**Title:** Interactions between nutrients and fruit defensive metabolites shape bat foraging behavior and protein absorption

**Author names:** Mariana Gelambi1,3\*, Estefania Morales-M.2,3, Susan R. Whitehead1,3

**Affiliations and addresses:** 1Department of Biological Sciences, Virginia Polytechnic Institute and State University, Latham Hall RM 427, 220 Ag Quad Lane, Blacksburg, VA 24060

2Ingenería en Ciencias Forestales y Vida Silvestre, Universidad Técnica Nacional, Barrio Lourdes, Provincia de Alajuela, Cd Quesada, Costa Rica

3La Selva Biological Station, Organization for Tropical Studies, Puerto Viejo de Sarapiquí, Heredia Province, Costa Rica

**\*Corresponding author:** Mariana Gelambi, gelambi@vt.edu, 0000-0002-6443-5420

**Abstract**

Frugivore foraging behavior is largely influenced by two key groups of chemical traits: nutrients and secondary metabolites. In fleshy fruit, nutrients incentivize consumption by mutualists (i.e., effective seed dispersers), while secondary metabolites often function to defend the pulp against antagonists. The consumption of some defensive metabolites can negatively impact both mutualistic and antagonistic frugivores, often due to toxic properties of the metabolites or through nutrient absorption interference. Frugivores are assumed to maximize nutrient acquisition while avoiding or minimizing defensive metabolite intake, but the relative roles of nutrients and defensive compounds in foraging behavior are not well understood. Here, we used a neotropical fruit bat to investigate the interactive effects of nutrients and a fruit defensive metabolite, piperine, on two essential processes in nutrient acquisition, namely foraging behavior and nutrient absorption. Through the manipulation of nutrient and piperine concentrations in artificial diets, we discovered that captive fruit bats prioritize nutrient concentrations regardless of the levels of defensive metabolites, even though piperine is a deterrent on its own. Furthermore, our findings reveal that while piperine has no detectable influence on sugar absorption, it reduces protein absorption, which is a crucial and limited nutrient in the frugivore diet. Overall, our results demonstrate the importance of considering the interaction between co-occurring chemical traits in fruit pulp to better understand frugivore foraging and physiology.

**Keywords:** *Carollia*, chemical ecology, foraging, frugivores, nutrient absorption, nutritional ecology, piperine

**Introduction**

The mutualism between fleshy fruits and seed dispersers is a critical process that promotes and maintains forest diversity [(Levey et al., 2002)](https://www.zotero.org/google-docs/?TVWtAD). To attract seed dispersers, fleshy fruits contain sugars, proteins, and lipids [(C. M. Herrera, 2002; Jordano, 2000)](https://www.zotero.org/google-docs/?wpcA5u), which can be advertised by scent (Nevo & Ayasse, 2020; Rodríguez et al., 2013) and coloration (Nevo et al., 2018; Valenta et al., 2017). However, fleshy fruits do not interact solely with seed dispersers, but with a broad community of antagonist frugivores that do not disperse seeds, including pathogens and pests [(Whitehead et al., 2021)](https://www.zotero.org/google-docs/?a1Uz74). To defend against antagonists, many fleshy fruits are rich in complex mixtures of secondary or specialized metabolites, many of which have deterrent, toxic, or antimicrobial properties (Schoonhoven et al., 2005). In some cases, defensive secondary metabolites in ripe fruit pulp also have broad bioactivity toward mutualists (Maynard et al., 2020; Whitehead et al., 2016; [Baldwin & Whitehead, 2015](https://www.zotero.org/google-docs/?YJTbtf); but see [Tewksbury & Nabhan, 2001)](https://www.zotero.org/google-docs/?W2lx1v). Thus, both mutualist and antagonist frugivores face complex foraging decisions, balancing the potential benefits of nutrient acquisition against the costs of fruit defensive metabolite ingestion.

For many frugivores, nutrients encourage consumption [(Blendinger et al., 2022; Schaefer, Schmidt, & Bairlein, 2003)](https://www.zotero.org/google-docs/?ph1vLo), while defensive metabolites typically act as deterrents [(Maynard et al., 2020; Schaefer, Schmidt, & Winkler, 2003; Whitehead et al., 2016)](https://www.zotero.org/google-docs/?jljVCo). However, frugivore foraging behavior and its physiological consequences are influenced by complex interactions among co-occurring nutrients and defensive metabolites [(Cazetta et al., 2008; Rojas et al., 2021)](https://www.zotero.org/google-docs/?NWSTGs). For instance, the role of nutrients in modulating the effects of defensive metabolites on several performance metrics in both invertebrate and vertebrate herbivores is well-documented [(Deans et al., 2016, 2017; Perkovich & Ward, 2020; Simpson & Raubenheimer, 2001; Wang et al., 2021)](https://www.zotero.org/google-docs/?cUtm7o). Nevertheless, most studies on frugivore preference have independently evaluated the effect of nutrients or defensive metabolites, leaving the tradeoffs that frugivores face between nutrient acquisition and toxin avoidance poorly explored.

