A Geometric Analysis of the Regulation of Inorganic Nutrient Intake by the Subterranean Termite Reticulitermes flavipes Kollar

Timothy M. Judd *, James R. Landes, Haruna Ohara and Alex W. Riley

Department of Biology, Southeast Missouri State University, Cape Girardeau, MO 63048, USA
* Correspondence: tjudd@semo.edu; Tel.: +1-573-651-2365

Academic Editors: Changlu Wang and Chow-Yang Lee
Received: 20 July 2017; Accepted: 2 September 2017; Published: 6 September 2017

Abstract: Most studies on termite food selection have focused on a single nutrient per choice, however, termites, like all animals, must balance multiple nutrients in their diet. While most studies that use multi-nutrient approaches focus on macromolecules, the ability to balance the intake of inorganic nutrients is also vital to organisms. In this study, we used the geometric framework to test the effects of multiple inorganic nutrients on termite feeding. We presented the subsets of Reticulitermes flavipes colonies with food enriched with varying levels of KCl, MgSO$_4$, and FePO$_4$. Each trial varied two of the three nutrients while the third nutrient was kept constant. The amount of food consumed was measured over two weeks. The termites’ feeding patterns during the study suggested that they fed until they reached a limit for MgSO$_4$. This result suggests that the termites were using the rule of compromise such that the termites would over consume KCl or FePO$_4$ in order to avoid overeating MgSO$_4$. Thus, the termite colonies are able to regulate the intake of inorganic nutrients, and by doing so, adjust their intake from multiple resources in order to maintain an intake target.

Keywords: termites; Reticulitermes flavipes; geometric framework; inorganic nutrients; feeding regulation

1. Introduction

Social insect colonies must regulate the intake of nutrients in order to optimize their foraging force. Rather than single individuals making food selections, colonies draw from multiple food sources in order for the colony as a whole to meet its nutritional needs. Bee and ant colonies will distribute their foraging force to their maximize energy intake [1,2] and also regulate the flow of different nutrients [3–6] into their colonies based on season and colony composition. Ant colonies have been specifically shown to be capable of regulating their feeding to maintain a balance of multiple nutrients [7]. Most of foraging regulation studies on social insect colonies have focused on social Hymenoptera. However, most feeding groups of termites also access nutrients from multiple sources [8–10] and individuals can recruit others to a food source [11]. Termites, like social Hymenoptera, are able to distribute nutrients to other colony members [12–15]. Thus, it would seem likely that termite colonies should also maintain the balance of multiple nutrients and regulate their feeding accordingly.

A number of studies have examined the effects of individual nutrients and other substances on the food selection of termites. Sugars, amino acids, ions, and other small compounds have been found to influence the feeding of different species of termites [16–22]. However, it has been found that different species have different responses to the same molecules [18,23,24], and in some cases the same species will have seasonal or even geographical differences in its response [25]. The effect of the interaction of different nutrients on feeding responses in termites has had very little attention in termite foraging studies, even though it is unlikely that termites are responding to a single nutrient when regulating.
their feeding. One model that was developed to examine the regulation of multiple nutrients is the geometric framework [26]. This framework examines the ratio of nutrients that organisms consume to reach an intake target, the ideal ratio, and amount of all the nutrients. When presented with multiple foods that do not have the same ratio as the intake target, organisms will use multiple food sources to reach the target or use different strategies in order to approximate the target as close as possible [27]. This model has been successfully used to demonstrate that a number of species from various trophic levels, are regulating their diets based on the availability of several nutrients in some cases from multiple food sources [28–33]. Up to this point, most of the geometric framework studies have focused on macromolecules (carbohydrates, lipids, and protein), however the model could be applied to other nutrients.

Termites are excellent models for examining inorganic nutrient balance because they feed on cellulose based sources [8] and can access monosaccharides found in cellulose and hemicelluloses with the aid of microorganisms or through the production of their own enzymes [34–36]. Thus, the energy compounds are not a limiting factor in the termite diet. However, cellulose-based foods are generally a poor source of other nutrients [12]. How termites are able to acquire the correct levels of other nutrients is of interest. In many cases, the termites access nitrogen with the aid of nitrogen fixing bacteria [37–41] or consuming fungi [12,15]. Thus, other inorganic nutrients would be ideal candidates to examine the nutrient regulation. Subterranean termites have two potential sources for inorganic nutrients, the soil and food [42]. It is from these sources that termites can balance the intake of the other nutrients required in their diet.

