Mosquitoes self-medicate according to the dynamics of a microsporidian infection

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Abstract

Immune responses protect against infectious diseases but often incur costs, such as oxidative stress. In mosquitoes, these costs may drive behaviours that manage oxidative homeostasis, such as self-medicating by consuming nectar containing biologically active substances, including prooxidants and antioxidants. We examined whether *Anopheles gambiae* mosquitoes adjust their dietary preferences when infected with the microsporidian parasite *Vavraia culicis*. Using sugar solutions supplemented or not with hydrogen peroxide (a prooxidant) or ascorbic acid (an antioxidant), we assessed the feeding preferences of uninfected and infected mosquitoes at different ages and measured the effects of these diets on oxidative homeostasis and parasite load. Uninfected mosquitoes preferred sugar alone, possibly to avoid oxidative stress caused by the supplemented diets. In contrast, infected mosquitoes initially preferred the prooxidant diet, which reduced parasite load and oxidative stress, but later shifted towards the antioxidant diet, which had little effect on parasite load or oxidative stress. These findings demonstrate that mosquitoes can self-medicate by consuming nectar with appropriate levels of prooxidants and antioxidants, adjusting their preference dynamically with infection progression. Given the role of mosquitoes as major vectors of infectious diseases, these results may have significant public health implications.

Introduction

Many animals change their behaviour when they are infected by a parasite. While this is often interpreted as manipulation by the parasite in an attempt to increase its transmission, it can also be an adaptive response of the infected animals to increase their reproductive fitness. Anti-parasitic behaviour has indeed been observed in many animals, including arthropods [1] and primates [2]. One such behaviour is self-medication, that is, the use of secondary plant chemicals or other non-nutritive substances [3], either therapeutically as a response to parasitic infection [4] or prophylactically (often in anticipation of a high parasite risk [5–8]). However, the boundaries between nutrients, medicines and toxins are flexible and are often determined solely by the ingested dose of a chemical [9].

Self-medication was already described by Aristotle in his work “Historia animalium” (384-322 B.C.) [10]. It was initially thought to require observation, learning and conscious decision-making [2,11], and thus restricted to animals with high cognitive abilities [12]. A typical example is a chimpanzee seeking medicinal plants to treat its diseases [3,13]. However, self-medication has been observed in “less intelligent mammals” [14], but also in insects (like moths [4,15], ants [5] and fruit flies [16]), indicating innate-based medication.

Mosquitoes are intriguing species to study self-medication. As vectors carrying parasites with significant implications for human health, mosquitoes feed on nectar [17] and thereby ingest many biologically active compounds, some of which possess anti-parasitic properties [18]. In addition, the

compounds can have oxidative or antioxidative properties, thus affecting the production of reactive oxygen species (i.e., ROS, metabolic by products) and the development of oxidative stress (i.e., OS) in mosquitoes [19], both of which mediate life-history traits [20,21] and the immune response [22]. Therefore, the protective mechanisms that shield mosquitoes from parasites come at a cost in the form of OS, increasing the selective pressure for them to manage their oxidative homeostasis.

Finally, the plants that mosquitoes feed on have a strong influence on the outcome of their infection by, for example, malaria parasites [23], probably because of a combination of the toxic secondary metabolites and the nutritional quality of the nectar.

Here, we tested whether the mosquito *Anopheles gambiae* engages in therapeutic self-medication after infection with the microsporidian parasite *Vavraia culicis* [24] by feeding on sugar sources supplemented with biologically relevant compounds. As nectars naturally consumed by mosquitoes vary in their composition of prooxidants, such as hydrogen peroxide, and antioxidants, such as ascorbic acid, we compared a standard sugar solution with two supplemented alternatives: one containing hydrogen peroxide and the other ascorbic acid, both at concentrations typically found in nectar [25–28]. Prooxidants can enhance immune responses by increasing oxidative stress, albeit at a physiological cost, whereas antioxidants may mitigate these costs but risk impairing parasite defence mechanisms. These dual and potentially opposing effects made these compounds particularly relevant for studying self-medication in mosquitoes.

Additionally, we examined whether the mosquitoes' choice was influenced by their age (and, consequently, the parasite's developmental stage). We further evaluated whether this behaviour adhered to all the criteria for therapeutic medication previously reported [6,29]: **(i)** the behaviour must be initiated by a parasite infection, **(ii)** it must be avoided by uninfected individual because of the associated cost, **(iii)** it must consist on the ingestion of a chemical compound, **(iv)** the compound must be relevant in the natural environment of the host, and **(v)** the behaviour must increase the reproductive success (that is, fecundity or longevity) of infected individuals, either by killing the parasite or increasing tolerance to infection. Finally, we explored the potential broader implications of such behaviour, which may be more widespread than previously anticipated, and highlighted the direct public health relevance of these findings given the role of mosquitoes as vectors for infectious diseases.