A key mechanism through which nutrients and secondary metabolites can interact is when defensive metabolites alter nutrient absorption for the consumer [(Kessler & Kalske, 2018; Mithöfer & Boland, 2012)](https://www.zotero.org/google-docs/?CsHGox). Research on the effects of defensive metabolites on nutrient absorption has primarily come from the agricultural sciences, with tannins being the most extensively studied class of defensive metabolites [(Foley & Moore, 2005)](https://www.zotero.org/google-docs/?MmGPC0). Tannins, a diverse group of water-soluble polyphenols, are widely recognized for their property of interacting with and precipitating dietary proteins [(Iason, 2005; Mueller-Harvey, 2006)](https://www.zotero.org/google-docs/?VKEG0z). Studies have shown that tannins alter the foraging behavior and nutrient absorption of ruminants in a dose-dependent manner [(Mueller-Harvey, 2006; Villalba, Provenza, & Banner, 2002; Villalba, Provenza, & Bryant, 2002; Villalba & Provenza, 2005)](https://www.zotero.org/google-docs/?6TT2oF). However, the narrow focus on tannins has limited our understanding of how other classes of defensive metabolites may affect nutrient absorption of non-ruminant animals.

Here, we investigate the interactive effect of nutrients and fruit defensive metabolites on the Seba's short-tailed bat, *Carollia perspicillata* (Linnaeus, 1758), a key seed disperser of *Piper* spp. plants throughout the tropics in Central and South America [(Fleming, 1988)](https://www.zotero.org/google-docs/?e2yUM8). First, we determined the relative role of nutrients and defensive metabolites in bat preference (objective 1). Specifically, we asked (1.1) Do bats distinguish between a high- and low-nutrient diet?, (1.2) Do bats avoid fruit defensive metabolites?, and (1.3) Can a high-nutrient diet offset the impact of high concentrations of defensive metabolites? Then, we investigate the effect of a defensive metabolite on nutrient absorption (objective 2). Specifically, we asked (2.1) Do fruit defensive metabolites modify the total and individual sugar absorption?, and (2.2) Do fruit defensive metabolites modify protein absorption?

**Methods**

*Study site and study organisms*

Preference and absorption experiments were conducted at La Selva Biological Station, province of Heredia, Costa Rica during June and July 2021. The station comprises 1536 ha of lowland protected area managed by the Organization for Tropical Studies (OTS). La Selva is a mosaic of primary and secondary forests and abandoned plantation areas [(McDade et al., 1994)](https://www.zotero.org/google-docs/?XRz0gp). In La Selva, three *Carollia* bats coexist, *Carollia perspicillata, C. sowelli*, and *C. castanea* [(York et al., 2019)](https://www.zotero.org/google-docs/?MHD2dq). *Carollia* is one of the most abundant bat genera in the Neotropics [(Fleming, 2004)](https://www.zotero.org/google-docs/?Vt2nsi) and their diet is mainly ripe infructescences (hereafter fruits) of *Piper* spp. [(York & Billings, 2009)](https://www.zotero.org/google-docs/?NnWiqg). When feeding on ripe *Piper* fruits, bats encounter numerous structurally diverse secondary metabolites, including amides, lignans, neolignans, phenylpropanoids, and chalcones [(Dyer et al., 2004; Schneider et al., 2021; Uckele et al., 2021)](https://www.zotero.org/google-docs/?1zSXJf).