Here, we used the geometric framework [26] to compare the effects of several inorganic nutrients on the feeding regulation of the eastern subterranean termite Reticulitermes flavipes. Lab colonies were placed in nutrient poor soil so they were forced to draw inorganic nutrients from artificial food sources enriched with different levels of KCl, MgSO$_4$, and FePO$_4$. KCl was chosen because both potassium (K) and chloride (Cl) are used for homeostasis in insects [43,44]. Furthermore, termites have been shown to be attracted to K in the soil [45]. Magnesium (Mg) is an important cofactor in glycolysis and sulfur (S), and is necessary for the production of proteins. Several symbionts in termite guts process sulfates [46–48]. FePO$_4$ is a source of phosphates which are important for the production of nucleic acids. Shortages in phosphates can potentially limit protein production [49]. R. flavipes has been shown to select foods with phosphates in the fall [17]. Iron (Fe) is important for protein function and essential for the nitrogen fixation process used by termite symbionts [50]. It is likely that these nutrients are not needed in similar amounts and termites’ ability to process these nutrients differ. Both factors could affect how termite colonies reach their intake targets. It was not the goal of this study to isolate the effects of individual nutrients, but instead to examine the interplay of these nutrients when the termites are faced with food varying levels of multiple nutrients.

2. Materials and Methods

2.1. Collections

Ten colonies were sampled from the Juden Creak Natural Area, Gape Girardeau, MO using termite traps as described in [25]. Colonies were removed from the traps in the laboratory and placed in a sealable container half filled with soil. All colonies were used within 48 h of collection.

2.2. Feeding Trials

All food was prepared by dissolving 1.5 g of agar (Sigma-Aldrich, Arvada, CO, USA) in 100 mL of hot deionized (ddi) H$_2$O. Each food type had different levels of KCl, MgSO$_4$, and FePO$_4$ (Sigma-Aldrich, Arvada, CO, USA, Table 1) dissolved into the solution followed by the addition of 7.5 g of α-cellulose (Sigma-Aldrich, Arvada, CO, USA). The mixtures were then poured into petri dishes and because α-cellulose is non-soluble, the petri dishes were placed on a shaker until the mixture solidified in order to ensure the cellulose remained suspended and evenly distributed throughout the food.
Insects 2017, 8, 97 3 of 10

Table 1. Total amounts of KCl, MgSO₄, and FePO₄ added to each food type. Amounts are listed as mg and resulting mM for each diet. For each food label, the element in lower case refers to the diet that was the lowest level in the enrichment and the element in upper case refers to the diet that was highest in the enrichment.

| Food Type | KCl mg (mM)         | MgSO₄ mg (mM)         | FePO₄ mg (mM)         |
|-----------|---------------------|-----------------------|-----------------------|
| kFE       | 0.025 (3.35 × 10⁻³) | 0.050 (4.15 × 10⁻³)   | 0.100 (6.63 × 10⁻³)   |
| kMG       | 0.025 (3.35 × 10⁻³) | 0.100 (8.31 × 10⁻³)   | 0.050 (3.32 × 10⁻³)   |
| mgFE      | 0.050 (6.71 × 10⁻³) | 0.025 (2.08 × 10⁻³)   | 0.100 (6.63 × 10⁻³)   |
| MgFe      | 0.050 (6.71 × 10⁻³) | 0.100 (8.31 × 10⁻³)   | 0.025 (1.66 × 10⁻³)   |
| Kmg       | 0.100 (13.4 × 10⁻²) | 0.025 (6.63 × 10⁻³)   | 0.050 (3.32 × 10⁻³)   |
| Kfe       | 0.100 (13.4 × 10⁻²) | 0.050 (4.15 × 10⁻³)   | 0.025 (1.66 × 10⁻³)   |

Three separate bins were created for each colony and designated kFE/Kfe, kMg/Kmg, and mgFE/Mgfe based types of food used in each trial. Two of the three nutrients were varied, while the third was kept constant. Each bin was a 17.8 × 17.8 × 5 cm sealable plastic container 1/4 filled with play-sand. Sand was used to minimize any uptake of inorganic nutrients from the soil [42]. After the sand was added, 100 worker termites, hereafter called a “subcolony”, were added from the main colony (Figure 1). 3.0 g of each of two food types were placed on a 3.5 × 3.5 cm note card and placed in the opposite corners of the bin. R. flavipes creates a small hole through the card to get to the food. For each of the three trials, three control bins were created containing everything except termites to control for water loss in the food [51]. Food was weighed every two days to determine loss of mass using a balance (Denver Instrument XP-300, Bohemia, NY, USA). All termites and sand were removed from the food prior to weighing. The feeding trials were terminated after two weeks. All three trials were run simultaneously. The termites used in the trials were not returned to the main colony.

