WHOLE TERMITES AND OF THEIR FECES.

| Species              | Time in captivity | Percent uric acid/dry fecal sample | Percent uric acid/dry worker equivalent <sup>1</sup> | Average live weight/worker equivalent (mg) |
|----------------------|-------------------|------------------------------------|--|--|
| <i>R. flavipes</i>   | 24 h              | ND <sup>2</sup>                    | 1.7  | 2.5  |
| <i>R. virginicus</i> | 24 h              | ND                                 | 1.4  | 2.4  |
| <i>R. virginicus</i> | 24 h              | ND                                 | 1.4  | 2.4  |
| <i>R. virginicus</i> | 24 h              | ND                                 | 1.8  | 2  |
| <i>R. virginicus</i> | 1 year            | 0.13                               | 17.5   | 2.3  |
| <i>R. flavipes</i>   | 1 year            | 0.16                               | 10.4   | 2.5  |
| <i>C. formosanus</i> | 24 h              | ND                                 | 1.3  | 3.3  |
| <i>R. flavipes</i>   | 24 h              | ND                                 | 1.5  | 2.4  |
| <i>R. flavipes</i>   | 24 h              | ND                                 | 1.6  | 2.7  |

<sup>1</sup>The weight of one termite equivalent was determined from the average of 3 groups of 10 workers each.

<sup>2</sup>ND: uric acid not detected.

TABLE 2. SOLUBLE PROTEIN CONTENTS OF WHOLE TERMITES AND OF THEIR FECES.

| Species              | Time in captivity | Percent protein/fresh fecal sample | µg protein/mg termite | Average live weight/one worker equivalent <sup>1</sup> (mg) |
|----------------------|-------------------|------------------------------------|-----------------------|---|
| <i>R. flavipes</i>   | 24 h              | 0.9                                | X <sup>2</sup>        | 2.6   |
| <i>R. flavipes</i>   | 24 h              | 0.7                                | 22.2                  | 2.4   |
| <i>C. formosanus</i> | 24 h              | 4.7                                | 35.7                  | 3.4   |
| <i>R. virginicus</i> | 1 year            | 0.8                                | 16.6                  | 2.3   |
| <i>R. virginicus</i> | 24 h              | 4.5                                | 20.6                  | 2   |
| <i>R. flavipes</i>   | 24 h              | 3.2                                | 26.9                  | 2.5   |
| <i>R. virginicus</i> | 24 h              | 2.1                                | 22.5                  | 2.4   |
| <i>R. flavipes</i>   | 1 year            | 2.1                                | 16.2                  | 2.8   |

<sup>1</sup>The average live weight of one termite equivalent was determined from the average of 3 groups of 10 workers each.

<sup>2</sup>X: no data.

Although uric acid was initially described as being present in termite feces by Noirot & Noirot-Timothee (1969), a later study with *R. flavipes* that assayed uric acid in their feces found it to be present only in trace amounts (Potrikus & Breznak 1980a). This was attributed to digestion of urates by uricolytic bacteria in the hindgut (Potrikus & Breznak 1980b, 1980c). We have found that 2 additional subterranean species, *R. virginicus* and *C. formosanus*, also excrete little or no uric acid (Table 1). Trace levels of uric acid detected in fecal samples of respective *R. flavipes* and *R. virginicus* populations (Table 1) were comparable to levels reported by Potrikus & Breznak (1980a). Insects obtained from these 2 populations were distinct from the others tested from having been reared in a laboratory in the original nest wood for an extended time period, and they had accumulated high levels of uric acid in the fat body (Table 1). High uric acid from fat body of cannibalized termites could have been above the capacity for the uricolytic bacteria in hindgut to process, resulting in some of the molecule being passed out undigested in feces. Alternately, because the nitrogen content of the basic wood diet is very low, synthesis of new proteins as well as uric acid formed from protein breakdown may occur at a slower rate than in insects that have ample protein in their diet. A low rate of uric acid synthesis may result in small quantities of the molecule reaching uricolytic bacteria in the hindgut, and with complete digestion occurring before the uric acid can be eliminated in their feces.

We could not establish a relationship between protein amounts in feces and levels in whole termites (Table 2). Populations that had been in captivity for 1 yr had the lowest whole body soluble protein content among the populations assayed for this study, but one such *R. flavipes* population had protein levels in feces near the average for all populations measured. Also, a high protein level was determined in the feces of *C. formosanus* workers with high body protein levels, while a *R. virginicus* population with similar fecal protein levels had a body protein content that was average among the groups (Table 2).

A source of protein in termite feces could be bacteria (Nation 2002). Other possible sources of protein in the feces include those that pass through the gut undigested, such as enzymes and food proteins, and those released into hindgut fluid from ruptured protozoa. The current study found that protein levels in termite feces are high compared to wood. Therefore, coprophagy may provide these termite species with dietary nitrogen.

#### SUMMARY

Levels of soluble proteins and uric acid were determined for workers and feces of 3 subterranean termite species, i.e., *Reticulitermes virginicus* Banks, *R. flavipes* Kollar, and *Coptotermes formosanus* Shiraki. Traces of uric acid were measured in the feces of workers that had been reared in a laboratory, but no detectable uric acid was measured in feces from freshly collected termites. Soluble protein levels of feces were high compared to wood for all samples tested, demonstrating that subterranean termites may obtain dietary nitrogen from ingestion of their feces.

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