As a representative fruit defensive metabolite, we used the piper amide piperine. Piper amides are a group of nitrogenous metabolites found in several plant species from the genus *Piper* [(Salehi et al., 2019; Uckele et al., 2021; Whitehead et al., 2013)](https://www.zotero.org/google-docs/?MTAxUZ). In several neotropical *Piper* species, amides play a key role in the meditation of ecological interactions with mutualistic bats, ants and antagonist pests [(Dyer et al., 2003; Nelson et al., 2023; Whitehead et al., 2016; Whitehead & Bowers, 2014)](https://www.zotero.org/google-docs/?OIJExR). Piperine, an amide present in black pepper (*Piper nigrum*) and other *Piper* species, is commercially available and has been extensively investigated for its pharmacological effects on mammalian physiology [(Haq et al., 2021)](https://www.zotero.org/google-docs/?78rjsG). Multiple studies suggest that piperine can modify nutrient metabolism in mammals through different mechanisms, including the inhibition of key digestive enzymes [(Haq et al., 2021; Suresh & Srinivasan, 2010)](https://www.zotero.org/google-docs/?5t68cE). Although piperine has not been detected in the more abundant native *Piper* at La Selva, it has been reported in other neotropical species [(de O. Chaves et al., 2003; Leitão da-Cunha & de Oliveira Chaves, 2001)](https://www.zotero.org/google-docs/?Td3c9b), and *Carollia* bats may also frequently encounter piperine when consuming *Piper nigrum* from cultivated plantings ([Hernández-Leal & Sánchez, 2021](https://www.zotero.org/google-docs/?4O3a39)). It is also structurally similar to other piper amides that are an abundant part of the *Carollia* diet [(Salehi et al., 2019; Whitehead et al., 2013)](https://www.zotero.org/google-docs/?LOsvHY).

*Bat capture and maintenance*

Two males and eighteen non-reproductive female *C.* *perspicillata* bats were captured using mist nets placed in clearings and secondary forest sites in June 2021. We kept two rounds of eight and ten bats in captivity, housed in individual flight cages (2x1x1m) located in the forest. Bats acclimated for at least two nights when we fed them with a synthetic maintenance diet based on the formulation described in [Denslow et al., (1987)](https://www.zotero.org/google-docs/?z2cykw) consisting of water, agar powder (Eco-Taste), mashed ripe banana, soy protein isolate powder (Bulk Supplements), NaCl, CaHPO4 (Eisen-Golden Laboratories), vegetable oil, and wheat germ (Bob's Red Mill). Consecutive trials were then conducted with each bat over a period of nine days (see below), and, after every night’s trial, we offered water and 35 g of an unsupplemented maintenance diet to each bat. After finishing all the trials, we released the bats at the site of capture. All experiments were approved by the Comisión Nacional para la Gestión de la Biodiversidad (resolution number R-007-2021-OT-CONAGEBIO) and Virginia Tech Institutional Animal Care and Use Committee protocols (approval no. IACUC 20-212).

*Objective 1. Determine the relative role of nutrients and defensive metabolites in bat preference*

We conducted three sets of trials, each one consisting of two-choice preference experiments comparing: (1.1) low nutrients versus high nutrients, both without piperine; (1.2) low piperine versus high piperine, both with high nutrients and (1.3) high nutrients, high piperine versus low nutrients, low piperine. The first group (eight bats) participated in trials 1.1 and 1.3, whereas the second group (10 bats) participated in trials 1.1, 1.2, and 1.3. Each bat participated in two or three paired choice tests in random order on consecutive nights. We started the trials at 6:00 PM by placing two Petri dishes approximately 30 cm inside the flight cage door. To avoid the influence of previous experience, we randomized the position (left or right) of the dishes for each trial. After 30 min, we quantified the amount of food eaten in each dish and used these values as a measure of preference.

Each petri dish in the trials contained 5 g of the artificial diet described above with different amounts of nutrients and toxins. The high-nutrient option was prepared with the same proportions of ingredients used for the maintenance diet. Based on the average nutritional values reported for bananas in previous studies [(Anyasi et al., 2013)](https://www.zotero.org/google-docs/?mC5EAR), and the nutritional information provided in the packages of the protein powder and wheat germ, we estimate that 5 g (wet weight) of diet contained approximately 96 mg of sugars, 76 mg of proteins, and 5 mg of lipids. The low-nutrient option was prepared based on the same ingredients, maintaining the same ratios for NaCl, CaHPO4, oil, and wheat germ, but leaving out the protein powder and reducing the bananas by half. Thus, the 5 g of diet contained approximately 54 mg of sugars, 7 mg of proteins, and 4 mg of lipids. We added 0.1% dry weight of piperine (≥95%, Sigma-Aldrich) in the low piperine option, and 2% dry weight in the high piperine option, which represents the range of natural variation of amides in ripe neotropical *Piper* spp. fruits [(Baldwin & Whitehead, 2015; Whitehead et al., 2013)](https://www.zotero.org/google-docs/?WeyPk6). After completing all trials, bats were fed the unsupplemented maintenance diet for one night and then participated in the experiments for objective 2.