Materials and Methods

Experimental system

We used the Kisumu strain of *An. gambiae s.s* [30], which had been maintained at our standard laboratory conditions (about 600 individuals per cage, constant access to 6% sucrose solution, 26 ± 1ºC, 70 ± 5% relative humidity and 12 h light/dark) for many years before the experiments.

The microsporidian *V. culicis floridensis* was provided by J.J Becnel (USDA, Gainesville, FL, USA). Originally discovered in *Aedes albopictus*, this parasite was later found to parasitize several mosquito genera, including *Aedes*, *Culex*, *Anopheles*, *Culiseta*, *Ochlerotatus* and *Orthopodomyia* [31–33]. We maintained it by alternately infecting *Ae. aegypti* and *An. gambiae* to ensure its status as a generalist parasite, as described previously [24].

V. culicis is an obligatory, intracellular parasite. Mosquitoes are infected when, as larvae, they ingest the spores along with their food. After several rounds of replication, the parasite produces new infectious spores that spread to gut and fat body cells. The spores are released to larval sites when infected larvae die, when adults die on the surface of water, or when eggs covered with spores are laid onto the surface of water.

Mosquito rearing and maintenance

Freshly hatched (0-3 hours old) larvae were individually placed into 12-well culture plates, each containing 3 ml of deionized water. The larvae received Tetramin Baby® fish food every day according to their age (0.04, 0.06, 0.08, 0.16, 0.32 and 0.6 mg/larva respectively on days 0, 1, 2, 3, 4 and 5 or older [34]).

Two-day-old larvae were exposed to either 0 or 10,000 spores of *V. culicis*. Pupae were transferred to individual 50 ml falcon tubes with approximately 10 ml of deionized water [35]. Female adults were individually moved to 150 ml cups (5.5 cm Ø x 10 cm) covered with a net. The cups contained a 50 mm Ø petri dish (to prevent mosquitoes from drowning) floating on the surface of 50 ml deionized water, along with a 10 x 7 cm rectangular filter paper to maintain humidity. The mosquitoes were provided with uncoloured cotton balls soaked in a 10% sucrose solution, which were replaced daily up to the day before the assay.

Diets

We compared three diets: 10% sucrose (referred to as sugar diet), 10 % sucrose supplemented with 8 mM of hydrogen peroxide (prooxidant diet) and 10% sucrose supplemented with 1mg/mL of ascorbic acid (vitamin c; antioxidant diet). The doses used for the different treatments correspond to concentrations found in nectar [25–28], and had been tested before the experiment to ensure they did not kill the mosquitoes. Redox cycles induced by hydrogen peroxide and ascorbic acid have been suggested as the most likely defence mechanism against a broad spectrum of bacteria and fungi in nectar [36,37].

Preference

The preferences of infected and uninfected mosquitoes were tested 0, 4 and 8 days after their emergence as adults. The diets were compared pairwise, that is, mosquitoes were put individually into cups with two food choices: prooxidant and sugar, antioxidant and sugar, or prooxidant and antioxidant. The

solutions were coloured with pink or green food dye (Trawosa®). An equal number of mosquitoes were tested for each diet in pink and green to prevent a possible preference for the colour from biasing the results.

The mosquitoes were starved for 12 hours before the test and were given 30 minutes to choose. They were then collected in a 2 ml Eppendorf tube and frozen at -20º C to prevent any digestion, and their choices were assessed from the colour of their abdomen observed under a dissecting scope.

On the right wing of every mosquito we measured the distance from the axillary incision to the tip [38,39] with the software ImageJ v1.54 [40]. The remaining portion of the mosquito, together with a stainless-steel bead (Ø 5 mm), was put into a 2 ml Eppendorf tube containing 0.1 ml of deionised water, and homogenised with a Qiagen TissueLyser LT at a frequency of 30 Hz for two minutes. 0.1 µl of each sample was added to a haemocytometer and the spores were counted at 400x magnification. All of the exposed mosquitoes harboured spores.