![Figure 1](image_url) Setup of the experiment. The top portion outlines the experimental procedure for each of the ten colonies used in the experiment. Large boxes represent a subcolony and small boxes represent diets fed to the subcolonies. Each subcolony received two diets simultaneously. The lower portion of the figure indicates the relative levels of KCl, MgSO₄, and FePO₄ in each diet.

Calculations for water loss were performed in the same manner as Judd et al. 2009 [51]. The mean weight change of the controls was subtracted from the change in weight for each food type. The result was the weight loss due to termite feeding. In rare instances (a total of 5 times throughout the experiment, the difference was less than 0.06 g) the calculated consumption was negative and they
were changed to a zeros to indicate that none of the weight loss was due to feeding. Although the possibility existed that the termites could have transported pellets of agar to the surface rather than eating them, this was not noted during the study. Previous studies have shown that the termite workers do not readily create pellets of agar less than 2% [52]. The level of agar in the solution used in this study was less than 2% making it unlikely that the transportation of agar contributed to the weight loss in the food.

Once the weight loss due to termite feeding was calculated, the total amount of each diet (KCl, MgSO₄, and FePO₄) that was consumed by each subcolony was determined from a proportion of the mass of each diet represented in the food. The result was converted to mmol. Nutrient rails, which represent the ratio of the diets in individual food types, were also determined by converting the amounts of each nutrient in the diets to mmols.

2.3. Data Analysis

The results of each individual trials were compared using the Wilcoxon Signed Rank Test (WSR) [53] to determine if there was a preference for either diet within a trial. In order to estimate the intake target, only colonies that were represented in all of the three experiments were used (N = 7). The total amounts of KCl, MgSO₄, and FePO₄ consumed were calculated based on the percent amount of each nutrient in each of the food consumed. The intake target was estimated by taking the centroid of the triangle formed by the average intake of nutrients in all three trials in three-dimensional spaces.

3. Results

3.1. Results of Individual Tests

3.1.1. Kfe vs. kFE Trial

There was no significant difference between the amount of Kfe or kFE foods consumed (T = 25, p > 0.05, WSR, Figure 2A). Based on the daily trajectory, the termites primarily took in KCl for the first two days, and then abruptly switch to consuming both KCl and FePO₄ equally (Figure 2B). The final trajectory was a balance between KCl and FePO₄.

Figure 2. Results of the feeding trial in termites exposed to the food varying in KCl and FePO₄ (Kfe and kFE). (A) Medians (blue bar) and quartiles (error bars) of the total amount of food eaten for all colonies (N = 10) after 14 days; (B) The nutritional intake of the termites for KCl and FePO₄ over the 14 d period. The large blue circle with error bars indicates the average total intake of KCl and FePO₄ for all colonies. The small x’s indicate the average cumulative intake per day (including day 0) for all colonies and are connected by the orange dashed line to represent the average intake trajectory. The black lines represent the nutrient rails, the ratio of FePO₄ and KCl in each diet (Kfe and kFE). These rails represent the intake trajectory if the termites were to exclusively feed on that diet. The closer the feeding trajectory is to a rail, the more similar the nutrient intake was to the diet represented by that rail.
3.1.2. MGfe vs. mgFE Trial

Termites in this study fed significantly more on the mgFE food source than the MGfe source (T = 0, p < 0.01, WSR, Figure 3A). The termites initially consumed equal amounts of MgSO₄ and FePO₄. After day four, the termites increased the intake of FePO₄ (Figure 3B). Thus, the termites appeared to be limiting the intake of MgSO₄ in favor of FePO₄.

![Figure 3](image1.png)

Figure 3. Results of the feeding trial in termites exposed to the food varying in MgSO₄ and FePO₄ (MGfe and mgFE diets). (A) Medians (red bar) and quartiles (error bars) of the total amount of food eaten for all colonies (N = 10) after 14 days. The "*" indicates a significant difference between the amount of each diet consumed (p < 0.01); (B) The nutritional intake of the termites for MgSO₄ and FePO₄ over a 14 days period. The lines and symbols are the same as in Figure 2, except for the large circle and x’s are in purple and the nutrient rails represent the ratio of MgSO₄ and FePO₄ in both food types.

3.1.3. Kmg/kMG Trial

There was no significant difference in the overall consumption of the Kmg or kMG foods (T = 13, p > 0.05, WSR, Figure 4A). However, when the intake of both KCl and MgSO₄ are compared, the termite colonies seemed to consume more of the KCl then MgSO₄ (Figure 4B). Thus, the termite colonies were on track to limit the intake of MgSO₄ relative to KCl.