*Objective 2. Investigate the effect of a defensive metabolite on sugar and protein absorption*

We added four different concentrations of piperine (≥95%, Sigma-Aldrich), 0.1%, 0.5%, 1.5%, and 2% dry weight, directly to 10 g of the artificial diet described above. Each bat was randomly assigned one of the four concentrations and a control (unsupplemented diet) for five consecutive nights. After consuming the 10 g of diet within approximately 2-3 hours, we collected fecal samples from the bottom of the cage using a clean spatula. Nutrients remaining in the feces provide an indication of the amount of the nutrient that has been excreted and therefore has not been absorbed. We stored fecal samples at -80 °C for later analysis in the laboratory, with the exception of transport from Costa Rica to Virginia, USA on dry ice. We split each fecal sample collected in two to quantify (2.1) individual and total sugars and (2.2) total proteins. Because bat fecal samples are potentially contaminated with the respiratory pathogen *Histoplasma capsulatum*, we decontaminated samples prior to analysis by adding 1 mL of isopropanol, vortexing the mix, and evaporating the isopropanol in a SpeedVac (vacuum: 100 mTorr, temperature: 65 °C). Finally, samples were then freeze-dried and ground to a fine powder.

*Sugar extraction and quantification*

We modified the extraction and quantification of sugars using high-performance liquid chromatography (HPLC) described in [Lord et al., (2021](https://www.zotero.org/google-docs/?D7Sk1F)). We weighed 10 mg of dried fecal sample and added 1 mL of HPLC water as the extraction solvent. Subsequently, we sonicated the samples for 20 minutes. Then, we centrifuged the homogenates at 10,000×g for 10 minutes at room temperature. We transferred 0.5 mL of supernatant into another microcentrifuge tube and added 0.7 mL of acetonitrile (ACN), and let the mix sit for 1 h at room temperature. We centrifuged the homogenates at 10,000×g for 10 minutes. We mixed 100 µL of the supernatant with 900 µL of 65% ACN, and the solution was filtered into a vial using a syringe with an IC Millex-LG 13 mm mounted 0.2 µm hydrophilic millipore (PTFE) membrane filter (Millipore). Samples were injected into an HPLC instrument, Agilent 1260 Infinity series (Agilent Technologies) with an apHera NH2 Polymer, 5-μm analytical column, and a 1260 Infinity ELSD detector (Agilent Technologies). We used acetonitrile/water (65:35, v/v) as the elution solvent with a flow rate of 1.0 mL min−1. To calculate the area and retention time of the peaks, we used the software ChemStation using the default integration parameters. We aligned the peaks based on the retention time using the R package GCalignR [(Ottensmann et al., 2018)](https://www.zotero.org/google-docs/?2x8L3G). We calculated the total amount of soluble sugars adding the areas of all the 12 individual sugar peaks found in the sample and expressed the concentration as glucose equivalents based on a standard curve for glucose. Each sample was extracted and analyzed once.

*Protein extraction and quantification*

We modified the extraction in [Bonjoch & Tamayo (2001)](https://www.zotero.org/google-docs/?Iufc3K), a specific protocol to extract and quantify protein from plant tissues. We weighed 10 mg of dried fecal sample and added 1 mL of 0.05 M Tris buffer (pH 8.0), containing ascorbic acid 0.1% (w/v), cysteine hydrochloride 0.1% (w/v), polyethylene glycol (1%), citric acid monohydrate 0.1% (w/v), 2-mercaptoethanol 0.008% (v/v). We sonicated the samples for 20 min and centrifuged the homogenates at 10,000×g for 20 min at 4°C. We quantified the total amount of protein in the supernatant using a commercial colorimetric assay (Bio-Rad, 5000002) based on the Bradford method [(Bradford, 1976)](https://www.zotero.org/google-docs/?5W5YXY), adapted to a 96-well plate. We expressed the total protein concentration as bovine serum albumin equivalents. Each sample was extracted and analyzed in duplicate, and the two duplicate measures were averaged.

*Statistical analysis*

We performed all statistical analyses in R v. 4.1.3 [(R Core Team, 2021)](https://www.zotero.org/google-docs/?xBCm31). Generalized linear mixed models (GLMMs) were performed using the glmmTMB package [(Magnusson et al., 2017)](https://www.zotero.org/google-docs/?qMMWxI) and multivariate analyses were performed using the vegan package [(Oksanen et al., 2020)](https://www.zotero.org/google-docs/?UkwkBO). To address the non-normality of residuals in the GLMMs, we used the beta distribution and the logit link function, which are suitable for response variables expressed as proportions [(Smithson & Verkuilen, 2006)](https://www.zotero.org/google-docs/?MvEWAk).

For objective 1, we fitted three separate GLMMs to estimate the differences in the proportion of food eaten by the bats. Each model consisted of the proportion of food eaten in 30 min as the response variable, and each treatment as the predictor variable, namely (1.1) low nutrients versus high nutrients, both without piperine; (1.2) low piperine versus high piperine, both with high nutrients or (1.3) high nutrients, high piperine versus low nutrients, low piperine. To account for the paired structure of the response variable (proportion consumed from each dish in a choice trial), we included the bat identity as a random effect in all models.