Oxidative stress

Female adults received one of the diets (uncoloured cotton balls soaked in the sugar, the prooxidant or the antioxidant solution) up to seven days after emergence. The next day, alive individuals were collected in a 2 ml Eppendorf tube, weighed, snap-frozen and then stored at -80º C until we measured oxidative stress and spore load.

We assessed oxidative stress [41,42] with a combination of the reduced (GSH) and the oxidised (GSSH) form of glutathione using an HPLC-MS. GSH neutralizes reactive oxygen species with a chemical reaction that converts it into GSSG [43]. Our three measures of oxidative stress were therefore the amount of GSH, the amount of GSSG and, the proportion of GSSG over the total glutathione (i.e., tGSH = GSH + GSSG), which is a reliable indicator of cellular oxidative stress [44]. Note that an analysis of the total glutathione content (GSH + GSSG) would be biased by the reduced form, since the concentration of GSH is usually much higher than that of GSSG; in our experiment there was a 25-fold difference between the two.

To measure GSH and GSSG [45] we kept the samples and ice. We added a stainless-steel bead (Ø 5 mm) and 80 µl of PBS (pH = 7.4) to each sample, and homogenised it with a Qiagen TissueLyser LT at 45 Hz for four minutes. The homogenates were centrifuged at 10 000 RPM at 4º C, and 12 µl of the supernatant was transferred to a 2 ml Eppendorf tube; the rest of the homogenate was stored at -20º C for later spore load quantification, as described for the experiment on preference. We added 10 µl of formic acid (1%), 181 µl of mqH_2O and 5 µl of glutathione reduced ethyl ester (800ng/ml, which serves as the internal standard; Sigma-Aldrich®), and vortexed the samples for 10 seconds. After centrifuging the samples for 15 min at 13 000 RPM at 4º C, 180 µl of the extract was pipetted into a syringe with a filter, and the extract was gently blown into a 250 µl conical glass insert placed inside a vial.

The samples were then injected into a HPLC-MS system for quantification. The amounts of GSH (Sigma-Aldrich®) and GSSG (Sigma-Aldrich®) were based on linear regression from ten calibration points (0.5–500 ng/mL). The liquid chromatography-mass spectrometry analyses were performed on a QTRAP 6500+ (Sciex) coupled to Acquity UPLC® I-class (Waters). The column used was an Acquity UPLC® HSS T3 (1.8 µm, 2.1 x 100 mm) at a temperature of 25°C. The mobile phases were milliQ water-1mM ammonium formate-0.05% formic acid (A) and acetonitrile-0.05% formic acid (B), with the following gradient elution: 100% A to 95% A in 2 min, at 4.7 min 100%B until 6.5 min, and at 6.7 min 100% A, then re-equilibration to 100% A for 4.8 min for a total run time of 11.5 minutes. The flow rate was 0.4 mL/min, with the volume injected equal to 2 µL.

With these settings, the limit of detection (LOD) for GSH was 0.07 ng/ml while the limit of quantification (LOQ) was 0.25 ng/ml. The LOD for GSSG was 0.5 ng/ml, and the LOQ was 2 ng/ml. The retention times were for GSH 2.06 min, GSSG 2.83 min and IS (GSH-ee) 3.60 min. Detection in ESI positive mode and for the quantification MRM (Multiple Reaction Monitoring) mode was used.

Table 1. MRM Transitions, Declustering Potential, Collision Energy, and Collision Cell Exit Potential for GSH, GSSG, and IS detection and quantification.

Compound	MRM transition	DP (V)	CE (V)	CXP (V)	Dwell time (msec)
GSH Quanti	308.0 → 179.0	56	17	14	30
GSH Quali	308.0 → 162.0	66	33	14	30
GSSG Quanti	613.2 → 355.1	16	33	24	30
GSSG Quali	613.2 → 484.2	21	31	12	30
IS	336.0 → 190.0	71	21	14	30

DP, declustering potential. CE, collision energy. CXP, collision cell exit potential.

Source (ESI) parameters
Ion spray voltage: 5500 V
Curtain gas: 35
Ion source gas: GS1 40 and GS2 35
Temperature: 550 °C

Statistical analyses

All analyses were conducted with the R software [46] version 4.4.2, using the packages DHARMa [47], car [48], glmmTMB [49], emmeans [50] and multcomp [51]. Significance was assessed with the "Anova" function of the "car" package [48]. We used a type III ANOVA in the case of a significant interaction and a type II ANOVA otherwise. When relevant, we performed post-hoc multiple comparisons with the package "emmeans", using the default Tukey adjustment.