![Figure 4](image2.png)

Figure 4. Results of the feeding trial in termites exposed to the food varying in MgSO₄ and KCl (Kmg and kMG diets). (A) Medians (purple bar) and quartiles (error bars) of the total amount of food eaten for all colonies (N = 10) after 14 days; (B) The nutritional intake of the termites for MgSO₄ and KCl over a 14 days period. The lines and symbols are the same as in Figure 2, except for the large circle and x’s are in purple and the nutrient rails represent the ratio of MgSO₄ and KCl in both food types.
3.2. Combined Analysis and Intake Target

Due to a labeling error for three of the colonies in one of the trials, seven colonies were represented in the results of all three trials to ensure the same colonies were represented in all trials. The means and estimated intake target are plotted in Figure 5. The colonies from the Kfe/kFE trials came closest to the intake target as compared to the other two trials.

![Figure 5](image_url)

**Figure 5.** Equilateral mixture triangle summarizing the intake of KCl, MgSO4, and FePO4 for all three trials. The blue, red, and purple dots represent the average intake of the same seven colonies from the Kfe/kFE, MGfe/mgFE, and Kmg/kMG trials, respectively. The light blue area is the area of possible accessibility from the combined trials. The gold diamond is the estimated intake target based on the results for all 7 colonies.

4. Discussion

The results of this study support the hypothesis that termites are regulating their feeding based on the nutrient content in available foods. If the termites were not regulating their feeding we should have seen termites eating all foods equally [54]. Instead, the termites in this study did not feed on all of the food equally. The termites in this study preferentially fed on the FeMg food to the FeMG food.

The termites fed until they reached an upper threshold for the intake of MgSO4. Based on the MGfe/mgFE and Kmg/kMG trials, the ceiling is around 0.001 mmol. In the Kfe/kFE trial, both foods had an equal amount of MgSO4. The final result was an approximate of the estimated intake target (Figure 5). It is unknown if it is Mg or SO4 (or both) that are responsible for the ceiling, a question for further study. In the MGfe/mgFE and Kmg/kMG trials, the termites increased the intake of FePO4 and KCl, respectively, before hitting the MgSO4 ceiling (Figure 5). One possible explanation for these results is the rule of compromise [27]. In studies using macronutrients, many carnivores will consume extra protein in order to gain enough carbohydrates [29,30,32]. Domestic cats, for example, will consume extra protein and fats, but will limit the level of carbohydrates in their diet [29]. It appears that a similar response is occurring in termites with the inorganic nutrients used here. The overconsumption of KCl and FePO4 is similar to what occurred with protein and fats in the domestic cats [29]. In both the MGfe/mgFE and Kmg/kMG trials, the intake trajectories were moving away from the food enriched with high levels of MgSO4 (MGfe and kMG, Figures 3B and 4B). Thus, it is possible the termites were operating under the rule of compromise as well.
The threshold for MgSO$_4$ can potentially be caused by either Mg or SO$_4$. Mg is an important cofactor in glycolysis [43]. However, an excess of Mg may be unhealthy for termites. Mg is regulated by the Malpighian tubules [55,56], and in *Hypiphora cercropia* it is also been shown to be stored in the midgut [55], thus it is possible that the same is true for termites. If the levels of Mg in the hemolymph and midgut epithelia reach capacity, it may cause termites to reduce their intake. On the other hand, sulfates are mainly processed by symbiotic bacteria *Desulfovibrio* in termite guts [46,48]. Increases in the sulfate content of termite diets will cause an increase in populations of these bacteria in termite guts [48]. Thus, it is possible that these bacteria are essential for processing sulfates and the size of the population of *Desulfovibrio* in termites may affect the levels of sulfates that termites can consume. If the bacteria are involved in setting the intake target for sulfates, then changes in the population of these bacteria could change the intake target. Whether or not bacteria can influence the intake of nutrients by its host remains to be seen.

Potassium is found in higher concentrations in termites than most other elements, including Mg and Fe [14,57]. Although Cl levels have not been measured in termites, Cl and phosphates are generally the most common anions in insects [44]. Both K and Cl are important for homeostasis in insects and can be processed by multiple tissues, thus, consuming excess amounts of both nutrients may be more tolerable [58,59]. Phosphorus levels have been shown to be higher than Mg levels in *Nasutitermes* [57]. Phosphates are needed for energetic functions, protein synthesis, and even used to store Mg [44,55]. Iron could be potentially toxic at higher levels, but insects generally have transferrins and ferritins to transport and store Fe, respectively [50]. These molecules also are involved in the immune defense in insects [60]. Thus, it is possible that termites may be able to handle a larger intake of Fe. Iron is also important for metabolic enzymes for several symbiotic bacteria [61].