For objective 2, we fitted two separate GLMMs to estimate the differences in sugar and protein excretion between the control and the four concentrations of piperine tested. Each model consisted of the proportion of total proteins or total sugars excreted by bats as the response variable, and each piperine concentration, namely 0, 0.1, 0.5, 1, and 2%, as the predictor variable. As we conducted repeated measurements on the same bats and used a fresh artificial diet every evening with a different banana, we included the bat identity and the trial date as random effects in all the models. We used the function ‘emmeans()’ to obtain estimated marginal means for each level of the treatment variable. In addition, to assess the effect of piperine on the relative composition of individual sugars in fecal samples, we visualized the multivariate differences in sugar composition across samples using a non-metric multidimensional scaling (NMDS) analysis. To test for statistical significance among different piperine concentrations, we first checked for homogeneity of variances using the function ‘betadisper()’ with the centroid type, then conducted a permutational multivariate analysis of variance (PERMANOVA) using the function ‘adonis2()’ with 999 permutations.

**Results**

*Objective 1. The relative role of nutrients and defensive metabolites in bat preference*

We found that the relative concentrations of fruit defensive metabolites and nutrients modify bat preferences (Table 1, Figure 1). In the first trial (1.1), comparing different nutrient concentrations, we found that bats consumed on average 2.2-fold (117%) more of the high-nutrient option relative to the low-nutrient option (*P* = 0.005). In the second trial (1.2), comparing different piperine concentrations, bats consumed on average 3.5-fold (250%) more of the low-piperine option relative to the high-piperine option (*P* = 0.002). For the final trial (1.3), we simulated a trade-off between the acquisition of nutrients and avoidance of defensive metabolites and we found that bats consumed 3.2-fold (220%) more of the high-nutrient, high-piperine option, relative to the low-nutrient, low-piperine option (*P* < 0.001).

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**Figure 1.** Two-choice feeding experiments with different nutrient concentrations and defensive metabolites. Preference was quantified as the proportion of food eaten from each option in 30 minutes. Top to bottom: Low nutrients versus high nutrients (*N* = 18 bats), 0.1% piperine versus 2% piperine (*N* = 10 bats), and low nutrients, 0.1% piperine versus high nutrients, 2% piperine (*N* = 18 bats). Each point is colored by bat ID and represents the proportion of food eaten by one individual bat. Gray lines connect the amount of food eaten by each individual bat. Black points and error bars represent the estimated marginal means and 95% confidence intervals predicted by the generalized linear mixed models (GLMMs), back-transformed from the logit scale.

**Table 1.** GLMMs output investigating bat preference for different concentrations of nutrients and defensive metabolites. One independent GLMM was run per treatment. P-values less than 0.05 are bolded.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Response variable | Treatment | Coefficient | SE | CI 95%  [Low, High] | z | P-value | MarginalR2 |
| GLM 1:  Amount of food eaten in the first trial (1.1) | High nutrients versus low nutrients | −1.23 | 0.44 | [−2.09, −0.37] | −2.79 | **0.005** | 0.558 |
| GLM 2:  Amount of food eaten in the second trial (1.2) | 2% piperine versus 0.1% piperine | 1.91 | 0.60 | [ 0.72, 3.09] | 3.16 | **0.002** | 0.714 |
| GLM 3:  Amount of food eaten in the third trial (1.3) | High nutrients, 2% piperine versus low nutrients, 0.1% piperine | −1.78 | 0.42 | [−2.60, −0.96] | −4.23 | **<0.001** | 0.690 |

*Objective 2. The effect of piperine on sugar and protein absorption*

Our results suggest that 2% piperine interferes with protein absorption in fruit bats (Table 3, Figure 2). We found that bats excreted, on average, 1.3-fold (34%) more protein when consuming 2% of piperine, relative to the control (*P =* 0.009). The lower piperine concentrations tested did not differ significantly from the control in the amount of protein excreted (Table 3).

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**Figure 2.** Proportions of sugars (left) and total proteins (right) excreted in fecal samples from bats feed on different experimental diets with varying concentrations of piperine. Each point is colored by bat ID. Black points and error bars represent the estimated marginal means and 95% confidence intervals predicted by the generalized linear mixed models (GLMMs), back-transformed from the logit scale.