The preference for a diet of each mosquito was coded as a binary response. Preference in each choice test (prooxidant vs. sugar, antioxidant vs. sugar, prooxidant vs. antioxidant) was analysed with a generalised linear model with a binomial distribution of errors, where the explanatory variables were age, infection status and their interaction. In a preliminary analysis we had included wing length and colour of the diet, but omitted both factors from the results shown here, for they explained very little of the variance.

We also considered the impact of spore load on this preference by analysing only infected individuals with a generalised linear model with a binomial distribution of errors, in which infection status was replaced by spore load, and wing length was added as a covariable. Because spore load did not affect this preference, we omitted it from the analyses and results presented here.

The GSH content was analysed with a linear model with a Gaussian distribution of the errors, and the GSSG content was analysed with a generalised linear model with negative binomial distribution of errors, both with infection status, diet and their interaction as explanatory variables, and mosquito weight as a covariable.

The ratio of GSSG to total glutathione (tGSH) was analysed with a generalised linear model with Gamma distribution of errors, where infection status, diet and their interaction, were the explanatory variables and mosquito weight was a covariable.

The presence of spores was coded as a binary response (presence or absence of detectable spores), and analysed with a generalized linear model with a binomial distribution of errors; the spore load was analysed with a generalised linear model with a quasi-Poisson distribution of errors. In both analyses the explanatory variables were diet, GSH content, GSSG, diet and their interactions with each of these variables, and weight was included as a covariable. Because we counted the number of spores in a haemocytometer containing 0.1 µl of the sample (i.e. 1/1000 of the total volume), the detection threshold was estimated to be 1000 spores.

Results

Preference

Prooxidant vs Sugar: Overall, about 56 % of the mosquitoes chose the sugar diet and 44% the prooxidant diet. Infected individuals, however, were more likely to feed on the prooxidant diet than uninfected individuals (57% vs 31%, $\chi^2 = 23.85$, df = 1, $p < 0.001$). This preference was only observed 0 days (67%) and 4 days (70%) after emergence, and decreased to 33% 8 days after emergence ($\chi^2 = 18.33$, df = 2, $p < 0.001$, **Fig. 1a**).

Antioxidant vs Sugar: 62% of the mosquitoes chose the sugar diet and 38% the antioxidant diet. Infected individuals, however, were more likely to feed on the antioxidant diet than uninfected

individuals (47% vs 29%, χ^2 = 11.54, df = 1, p < 0.001), but this preference was only observed 8 days (65%) after emergence, and increased with the age of the mosquitoes (χ^2 = 7.69, df = 2, p = 0.021, **Fig. 1b**).

Prooxidant vs Antioxidant: 51% of the mosquitoes chose the prooxidant diet and 49% the antioxidant diet. Averaged across ages, infected individuals (54%) and uninfected individuals (49%) were as likely to feed on the prooxidant (χ^2 = 0.90, df = 1, p = 0.343). Younger individuals, however showed a stronger preference for the prooxidant and lost their preference as they got older (χ^2 = 5.35, df = 2, p = 0.068, **Fig. 1c**).