Termite colonies involve multiple individuals, and the intake target represents the combination of the nutritional needs of all of the individuals. Termites will pass inorganic nutrients to other individuals and different castes will retain different levels of these nutrients [14]. Thus, the intake target is reached when all individuals have reached their individual target. The presence of soldiers and reproductives that are not present in this study could affect the intake target. The maintenance of an intake target requires internal sensing mechanisms that would regulate foraging behavior. Insects have been shown to forage for inorganic nutrients to meet nutritional demands. A number of insects will collect sodium from water sources [62–65] until their needs are met. Honeybees will forage for several inorganic nutrients from water sources when they cannot get those nutrients from pollen or nectar sources [66]. In this study, the termite workers were forced to gain their nutrients from the food sources because they were housed in nutrient poor soil (sand). They too changed their foraging patterns during the course of the study, presumably as members of the subcolony met their intake targets. The change in food consumption suggests that termites also have internal systems to detect the levels of several inorganic nutrients, and these levels may influence foraging behavior and recruitment. It is possible that the termites in this study simply prefer foods with higher levels of FePO$_4$ or possibly KCl, regardless of their nutritional state. However, the fact that in the MGfe/mgFE and Kmg/kMG trials the threshold of the consumption of MgSO$_4$ was extremely close (Figure 5), this suggests that some form of regulation may be taking place.

The development of baits for monitoring and control is an important aspect of applied termite nutritional ecology because some species are pests. The single-attractant approach may not be the most effective method to produce a reliable bait. Subterranean termites are drawing nutrients from multiple sources, and thus other dietary components may limit the amount of bait termites they are able to consume even if there is a viable phagostimulant or important nutrient in the bait. Analyzing the foraging behavior of termites using a geometric approach may lead to a better understanding on how to detect termites using baits.
Acknowledgments: We thank three anonymous reviewers and the academic editor for their helpful comments. This work was supported by Southeast Missouri State University.

Author Contributions: Timothy M. Judd conceived and designed the experiments, analyzed the data and wrote the paper. James R. Landes, Hanruna Ohara and Alex W. Riley performed the experiments and are listed in alphabetical order, not by level of contribution.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Seeley, T.D.; Camazine, S.; Sneyd, J. Collective decision-making in honey bees: How colonies choose among nectar sources. Behav. Ecol. Sociobiol. 1991, 28, 277–290. [CrossRef]
2. Sudd, J.H.; Sudd, M.E. Seasonal changes in the response of wood-ants (Formica lugubris) to sucrose baits. Ecol. Entomol. 1985, 10, 89–97. [CrossRef]
3. Cook, S.C.; Eubanks, M.D.; Gold, R.E.; Behmer, S.T. Colony-level macronutrient regulation in ants: mechanisms, hoarding and associated costs. Anim. Behav. 2010, 79, 429–437. [CrossRef]
4. Cook, S.C.; Eubanks, M.D.; Gold, R.E.; Behmer, S.T. Seasonality directs contrasting food collection behavior and nutrient regulation strategies in ants. PLoS ONE 2011, 6, e25407. [CrossRef] [PubMed]
5. Judd, T.M. Relationship between food stores and foraging behavior of Pheidole ceres (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 2006, 99, 398–406. [CrossRef]
6. Sorensen, A.A.; Busch, T.M.; Vinson, S.B. Behavioral flexibility of temporal subcastes in the fire ant, Solenopsis invicta in response to food. Psyche 1985, 91, 316–331. [CrossRef]
7. Dussutour, A.; Simpson, S.J. Carbohydrate regulation in relation to colony growth in ants. J. Exp. Biol. 2008, 211, 2224–2232. [CrossRef] [PubMed]
8. Donovan, S.E.; Eggleton, P.; Bignell, D.E. Gut content analysis and a new feeding group classification of termites. Ecol. Entomol. 2001, 26, 356–366. [CrossRef]
9. Inward, D.J.G.; Vogler, A.P.; Eggleton, P.A. Comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. Mol. Phylogenet. Evol. 2007, 44, 953–967. [CrossRef] [PubMed]
10. Eggleton, P.; Tayasu, I. Feeding groups, lifetypes and the global ecology of termites. Ecol. Res. 2001, 16, 941–960. [CrossRef]
11. Reinhard, J.; Kaib, M. Trail communication during foraging and recruitment in the subterranean termite Reticulitermes santontensis De Feytaud (Isoptera, Rhinotermitidae). J. Insect Behav. 2001, 14, 157–171. [CrossRef]
12. La Fage, J.P.; Nuttering, W.L. Nutrient dynamics of termites. In Production Ecology of Ants and Termites; Brian, M.V., Ed.; Cambridge University Press: Cambridge, UK, 1978; pp. 165–232.
13. Suárez, M.E.; Thorne, B.L.; Rate, A.B. Size, amount, and distribution pattern of alimentary fluid transfer via trophallaxis in three species of termites (Isoptera: Rhinotermitidae, Termopsidae). Ann. Entomol. Soc. Am. 2000, 93, 145–155. [CrossRef]
14. Judd, T.M.; Fasnacht, M.P. Distribution of micronutrients in social insects: A test in the termite Reticulitermes flavipes (Isoptera: Rhinotermitidae) and the ant Myrmica punctiventris (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 2007, 100, 893–899. [CrossRef]
15. Waller, D.A.; La Fage, J.P. Nutritional ecology of termites. In Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates; Slansky, F., Jr., Rodriguez, J.G., Eds.; John Wiley & Sons: New York, NY, USA, 1987; pp. 487–532.
16. Abushama, F.T.; Kamal, M.A. The role of sugars in the food-selection of termite Microtermes traegardhi (Sjostedt). J. Appl. Entomol. 1977, 84, 250–255.
17. Botch, P.S.; Brennan, C.L.; Judd, T.M. Seasonal effects of calcium and phosphate on the feeding preference of the termite Reticulitermes flavipes (Isoptera: Rhinotermitidae). Sociobiology 2010, 55, 42–56.
18. Haifig, I.; Costa-Leonardo, A.M.; Marchetti, F.F. Effects of nutrients on feeding activities of the pest termite Heterotermes tenius (Isoptera: Rhinotermitidae). J. Appl. Entomol. 2008, 132, 497–501. [CrossRef]
19. Haifig, I.; Marchetti, F.F.; Costa-Leonardo, A.M. Nutrients affecting food choice by the pest subterranean termite Coptotermes gestroi (Isoptera: Rhinotermitidae). Int. J. Pest Manag. 2010, 56, 371–375. [CrossRef]
20. Saran, R.K.; Rust, M.K. Feeding, uptake, and utilization of carbohydrates by western subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 2005, 98, 1284–1293. [CrossRef] [PubMed]
21. Swoboda, L.E.; Miller, D.M.; Fell, R.J.; Mullins, D.E. The effect of nutrient compounds (sugars and amino-acids) on bait consumption by Reticulitermes spp. (Isoptera: Rhinotermitidae). *Sociobiology* 2004, 44, 547–563.

