Acute bacterial challenge in Drosophila reveals age- and sex-dependent feeding and macronutrient choice without generalised anorexia

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Abstract

Sickness behaviours are often interpreted as adaptive host responses that reallocate resources from performance to defence. Anorexia - a reduction in food intake - is one of the most frequently cited examples, yet evidence across insects is variable and rarely separates the effects of wounding, immune stimulation, and live infection. Here we use a factorial design in *Drosophila melanogaster* to disentangle these components and quantify both feeding amount and macronutrient choice during the first four hours following acute bacterial challenge. Using the FlyPAD system, we recorded cumulative sips from protein- and carbohydrate-rich diets in young (2-4 days old) and older (22-24 days old) male and female flies. We compared naïve flies to those injured, immune stimulated with heat-killed *Pseudomonas entomophila*, or infected with live *P. entomophila*. Across sexes and ages, neither live infection nor sterile immune stimulation reduced total food intake during the first 4 h; notably, in old females and young males, live infection increased feeding. In contrast, immune stimulation alone elicited sex- and age-dependent shifts in macronutrient choice: in old females, injury and immune stimulation reduced protein preference relative to naive, whereas in old males they increased it. Thus, 'sickness feeding' in Drosophila manifests primarily as an increase in feeing rate or a host-driven macronutrient rebalancing rather than anorexia.

Keywords: sickness behaviour; anorexia; protein; carbohydrate; infection; feeding; FlyPAD

Data availability: The raw data and full analysis script accompanying the dataset is available in supplementary material.

Introduction

Animals confronted with infection frequently exhibit a coordinated suite of behavioural and physiological changes, termed "sickness behaviours", that include lethargy, reduced social interaction and exploration, altered thermoregulation, and changes in feeding (Hart 1988; Dantzer and Kelley 2007). Although this phenotype was first formalised in vertebrates (Dantzer and Kelley 2007; Lopes et al. 2021), analogous responses occur in invertebrates, including insects, indicating deep conservation of neuro-immune crosstalk linking peripheral immune activation to central motivational states (Ayres and Schneider 2009; Kazlauskas et al. 2016; Sullivan et al. 2016; Vale et al. 2018). These behavioural changes are often interpreted as adaptive because they can promote recovery, reduce risk, and conserve resources for defence and repair (Dantzer and Kelley 2007; Lopes 2014; Lopes et al. 2021) .

Anorexia (reduced food intake) is among the most widely cited sickness behaviours(Murray and Murray 1979; Exton 1997; Ayres and Schneider 2009; Jindal et al. 2024). Evolutionary interpretations of sickness behaviours posit that anorexia limits foraging and therefore reduces exposure to predators and injury during a vulnerable period and may also deprive pathogens of key nutrients (e.g. iron), thereby constraining growth (Hart 1988; Exton 1997; Lopes 2014; van Niekerk et al. 2016). Empirical observations of reduction in food intake in response to actual or perceived infection are common: for example, reduced intake after lipopolysaccharide (LPS) has been reported in mammals and birds, and in insects such as *Manduca sexta* and honey bees (Owen-Ashley et al. 2006; Kazlauskas et al. 2016; Wilson et al. 2018). However, anorexia is far from universal. In orthopterans, immune challenge does not reliably reduce activity or feeding (Sullivan et al. 2016; Kelly and Mc Cabe Leroux 2020), and in social insects immune stimulation can even increase food consumption (Tyler et al. 2006). This heterogeneity suggests that the expression and value of anorexia are conditional on context, pathogen, dose, host state, and time since challenge.

Beyond "how much" is eaten, infection can also change "what" is eaten. A growing body of work shows that sick individuals can shift macronutrient balance in ways that affect immunity, tolerance, and survival, but the direction of change is not universal (Ponton et al. 2013). In several lepidopterans, resistance to baculoviruses is higher on protein-rich diets, and infected caterpillars self-select higher protein:carbohydrate (P:C) ratios compared to uninfected controls (Lee et al. 2006; Povey et al. 2014). In Drosophila, by contrast, carbohydrate-biased diets can enhance survival to bacterial infection, consistent with a strategy that fuels energetically costly defences, and indicating that the optimal macronutrient balance for defence need not be protein-rich(Graham et al. 2014; Ponton et al. 2020; Savola et al. 2021). Taken together, these findings indicate that there is no single directional "sickness diet".

A persistent challenge in interpreting changes in feeding is that many studies do not separate effects of wounding, immune stimulation, and infection-specific pathology. Here we address this explicitly. Using *Drosophila melanogaster* and the FlyPAD system (Itskov et al. 2014), we quantify both total intake and macronutrient choice during the first four hours after challenge, in males and females at two ages. We asked whether feeding changes after bacterial challenge reflect a generalised "sickness behaviour" of the host, such as anorexia or a shift in

macronutrient intake, or whether they can instead be attributed to other components of the challenge, including wounding, immune stimulation, or processes specific to live infection (including pathology). To disentangle these possibilities, we compared flies pricked with live *Pseudomonas entomophila* to three matched controls: naïve (no prick), PBS-injured (wounding/handling), and heat-killed *P. entomophila* (sterile immune stimulation without pathogen replication). Because feeding behaviour can depend on sex, age, and time since challenge, we analysed males and females at two ages: 2-4 days old (young) and 22-24 days old (old) and followed feeding over the first four hours following challenge.