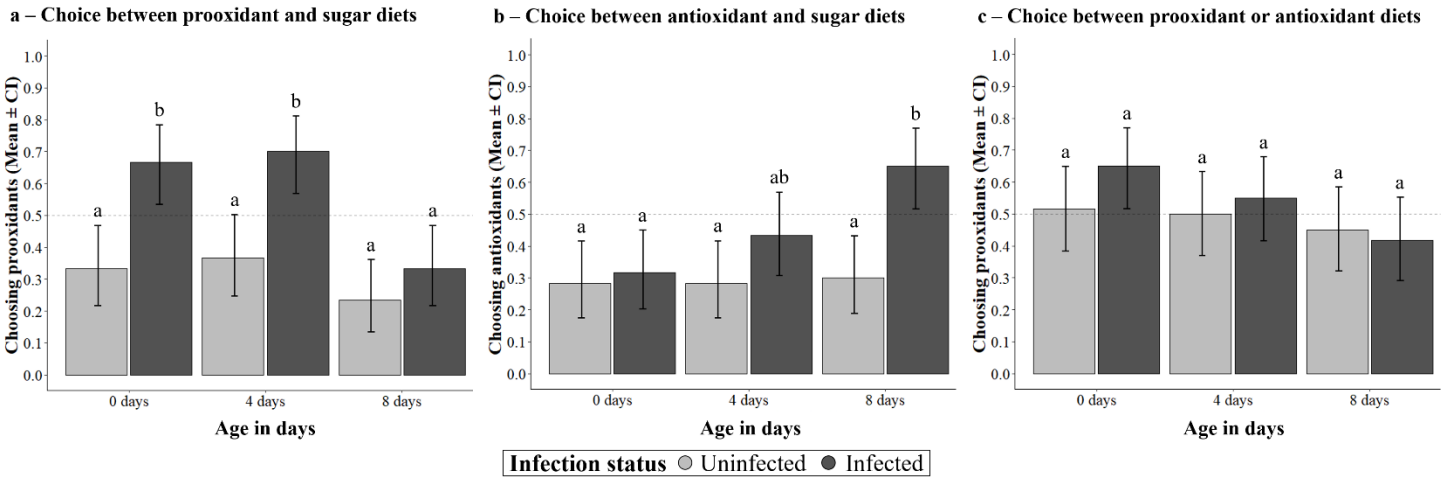

Figure 1. Food preferences of infected and uninfected mosquitoes across different age classes when given two food choices. The proportion of mosquitoes **(a)** choosing the prooxidant diet when offered a choice between prooxidant and sugar diets, **(b)** choosing the antioxidant diet when offered a choice between antioxidant and sugar diets, and, **(c)** choosing the prooxidant diet when offered a choice between prooxidant and antioxidant diets. The sample size underlying each bar was 60, and error bars show the 95% confidence intervals of the means. Letters indicate statistically significant differences from multiple comparisons within each choice.

Oxidative stress

Glutathione reduced (GSH): Infected individuals contained less GSH than uninfected individuals (1785 ng vs 1980 ng, χ^2 = 4.74, df = 1, p = 0.029), independently of the diet (χ^2 = 0.63, df = 2, p = 0.730) and the interaction between infection status and diet (χ^2 = 3.67, df = 2, p = 0.160, **Fig. 2a**).

Glutathione oxidised (GSSG): In contrast, the amount of GSSG was not affected by infection status (infected: 72 ng; uninfected: 73 ng, χ^2 = 0.32, df = 1, p = 0.570), but was greater if the diet was supplemented with either the prooxidant or the antioxidant (prooxidant: 92 ng; antioxidant: 89 ng; sugar: 46 ng; χ^2 = 101.05, df = 2, p < 0.001) and was affected by the combination of infections status and diet (χ^2 = 41.80, df = 2, p < 0.001, **Fig. 2b**). Thus, infected individuals contained more GSSG than uninfected

ones if they fed on the sugar diet (61 ng vs 36 ng), a similar GSSG if they fed on the antioxidant (93 ng vs 86 ng) and less GSSG content if they fed on the prooxidant (66 ng vs 127 ng).

GSSG/tGSH: Combining these results to consider a measure of oxidative stress - the ratio of GSSG over total glutathione – showed that oxidative stress was similar in infected an uninfected individuals (0.0458 vs 0.0364, χ^2 = 1.90, df = 1, p = 0.168), that individuals taking the sugar diet had a lower level of oxidative stress than those feeding on either supplement (sugar: 0.0256; prooxidant: 0.0508; antioxidant: 0.0521; χ^2 = 82.53, df = 2, p < 0.001) and that the effect of the diet was influenced by infection status (χ^2 = 35.05, df = 2, p < 0.001, **Fig. 2c**). Infected individuals had a higher level of oxidative stress than uninfected individuals when consuming the sugar diet (0.0400 vs 0.0164), a similar level when consuming the antioxidant diet (0.0581 vs 0.0468), and a lower level when consuming the prooxidant diet (0.0414 vs 0.0625).

