High sugar diets can increase susceptibility to bacterial infection in *Drosophila melanogaster*

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Abstract

Overnutrition with dietary sugar can worsen infection outcomes in diverse organisms including insects and humans, generally through unknown mechanisms. In the present study, we show that adult *Drosophila melanogaster* fed high-sugar diets became more susceptible to infection by the Gram-negative bacteria *Providencia rettgeri* and *Serratia marcescens,* although diet had no significant effect on infection by Gram-positive bacteria *Enterococcus faecalis* or *Lactococcus lactis.* We found that *P. rettgeri* and *S. marcescens* proliferate more rapidly in *D. melanogaster* fed a high-sugar diet, resulting in increased probability of host death. *D. melanogaster* become hyperglycemic on the high-sugar diet, and we find evidence that the extra carbon availability may promote *S. marcescens* growth within the host. However, we found no evidence that increased carbon availability directly supports greater *P. rettgeri* growth. *D. melanogaster* on both diets fully induce transcription of antimicrobial peptide (AMP) genes in response to infection, but *D. melanogaster* provided with high-sugar diets show reduced production of AMP protein. Thus, overnutrition with dietary sugar may impair host immunity at the level of AMP translation. Our results demonstrate that dietary sugar can shape infection dynamics by impacting both host and pathogen, depending on the nutritional requirements of the pathogen and by altering the physiological capacity of the host to sustain an immune response.

Introduction

Nutritional status and diet are important factors for immune defense in both mammals and insects (1–5). Hyperglycemia and diets excessively high in sugar have adverse effects on metabolic homeostasis and infection outcome (6–10). For example, human patients admitted to the intensive care unit with sepsis have a higher probability of death if they have elevated blood sugar (11) and non-diabetic hyperglycemia increases the severity of *Mycobacterium*

47 *tuberculosis* infections in guinea pigs (1). A similar phenomenon is observed in the fruit fly
48 *Drosophila melanogaster*, where adults reared on high sugar diets experience higher pathogen
49 burden (3) and increased mortality from systemic bacterial infection (2,12). Despite these clear
50 effects of diet and nutritional state on infection outcome, the molecular and physiological
51 mechanisms by which high sugar impacts infections is often unclear. By uncovering these
52 mechanisms, we can clarify the role that diet has on shaping organismal physiology during an
53 active infection and identify strategies to alleviate consequences of infection that are
54 exacerbated by obesogenic diets.

55 *Drosophila* is an excellent model to understand the mechanisms by which high-sugar diets
56 affect infection dynamics, with extensive prior study on infection, diet, and metabolism (e.g., 13–
57 16). Lacking adaptive immune systems, *Drosophila* rely on innate immune defenses that include
58 humoral production and secretion into circulation of antimicrobial peptides (AMP) and cytokines
59 secreted that share homologous function with innate immune defenses in mammals and other
60 invertebrate species (17,18). The Toll and the immune deficiency (IMD) pathways are the two
61 major regulators of the *Drosophila* humoral immune response to infection (14). Gram-positive
62 bacterial and fungal infections predominantly stimulate Toll activity (19,20) while Gram-negative
63 bacterial infections activate the IMD pathway (19,21). Once activated, both pathways lead to the
64 nuclear translocation of nuclear factor-κB (NF-κB) family transcription factors to drive expression
65 of hundreds of infection-responsive genes, including those encoding antimicrobial peptides
66 (22,23). Infection and/or constitutive activation of these pathways stimulates major shifts in
67 metabolic processes to support the immune response, including suppressed insulin signaling,
68 reduction in energetic stores like glycogen and triglycerides, and alterations in carbohydrate and
69 lipid metabolism (24–28).

70 Both high-sugar diets and infection alter metabolism and energetic usage in *Drosophila* (29).
71 In the absence of infection, high-sugar diets can also upregulate genes involved in the immune

72 response, including antimicrobial peptides (12,30), which is similar to high levels of dietary
73 sugar stimulating low-grade inflammation responses in humans (31). Additionally, uninfected
74 *Drosophila* larvae reared on high-sugar diets exhibit impaired melanization and reduced
75 phagocytic capacity of fungal spores (12,32). While there is evidence that high-sugar diets affect
76 the *Drosophila* immune response in uninfected states, it is less clear whether high-sugar diets
77 impact immune activity during a live infection. Resistance to infection can be measured as
78 immune system activity or control of pathogen burden (33). Feeding *Drosophila* high-glucose
79 diets results in higher pathogen burden after systemic infection with the Gram-negative bacteria
80 *Providencia rettgeri* (2,3) and *Pseudomonas aeruginosa* (6). This could suggest that high-sugar
81 diets impair host immune system activity, but *Pseudomonas aeruginosa* is additionally able to
82 use glucose in hyperglycemic hosts to establish higher pathogen burdens (6). Thus, it has been
83 difficult to discern whether elevated pathogen burden in *Drosophila* provided with high-sugar
84 diets is due to impaired host immune responses or to bolstered pathogen growth capacity.
85 These are not mutually exclusive alternatives. Furthermore, prior *D. melanogaster* studies that
86 investigated the effects of high dietary sugar on systemic infection outcome were performed
87 using flies that were reared throughout their entire development on high-sugar diets (2,3,12,32).
88 Rearing on elevated dietary sugar diets causes hyperglycemia, lipidemia, and reduced body
89 weight in both larvae and as adults, and prolongs larval developmental time (12,34). Thus, it is
90 difficult to determine in those experiments whether effects of diet on adult infection outcome
91 result from altered metabolism and immunity or from indirect consequences of altered
92 development.

93 In the present study, we specifically focus on the immediate effects of high dietary sugar on
94 infection dynamics in adult *D. melanogaster*. We do this by rearing *D. melanogaster* larvae on a
95 common base diet, then switching adults to diets that vary in sugar content prior to delivering
96 systemic bacterial infection. To determine whether adverse effects from high-sugar diets are

97 universal across pathogenic infections, we test infection with four different bacterial pathogens
98 representing Gram-positive and Gram-negative bacteria. We find that flies provided with high-
99 sugar diet as adults are particularly susceptible to Gram-negative bacterial infection, suffering
100 increased mortality and more rapid pathogen proliferation during the early hours of infection. We
101 find evidence that increased carbon availability in high-sugar hosts can accelerate the growth of
102 certain pathogens, and that high-sugar diets impaired immune system function at the level of
103 antimicrobial peptide translation without affecting transcription levels.

104

105 **Results**

106 **High-sucrose diets increase mortality from infection with some bacterial pathogens**

107 We first wanted to test whether high-sugar diets reduce immune defense in adult *D.*
108 *melanogaster* independent of any developmental consequences. We therefore reared larvae on
109 a yeast-cornmeal diet containing 4% sucrose, then split the population across the experimental
110 diets during the non-feeding pupal stage such that eclosing adults emerged onto experimental
111 diets that were 0%, 2%, 4%, 8%, 16%, or 24% (w/v) sucrose (Fig. 1A), which covers the range
112 used in prior studies (3,12,32). After 3-5 days on the experimental diets, adult female flies were
113 given a systemic infection with one of four bacteria: *Providencia rettgeri*, *Serratia marcescens,*
114 *Enterococcus faecalis, Lactococcus lactis*. These bacteria were chosen as natural pathogens of
115 *D. melanogaster* (35,36) and include both Gram-positive and Gram-negative bacteria, which are
116 respectively expected to predominantly activate the Toll and IMD immune response pathways
117 (19).

118 We found that flies provided with high-sucrose diets experienced higher mortality after
119 infection with the Gram-negative bacteria *S. marcescens* and *P. rettgeri* than flies fed low sugar
120 (Figs. 1B and 1C). This increased mortality occurred in a concentration-dependent manner

121 depending on the pathogen. Flies provided with 0%, 2%, or 4% sucrose experienced low
122 mortality and flies provided with 16% or 24% sucrose experienced high mortality after infection
123 with both pathogens. However, on the 8% sucrose diet, flies infected with *S. marcescens*
124 exhibited high survival (Fig. 1B), but flies infected with *P. rettgeri* exhibited low survival (Fig.
125 1C). Overall, there was no effect of diet on survivorship of infection with the Gram-positive
126 bacteria *L. lactis* and *E. faecalis* (Figs. 1D and 1E). Although flies provided with the 24%
127 sucrose diet experienced the highest mortality after *L. lactis* infection (Fig. 1D), we attribute this
128 to the diet being generally poor as sham-infected control flies fed on the 24% sucrose diet also
129 experienced elevated mortality (Fig. S1A). Varying the diet of the host can alter the composition
130 of the gut microbiota (37–39), which can yield effects on the host's metabolic status (40–42).
131 Flies provided with 16% sucrose in our experiments showed a reduced abundance of gut flora
132 compared to flies provided with 2% sucrose diet (Fig S2A). However, we found that high-sugar
133 diets increased the probability of death from systemic *P. rettgeri* infection even in axenic flies
134 with no microbiota (Fig S2B), suggesting that our observed effect of diet on immunity is not
135 mediated by effects on gut flora.

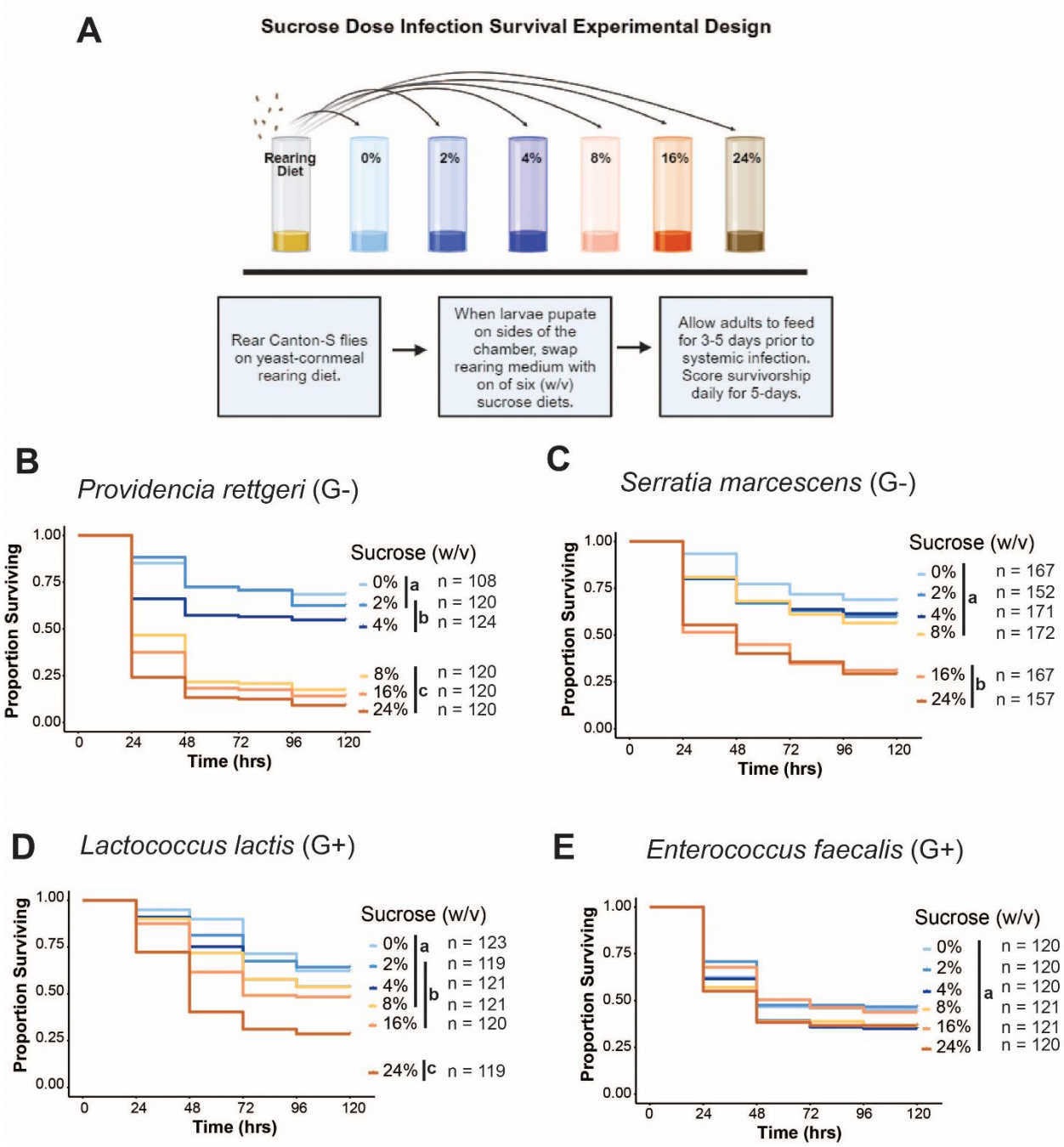

Fig 1. High-sugar diet increases mortality after infection with some pathogens. (A) Experimental setup for assaying effects of high-sugar diets on adult survival of infection. The six experimental diets used have the same yeast and cornmeal content, but they varied in sucrose (w/v). Diets with 0%, 2%, and 4% are represented in shades of blue while diets with 8%, 16%, 24% sucrose are represented in shades of orange. The rearing diet contains 4% sucrose. (B) Flies fed 8%, 16%, and 24% sucrose diets exhibit significantly higher mortality after *Providencia rettgeri* infection than flies given 0%, 2%, and 4% sucrose diets ($p<0.0001$; Cox Mixed Effects Model). There was no difference in survival among flies provided with the three high-sugar diets, while flies provided with the 4% sucrose diet exhibited significantly lower survivorship than flies provided with 0% added sucrose ($p = 0.035$; Cox Mixed Effects Model). (C) Flies

146 fed 16% and 24% sucrose diets exhibit significantly higher mortality after *Serratia marcescens* than flies
147 given diets with 8% sucrose or lower (p<0.0001; Cox Mixed Effects Model). (D) Flies fed the 24% sucrose
148 diet have the highest mortality after *Lactococcus lactis* infection compared to all other diets (p<0.001, Cox
149 Mixed Effects Model). Flies fed 16% sucrose died significantly faster than flies fed 0% sucrose diets (p =
150 0.047; Cox Mixed Effects Model) but there were no other significant pairwise differences in survivorship
151 among flies provided the 16% sucrose diet or lower. (E) There was no effect of diet on survivorship after
152 infection with *Enterococcus faecalis*. Letters denote significant pairwise differences between diets
153 (p<0.05).