22. Waller, D.A.; Morlino, S.E.; Matkins, N. Factors affecting termite recruitment to baits in laboratory and field studies. In Proceedings of the 3rd International Conference on Urban Pests, Czech University of Agriculture, Prague, Czech Republic, 19–22 July 1999; Robinson, W.M., Rettich, F., Rambo, G.W., Eds.; Grafické Závody Hronov: Prague, Czech Republic, 1999; pp. 597–600.

23. Castillo, V.P.; Sajap, A.S.; Sahri, M.H. Feeding response of subterranean termites Coptotermes curvignathus and Coptotermes gestroi (Blattodea: Rhinotermitidae) to baits supplemented with sugars, amino acids, and cassava. *J. Econ. Entomol.* 2013, 106, 1794–1801. [CrossRef] [PubMed]

24. Haifig, I.; Jost, C.; Fourcassie, V.; Zana, Y.; Costa-Leonardo, A. Dynamics of foraging trails in the Neotropical termite Velocitermes heteropterus (Isoptera: Termitidae). *Behav. Proc.* 2015, 118, 123–129. [CrossRef] [PubMed]

25. Wallace, B.A.; Judd, T.M. A test of seasonal responses to sugars in four populations of the termite Reticulitermes flavipes. *J. Econ. Entomol.* 2010, 103, 2126–2131. [CrossRef] [PubMed]

26. Simpson, S.J.; Raubenheimer, D. A multi-level analysis of feeding behaviour: The geometry of nutritional decisions. *Philos. Trans. R. Soc. B* 1993, 342, 381–402. [CrossRef]

27. Simpson, S.J.; Raubenheimer, D. The geometric analysis of feeding and nutrition: A user’s guide. *J. Insect Physiol.* 1995, 41, 545–553. [CrossRef]

28. Behmer, S.T.; Raubenheimer, D.; Simpson, S.J. Frequency-dependent food selection in locusts: a geometric analysis of the role of nutrient balancing. *Anim. Behav.* 2001, 61, 995. [CrossRef]

29. Hewson-Hughes, A.K.; Hewson-Hughes, V.L.; Miller, A.T.; Hall, S.R.; Simpson, S.J.; Raubenheimer, D. Geometric analysis of macronutrient selection in the adult domestic cat, *Felis catus*. *J. Exp. Biol.* 2011, 214, 1039–1051. [CrossRef] [PubMed]