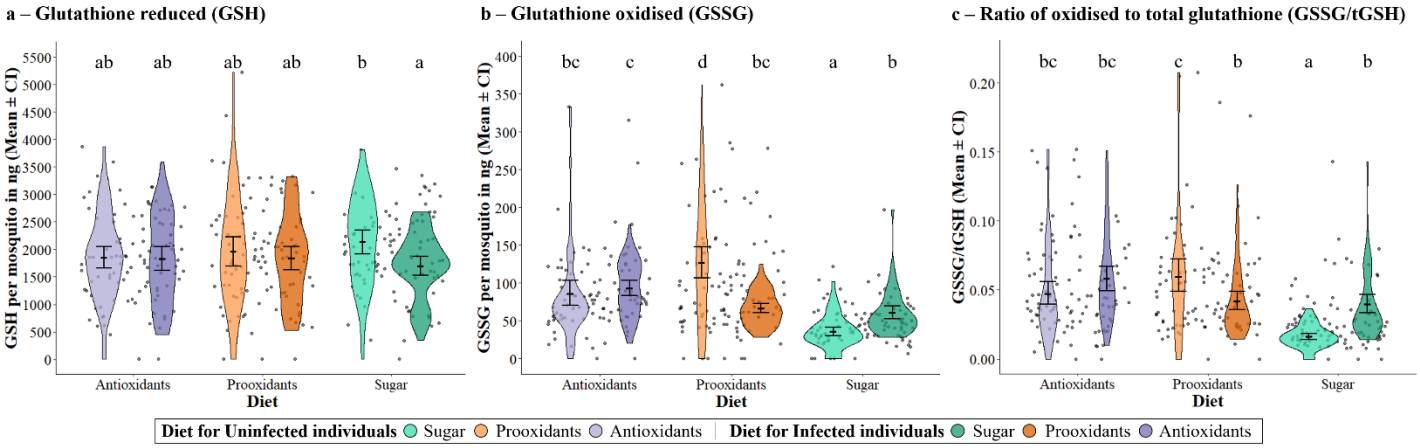

Figure 2. Oxidative stress of infected and uninfected mosquitoes after consuming a specific diet for 7 days. (a) GSH and **(b)** GSSG content in ng per mosquito. Note that the scales differ between GSH and GSSG content. The proportion of **(c)** oxidised glutathione per mosquito, calculated by dividing the GSSG content over the total glutathione (i.e., tGSH = GSH + GSSG). The sample sizes were 59, 48, 60, 50, 55 and 48. Error bars show the 95% confidence intervals of the means. Letters indicate statistically significant differences from multiple comparisons.

Spore load

All of the individuals feeding on sugar harboured spores, while only 82% were infected if they fed on the prooxidant and 87% if they fed on the antioxidant (χ^2 = 10.70, df = 2, p = 0.005, **Fig. 3a**). The proportion of mosquitoes harbouring spores decreased with the amount of GSSG (χ^2 = 5.03, df = 1, p = 0.025), but was not affected by the amount of GSH (χ^2 = 2.95, df = 1, p = 0.086) or any of the interactions between diet, GSSG and GSH (diet * GSH content: χ^2 = 0.68, df = 2, p = 0.712; diet * GSSG content: χ^2 = 0.06, df = 2, p = 0.970; GSH content * GSSG content: χ^2 = 1.49, df = 1, p = 0.221; diet * GSH content * GSSG content: χ^2 = 0.00, df = 2, p = 0.998).

If the mosquitoes were infected the spore load was higher if they fed on sugar (8.2 x 10^4) than on either of the supplements (prooxidant: 4.5 x 10^4; antioxidant: 4.7 x 10^4; χ^2 = 8.11, df = 2, p = 0.017, **Fig. 3b**). Spore load decreased with GSH content if the mosquitoes fed on the prooxidant, but not on the other diets (diet * GSH content: χ^2 = 7.81, df = 2, p = 0.020, **Fig. 3c**), and it decreased with GSSG content if they had fed on either supplemented diet (diet * GSSG content: χ^2 = 5.78, df = 2, p = 0.050, **Fig. 3d**).