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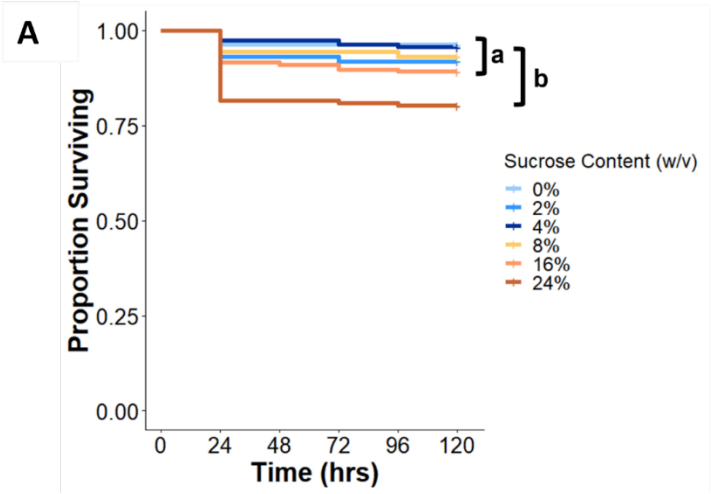

155

156 **Fig. S1**. A) Survivorship of PBS injury controls across all infection experiments pooled together.
157 Flies fed 24% (w/v) sucrose diet have higher proportion of death after sham-infection with sterile
158 PBS compared to all other diets (p<0.05; Cox Proportional Hazards Mixed effects model).

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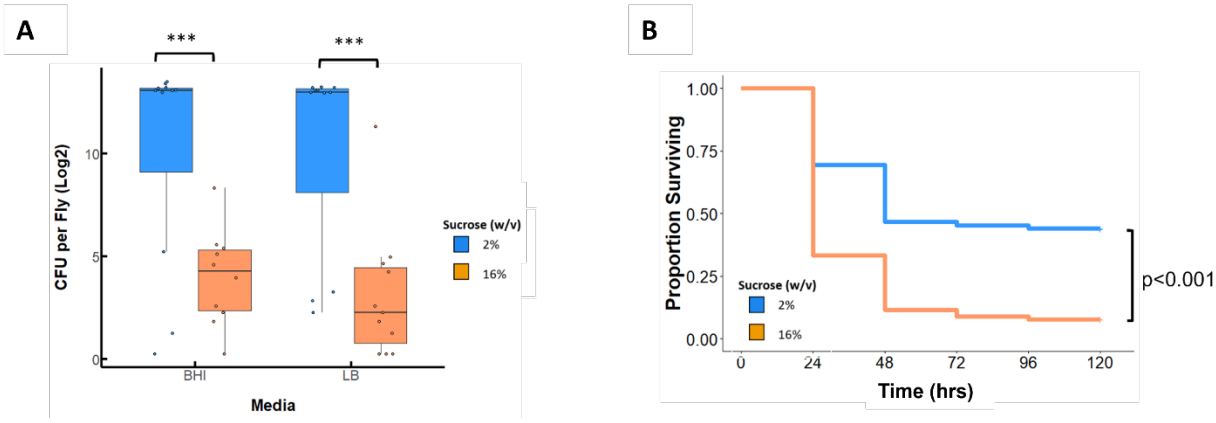

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161

162 **Fig. S2**. A) To measure the abundance of the endogenous gut microbiota in uninfected flies fed on
163 the 2% (w/v) sucrose or 16% sucrose diets, 10 whole flies were pooled and homogenized in 250 µl
164 of PBS, and 50 µl of the homogenate was plated on lysogeny broth (LB) or brain heart infusion (BHI)
165 agar. Flies fed on the 2% diets exhibit higher microbiota loads on LB (p<0.001, n = 15 pools of 10

flies per diet) and BHI ($p<0.001$, n = 15 pools of 10 flies per diet) plates than flies fed 16% sucrose. C) Axenic flies were infected with *P. rettgeri*, and flies fed 16% sucrose diets exhibited higher mortality than flies given 2% sucrose ($p<0.001$; Cox Proportional Hazards model).

Flies fed on high-sucrose diets become hyperglycemic despite eating less

D. melanogaster will modulate feeding behavior depending on the content of their diet (43–45), and the nutritional composition of the diet influences metabolic state (3,34,46). We therefore first assessed whether varying sucrose levels in the diet impacts feeding rate. We performed an excreta quantification assay (ExQ) (47) on uninfected females to measure feeding across the six experimental diets shown in Figure 1A. Flies were housed in ExQ chambers over a 2-day period with experimental diets containing the dye erioglaucine (1% w/v) and their excreta was collected every 24-hours. We found that flies ate significantly more of the three diets with the lowest sucrose (0%, 2%, and 4%) than the three diets with the highest sucrose (8%, 16%, 24%; $p < 0.0001$, Fig. 2A). There was no significant difference in feeding rate among the three lowest-sucrose diets or among the three highest-sucrose diets. In subsequent experiments, we focus on the 2% and 16% sucrose diets as representative the low- and high-sugar diets.

To assess the effects of diet on the metabolic status of adults, we measured glucose, trehalose, glycogen, and soluble protein in the whole body of uninfected flies provided with either 2% or 16% sucrose diets. We found that uninfected flies fed the high-sucrose diet exhibit higher levels of glucose ($p < 0.001$, Fig. 2B), trehalose ($p = 0.001$, Fig. 2C), and glycogen (Fig. 2D, $p= 0.0479$) than flies fed the low-sugar diet (Fig. 2B-D). Given that infection can stimulate shifts in carbohydrate levels (26,48), we also tested whether flies fed high-sugar continue to exhibit elevated carbohydrate levels after infection. We indeed found that flies fed the high-sucrose diet exhibited higher levels of glucose ($p<0.01$, Fig. 2B), trehalose ($p < 0.01$, Fig. 2C), and glycogen ($p =0.019$, Fig. 2C) than flies fed the low-sucrose diet at 24 hours after *P. rettgeri*

infection. Interestingly, there was no difference in glucose content (Fig. 2B) or trehalose content (Fig. 2C) between flies fed low-sucrose or high-sucrose at 24 hours after infection with *S. marcescens*. However, flies given a high-sucrose diet exhibited higher glycogen stores after *S. marcescens* infection than flies given low sucrose (p = 0.035 Fig. 2D). Despite reduced feeding on high sugar, flies on the high-sucrose diet still sustain higher levels of carbohydrate stores in both uninfected and infected states.

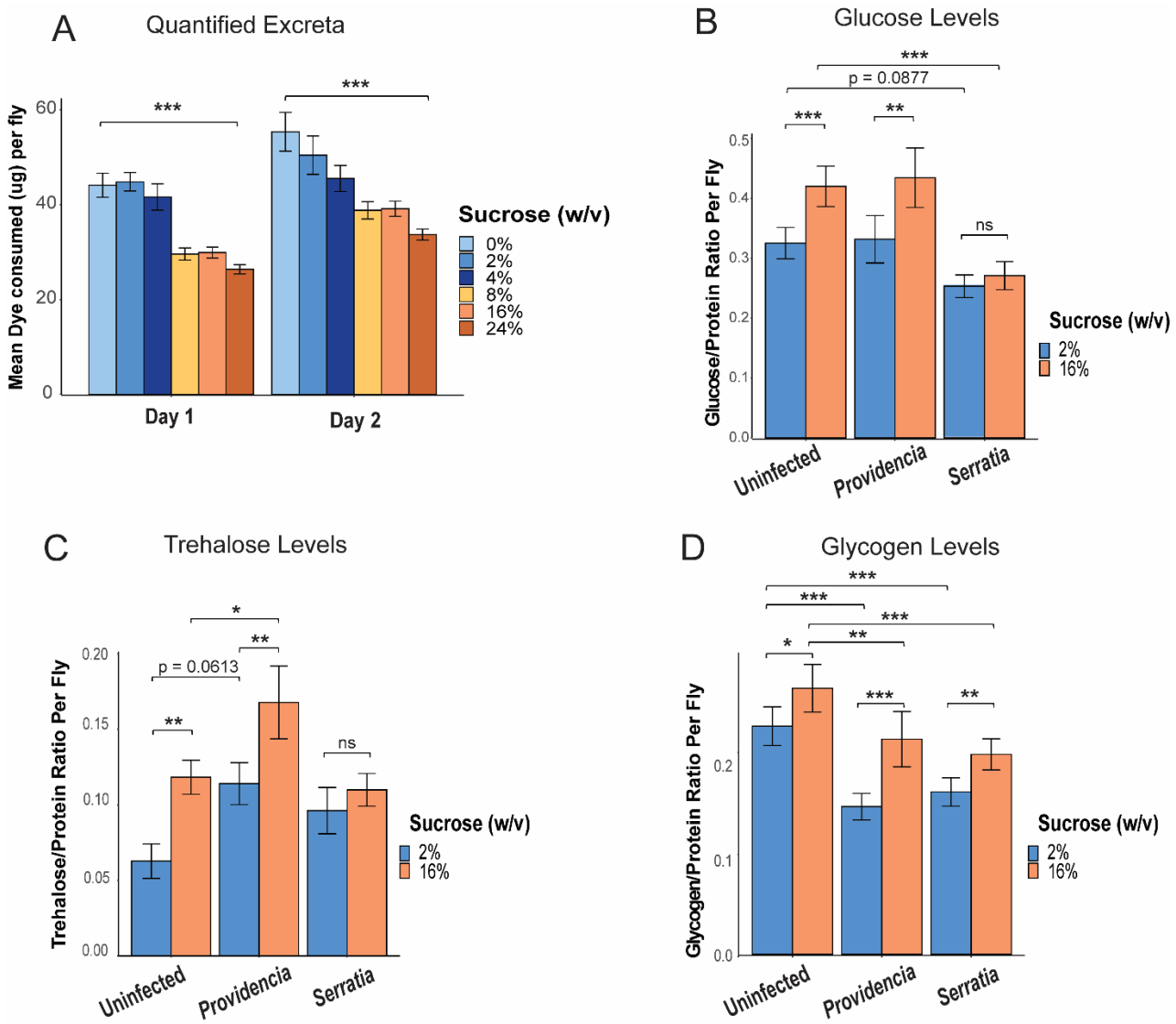


Fig. 2. Flies fed high sugar sustain higher carbohydrate stores despite reduced feeding. (A) Excreta quantification of uninfected female flies fed on diets ranging from 0%-24% sucrose was measured over two days. Flies feeding on the 8%, 16%, and 24% diets consumed significantly lower dye than those fed on the 0%, 2%, 4% sucrose diets (p < 0.001). (B) Uninfected flies fed on the 16% diet had higher glucose levels compared to flies fed on 2% sucrose (p < 0.001; ANOVA with Tukey HSD post-hoc). After 24 hours of *P. rettgeri* infection, flies fed on high sugar continued to exhibit higher

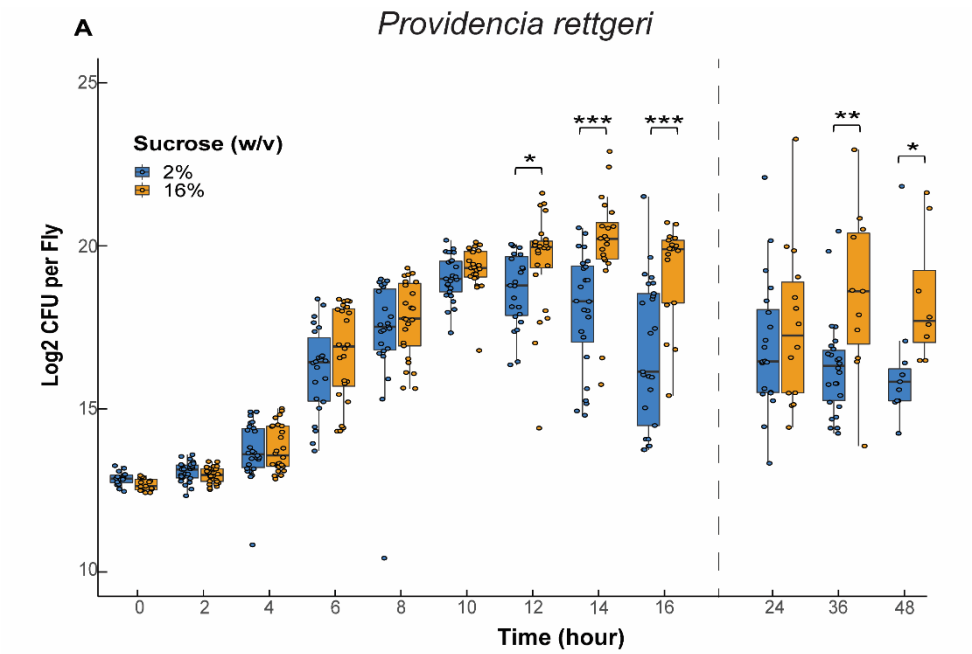
glucose levels compared to flies fed 2% sucrose ($p < 0.01$; ANOVA with Tukey HSD post-hoc). However, there was no difference in glucose levels between flies provided with 2% and 16% diets 24-hours after *S. marcescens* infection ($p=0.658$). Flies fed 16% sucrose exhibit significantly reduced glucose levels after *S. marcescens* infection ($p<0.001$), and there was a nearly significant decrease in glucose levels of flies fed 2% sucrose after *S. marcescens* infection ($p = 0.0877$) (C) Trehalose levels were significantly higher in flies fed 16% sucrose than in flies fed 2% sucrose when the flies were uninfected ($p<0.001$) or when they were infected with *P. rettgeri* ($p<0.01$). However, *S. marcescens* infection had no effect on trehalose levels between flies given the 2% and 16% diets ($p = 0.38$). *P. rettgeri* infection led to higher trehalose levels in flies on both 2% ($p = 0.0554$) and 16% ($p = 0.0446$) sucrose diets compared to uninfected flies, while *S. marcescens* had no effect on trehalose levels on either diet. (D) Glycogen levels were significantly higher in flies on 16% versus 2% diets when flies were uninfected ($p = 0.0479$), infected with *P. rettgeri* ($p = 0.019$), or infected with *S. marcescens* ($p = 0.035$). On both diets, infection with both pathogens led to reduced glycogen levels compared to uninfected (2% *Providencia*, $p<0.01$; 16% *Providencia,* $p = 0.036$; 2% *Serratia*, $p<0.01$; 16% *Serratia*, $p < 0.01$). Legend for panel figure: * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

Feeding on high sucrose increases pathogen load in the early stages of infection

High pathogen load is associated with higher mortality from infection (49,50). Since we observed that high-sugar diets lead to higher mortality during infection with the Gram-negative bacteria *S. marcescens* and *P. rettgeri*, we wanted to establish whether this high mortality arises from higher bacterial load. To test whether flies fed on high sugar have higher pathogen loads, we performed an *in vivo* bacterial growth assay where we sampled infected flies at two-hour intervals from 0-16 hours post-infection and at 24, 36, and 48 hours post-infection. At each time of sampling, individual flies were homogenized and plated on agar plates to estimate the number of live bacterial cells in the fly.