**Table 2.** GLMM output estimating the effect of sugar excretion by bats fed on different piperine concentrations. The bat identity and trial date were included as random effects in the models. P-values less than 0.05 are bolded.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Predictor variable:  Treatment | Coefficient | SE | CI 95%  [Low, High] | z | P-value |
| Control, intercept | −1.76 | 0.09 | [−1.98, −1.58] | −18.85 | **<0.001** |
| 0.1% piperine | 0.03 | 0.03 | [−0.03, 0.09] | 1.04 | 0.297 |
| 0.5% piperine | 0.05 | 0.03 | [−0.01, 0.11] | 1.49 | 0.135 |
| 1.5% piperine | 0.03 | 0.03 | [−0.03, 0.09] | 1.05 | 0.292 |
| 2% piperine | −0.02 | 0.03 | [−0.08, 0.04] | −0.59 | 0.557 |
| Conditional R2 | 0.959 |  |  |  |  |
| Marginal R2 | 0.005 |  |  |  |  |

**Table 3.** GLMM output estimating the effect of protein excretion by bats fed on different piperine concentrations. The bat identity and trial date were included as random effects in the models. P-values less than 0.05 are bolded.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Predictor variable:  Treatment | Coefficient | SE | CI 95%  [Low, High] | z | P-value |
| Control, intercept | −4.12 | 0.16 | [−4.43, −3.80] | −25.93 | **<0.001** |
| 0.1% piperine | -0.03 | 0.12 | [−0.27, 0.20] | −0.30 | 0.766 |
| 0.5% piperine | 0.06 | 0.11 | [−0.16, 0.28] | 0.52 | 0.605 |
| 1.5% piperine | −0.10 | 0.12 | [−0.33, 0.13] | −0.82 | 0.410 |
| 2% piperine | 0.29 | 0.11 | [ 0.07, 0.51] | 2.59 | **0.009** |
| Conditional R2 | 0.697 |  |  |  |  |
| Marginal R2 | 0.053 |  |  |  |  |

Similarly, none of the piperine concentrations tested significantly affected total sugar excretion (Table 2) or the composition of 12 unique individual sugars (PERMANOVA, P = 0.969; Figure 3).

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**Figure 3.** Non-metric Multidimensional Scaling (NMDS) plot of different sugars excreted by bats fed on different concentrations of piperine. There was no effect of the amount of piperine ingested on the composition of sugars that were excreted by bats (final stress = 0.0838, PERMDISP2, P = 0.941; PERMANOVA, P = 0.969, F = 0.335, R2 = 0.018).

**Discussion**

Fleshy fruits contain complex mixtures of nutrients and secondary metabolites, but little is known about the interactive effects of these two key chemical traits on frugivores. Our findings suggest that nutrients and defensive metabolites can interact in complex ways to modify both animal foraging strategies and physiology. Our focal species, *Carollia perspicillata*, makes foraging decisions based on both nutrients and secondary metabolites. Our behavioral trials demonstrated that nutrients and piperine have independent effects on bat preferences, with nutrients promoting increased food consumption and piperine decreasing consumption. Our trials also showed that nutrients appear to be the primary foraging driver and a relatively high nutrient concentration can mask the deterrent effect of piperine. Finally, in our absorption experiments, we found that at relatively high concentrations of 2%, piperine significantly increased protein excretion by 34%, while none of the tested piperine concentrations had an effect on sugar absorption.

Our first trial revealed that bats preferred a high-nutrient diet (Fig. 1, Table 1). The ability to distinguish between different nutrient concentrations and optimize nutrient intake has been reported for different frugivores, including fruit bats [(Nelson et al., 2005)](https://www.zotero.org/google-docs/?AdWOlZ) and birds [(Bosque & Calchi, 2003; Schaefer, Schmidt, & Winkler, 2003)](https://www.zotero.org/google-docs/?0g2z2g). To our knowledge, this study presents the first evidence that *Carollia* bats, a crucial disperser in tropical ecosystems, may select and optimize food consumption based on its nutritional content. The biggest difference between our high-nutrient and low-nutrient diet treatments was a 90% reduction in protein content, but a simultaneous reduction in the banana added to the diet meant that several other macronutrients (soluble carbohydrates, proteins, and lipids), micronutrients, such as vitamins and minerals, and volatile organic compounds may have also changed between the two treatments. Thus, further investigation is required to unravel the specific role of different macronutrients and other attractants in shaping bat preferences. Additionally, under natural conditions, the impact of nutrient concentration on foraging behavior may be more pronounced than what we observed in our experiments with captive bats, due to the potential added costs of flying long distances and foraging.