30. Hewson-Hughes, A.K.; Hewson-Hughes, V.L.; Coyler, A.; Miller, A.T.; McGrane, S.J.; Hall, S.R.; Butterwick, R.F.; Simpson, S.J.; Raubenheimer, D. Geometric analysis of macronutrient selection in breeds of the domestic dog, *Canis lupus familiaris*. *Behav. Ecol.* 2013, 24, 293–304. [CrossRef] [PubMed]

31. Huang, X.; Hancock, D.P.; Gosby, A.K.; McMahon, A.C.; Solom, S.M.C.; Le Couteur, D.G.; Conigrave, A.D.; Raubenheimer, D.; Simpson, S.J. Effects of dietary protein to carbohydrate balance on energy intake, fat storage, and heat production in mice. *Obesity* 2013, 21, 85–92. [CrossRef] [PubMed]

32. Jensen, K.; Mayntz, D.; Toft, S.; Clissold, F.J.; Hunt, J.; Raubenheimer, D.; Simpson, S.J. Optimal foraging for specific nutrients in predatory beetles. *Proc. Biol. Sci.* 2012, 279, 2212–2218. [CrossRef] [PubMed]

33. Lee, K.P.; Behmer, S.T.; Simpson, S.J.; Raubenheimer, D. A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisdvulu). *J. Insect Physiol.* 2002, 48, 655–665. [CrossRef]

34. Brune, A. Symbiotic digestion of lignocellulose in termite guts. *Nat. Rev. Microbiol.* 2014, 12, 168–180. [CrossRef] [PubMed]

35. König, H.; Li, L.; Fröhlich, J. The cellulolytic system of the termite gut. *Appl. Microbiol. Biotechnol.* 2013, 97, 7943–7962. [CrossRef] [PubMed]

36. Ni, J.; Tokuda, G. Lignocellulose-degrading enzymes from termites and their symbiotic microbiota. *Biotechnol. Adv.* 2013, 31, 838–850. [CrossRef] [PubMed]

37. Bentley, B.L. Nitrogen fixation in termites: Fate of newly fixed nitrogen. *J. Insect Physiol.* 1984, 30, 653–655. [CrossRef]

38. Breznak, J.A.; Brill, W.J.; Mertins, J.W.; Coppel, H.C. Nitrogen fixation in termites. *Nature* 1973, 244, 577–580. [CrossRef] [PubMed]

39. Curtis, A.D.; Waller, D.A. Seasonal patterns of nitrogen fixation in termites. *Funct. Ecol.* 1998, 12, 803–807. [CrossRef]

40. Ohkuma, M.; Noda, S.; Kudo, T. Phylogenetic diversity of nitrogen fixation genes in the symbiotic microbial community in the gut of diverse termites. *Appl. Environ. Microbiol.* 1999, 65, 4926–4934. [PubMed]

41. Prestwich, G.D.; Bentley, B.L.; Carpenter, E.J. Nitrogen sources for neotropical nasute termites: Fixation and selective foraging. *Oecologia* 1980, 46, 397–401. [CrossRef] [PubMed]

42. Janzow, M.P.; Judd, T.M. The termite Reticulitermes flavipes (Rhinotermitidae: Isoptera) can acquire micronutrients from soil. *Environ. Entomol.* 2015, 44, 814–820. [CrossRef] [PubMed]

43. Cohen, A.C. *Insect Diets Science and Technology;* CRC Press: New York, NY, USA, 2004.

44. Nation, J.L. *Insect Physiology and Biochemistry;* CRC Press: Boca Raton, FL, USA, 2002.
45. Botch, P.S.; Judd, T.M. The effects of soil cations on the foraging behavior of the termite Reticulitermes flavipes. *J. Econ. Entomol.* **2011**, *104*, 425–435. [CrossRef] [PubMed]

46. Brauman, A.; Koenig, J.F.; Dutreix, J.; Garcia, J.L. Characterization of two sulfate-reducing bacteria from the gut of the soil-feeding termite, Cubitermes speciosus. *Antonie Van Leeuwenhoek* **1990**, *58*, 271–275. [CrossRef] [PubMed]

47. Droge, S.; Limper, U.; Emittiazi, F.; Schonig, I.; Pavlus, N.; Dryzga, O.; Fischer, U.; König, H. In vitro and in vivo sulfate reduction in the gut contents of the termite Mastotermes darwiniensis and the rose-chaffer Pachnoda margarita. *J. Gen. Appl. Microbiol.* **2005**, *51*, 57–64. [CrossRef] [PubMed]

48. Kuhnigk, T.; Branke, J.; Krekeler, D.; Cypionka, H.; König, H. A feasible role of sulfate-reducing bacteria in the termite gut. *Syst. Appl. Microbiol.* **1996**, *19*, 139–149. [CrossRef]