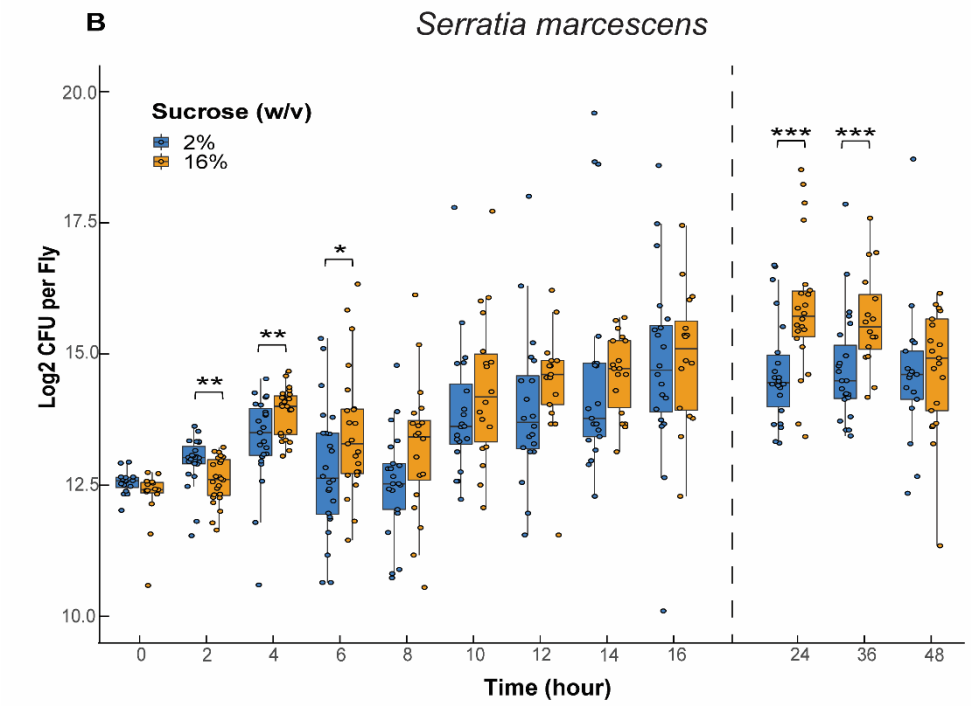
Overall, the bacteria proliferated more quickly in flies fed on the high-sugar diet after both *P. rettgeri* (Fig. 3A) and *S. marcescens* (Fig. 3B) infection. However, the divergence in load between the two diets occurs at different times for the two pathogens. *S. marcescens* infections start to exhibit a higher pathogen burden as soon as 6 hours into the infection, while *P. rettgeri* burden becomes higher by 12 hours into the infection. Flies that died during the sampling period were not included in this assay, which may mean that pathogen burdens are underestimated if the surviving flies at any given timepoint tend to be the ones with lower pathogen burdens. At 10

238 hours and later post-infection with *S. marcescens*, we observed a lower proportion of surviving
239 flies on the high-sugar diet than on the low-sugar diet (Fig. 3B), which may explain the apparent
240 absence of difference in pathogen burden 10-16 hours into the infection. We similarly observe a
241 lower proportion of flies provided with high-sugar diet surviving *P. rettgeri* infection 10 hours and
242 later, although the difference in pathogen burden between flies on the two diets remains
243 significant (Fig. 3A).



Proportion of flies alive at each time point

Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	0.97	0.93	0.93	1.0	0.93	0.90	0.90	0.97	0.76	0.76	0.71
16%	1.0	0.97	1.0	0.89	1.0	0.83	0.85	0.81	0.59	0.47	0.29	0.29



Proportion of flies alive at each time point

Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	1.0	1.0	0.96	0.90	0.93	0.85	0.90	0.83	0.80	0.74	1.0
16%	1.0	1.0	0.93	1.0	0.83	0.69	0.76	0.67	0.63	0.62	0.42	0.44

244

245

246 **Fig 3.** Pathogens proliferate faster in flies fed high sugar. (A) Pathogen load was assayed at 2-hour
247 intervals from 0-16 hours post-infection and at 24, 36, and 48 hr post-infection in flies fed 2% and
248 16% sucrose diets. After *P. rettgeri* infection, flies provided with high-sugar diets have higher
249 pathogen loads at 12, 14, 16, 36, and 48 hr than flies given low-sugar diet (12 hr, $p = 0.031$; 14 hr, p
250 < 0.001, 16 hr, $p < 0.001$; 36 hr, $p < 0.01$; 48 hr, $p = 0.022$; Generalized Least Square Means (GLS)
251 with Tukey HSD post-hoc for pairwise comparisons). Flies given high-sugar diets have consistently
252 lower survival to time of sampling at hour 10 and onward. (B) After *S. marcescens* infection, flies
253 given high-sugar diets have higher pathogen loads than flies given low-sugar diet at 4 hr, 6 hr, 24 hr,
254 and 36 hr post-infection (4 hr, $p = 0.011$; 6 hr, $p = 0.044$, 24 hr, $p < 0.001$; 36 hr, $p < 0.01$;
255 Generalized Least Square Means (GLS) with Tukey HSD post-hoc for pairwise comparisons). Flies
256 given high-sugar diets have consistently lower survival to time of sampling at hour 8 and beyond. At
257 2 hours post-infection, flies given low-sugar diets have higher pathogen load than flies given high-
258 sugar diets ($p < 0.01$). Each dot represents the pathogen burden of an individual fly. Legend for
259 panel figure: * = $p<0.05$, ** = $p<0.01$, *** = $p <0.001$.

260

261 **Nutrient availability promotes *S. marcescens* proliferation within hosts on high-sugar**

262 **diets**

263 Having determined that *D. melanogaster* provided with high-sucrose diets are both more
264 susceptible to infection and sustain higher internal carbohydrate stores, we next sought to
265 understand whether the increased susceptibility to infection on the high-sugar diet is due to
266 impaired immune system activity or to an altered nutritional environment experienced by the
267 infecting pathogen. To test this, we measured pathogen proliferation during *in vivo* infection of
268 immune compromised *D. melanogaster* on the low- and high-sugar diets. We predicted that the
269 effect of diet on sensitivity to infection would be eliminated in immunocompromised flies if the
270 dietary effect is due to impaired immunity, but it would remain if the dietary effect were due to
271 altered nutritional environment for the pathogen. We used the *D. melanogaster* strain ΔAMP10
272 (51), which has intact Toll and IMD signaling but is missing 10 genes encoding major infection-
273 inducible antimicrobial peptides. Antimicrobial peptides are small, cationic peptides that are
274 secreted from the insect fat body in response to a systemic infection, and they are critical for
275 killing pathogens by disrupting function of specific microbial processes (52–55). The ΔAMP10

276 flies have mutations that eliminate *Diptericin A*, which is critical for controlling *P. rettgeri*
277 infection (51,56), and the four *Attacin* genes, three of which are critical for the control of *S.*
278 *marcescens* infection (P. Nagy, N. Buchon, and B.P. Lazzaro, unpublished). We can use these
279 flies to monitor pathogen growth in response to diet without any interference from the humoral
280 immune response. There was no difference in the rate of *P. rettgeri* proliferation in ΔAMP10
281 hosts provided with either low or high sucrose (Fig. 4A), indicating that the typically observed
282 effect of diet on sensitivity to this infection may be a consequence of diet-dependent immune
283 impairment. In contrast, *Serratia marcescens* continued to proliferate faster in ΔAMP10 flies fed
284 high sucrose than in those fed low sucrose (Fig. 4B), indicating an effect of diet beyond direct
285 mediation of the immune system.

286 As an independent, albeit indirect, test of whether *S. marcescens* might utilize excess
287 available carbon to grow faster in hosts provided with high-sugar diets, we measured *in vitro*
288 growth curves of *P. rettgeri* and *S. marcescens* in minimal medium supplemented with either a
289 carbon source or a nitrogen source. We performed an 18-hour growth curve assay in M9
290 minimal media supplemented with different combinations of casamino acids, sucrose, and
291 glucose. We found that supplementation with 1% casamino acids enabled *P. rettgeri* growth, but
292 there was no further change in the growth trajectory or final OD with supplementation with 1%
293 glucose, 1% sucrose, or no additional sugar ($p > 0.05$, Fig. 4C). These data indicate that *P.*
294 *rettgeri* is nitrogen-limited but not carbon-limited in minimal media. The addition of either
295 glucose or sucrose dramatically increased the growth of *S. marcescens* in minimal media and
296 resulted in a significantly higher OD at the end of the assay ($p<0.01$, Fig. 4D), indicating a
297 greater dependence on environmental carbon under these growth conditions. While we do not
298 know precise bioavailability of carbon to bacteria infecting *D. melanogaster*, the data in Figure 2
299 show that flies on high-sugar diets have higher levels of circulating and stored sugars, and in
300 combination these data offer conceptual support to the hypothesis that the increased

301 proliferation of *S. marcescens* within hosts provided with a high-sugar diet may arise in part
302 from increased availability of carbon to the infecting bacteria.

303 We had observed that flies provided with high-sucrose diets show elevated glucose and
304 trehalose levels after infection with *S. marcescens* (Fig. 2B, 2C) despite reduction in glycogen
305 stores in both diets (Fig. 2D), leading us to hypothesize that *S. marcescens* might consume
306 excess circulating sugar within the host. We measured the change in carbohydrate levels after
307 infection with either *P. rettgeri* or *S. marcescens* relative to uninfected flies on both diets using
308 the data in Fig. 2B-D. We found that flies infected with *S. marcescens* have a significantly higher
309 loss in glucose levels compared to flies infected with *P. rettgeri* on both the low-sugar ($p < 0.01$)
310 and high-sugar ($p<0.001$, Fig. 4E) diets, although they exhibit similar relative loss in glycogen (p
311 > 0.05, Fig. 4F). Interestingly, we observed significantly higher loss of glycogen in flies provided
312 with low-sugar compared to high-sugar diets after both *P. rettgeri* ($p = 0.0178$) and *S.*
313 *marcescens* infection ($p = 0.029$, Fig. 4F). There was no significant difference between the
314 infections in proportional change in trehalose levels on either diet (Fig. 4G). Our observation
315 that both infections stimulate loss in glycogen stores but only *S. marcescens* infection results in
316 a significant reduction in glucose levels is consistent with *S. marcescens* consumption of free
317 sugars during infection.

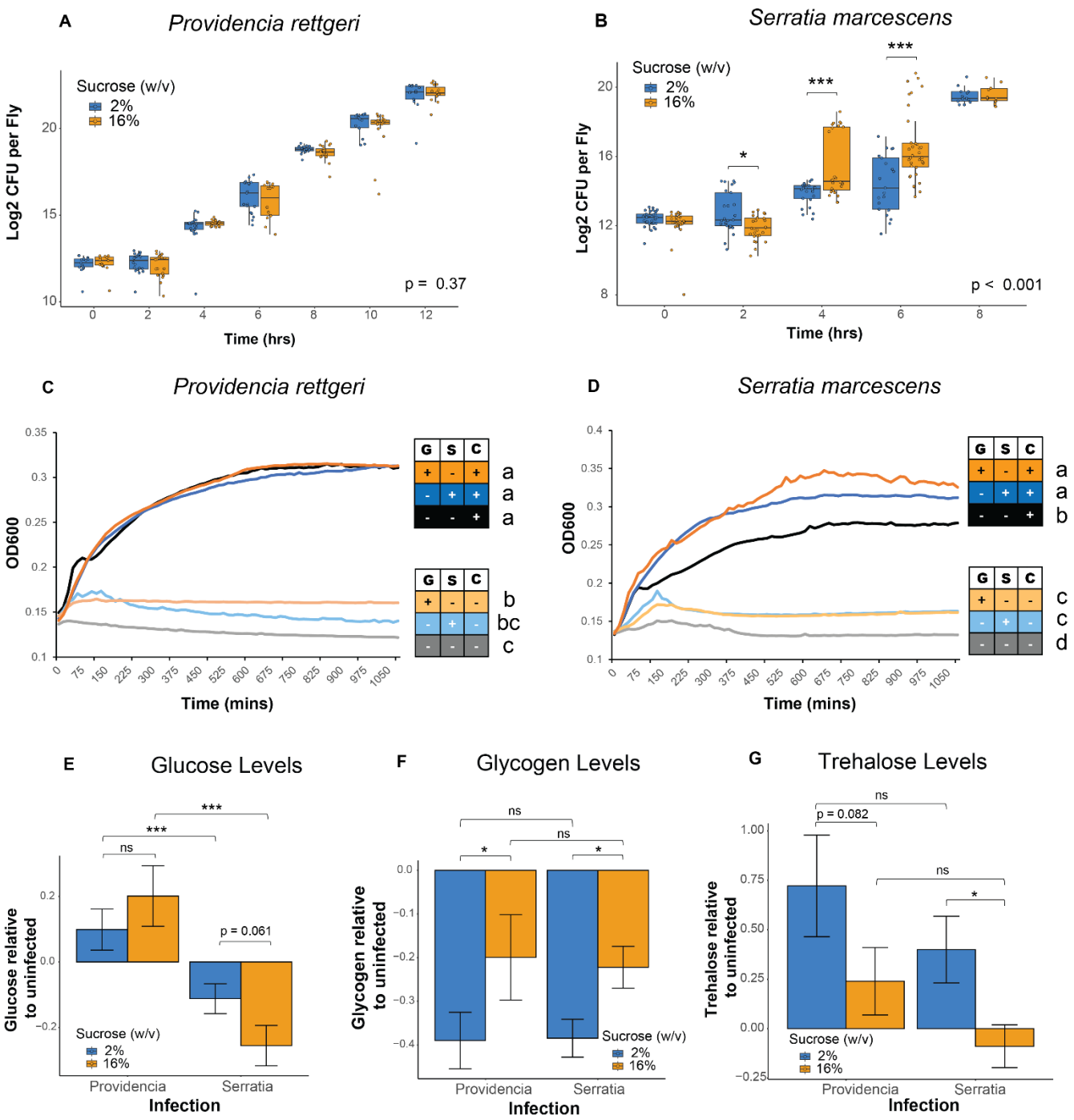


Fig 4. Nutrient availability promotes *Serratia marcescens* proliferation within hosts on high-sugar diets. (A) Diet did not affect the proliferation of *P. rettgeri* in flies lacking 10 major inducible antimicrobial peptides (p> 0.05 time*diet; GLS model). (B) *S. marcescens* proliferate significantly more rapidly in ΔAMP10 flies fed high sugar (p<0.001 time*diet; GLS model). Two hours into the infection, flies fed high sugar exhibit a lower *Serratia* burden (p < 0.01; GLS model followed by Tukey HSD post-hoc), but flies fed high sugar exhibit significantly higher loads by hours by hours 4 (p<0.001) and 6 (p<0.0001; GLS model followed by Tukey HSD-post-hoc). (C) There was no effect of