In our second (1.2) and third (1.3) trials, we found that bats were deterred by piperine, but its negative effect was completely removed under high-nutrient conditions. Previous studies have also shown a deterrent effect of piper amides [(Whitehead et al., 2016)](https://www.zotero.org/google-docs/?99UlKZ) and other classes of secondary metabolites found in ripe *Piper* spp. pulp [(Hernández-Leal & Sánchez, 2021; Maynard et al., 2020)](https://www.zotero.org/google-docs/?ReYI8v). These studied compounds represent only a small fraction of the secondary metabolites that *Carollia* spp. encounter when consuming *Piper* [(Maynard et al., 2019; Santana et al., 2021)](https://www.zotero.org/google-docs/?qWIoKn), which are notably diverse in terms of the metabolite classes that occur in ripe fruit pulp [(Salehi et al., 2019; Uckele et al., 2021)](https://www.zotero.org/google-docs/?CPWJzW). Although fruit bats likely possess physiological adaptations that enable them to tolerate and/or detoxify some secondary metabolites, it is clear that many secondary metabolites do deter consumption. Our third preference trial revealed that bats are less deterred by high piperine concentrations when the nutrient concentrations are also relatively high. This shift in bat preference could occur through two mechanisms; either higher nutrient concentrations mask the ability of bats to detect secondary metabolites, or bats make flexible foraging decisions depending on the relative abundances of nutrients and secondary metabolites.

The influence of nutrients on taste perception in the consumption of defensive metabolites is a crucial factor in determining feeding preferences. Nutrients can alter the consumer taste perception of potentially deterrent defensive metabolites, reducing or masking unpleasant tastes such as bitterness and astringency. In nectar, for instance, high concentrations of sugars conceal the presence of toxins in the animal taste receptors, promoting the consumption of high-sugar, high-toxin diets [(Lerch-Henning & Nicolson, 2015)](https://www.zotero.org/google-docs/?A06OYk). Similarly, the interaction between proteins and tannins can impede consumer taste receptors from detecting tannins [(Behmer et al., 2002; Simpson & Raubenheimer, 2001)](https://www.zotero.org/google-docs/?jrwVWx). It is unlikely that this mechanism operates in our study system because bats were able to avoid high piperine concentrations in the second preference trials, where the diet nutrient concentration was comparable to the high-nutrient diet in the third trial.

Alternatively, frugivores can exhibit flexible foraging decisions, even when detecting secondary metabolites, likely based on the relative costs and benefits of consuming different ratios of secondary metabolites and nutrients. A higher nutrient intake during the digestion of well-defended plant material may provide the necessary energy for detoxification processes, which are often energetically demanding [(Iason, 2005)](https://www.zotero.org/google-docs/?orjEEG). As reported for some mammal species consuming different toxin classes [(Iason, 2005)](https://www.zotero.org/google-docs/?140S00), piperine may also impose an additional energetic cost allocated to detoxification pathways. Therefore, when feeding on a well-defended fruit, bats might optimize nutrient intake to fuel detoxification reactions. Defensive metabolite consumption can lead to amino acid losses, which often stimulate the consumer to forage for additional protein [(Au et al., 2013; Wang et al., 2021)](https://www.zotero.org/google-docs/?RVLZky). A similar mechanism might be operating in our study system because our absorption experiment showed that bats that consume a relatively high concentration of piperine showed a significant increase in protein excretion.

Our results reveal that while the tested piperine concentration did not significantly affect sugar absorption, 2% piperine increased protein excretion by 34%, indicating a significant interference with protein absorption (Fig. 2). Piperine could alter protein absorption in fruit bats by several mechanisms. First, piperine may affect enzymes involved in protein absorption. Evidence from pharmaceutical trials in mice and other mammals suggests that piperine inhibits a number of different digestive enzymes [(Db et al., 2018)](https://www.zotero.org/google-docs/?a62X3u). Second, it has been shown that amides cause a significant reduction in gut retention time in *Carollia* bats [(Baldwin & Whitehead, 2015)](https://www.zotero.org/google-docs/?CJFDW1), and other mammals [(Srinivasan, 2007)](https://www.zotero.org/google-docs/?YvDAzu), and shorter retention times can reduce nutrient absorption [(Karasov, 2011)](https://www.zotero.org/google-docs/?GiYElK). Regardless of the mechanism, interfering with protein absorption can have ecological implications for frugivores, as numerous studies indicate that fleshy fruits are often nutritionally imbalanced, being high in carbohydrates but low in proteins [(Lei et al., 2021)](https://www.zotero.org/google-docs/?n3QhI3). To compensate for the low-protein fruit pulp, many fruit bat species occasionally consume insects, pollen, and leaves as dietary supplements [(Pereira et al., 2018; Stevens, 2022)](https://www.zotero.org/google-docs/?YTZmDL), though others rely mainly on dietary protein from fruit [(Herrera et al., 2001)](https://www.zotero.org/google-docs/?SjenyW). In addition, choice experiments indicate that frugivorous and nectarivorous bats do not consistently prefer the highest protein options, which suggests that bats may have relatively low protein requirements [(Mqokeli & Downs, 2014; Rodríguez-Peña et al., 2013)](https://www.zotero.org/google-docs/?YrTQeZ). More research is needed on the protein requirements of fruit bats to understand the broader ecological consequences of interactions between plant defensive metabolites and protein absorption.