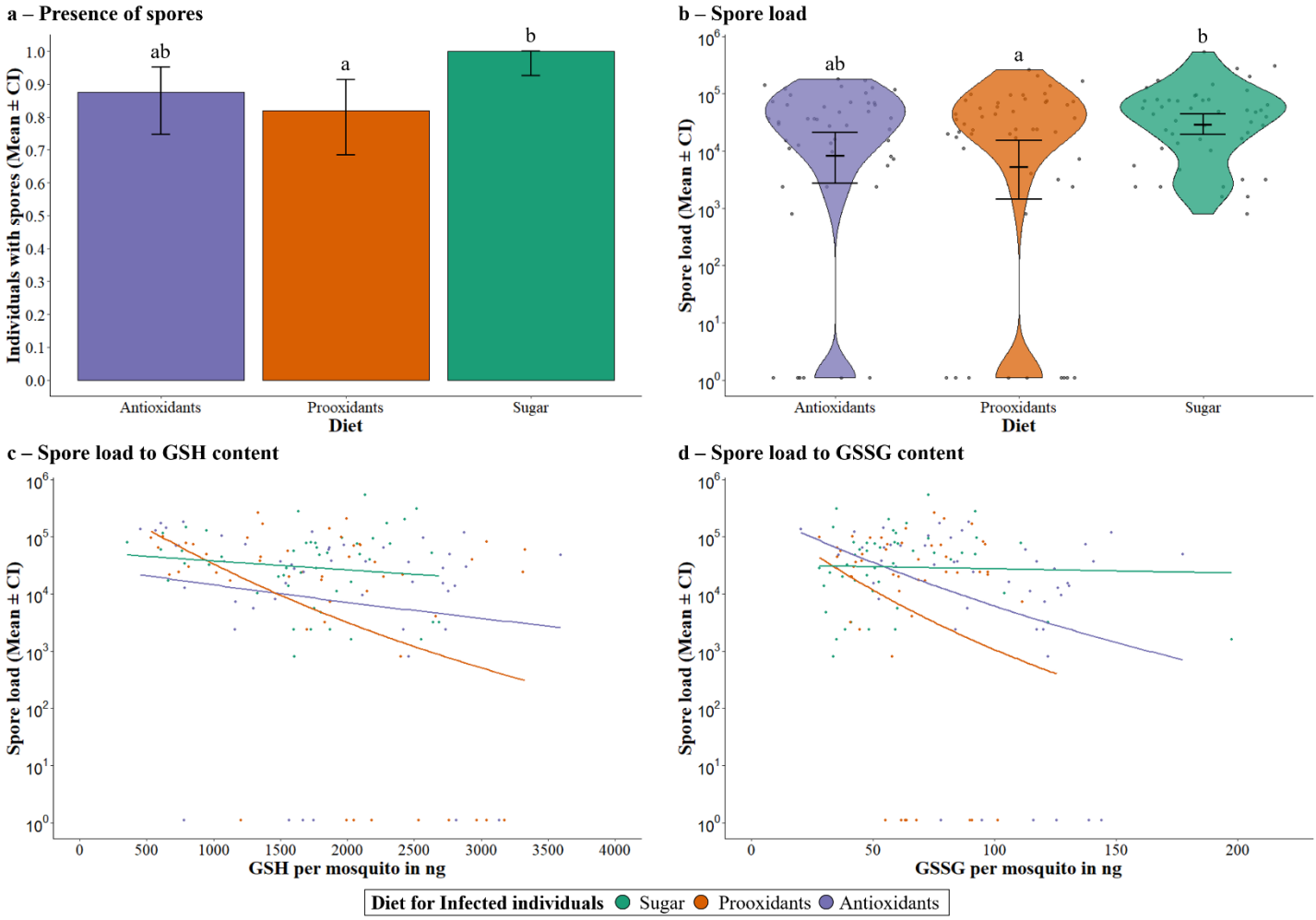

Figure 3. Parasite load of infected mosquitoes after consuming a specific diet for 7 days. The proportion of mosquitoes that had detectable spores for **(a)** each diet, and the spore load for **(b)** each diet, **(c)** GSH or **(d)** GSSG content in ng per mosquito. The sample sizes were 48, 50 and 48 and for ease of visualisation, a smooth line was drawn to show the evolution of the spore load with an increase of GSH or GSSG content, using the "glm" method, family = "quasipoisson" in ggplot2. Error bars show the 95% confidence intervals of the means. Letters indicate statistically significant differences from multiple comparisons.

Discussion

We demonstrated that infection of mosquitoes by *V. culicis* induced significant shifts in their dietary choices. These shifts disrupted the mosquitoes' oxidative homeostasis and delayed parasite

development, supporting the hypothesis of therapeutic self-medication. Importantly, uninfected mosquitoes actively avoided these diets, further reinforcing this interpretation. In the following sections, we delve deeper into our results, exploring the potential mechanisms by which these dietary changes influence infected and uninfected mosquitoes, emphasizing their effects on oxidative homeostasis. Our findings align with established criteria for therapeutic self-medication [6,52], providing strong evidence for this behaviour in *An. gambiae*. Additionally, we consider the broader epidemiological implications of this phenomenon, particularly its potential impact on disease transmission dynamics and host-parasite coevolution.

Uninfected mosquitoes preferred the sugar diet, actively avoiding both the prooxidant (**Fig. 1a**) and the antioxidant (**Fig. 1b**) diets, while displaying random preferences when the sugar diet was unavailable. This avoidance may stem from the oxidative stress induced by these diets, as our data indicate a three-fold increase in the oxidative stress marker (GSSG/tGSH ratio) with antioxidants and a four-fold increase with prooxidants (**Fig. 2c**).