326 sugar supplementation on *P. rettgeri* growth over 1080 mins in M9 minimal media supplemented with
327 1% casamino acids (CAS; p> 0.05; ANOVA followed by Tukey HSD post-hoc). CAS supplementation
328 significantly increased OD at 1080 minutes compared to media without casamino acids (p<0.001;
329 ANOVA). (D) *S. marcescens* grew significantly more in minimal media supplemented with the
330 combination of casamino acids and glucose (p<0.001; ANOVA with Tukey HSD post-hoc) or sucrose
331 (p < 0.01, ANOVA with Tukey HSD post-hoc) than in media supplemented with casamino acids alone.
332 *S. marcescens* OD at 1080 mins was significantly lower in media supplemented only with glucose (p
333 < 0.001; ANOVA with Tukey HSD). Each curve represents 6-8 replicates per treatment, pooled.
334 Letters in figures denote significant pairwise differences with p <0.05. Legend: G = glucose, S =
335 sucrose, C = casamino acids. Colors for *in vitro* curves: 1% glucose (light orange), 1% sucrose (light
336 blue), 1% casamino acids (black), or 1% glucose & casamino acids (dark orange), and 1% sucrose &
337 casamino acids (dark blue). (E) Glucose levels declined significantly more after infection with *S.*
338 *marcescens* but increased in flies infected with *P. rettgeri* on both 2% (p < 0.01; ANOVA followed by
339 Tukey HSD post-hoc) and 16% (p< 0.0001; ANOVA followed by Tukey HSD post-hoc) sucrose diets.
340 There was no significant difference in change in glucose levels after *P. rettgeri* infection between
341 diets (p = 0.24; ANOVA followed by Tukey HSD post-hoc). There was a trend for increased relative
342 loss of glucose after *S. marcescens* infections in flies fed the 16% sucrose diet compared to 2%,
343 however this was not statistically significant (p = 0.0601, ANOVA followed by Tukey HSD post-hoc).
344 (F) Glycogen levels were reduced after infection with both *P. rettgeri* and *S. marcescens*. Flies fed
345 2% sucrose had significantly higher loss of glycogen stores compared to flies fed 16% sucrose after
346 infection with both *P. rettgeri* (p = 0.0178, ANOVA with Tukey HSD post-hoc) and *S. marcescens* (p =
347 0.0291, ANOVA with Tukey HSD post-hoc). (G) There was no significant difference in change upon
348 infection in trehalose levels between *P. rettgeri* and *S. marcescens* on either the 2% diet (p = 0.197)
349 or the 16% sucrose diet (p = 0.215). There was a significant reduction in trehalose levels of flies
350 infected with *S. marcescens* on 16% sucrose diets compared to flies fed 2% sucrose (p= 0.0372,
351 ANOVA with Tukey HSD post-hoc). There was a trend toward reduced trehalose in flies provided with
352 16% sucrose diet after *P. rettgeri* infection, but it was not statistically significant (p = 0.0816, ANOVA
353 with Tukey HSD post-hoc). Legend for panel figure: * = p<0.05, ** = p<0.01, *** = p <0.001.

354

355 ***Drosophila* on high sugar diets produce less AMP peptide after infection**

356 We inferred that high-sugar diets increase sensitivity to *P. rettgeri* infection by impairing the
357 immune system because the effect of diet was eliminated in immunocompromised flies.
358 However, the inference that *S. marcescens* may consume excess carbohydrates present in flies
359 on high-sugar diets does not preclude the possibility that those flies might also have reduced
360 immune responses. To directly test whether high-sugar diets impair the immune response, we
361 first measured mRNA transcripts of a panel of genes encoding six *D. melanogaster*
362 antimicrobial peptides (AMPs). Gene expression was measured in flies fed low or high-sugar
363 diets at 8-hours after infection with either *S. marcescens* or *P. rettgeri*. We found that diet had
364 no effect on the infection-induced expression levels of *Diptericin A, Attacin A, Cecropin A1,*

365 *Drosocin, Drosomycin,* or *Defensin* after either *P. rettgeri* and *S. marcescens* infection (Figs 5A
366 and 5B).

367 Measuring the amount of mRNA transcript does not directly demonstrate the abundance of
368 translated peptide (57), and translation can be impaired by cellular stress during the *D.*
369 *melanogaster* response to infection (58). To test whether high-sugar diets impact AMP protein
370 levels in response to infection, we used sandwich ELISA quantify Drosocin and Cecropin A1
371 from flies that have hemagglutinin (HA) and FLAG epitopes tagged to the endogenous AMPs.
372 Infection-induced production of tagged AMP peptides is detectable as early as 3 hours post-
373 infection (Fig. S3A, S3B). We quantified tagged AMPs from individual flies at 6-hours after
374 infection with either *P. rettgeri* or *S. marcescens.* Flies fed on the high-sugar diet produced
375 significantly lower levels of tagged Drosocin than flies fed on the low-sugar diet after *P. rettgeri*
376 infection ($p = 0.008$, Fig. 5C). However, we observed no effect of diet on Drosocin levels in flies
377 infected with *S. marcescens* (Fig. 5D). An unexpectedly high number of flies did not have
378 detectable levels of tagged Cecropin peptide after infection with either *P. rettgeri* or *S.*
379 *marcescens* (Fig. S4A, S4B). We performed a hurdle model to statistically test for differences in
380 Cecropin abundance. The hurdle model first tests the probability that detectable levels of tagged
381 Cecropin are present using a binomial logistic regression, and subsequently tests whether there
382 are quantitative differences in peptide levels conditional on having observed a detectable level
383 of peptide. While the probability of detecting Cecropin was equivalent flies infected with *P.*
384 *rettgeri* on both diets (Fig. 5E), flies infected with *S. marcescens* had lower probability of
385 expressing detectable Cecropin when fed the high-sugar diet ($p < 0.01$, Fig. 5E). In the flies for
386 which Cecropin was detectable, peptide abundance was significantly lower in the flies on the
387 high-sugar sucrose diet than in the flies on the low-sugar diet after infection with *P. rettgeri* ($p =$
388 0.023, Fig. 5F). Diet had no effect on the abundance of detectable Cecropin after *S.*
389 *marcescens* infection ($p = 0.21$, Fig 5F). Overall, flies provided with 16% sucrose diets produce

less Drosocin and Cecropin after infection with *P. rettgeri* and have a lower probability of detectable Cecropin 6 hours post-infection with *S. marcescens*. Thus, the translational production of AMPs seems to be impaired in flies on high-sucrose diets even when there is no decrease in transcription level.

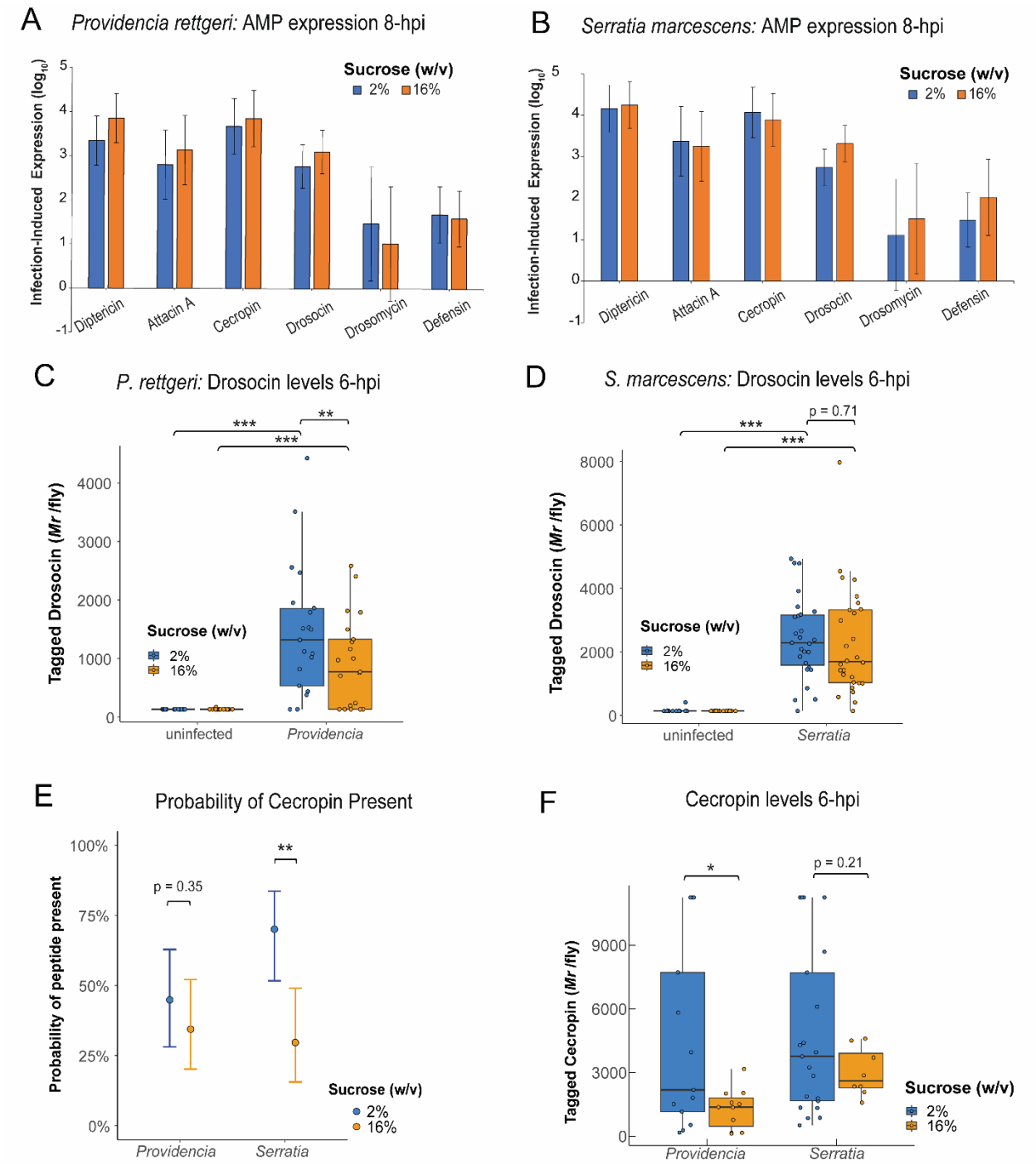


Fig 5. High sugar diets reduce production of AMP peptides in response to infection without affecting AMP gene expression. (A) There was no effect of diet on expression of genes encoding the AMPs Diptericin, Attacin A, Cecropin A1, Drosocin, Drosomycin, and Defensin at 8-hours post-infection with *P. rettgeri* or (B) *S. marcescens* ($p > 0.05$). (C) *P. rettgeri* infection resulted in significantly higher levels of tagged Drosocin than in uninfected flies on both 2% ($p < 0.0001$) and 16% ($p = 0.0002$) diets. Flies on 16% sucrose diet had significantly lower amounts of tagged Drosocin six hours after *P. rettgeri* infection than flies on 2% sucrose diet ($p = 0.008$). (D) Tagged Drosocin levels were higher at 6 hours after *S. marcescens* infection than in uninfected flies on both 2% ($p < 0.001$) and 16% ($p < 0.001$) diets, but there was no difference between the diets after *S. marcescens* infection ($p = 0.71$). (E) The probability of detectable Cecropin peptide was similar after *P. rettgeri* infection on both diets ($p = 0.45$), while flies fed the 2% diet were significantly more likely to have detectable peptide present after *S. marcescens* infection than those fed the 16% diet ($p = 0.003$). (F) Of the flies with detectable Cecropin levels, there were significantly higher peptide levels in flies fed the 2% diet compared to 16% diet after both *P. rettgeri* ($p = 0.0229$) and *S. marcescens* ($p = 0.211$) infections.

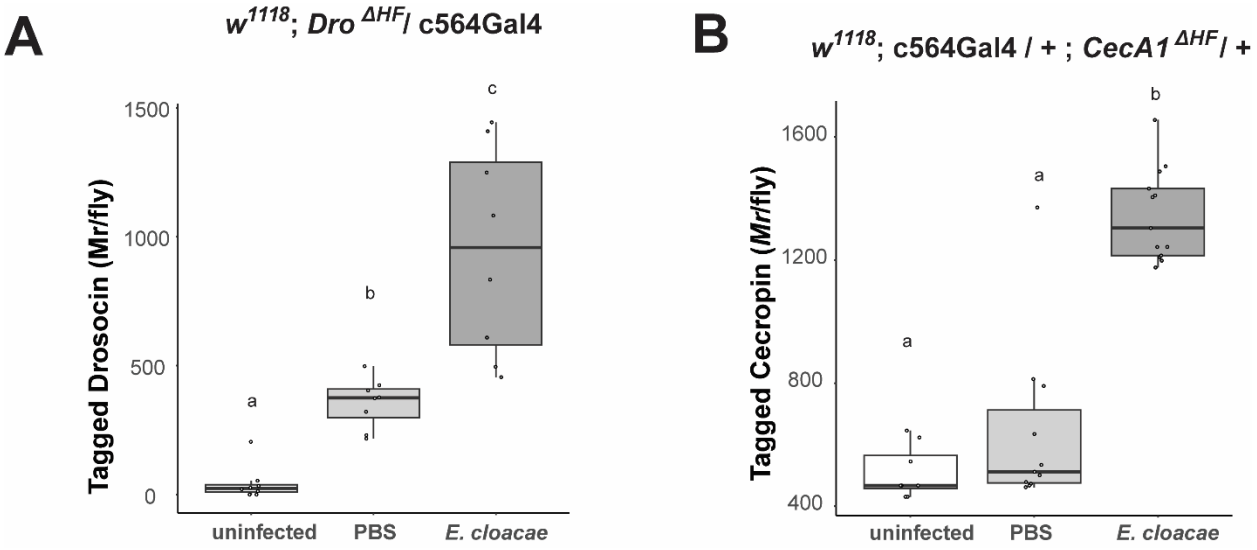


Fig S3. Demonstration that HA- and FLAG-tagged Drosocin and Cecropin can be detected by sandwich ELISA following bacterial infection. (A) Tagged Drosocin levels are significantly higher 3-hours after injection with PBS ($p = 0.0455$) or ~6.0×10^4 cells of the Gram-negative bacterium *Enterobacter cloacae* ($p<0.001$) than they are in uninjured flies. *E. cloacae* infection results in significantly higher Drosocin levels than PBS injection ($p<0.001$; linear model with Tukey post-hoc). (B) Tagged Cecropin levels are significantly higher 3-hours after infection with *E. cloacae* than after PBS injection ($p<0.001$) or in uninjured flies ($p<0.001$). There was no detectable difference in Cecropin levels between uninjured and PBS-injected flies ($p = 0.32$). Letters denote pairwise difference $p<0.05$.

A

	Providencia detected	Providencia undetected	Proportion in linear range
2%	13	16	0.45
16%	11	21	0.34

B

	Serratia detected	Serratia undetected	Proportion in linear range
2%	21	9	0.70
16%	8	19	0.30

Fig. S4. (A) The number of flies fed the 2% and 16% diets that have, or do not have, detectable tagged Cecropin six hours after *P. rettgeri* infection. (B) The number of flies fed the 2% and 16% sucrose diets that have, or do not have, detectable tagged Cecropin six hours after *S. marcescens* infection.