49. Elser, J.J.; Stern, R.W.; Gorokhova, E.; Fagan, W.F.; Markow, T.A.; Cotner, J.B.; Harrison, J.F.; Hobbie, S.E.; Odell, G.M.; Weider, L.W. Biological stoichiometry from genes to ecosystems. *Ecol. Lett.* **2000**, *3*, 540–550. [CrossRef]

50. Nichol, H.; Law, J.H.; Winzerling, J.J. Iron metabolism in insects. *Annu. Rev. Entomol.* **2002**, *47*, 535–559. [CrossRef] [PubMed]

51. Judd, T.M.; Corbin, C.C. Effect of cellulose concentration on the feeding preferences of the termite Reticulitermes flavipes (Isoptera: Rhinotermitidae). *Sociobiology* **2009**, *53*, 775–784. [CrossRef]

52. Zachariah, N.; Das, A.; Murthy, T.G.; Borges, R.M. Building mud castles: A perspective from brick-laying termites. *Sci. Rep.* **2017**, *7*, 4692. [CrossRef] [PubMed]

53. Daniel, W.W. *Applied Nonparametric Statistics*, 2nd ed.; PWS-Kent: Boston, MA, USA, 1990.

54. Emmans, G.C. Diet selection by animals: Theory and experimental design. *Proc. Nutr. Soc.* **1991**, *50*, 59–64. [CrossRef] [PubMed]

55. Jungreis, A.M. Distribution of magnesium in tissues of silkworm Hyalophora cecropia. *Am. J. Physiol.* **1973**, *224*, 27–30.

56. Kiceniuk, J.; Phillips, J.E. Magnesium regulation in mosquito larvae (Aedes compestris) living in waters of high MgSO4 content. *J. Exp. Biol.* **1974**, *61*, 749–760. [PubMed]

57. Oyarzun, S.E.; Crawshaw, G.J.; Valdes, E.V. Nutrition of the tamandua: I. Nutrient composition of termites (Nasutitermes spp.) and stomach contents from wild tamanduas (Tamandua tetradactyla). *Zoo Biol.* **1996**, *15*, 509–524. [CrossRef]

58. Blumenthal, E.M. Regulation of chloride permeability by endogenously produced tyramine in the Drosophila Malpighian tubule. *Am. J. Physiol.* **2003**, *284*, C718–C728. [CrossRef] [PubMed]

59. Harvey, W.R.; Cioffi, M.; Dow, J.A.; Wolfersberger, M.G. Potassium ion transport ATPase in insect epithelia. *J. Exp. Biol.* **1983**, *106*, 91–117. [PubMed]

60. Popham, H.J.; Sun, R.; Shelby, K.S.; Robertson, J.D. Changes in trace metals in hemolymph of baculovirus-infected noctuid larvae. *Bioll. Trace Elem. Res.* **2012**, *146*, 325–334. [CrossRef] [PubMed]

61. Inoue, J.I.; Saita, K.; Kudo, T.; Ui, S.; Ohkuma, M. Hydrogen production by termite gut protists: Characterization of iron hydrogenases of parabasalian symbionts of the termite Coptotermes formosanus. *Euakrpt. Cell* **2007**, *6*, 1925–1932. [CrossRef] [PubMed]

62. Arms, K.; Feeny, P.; Lederhouse, R.C. Sodium: Stimulus for puddling behavior by tiger swallowtail butterflies, *Papilio glaucus*. *Science* **1974**, *185*, 372–374. [CrossRef] [PubMed]

63. Barrows, E.M. Aggregation behavior and response to sodium chloride in females of a solitary bee, Augochlora pura (Hymenoptera: Halictidae). *Fla. Entomol.* **1974**, *57*, 189–193. [CrossRef]

64. Pivnick, K.A.; McNeil, J.M. Puddling in butterflies: Sodium affects reproductive success in Thymelicus lineola. *Physiol. Entomol.* **1987**, *12*, 461–472. [CrossRef]

65. Spring, J.H.; Hyatt, A.D.; Marshall, A.T. Uptake and release of sodium and potassium by the fat body of the American cockroach in vitro. *J. Insect Physiol.* **1986**, *32*, 439–444. [CrossRef]

66. Bonoan, R.E.; Tai, T.M.; Tagle Rodriguez, M.; Feller, L.; Daddario, S.R.; Czaja, R.A.; O’Connor, L.D.; Burruss, G.; Starks, P.T. Seasonality of salt foraging in honey bees (Apis mellifera). *Ecol. Entomol.* **2017**, *42*, 195–201. [CrossRef]