Infection, however, altered mosquito's preference away from the sugar diet, with younger individuals preferring the prooxidant diet (**Fig. 1a**) but shifting this preference towards the antioxidant diet as they grew older (**Fig. 1b**). This shift could be seen as a response to the infection, as our oxidative markers highlight an infection-induced two-fold increase in oxidative stress (**Fig. 2c**). Notably, the oxidative stress levels in infected mosquitoes were comparable across all diets, suggesting that the prooxidant and antioxidant diets were no more burdensome than the sugar diet for infected individuals.

Crucially, both supplements—particularly the prooxidant one—reduced the proportion of individuals harbouring spores (**Fig. 3a**) and the parasite burden (**Fig. 3b**) by day 8, indicating a potential fitness benefit. This is significant because *V. culicis* infection is known to reduce *An. gambiae* fecundity [53]. It is also worth noting that the differences in parasite load might have been more pronounced if measured earlier, as nearly all individuals harbour spores by day 4 [24]. These findings support the hypothesis that infected mosquitoes engage in self-medication to mitigate infection impacts, although further fitness measures (e.g., longevity, fecundity, parasite load at earlier and later stages) are needed for confirmation.

Another intriguing avenue for exploration would be to investigate whether the F1 generation from infected parents might exhibit pre-emptive self-medication (prophylactic self-medication) even in the absence of infection, or if they would adopt counter-medication strategies (such as taking antioxidants if their parents used prooxidants). The latter scenario could provide insights into the possible reduction of transgenerational residual oxidative stress resulting from their parents' choices.

Hydrogen peroxide and ascorbic acid, naturally found in wild nectar [25–28], play critical roles as natural defences against a wide range of bacteria and fungi [36,37]. Here, we propose that their consumption could help mosquitoes to counteract the negative effects of parasite infection, similarly to their demonstrated

efficacy in mitigating stressors such as insecticides [54]. This behaviour may be more widespread than previously recognized and could serve as a strategy for mosquitoes to manage the oxidative imbalance caused by blood meal ingestion [55,56] and digestion [57,58], processes essential for reproduction. Consequently, mosquitoes might already be adapted to dealing with the respective oxidative imbalance.

This study highlights therapeutic self-medication as a dynamic behaviour that evolves with the progression of infection, reflecting the parasite's infection dynamics. To our knowledge, this is the first report of such adaptive dietary changes during the course of infection. Mosquitoes initially favour the prooxidant diet during the parasite's exponential growth phase (prior to day 8, [24]), potentially hindering parasite replication. Later, they switch to the antioxidant diet during the slower growth phase (after day 8, [24]), possibly enhancing their ability to tolerate the infection and mitigate its associated costs.

Interestingly, while infection altered dietary preference, the parasite burden did not influence these choices, suggesting that mosquitoes respond qualitatively rather than quantitatively to infection. When offered both diets simultaneously, we observed a gradual shift from the prooxidant diet to the antioxidant diet. This smooth transition, particularly evident in 4- and 8-day-old mosquitoes, implies that individual conditions may mediate how these diets influence tolerance [59]. Such findings underscore the complexity of dietary choices during infection, driven by both the parasite's life cycle and the mosquito's physiological state.

Our findings suggest that therapeutic self-medication in mosquitoes could diminish the efficacy of microsporidians, such as *V. culicis*, as agents of biological control. Although *V. culicis* has long been proposed as a method to slow [60] or impede [61,62] the transmission of malaria, our observations indicate that by self-medicating, mosquitoes could mitigate the fitness reduction associated with the parasite infection [24,62–64]. This adaptation could counteract the intended impact of *V. culicis* in reducing mosquito populations or impairing their ability to transmit diseases.

Moreover, this behaviour may not be specific to *V. culicis* infections. It is plausible that *An. gambiae* might exhibit similar self-medicating responses to other pathogens, such as *Plasmodium falciparum*. This potential adaptability could alter infection dynamics and significantly influence malaria transmission, with critical epidemiological consequences.

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Author contributions

ARM conceived the overall idea. TGZ, ARM and JCK designed the experiments. TGZ collected, analysed, and interpreted the data and wrote the first draft of the manuscript. AV developed the method to analyse GSSG/tGSH ratio and analysed the samples. All authors contributed critically to the drafts.

Data availability

All data generated or analysed during this study are included as Supplementary Information files.

Additional Information

The authors declare no competing interests.