Discussion

In this study, we show that *D. melanogaster* provided with high-sugar diets exhibit higher mortality and elevated pathogen load after infection with the Gram-negative bacteria *Providencia rettgeri* and *Serratia marcescens*, even when the high-sugar diet is provided only to the adult stage after development is complete. *S. marcescens* may be able to take a growth advantage from the excess carbohydrates available in hosts provided with high-sugar diets, and the effect of diet on sensitivity to both infections seems to be mediated by impaired host immune response on high-sugar diet. Interestingly, we saw no effect of diet on susceptibility to infection with the Gram-positive bacteria *Enterococcus faecalis* and *Lactococcus lactis*. As Gram-negative (*S. marcescens* and *P. rettgeri*) infections should primarily activate the IMD signaling pathway whereas Gram-positive (*E. faecalis* and *L. lactis*) infections should primarily activate the Toll pathway (50,59), the much larger effect of diet on sensitivity to Gram-negative infections may implicate an interaction with the IMD pathway.

Our approach of feeding flies on diets that range from 0%-24% sucrose was based on previous studies of systemic infection outcome that defined “high” dietary sugar as ranging from 10% glucose (3) to 24% (w/v) sucrose (12). We predicted that there would be a concentration-responsive decrease in host survival post-infection as dietary sugar increased. However, what we observed was a threshold response where quantitatively variable low-sucrose diets (0%, 2% and 4%) were statistically equivalent to each other but different than high-sucrose diets (16% and 24%) with respect to host survivorship of infection. However, the precise threshold

447 depended on the pathogen, with 8% sucrose being a functionally high-sugar diet for a *P. rettgeri*
448 infection but low-sugar for a *S. marcescens* infection.

449 Our observation of immunological effects of diet that vary among pathogens is consistent
450 with previous reports in the literature. For example, Ayres and Schneider (2009) (60)
451 demonstrate that dietary restriction at 24 hours prior to infection of adult *D. melanogaster* is
452 protective against a *Salmonella typhimurium* infection, decreases resistance to *Listeria*
453 *monocytogenes*, and has no effect on *E. faecalis* infection (60). Similarly, Singh et al. (2022)
454 (61) found that *D. melanogaster* reared on a calorie-restricted diet were more susceptible to
455 *Pseudomonas entomophila* infection while diet had no effect on mortality after *E. faecalis*
456 infection. Our finding that diet had no effect on *E. faecalis* infection outcome is corroborated by
457 several studies (60–62) that suggest negligible impact of diet or relevant host physiology on
458 defense against this pathogen. Our data in combination with these previous studies suggest that
459 the effects of diet on immune outcome are in part dependent on the unique physiology of the
460 infecting pathogen.

461 We observed that *S. marcescens* and *P. rettgeri* pathogen burdens are both higher in *D.*
462 *melanogaster* fed high sugar. However, the increased pathogen load arises differently for the
463 two pathogens. The effect of diet on defense against *P. rettgeri* is mediated by the host immune
464 system, as flies provided with 16% sucrose diet produce less Drosocin after infection with *P.*
465 *rettgeri* and the effect of dietary sugar on *P. rettgeri* proliferation is eliminated in *D.*
466 *melanogaster* lacking a functional immune response. However, while flies infected with *S.*
467 *marcescens* produced less Cecropin when provided with 16% sucrose diet, we also observed
468 that *S. marcescens* continued to proliferate more rapidly in flies provided with 16% sucrose diets
469 even when those flies lack most inducible AMPs. We found that high-sugar diet increases the
470 level of circulating sugar within the fly, and that *S. marcescens* benefits more than *P. rettgeri*
471 from carbon supplementation when growing *in vitro.* These data suggest *S. marcescens* may

472 perhaps gain a growth benefit from the hyperglycemia of *D. melanogaster* feeding on high-sugar
473 diets. A similar phenomenon has been observed with the Gram-negative opportunistic pathogen
474 *Pseudomonas aeruginosa* infecting hyperglycemic mice. Wild-type *P. aeruginosa* proliferates
475 more quickly in hyperglycemic mice, but mutants for glucose uptake and metabolism genes
476 (*oprB, gltK, gtrS*, *glk)* exhibit similar growth in control and hyperglycemic mice (6). More detailed
477 future study of *S. marcescens* mutants deficient for glucose uptake or metabolism infecting *D.*
478 *melanogaster* mutant for key factors in metabolism and immune response could probe the
479 relationship between diet, host physiology, and pathogen physiology as determining infection
480 outcome.

481 Interestingly, we noticed that both wild-type and ΔAMP10 *D. melanogaster* genotypes had
482 lower *S. marcescens* burdens at 2 hours after infection on high-sugar diet than on low-sugar
483 diet (Figs. 3B and 4B). Phagocytosis is known to play a role in controlling *S. marcescens*
484 infection in some contexts (63) and infection has previously been shown to increase aerobic
485 glycolysis in *Drosophila* phagocytes (64). Since ΔAMP10 flies still have intact cellular immune
486 systems, it is possible that the elevated dietary sugar provides energy for phagocytes to use in
487 clearing pathogens sensitive to phagocytosis, although this process alone is not sufficient to
488 manage the infection delivered in our experimental context.

489 We found that elevated dietary sugar does not impact the transcription of antimicrobial
490 peptides genes (Fig. 5A, 5B), but it does reduce translation of the encoded peptides Drosocin
491 and Cecropin A1 (Fig. 5C-5E). A similar transcriptional effect was observed in larvae of the beet
492 army worm (*Spodoptera exigua*) and fall army worm (*Spodoptera frugiperda*), where infection-
493 induced expression of the AMPs *attacin* and *gloverin* transcripts were equivalent between a low
494 protein and control diet despite reduced resistance to infection on the low-quality diet (65). This
495 suggests that dietary impacts on the immune system may not occur at the transcriptional level
496 but at the level of translation. A recent proteomics study on *D. melanogaster* found that naive

497 flies subjected to dietary restriction exhibit lower peptide levels of the AMPs Attacin A and
498 Cecropin C (66), although those authors did not measure the effect of diet on production of
499 antimicrobial peptides in the context of a systemic infection.

500 Antimicrobial peptides are critical for controlling Gram-negative infections in *D.*
501 *melanogaster*. At the transcriptional level, there is a high degree of co-regulation of genes
502 encoding AMPs (19,67), and we would anticipate corresponding similarity in translational
503 efficiency (68). Given that high dietary sugar reduced production of Drosocin and Cecropin, we
504 anticipate it would also reduce production of other AMPs. It should also be noted that the IMD
505 and Toll immune regulatory pathways do more than simply stimulate production of AMPs.
506 Activation of these pathways alters host metabolism and is influenced by host metabolic status
507 (13), and even stimulates host sequestration of micronutrients to limit pathogens from accessing
508 them (69). Future experiments will be necessary to establish the mechanistic details of how
509 high-sugar diets impact the IMD and Toll pathways to shape resistance to infection.

510 Our present work establishes that adverse effects of high-sugar diets on infection outcome
511 are due to the combination of impaired host immune function as well as, in some cases, faster
512 proliferation of the infecting pathogen in a hyperglycemic environment. Notably, the immune
513 impairment is not necessarily at the level of transcriptional activation of the immune response
514 but appears to be due to reduced translation of antimicrobial peptides. It is also notable that
515 dietary effects on immune defense are pathogen-specific, and there is not one universal
516 immunological consequence of high-sugar diets. Our findings illustrate the importance of
517 understanding the nutritional requirements of the infecting pathogens as well as the
518 physiological intertwining of host metabolism and immune responses when interpreting effects
519 of diet on infection outcome. We expect this principle to be general across host-pathogen
520 infection systems.

521

522 **Methods**

523 **Fly stocks and husbandry**

524 All flies were reared and maintained at 25 °C on a 12-hour light/dark cycle. Mated females of
525 the wild-type strain Canton S (CS) were used for all experiments unless otherwise indicated.
526 Females were cohoused with males in culture chambers and were aged to 3-5 days post-
527 eclosion prior to infection for all experiments. Flies were anesthetized on CO_2 pads for collection
528 and during infection.

529 **Generation of Tagged AMP Flies**

530 Flies carrying tagged Cecropin A1 or Drosocin were generated using CRISPR-facilitated
531 homology-directed repair. In brief, guide sequences for *CecA1* or *Dro* were cloned into pCFD5
532 (*CecA1* guides: guide 1: CCATTGGACAATCGGAAGCT; guide 2:
533 ATAATTATAAATAATCATCG; *Dro* guides: guide 1: CAAAAACGCAAGCAAGCAGC; guide 2:
534 CAATCAATTGTGACACAATG) (70). Homology-directed repair cassettes were cloned into pHD-
535 Scarless-dsRed, encoding the replacement of endogenous *CecA1* and *Dro* with peptides that
536 included FLAG and HA tags (predicted sequences of mature peptides: $CECA1^{HF}$,
537 GWLDYKDDDDKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATYPYDVPDYAR; DRO^{HF},
538 GDYKDDDDKPRPYSPRPTSHPRPIYPYDVPDYARV) (71). These two plasmids were then
539 injected together into embryos of the genotype y^1 *P(nos-cas9, w-) M(RFP-.attP)ZH-2A w**. Male
540 flies emerging from this injection were crossed with females of the genotype w^{1118}; *If /* SM6a or
541 w^{1118}; TM2 / TM6c, Sb^1, as appropriate. Males expressing dsRed in the eye were then selected
542 and crossed to flies carrying *CyO, Tub-PBac* to enable excision of the 3xP3-dsRed cassette.
543 The final structure of each locus was verified by PCR.

544 **Experimental Diets**

545 All flies were reared from egg to pupation in rearing chambers with interchangeable food plates
546 on a standard base sucrose-yeast-cornmeal diet (per liter: 60 g yeast, 60 g cornmeal, 40 g

547 sucrose, 7 g *Drosophila* Agar, 0.5 mL phosphoric acid, 5 mL propionic acid, and 6 g
548 methylparaben dissolved in 26.5 mL 100% ethanol). *D. melanogaster* pupated on the chamber
549 walls and, prior to eclosion, the food plates containing the standard rearing media were
550 swapped with one of the experimental diets. Emerging adult flies were aged on the experimental
551 media for 3-5 days post-eclosion prior to infection for all experiments. Experimental diets were
552 identical to the rearing diet aside from the concentration of sucrose. Experimental diets
553 contained either 0%, 2%, 4%, 8%, 16%, and 24% (w/v) of sucrose.

554 **Infection Survival Assays**

555 Flies were anesthetized on CO_2 and injected in the thorax with either a bacterial suspension or
556 sterile PBS (as injury control) using a pulled glass capillary needle and nano-injector (72). The
557 following bacteria were injected in at least four experimental blocks: *Providencia rettgeri* strain
558 Dmel, *Serratia marcescens* strain 2698B, *Enterococcus faecalis* strain Dmel, *Lactococcus lactis*
559 strain Dmel. All bacteria were originally isolated as natural infections of *D. melanogaster* (36,73)
560 except *S. marcescens* 2698B, which is a clinical isolate (74). Cultures for *P. rettgeri* and *S.*
561 *marcescens* were started from a frozen glycerol stock then cultured overnight in liquid lysogeny
562 broth (LB) at 37 °C on a shaker, then subcultured the next morning in fresh LB to achieve
563 growth phase. *L. lactis* was cultured overnight from a frozen glycerol stock in liquid Brain Heart
564 Infusion (BHI) at 37 °C with shaking, then subcultured the next morning in fresh BHI to achieve
565 log-phase. *E. faecalis* was cultured from a frozen glycerol stock at room temperature (~24 °C) in
566 BHI. All bacteria were resuspended in 1x phosphate-buffered saline (PBS), diluted to A_{600} = 0.1,
567 and injected at a volume of 23 nL per fly. There were ~4000 *P. rettgeri* cells, ~3000 *S.*
568 *marcescens* cells, ~1000 cells *E. faecalis* cells injected, or ~ 1000 *L. lactis* cells injected per fly.
569 Flies were housed in groups of ten and each experimental block contained 3-4 vials of flies per
570 experimental diet. Survival curves were assessed using a Cox proportional hazards mixed effect
571 model (coxme) with the “CoxMe” package in R.

572 Model A: coxme(Surv(status, time) ~ diet + (1|block)

573 Diet was treated as a main effect and experimental block was treated as a random factor in our
574 model. This model was used to perform all pairwise comparisons using the “emmeans” package
575 in R with a Tukey p-value correction ($p<0.05$).

576 **Bacterial Load Trajectory Assay**

577 To assay bacterial proliferation during *in vivo* infection, flies were infected with *P. rettgeri* or
578 *S. marcescens* as described above, then collected for measurement of bacterial load at 0, 2, 4,
579 6, 8, 10, 12, 14, 16, 24, 36, and 48 hours post-infection. Single, live flies were homogenized in
580 500 µL sterile 1x-PBS with a metal bead and 50 µL of the homogenate was plated onto LB agar
581 using a spiral plater (Whitley Automated Spiral Plater-2). To ensure there was no overgrowth of
582 bacteria on the plates, fly homogenates were diluted with PBS at time points where we
583 anticipated higher bacterial growth. For wild-type CS flies, fly homogenates from timepoints 0, 2,
584 and 4 hours were directly plated, homogenates from timepoints 6-12 were diluted 1:10, and
585 homogenates from timepoints 14-48 were diluted 1:100. For ΔAMP10 mutants, fly homogenates
586 were directly plated for time points 0, 2, and 4, diluted 1:10 at hour 6, and 1:100 at time points 8,
587 10 and 12. Agar plates were incubated at 37°C overnight. The resulting colonies were counted
588 using PROTOCOL3 software to estimate the number of colony forming units (CFU) per mL of fly
589 homogenate. We then used the CFU/mL value to approximate the number of bacteria in the
590 individual fly at the time of sampling by using the following formula:

591 CFU/fly = CFU/mL * (dilution factor) * (0.5 mL/fly of original homogenate)

592 Two experimental blocks were completed for *P. rettgeri* and *S. marcescens* in CS flies, and
593 three experimental blocks were completed for ΔAMP10 mutants with each pathogen. A
594 generalized least squares (GLS) model from the “nlme” package in R was used to determine the
595 effect of diet on pathogen loads over time. Fixed effects in the model include time point sampled
596 (T) and diet (D). The function weights = varIdent(form = ~1|time point) from the “nlme” package

597 was incorporated into the model to account for unequal variances across time points, which
598 arises as the pathogen grows from the initial injection time point. The emmeans function was
599 used for pairwise comparisons between the experimental diets within each time point using the
600 Tukey method to determine p-values ($p<0.05$)

601 Model B: gls($\log_2$(pathogen load) ~ T * D, weights = varIdent(form = ~ 1|T))

602 **Excreta Quantification (ExQ) to measure feeding**

603 Methods for the ExQ assay are further detailed in Wu (2020) (47). Briefly, food was prepared by
604 melting down experimental diets and adding 1% (w/v) of dry erioglaucine powder (Sigma #
605 861146). The dyed food mixture was then dispensed into microcentrifuge tubes caps filled up to
606 50 µL volume. ExQ chambers were prepared from 50 mL conical vials (Cell Treat # 229421). Air
607 holes were poked along the sides of tubes and the lid using a pushpin (~0.5 mm diameter). A
608 precision knife was used to cut a hole in the lid of the conical vial to fit the diameter of the food
609 caps. Naïve flies were anesthetized with CO_2 and sorted into ExQ chambers in groups of 8-10.
610 Every 24 hours, the flies were transferred to new chambers with fresh food. After 48 hours, flies
611 were emptied from chambers and 3 mL of 1x-PBS was used to collect the excreta. Total dye
612 concentration was determined by using a spectrophotometer to measure absorbance of the dye
613 excreted from flies. A standard curve was prepared from a stock solution of erioglaucine by
614 dissolving 10 mg of powder dye in 10 mL of PBS. A serial 2-fold dilution was then created from
615 this initial stock, and the final range of standards used to create the standard curve was 312.5
616 µg/mL to 5 µg/mL. Absorbance was measured at 630 nm in a 96-well plate on a spectrometer
617 (Molecular Biosciences Spectra Max Series Plus 384). Standards and samples were run in
618 duplicates with 100 µL per well and then averaged for data analysis. Dye consumed per fly
619 within a single ExQ chamber was determined using the following equation:

620 Dye (µg) per fly = [(Absorbance – Slope)]/(Intercept * # flies)

621 A two-way Analysis of Variance (ANOVA) in R was used to test for differences in dye
622 consumed by flies on each diet on Day 1 and Day 2 of feeding. Then the emmeans function in R
623 was used for pairwise comparisons between diets within each day using the Tukey test to
624 determine p-values ($p<0.05$).