Taken together, the results from our preference and absorption experiments provide support for the ‘nutrient-toxin titration hypothesis’ [(Cipollini & Levey, 1997b)](https://www.zotero.org/google-docs/?0iqwNI), which predicts a positive correlation between nutrients and defenses in fruit pulp, arguing that nutritional rewards can compensate for any negative effects of toxins on mutualists. Past evidence from two frugivorous bird and two frugivorous mammal species also showed that the deterrent effects of glycoalkaloids, a class of defensive metabolites primarily limited to the Solanaceae family, are reduced at higher nutrient content [(Cipollini & Levey, 1997b)](https://www.zotero.org/google-docs/?PxZDQW). However, in contrast to our results, in that system, high nutrient concentrations did not fully compensate for the deterrent effects of the defensive metabolites, and frugivores remained strongly deterred by glycoalkaloids even at high nutrient concentrations [(Cipollini & Levey, 1997b)](https://www.zotero.org/google-docs/?TjyGU0). Similarly, evidence from a mammalian herbivore and the terpene 1,8-cineole, showed that increasing nutrients can partially, but not fully, compensate for the negative effects of defensive metabolites on preference [(Bedoya-Pérez et al., 2014)](https://www.zotero.org/google-docs/?FV6eeJ). Our study highlights the limited generalizability of patterns observed in how animals balance the trade-off between nutrients and potentially toxic defensive metabolites. The effects of defensive metabolites as deterrents often depend on the specific metabolites studied, and our findings suggest that fruit bat foraging is more likely to be driven by nutrients compared to other plant consumers.

In addition to the consequences for frugivores, interactions between nutrients and defensive metabolites can have complex consequences for plant fitness that may not always be obvious. For example, while deterring effective seed dispersers may initially appear detrimental to the plant reproductive success, a frugivore’s early departure can be beneficial in some cases. For instance, the 'protein assimilation hypothesis' proposes that certain secondary metabolites interfere with protein assimilation that may force frugivores to leave the plant earlier and search for alternative high-protein food sources [(Cipollini & Levey, 1997a)](https://www.zotero.org/google-docs/?ldjEKW). During the new foraging events, seeds would be dispersed over longer distances, away from the parent plant, reducing competition for resources. Our results are consistent with the ‘protein assimilation hypothesis’. Furthermore, we did not find a significant effect on sugars excreted. From the plant's perspective, selectively modifying nutrient absorption could benefit the seed dispersal process. By limiting the absorption of proteins while still providing high concentrations of easily assimilable sugars, the plant could energetically fuel the locomotion of seed dispersers, promoting future foraging bouts. Future foraging bouts, especially long-distance ones, would be less likely if fruit secondary metabolites interfered with the absorption of all the nutrients in the fruit pulp, as the energy available for flying would be reduced.

Future studies should focus on disentangling the individual effects of nutrients and other attractants to elucidate the primary drivers of bat foraging behavior, incorporating potential sources of variations in these drivers over time and space, including variation in bat reproductive stages, seasons, or bat populations. Furthermore, a comprehensive exploration of diverse secondary metabolites is crucial, considering the broad range of compounds found in species such as *Piper spp.* Our study focuses on piperine, as a representative defensive metabolite, demonstrating the broad potential for interactions between nutrients and defenses in shaping bat foraging behavior. Examining a wide range of ecologically relevant compounds will deepen our understanding of the diverse and complex consequences of these interactions for frugivory and seed dispersal.

In conclusion, our study shows complex relative and interactive effects between nutrients and a representative secondary metabolite, piperine, on fruit bat behavior and physiology. Taken together, our results highlight the importance of considering both the nutritional and defensive properties of food in understanding animal foraging behavior. Our results also showed that relatively high concentrations of defensive metabolites do not significantly affect sugar absorption but decrease protein absorption. Our findings capture the foraging dynamic of a fruit bat on chemically heterogeneous resources, and the physiological changes associated with it, highlighting the importance of integrating nutrients and defensive metabolites in chemical and nutritional ecology.

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**Data Accessibility Statement:** Upon acceptance, all data, metadata, and R scripts used to generate results and figures will be archived in a Zenodo repository.