625 Model C: aov(dye ~ diet + day)

626 **Quantitative real-time PCR (RT-qPCR) for Antimicrobial Peptide Gene Expression**

627 Female flies provided with 2% and 16% sucrose diets were injected with ~3000 CFU of log-
628 growth *S. marcescens* or *P. rettgeri* then sampled at 8 hours after injection for quantification of
629 gene expression. Flies were pooled in groups of 5 and frozen at -80 °C for subsequent RNA
630 extraction. In 1.5 mL microcentrifuge tubes, RNA was isolated and purified using a TRIzol
631 extraction procedure according to the manufacturer's instructions (Zymo Kit direct-Zol RNA kit
632 #R2050). Briefly, flies were homogenized in TRIZoL (Invitrogen #15596018) using a motorized
633 pestle in a microcentrifuge tube and RNA was then extracted using the Zymo Spin Column
634 (Zymo Research #C1078). Samples were then treated with the kit's DNAse and RNA was
635 dissolved 50 µL of molecular grade water. RNA concentration was determined using a Qubit4
636 Broad Sense RNA kit (Thermo #Q10210). cDNA was generated using 500 ng of total RNA with
637 the iSynthesis cDNA kit (Bio-Rad #1708891). RT-qPCR was performed on a CFX Connect
638 Real-Time Detection System (Bio-Rad) using PerfeCTa SYBER Green fast mix (VWR #101414-
639 270). Expression of the AMPs *Drosocin, Defensin, CecropinA, Dipterin, Attacin A, Drosomycin*
640 was normalized to the expression of the housekeeping gene *Actin 5C*. Uninfected controls were
641 not subject to injury. Primer sequences used are listed in Supplementary Table 1. A linear
642 model was used to test the contributions of diet and infection status to expression of each AMP
643 gene, standardized to the housekeeping gene (75).

644 Model D: lm(AMP_CT ~ *Actin5C*_CT + Diet + Infection Status * Diet)

645 Diet and Infection Status were fixed effects in the model. Estimated marginal mean CT values
646 for the Diet x Infection interaction terms were extracted using the emmeans package in R. Then
647 we subtracted the estimated marginal mean CT value for uninfected flies from the estimated
648 marginal means from the infected flies for each diet. This difference in estimated marginal mean
649 CT between uninfected and infected values indicates infection-induced change in AMP gene
650 expression on a $\log_2$ scale. Using the emmeans function, we compared infection-induced
651 expression values between experimental diets with Tukey's corrections test for p-values
652 ($p<0.05$).

653

654

Gene	Forward Primer	Reverse Primer
Actin-5C	AGCGCGGTTACTCTTTCACCAC	GTGGCCATCTCCTGCTCA AAGT
Attacin A	CGTTTGGATCTGACCAACG	AAAGTTCCGCCAGGTGTGAC
CecropinA1	CTCTCATTCTGGCCATCACC	TCTTGAGCGATTCCCAGTC
Defensin	GCGGATCATGTCCTGGTGCAT	TCGCTTTGGCGGCTATG
Diptericin A	GCGGCGATGGTTTTGG	CGCTGGTCCACACCTTCTG
Drosocin	TTTTCCTGCTGCTTGCTTGC	GGCAGCTTGAGTCAGGTGAT
Drosomysin	CTGCCTGTCCGGAAGATACAA	TCCCTCCTCCTTGCACACA

655 **Supplementary Table 1:** Primer sequences for AMP and housekeeping genes ('5-3').

656 **Sandwich ELISA to quantify tagged AMPs**

657 HA/FLAG tagged AMPs were quantified 6 hr post-infection. The fly line w^{1118} ; Dro^{HF}/Dro^{Hf}
658 was used to quantify tagged Drosocin and w^{1118} ; $Cec^{HF}/+$ was used to quantify tagged
659 Cecropin. Maxisorp Nunc 96-well ELISA plates (Thermo Scientific 232698) were coated with 2.5
660 µg/mL of anti-FLAG antibody (Sigma-Aldrich F1804) in coating buffer (0.2 M sodium

661 carbonate/bicarbonate buffer) overnight at 4 °C, then the plates were blocked for 2 hours at
662 room temperature with 2% bovine serum albumin (Sigma-Aldrich A2153) prior to use. At the
663 time of sampling, flies were homogenized in 200 µL of 0.2% Triton-X buffer and centrifuged at
664 10,000 RPM for 5 mins to pellet fly debris. Drosocin samples were diluted 1:20 and Cecropin
665 samples were diluted 1:10 so that they fell within linear range of a HA/FLAG peptide standard
666 curve (sequence NH2-DYKDD DDKGG GGGSY PYDVP DYA-NH2 made from AAPTec). The
667 standard curve was prepared by performing a 2-fold serial dilution starting from a stock solution
668 of 2 ng/mL of HA/FLAG peptide to yield 14 standard concentrations. For sample incubation, 50
669 µL of each standard and sample were added to the ELISA plate and then incubated overnight.
670 Any unused wells were filled with 50 µL of 0.2% Triton-X buffer to serve as blanks. The next
671 day, anti-HA diluted 1:5000 in 0.2% Triton-X was added to each well for another overnight
672 incubation at 4 °C. The following morning, 100 µL of room temperature 1-Step Ultra TMB-ELISA
673 Substrate Solution (ThermoFisher #34028) was added to each well and the plate was incubated
674 at 25 °C for 30 min. After incubation, 100 µL of 2M sulfuric acid was added to each well to stop
675 the reaction. Plates were then read on a spectrometer at 450 nm to measure absorbance. The
676 amount of peptide per fly was determined from the HA/FLAG standard curve with the following
677 equation:

678 Peptide per fly = [(Absorbance-Slope) * Dilution Factor]/ (Intercept * Fly Homogenate)

679 To determine whether diet affects Drosocin peptide levels, a linear mixed effects model was
680 applied, with diet and infection status as main factors and experimental block as a random
681 factor. The emmeans function in R was used to compare peptide yield from flies on each diet
682 within infection status.

683 Model E: lmer(Dro_levels ~ diet * infection + diet + (1|block))

684 To determine effects of diet on Cecropin peptide levels, a hurdle model was applied. In the first
685 step, a binomial generalized linear model (GLM) was used to test whether diet and infection
686 status (*Serratia* or *Providencia* infection versus uninjured) affected the probability of detecting
687 Cecropin peptide in the samples.

688 Model F: glm(Cec_present ~ diet * infection + diet, family = binomial)

689 In the second step, a GLM was performed on samples with detectable peptide to test
690 whether diet and infection resulted in quantitative differences in peptide abundance.

691 Model G: glm(Cec_level ~ diet *infection + diet)

692 The significance of the contrast between diets within each infection was determined with a
693 post-hoc Tukey test ($p<0.05$) using the emmeans package in R.

694 **Measurement of nutritional indices**

695 Nutritional indices were measured in naïve flies and in flies 24 hours post-infection with
696 either *P. rettgeri* or *S. marcescens.* Flies were homogenized in pools of three in 150 µL of cold
697 lysis buffer (10 mM Tris, 1 mM EDTA, pH 8.0 with 0.1% (v/v) Triton-X-100) with a motorized
698 pestle in 1.5 mL microcentrifuge tubes. Homogenates were centrifuged for 1 minute at 13,000
699 rpm at 4°C to pellet fly debris. The supernatants were transferred to fresh 1.5ml microfuge
700 tubes, and 10 µL of homogenized sample was removed for protein quantification. The remaining
701 samples were placed in a 72°C water bath for 20 minutes to denature endogenous enzymes.
702 The tubes were stored at -80 °C for later use.

703 <u>Protein measurements:</u> Protein was measured to normalize all carbohydrate measures. Non-
704 heated fly homogenates were diluted to 1:8 in lysis buffer. Protein was quantified according to
705 Bio-Rad Assay DC Protein Assay Kit instructions. Briefly, 5 µL of standard and sample were
706 incubated with 25 µL reagent A and 200 µL of reagent B. Bovine serum albumin was serially
707 diluted to generate a standard curve that was then used to determine the quantity of protein in

708 each sample. Samples and standards were all run in duplicates. Samples were incubated at
709 room temperature for 15 minutes and then read on a spectrometer plate reader at 750 nm.

710 Glucose, trehalose, and glycogen level measurements: Glucose, trehalose, and glycogen
711 levels were measured using reagents from the Sigma Aldrich Glucose (GO) Assay Kit
712 (GAGO20-1KT) and processed according to manufacturer's instructions. The same fly
713 homogenates were used across all nutrients assayed. Glucose was measured by mixing 5 μL of
714 standard or 5 μL of sample with 150 μL of glucose assay reagent. A glucose standard curve
715 was generated from 2-fold serial dilution from a 1 mg/mL glucose stock concentration. Each
716 sample and standard were run in duplicate. The plate was incubated for 30 minutes at 37°C,
717 then 150 μL of 12M sulfuric acid was added to each well to stop the reaction. Plates were then
718 measured for absorbance at 544 nm on a spectrometer. For trehalose measurements, 5 μL of
719 sample and standards were incubated with 2.5 μL of trehalase overnight at 37 °C. For each
720 sample, two replicates were treated with trehalase enzyme and two were not. Trehalose
721 standard curves were generated from a glucose standard curve, trehalose standards treated
722 with trehalase, and trehalose standards without enzyme. Glucose liberated from trehalose
723 digestion was quantified the next day using the procedure described above, and the abundance
724 of trehalose in the standard was inferred from the difference between the samples that were
725 treated with trehalase and those that were not. Glycogen measurements were conducted
726 similarly, except 5 μL of standard and 5 μL of sample were incubated overnight with
727 amylogucosidase instead of trehalase. The glycogen standard curve was prepared from a
728 glycogen stock solution over the range 1 mg/mL to 0.1 mg/mL. All estimates of carbohydrate
729 levels were normalized to measured protein level for each sample to correct for potential
730 differences in the size of the fly. To calculate change in metabolite level after infection, we
731 subtracted the average post-infection value of the metabolite level from the average level in
732 uninfected samples within an experimental block. A linear mixed-effects model was performed

733 to test for the main effects of diet and infection on carbohydrate/protein ratios with experimental
734 block as a random factor. The emmeans package was used to perform a post-hoc Tukey's test
735 to determine the significance of pairwise comparisons between diet and infection ($p<0.05$).

736 **Bacteria growth curves *in vitro***

737 *Serratia marcescens* and *Providencia rettgeri* were measured for *in vitro* growth using the
738 kinetic cycle in a spectrometer (Molecular Biosciences Spectra Max Series Plus 384) at room
739 temperature. Bacteria were first cultured overnight with shaking in liquid LB at 37°C. The next
740 morning, bacterial cells were resuspended in 1X M9 minimal media (M9) to achieve A_{600} = 1.0,
741 then further diluted to A_{600} = 0.20 in one of six different experimental M9 media conditions. The
742 combinations of M9 media used were 1% glucose, 1% sucrose, 1% casamino acids (CAS), 1%
743 casamino acids and 1% glucose, 1% casamino acids and 1% sucrose, and a M9 blank. CAS
744 was added to the media to provide a nitrogen source while glucose and sucrose provide a
745 carbon source. In a sterile 96-well plate with a clear flat bottom, 100 µL of bacteria resuspended
746 in the experimental media were pipetted into individual wells in 3-fold replicate. Two wells of
747 uninoculated experimental media were included to monitor for contamination. The plate was
748 covered with a lid and inserted into the spectrometer to measure A_{600} at 15-minute intervals over
749 18 hours at 37°C. We used a linear mixed effect model to test for the main effects of CAS and
750 sugar with block as a random factor to test whether the addition of CAS and/or sugar led to
751 increased bacterial growth, measured as a higher final A_{600}. A post-hoc Tukey HSD was
752 performed using the emmeans package in R to test pairwise comparisons ($p<0.05$) between
753 media containing sugars and CAS.

754 Model H: lmer(OD600 at 18 hours ~ sugar *CAS + sugar + (1|block))

755

756

757 **Preparation of Axenic Flies**

758 Axenic flies were prepared by embryo bleaching. Conventionally reared (microorganism
759 associated) flies were fed on lab standard yeast cornmeal diet (4% sucrose w/v), and
760 approximately 100-150 flies were placed into an egg laying chamber and allowed to lay eggs on
761 grape juice agar plates. Embryos were collected after 20 hours and removed from agar plates
762 with a clean paint brush into a cell strainer. In a six-well tissue culture plate, embryos were
763 washed within the cell strainer first in 10% bleach twice for 1.5 minutes each, followed by two
764 30-second washes in 70% ethanol. Lastly, embryos were washed twice for 10 seconds with
765 autoclaved 1X PBS. Embryos were continuously mixed throughout the process to prevent them
766 from sticking to each other or the cell strainer. Once sterilized, embryos were transferred with a
767 sterile brush onto autoclaved yeast-cornmeal rearing media in groups of 50. During pupation,
768 the rearing media was replaced with autoclaved 2% and 16% sucrose diets for adult flies to
769 eclose onto.

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773

774 **References**

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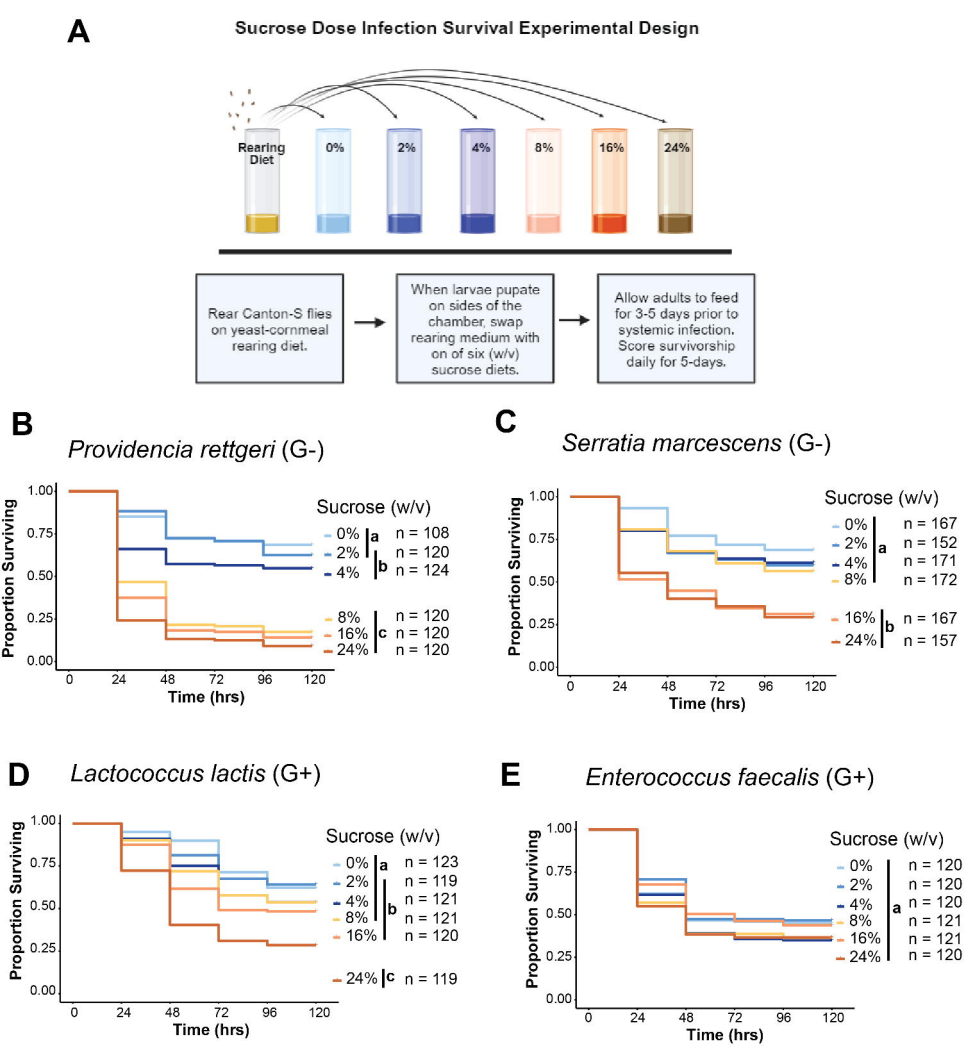
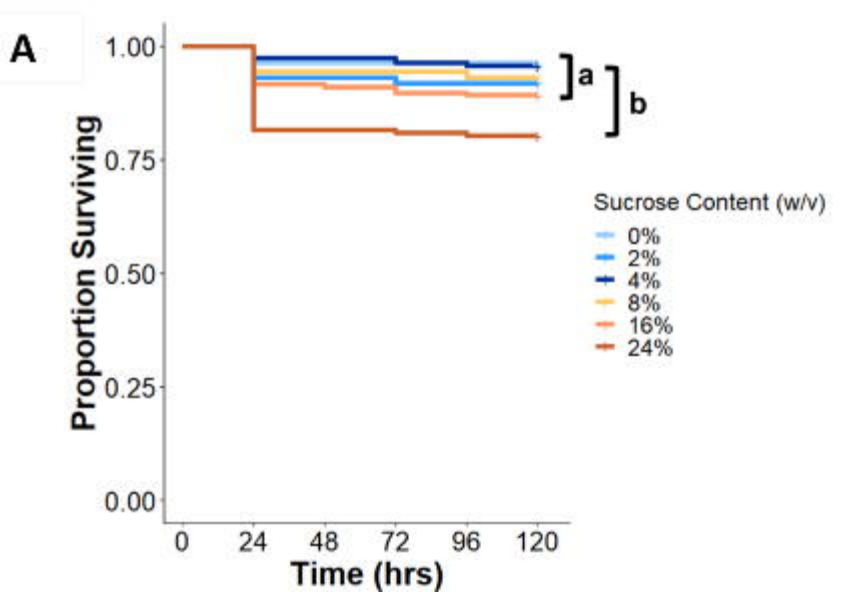
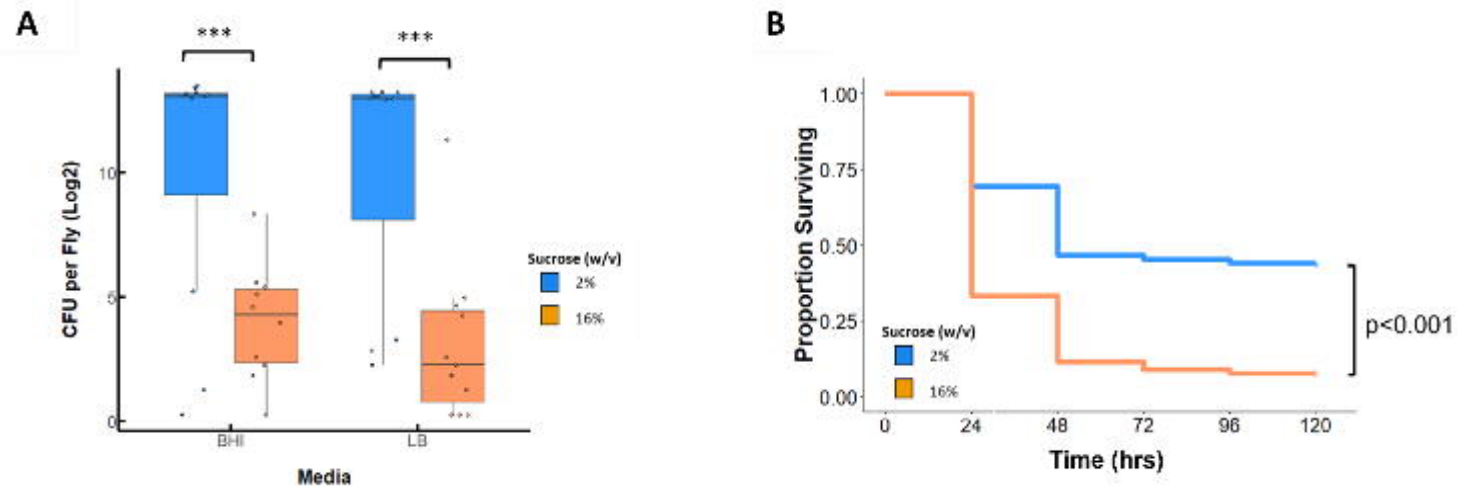
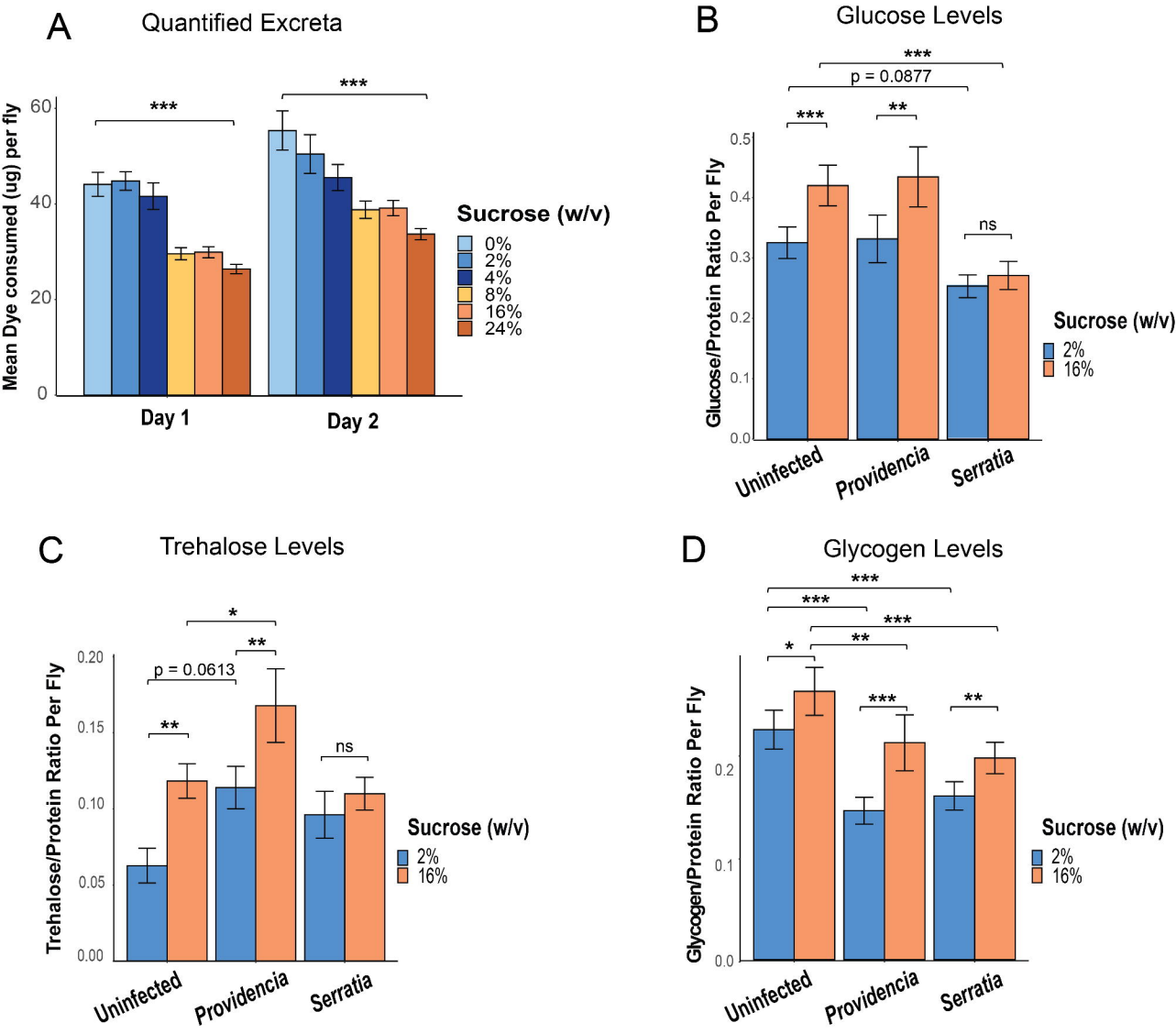
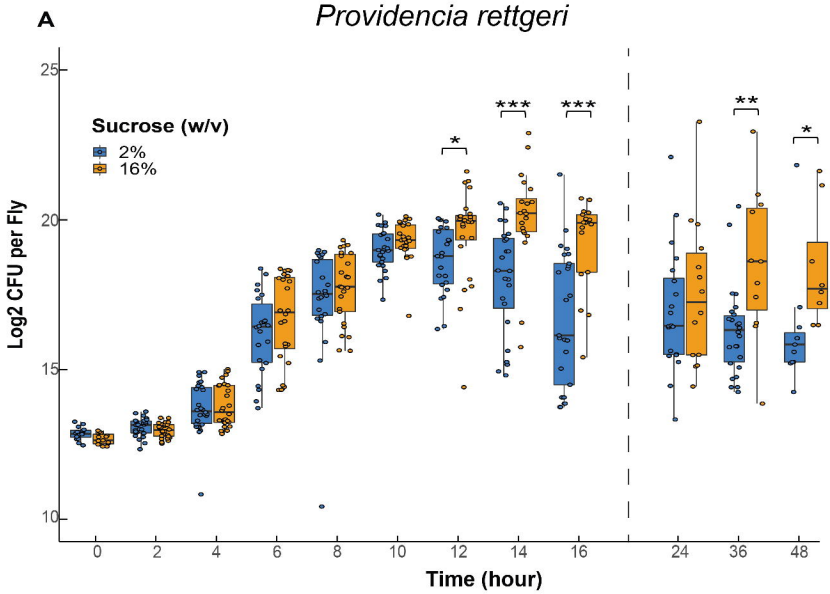

A
Sucrose Dose Infection Survival Experimental Design
Rearing Diet
0%
2%
4%
8%
16%
24%
Rear Canton-S flies on yeast-cornmeal rearing diet.
When larvae pupate on sides of the chamber, swap rearing medium with on of six (w/v) sucrose diets.
Allow adults to feed for 3-5 days prior to systemic infection. Score survivorship daily for 5-days.
B
Providencia rettgeri (G-)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% a n = 108
2% b n = 120
4% n = 124
8% c n = 120
16% n = 120
24% n = 120
C
Serratia marcescens (G-)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% a n = 167
2% n = 152
4% n = 171
8% n = 172
16% b n = 167
24% n = 157
D
Lactococcus lactis (G+)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% a n = 123
2% n = 119
4% b n = 121
8% n = 121
16% n = 120
24% c n = 119
E
Enterococcus faecalis (G+)
Proportion Surviving
Time (hrs)
Sucrose (w/v)
0% n = 120
2% n = 120
4% a n = 120
8% n = 121
16% n = 121
24% n = 120


A
1.00
0.75
0.50
0.25
0.00
Proportion Surviving
0
24
48
72
96
120
Time (hrs)
a
b
Sucrose Content (w/v)
0%
2%
4%
8%
16%
24%

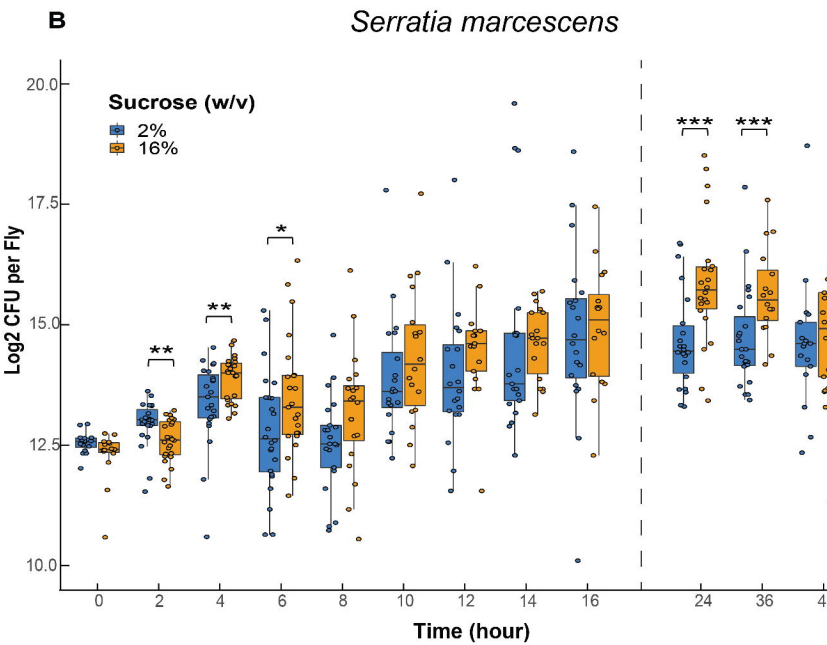

A
***
***
CFU per Fly (Log2)
10
5
0
BHI
LB
Media
Sucrose (w/v)
2%
16%
B
Proportion Surviving
1.00
0.75
0.50
0.25
0.00
0
24
48
72
96
120
Time (hrs)
Sucrose (w/v)
2%
16%
p<0.001


A
Quantified Excreta
Mean Dye consumed (ug) per fly
60
40
20
0
***
***
Day 1
Day 2
Sucrose (w/v)
0%
2%
4%
8%
16%
24%
B
Glucose Levels
Glucose/Protein Ratio Per Fly
0.5
0.4
0.3
0.2
0.1
0.0
***
p = 0.0877
***
**
ns
Uninfected
Providencia
Serratia
Sucrose (w/v)
2%
16%
C
Trehalose Levels
Trehalose/Protein Ratio Per Fly
0.20
0.15
0.10
0.05
0.00
*
**
p = 0.0613
**
ns
Uninfected
Providencia
Serratia
Sucrose (w/v)
2%
16%
D
Glycogen Levels
Glycogen/Protein Ratio Per Fly
0.2
0.1
0.0
***
***
***
**
*
***
**
Uninfected
Providencia
Serratia
Sucrose (w/v)
2%
16%



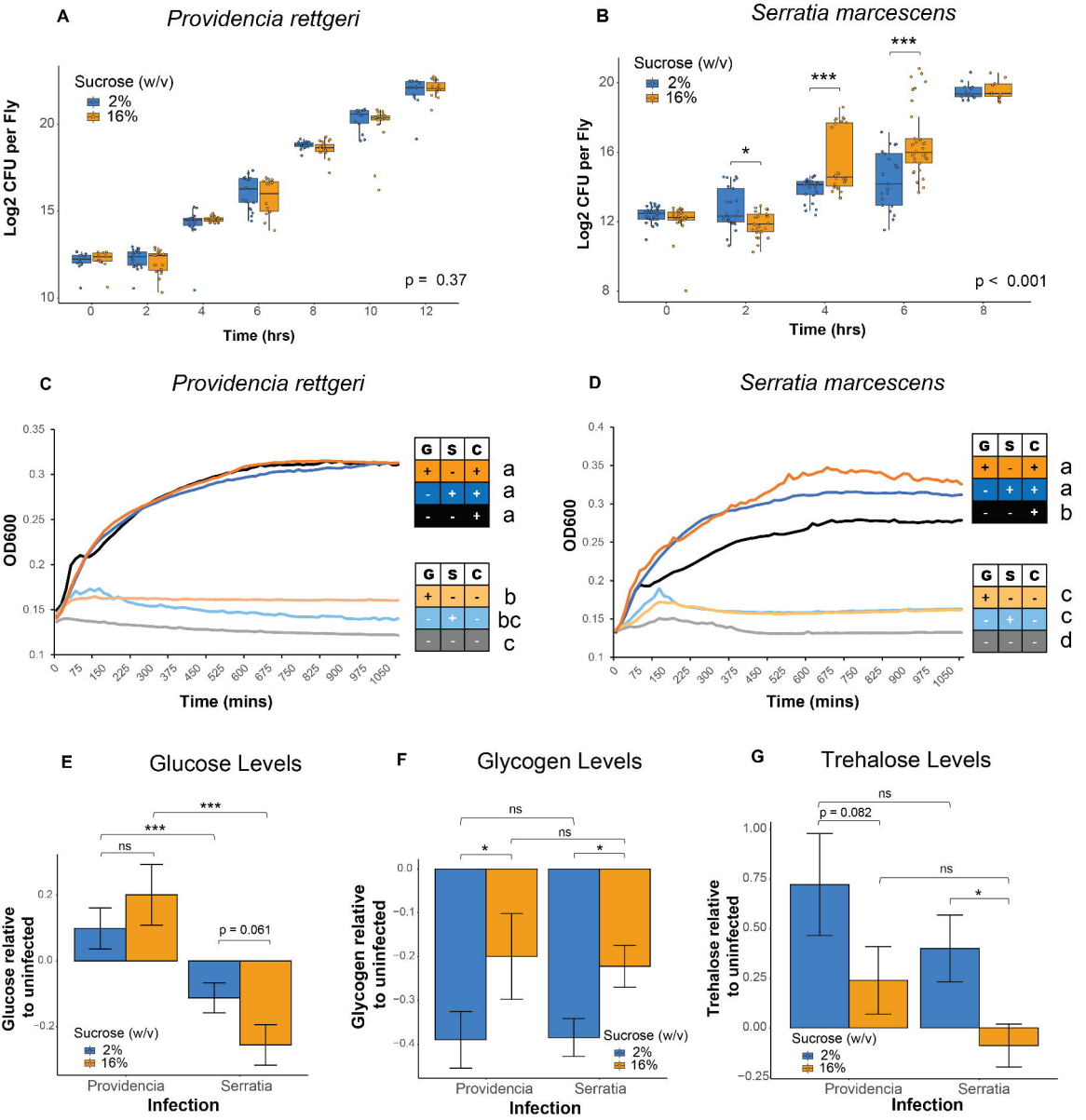
Proportion of flies alive at each time point

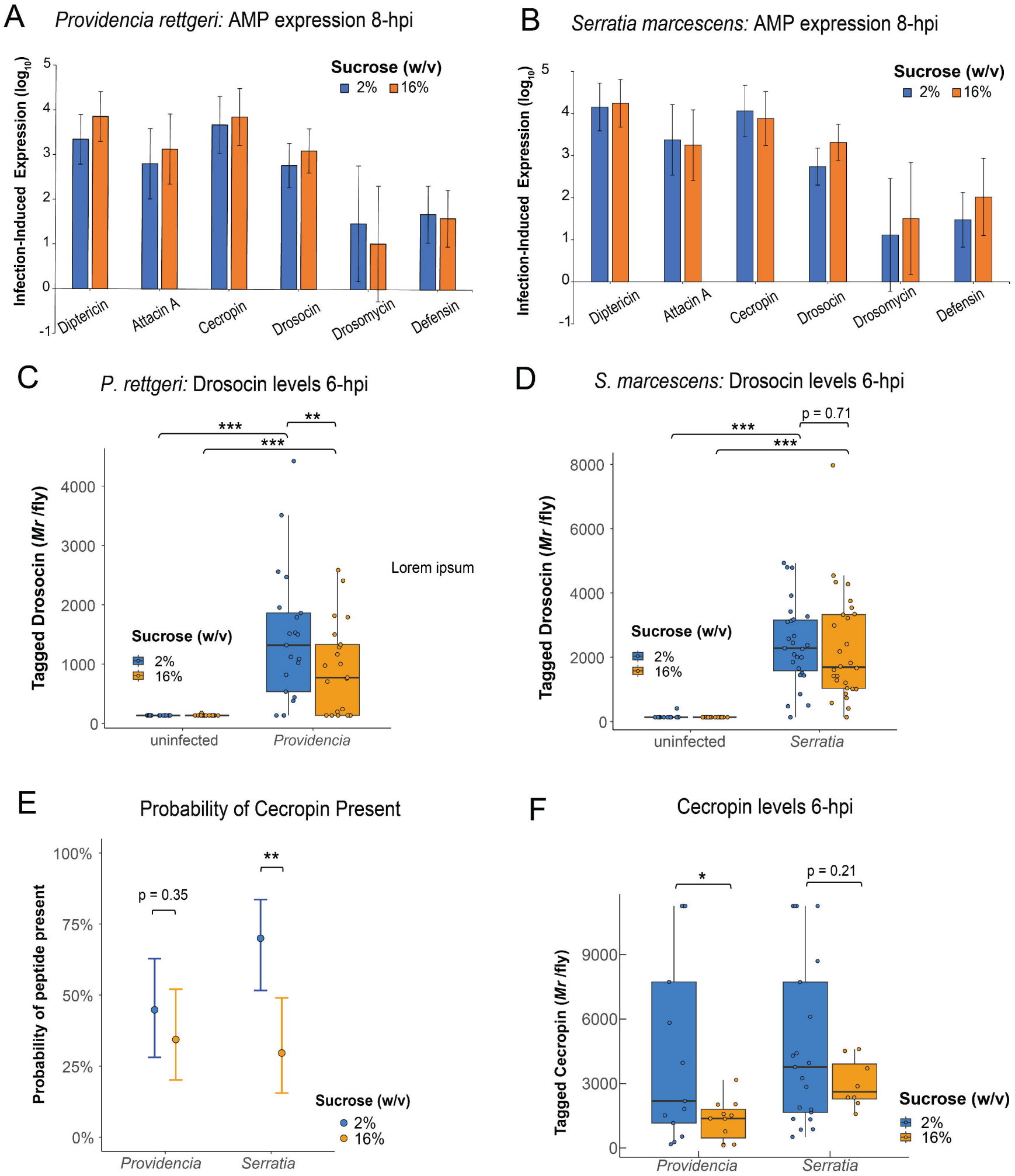
Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	0.97	0.93	0.93	1.0	0.93	0.90	0.90	0.97	0.76	0.76	0.71
16%	1.0	0.97	1.0	0.89	1.0	0.83	0.85	0.81	0.59	0.47	0.29	0.29

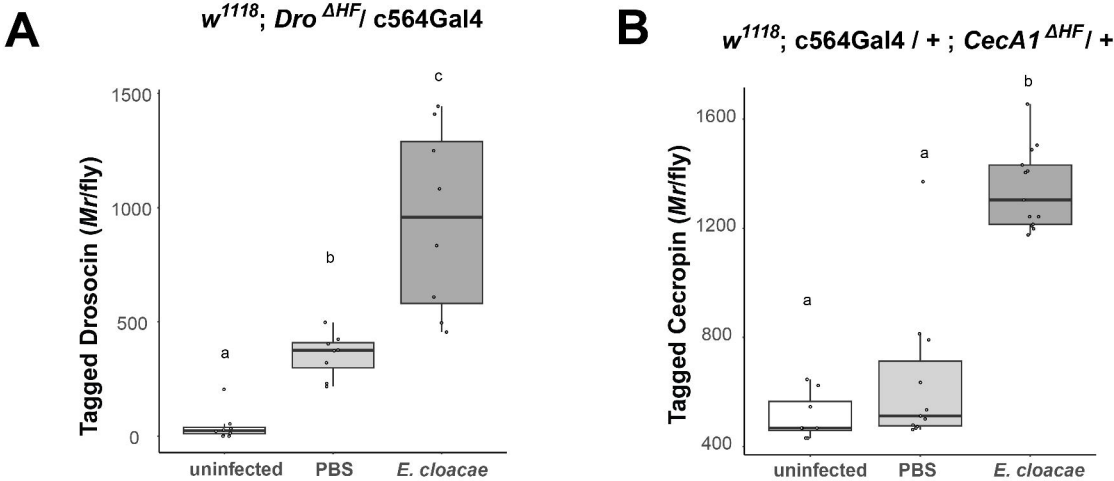


Proportion of flies alive at each time point

Hour	0	2	4	6	8	10	12	14	16	24	36	48
2%	1.0	1.0	1.0	0.96	0.90	0.93	0.85	0.90	0.83	0.80	0.74	1.0
16%	1.0	1.0	0.93	1.0	0.83	0.69	0.76	0.67	0.63	0.62	0.42	0.44


A
Providencia rettgeri
Sucrose (w/v)
2%
16%
Log2 CFU per Fly
Time (hrs)
p = 0.37
B
Serratia marcescens
Sucrose (w/v)
2%
16%
Log2 CFU per Fly
Time (hrs)
p < 0.001
C
Providencia rettgeri
OD600
Time (mins)
G S C
a
a
a
b
bc
c
D
Serratia marcescens
OD600
Time (mins)
G S C
a
a
b
c
c
d
E
Glucose Levels
Glucose relative to uninfected
Sucrose (w/v)
2%
16%
ns
***
***
p = 0.061
Providencia
Serratia
Infection
F
Glycogen Levels
Glycogen relative to uninfected
Sucrose (w/v)
2%
16%
ns
ns
*
*
Providencia
Serratia
Infection
G
Trehalose Levels
Trehalose relative to uninfected
Sucrose (w/v)
2%
16%
ns
p = 0.082
ns
*
Providencia
Serratia
Infection


A
Providencia rettgeri: AMP expression 8-hpi
Infection-Induced Expression (log10)
Sucrose (w/v)
2%
16%
Diptericin
Attacin A
Cecropin
Drosocin
Drosomycin
Defensin
B
Serratia marcescens: AMP expression 8-hpi
C
P. rettgeri: Drosocin levels 6-hpi
Tagged Drosocin (Mr /fly)
Lorem ipsum
uninfected
Providencia
D
S. marcescens: Drosocin levels 6-hpi
p = 0.71
Serratia
E
Probability of Cecropin Present
Probability of peptide present
p = 0.35
F
Cecropin levels 6-hpi
Tagged Cecropin (Mr /fly)
p = 0.21


A
w1118; Dro ΔHF/ c564Gal4
Tagged Drosocin (Mr/fly)
1500
1000
500
0
a
b
c
uninfected
PBS
E. cloacae
B
w1118; c564Gal4 / + ; CecA1 ΔHF/ +
Tagged Cecropin (Mr/fly)
1600
1200
800
400
a
a
b
uninfected
PBS
E. cloacae

A

	Providencia detected	Providencia undetected	Proportion in linear range
2%	13	16	0.45
16%	11	21	0.34

B

	Serratia detected	Serratia undetected	Proportion in linear range
2%	21	9	0.70
16%	8	19	0.30

Gene	Forward Primer	Reverse Primer
Actin-5C	AGCGCGGTTACTCTTTCACCAC	GTGGCCATCTCCTGCTCA AAGT
Attacin A	CGTTTGGATCTGACCAACG	AAAGTTCCGCCAGGTGTGAC
CecropinA1	CTCTCATTCTGGCCATCACC	TCTTGAGCGATTCCCAGTC
Defensin	GCGGATCATGTCCTGGTGCAT	TCGCTTTGGCGGCTATG
Diptericin A	GCGGCGATGGTTTTGG	CGCTGGTCCACACCTTCTG
Drosocin	TTTTCCTGCTGCTTGCTTGC	GGCAGCTTGAGTCAGGTGAT
Drosomycin	CTGCCTGTCCGGAAGATACAA	TCCCTCCTCCTTGCACACA