Methods

Fly stock maintenance

We used *Drosophila melanogaster* from the Ashworth Advanced Outcrossed (AOx) population, a large, laboratory-adapted outbred population derived from DGRP lines (Monteith et al. 2019; Savola et al. 2021). Stocks were maintained at 25°C under a 12:12 h light:dark cycle on a ~14-day generation cycle at a census size of 3,000–4,000 adults per generation. Flies were assayed at two adult ages and both sexes: Young (2–4 days post-eclosion) and Old (22–24 days).

Experimental treatments and injury procedure

Four treatments were used to separate effects of handling/injury, sterile immune stimulation, and active infection: Naïve (unhandled control), PBS-injured (septic injury with sterile PBS). *Pseudomonas entomophila*, a Gram-negative entomopathogen of Drosophila (Dieppois et al. 2015), was used for immune stimulation and live infection. Overnight cultures were grown with shaking under sterile conditions. For the live inoculum, the overnight culture was standardized to an equivalent of OD = 0.001 (OD=1 diluted 1:1000). This inoculation dose was chosen to induce acute sickness while permitting survival over the recording window (see (Prakash et al. 2023, 2025).The heat-killed inoculum, an aliquot of the same culture was heat-inactivated in a 60–70°C water bath for 1 h and returned to shaking overnight prior to use. Sterile control: phosphate-buffered saline (PBS). Immediately prior to treatment, flies were lightly anesthetized with CO_2. Each Sex × Age × Treatment combination was replicated 16-27 times (median n = 21).

Diets and FlyPAD assay

We used the FlyPAD system - an automated, high throughput system to measure feeding behaviour in Drosophila (Itskov et al. 2014) - to track and quantify feeding in real time as described previously (Itskov et al. 2014; Monteith et al. 2024). Each FlyPAD arena contains two independent capacitive sensors; each sensor comprises a pair of electrodes, one in contact with the food and one adjacent to where the fly stands. When a fly touches the food with its proboscis or legs, the resulting change in electric capacitance between the electrodes is detected and recorded as a discrete “sip.” Choice diets followed (Itskov et al. 2014) and were presented simultaneously in each arena in equal quantities: a carbohydrate-rich food (2% agar, 5% sucrose, 1% yeast, w/v) and a protein-rich food (2% agar, 1% sucrose, 5% yeast,

w/v). 1 µL of each substrate was pipetted onto the corresponding electrode in each arena. Flies were wet-starved on water-soaked cotton for 3 h prior to treatment and loading into the FlyPAD. Feeding was recorded for 4 h post-procedure, with cumulative readouts at 60, 120, 180, and 240 min from the FlyPAD software for each channel (carbohydrate and protein). The sum of channel-specific counts yields total sips. The experiment was staggered across 28 days and grouped into seven day-blocks. Within each combination, flies were assayed individually.

Statistical analysis

Total feeding was modelled using a negative binomial generalised linear mixed model (GLMM) fitted with 'glmmTMB' (Brooks ME et al. 2017) to accommodate overdispersed counts. Fixed effects were Sex, Age, Treatment, Hour (categorical: 1–4), and all interactions. Random intercepts accounted for repeated measures within fly (fly ID) and for day-block effects. Macronutrient preference was modelled using a binomial GLMM with a cbind(protein, carbohydrate) response per interval, with the same fixed and random effects structure. Model diagnostics included checks for overdispersion and zero inflation (glmmTMB), and simulation-based residual diagnostics (DHARMa). For specific pairwise comparisons between treatment levels, we obtained estimated marginal means (EMMs) from the fitted models using 'emmeans' (Lenth et al. 2022). P-values for these contrasts were adjusted by Holm's method within stratum. All analyses were performed in R (version 4.2). The raw data and full analysis script accompanying the dataset is available in supplementary material.

Results

Age- and sex-specific feeding patterns

We assayed 341 flies over the first 4 h after challenge (60, 120, 180, 240 min) (Figure 1A and B). Across all treatments, the food intake of older flies was substantially higher than young flies, and intake increased over time (age, $p < 0.001$; hour, $p < 0.001$; Table S1). In addition to a general age effect in feeding rate, we observed smaller, age-specific sex differences in feeding. In older flies, males ate more than females across treatments - at 240 min (Figure 1B), the mean cumulative sips ranged between 139–305 in old females and 209–330 in old males; In young flies the sex difference was small and varied with treatment (sex × age, $p <$ 0.001): 59–100 sips in young females and 34–85 sips in young males (see Table S2).

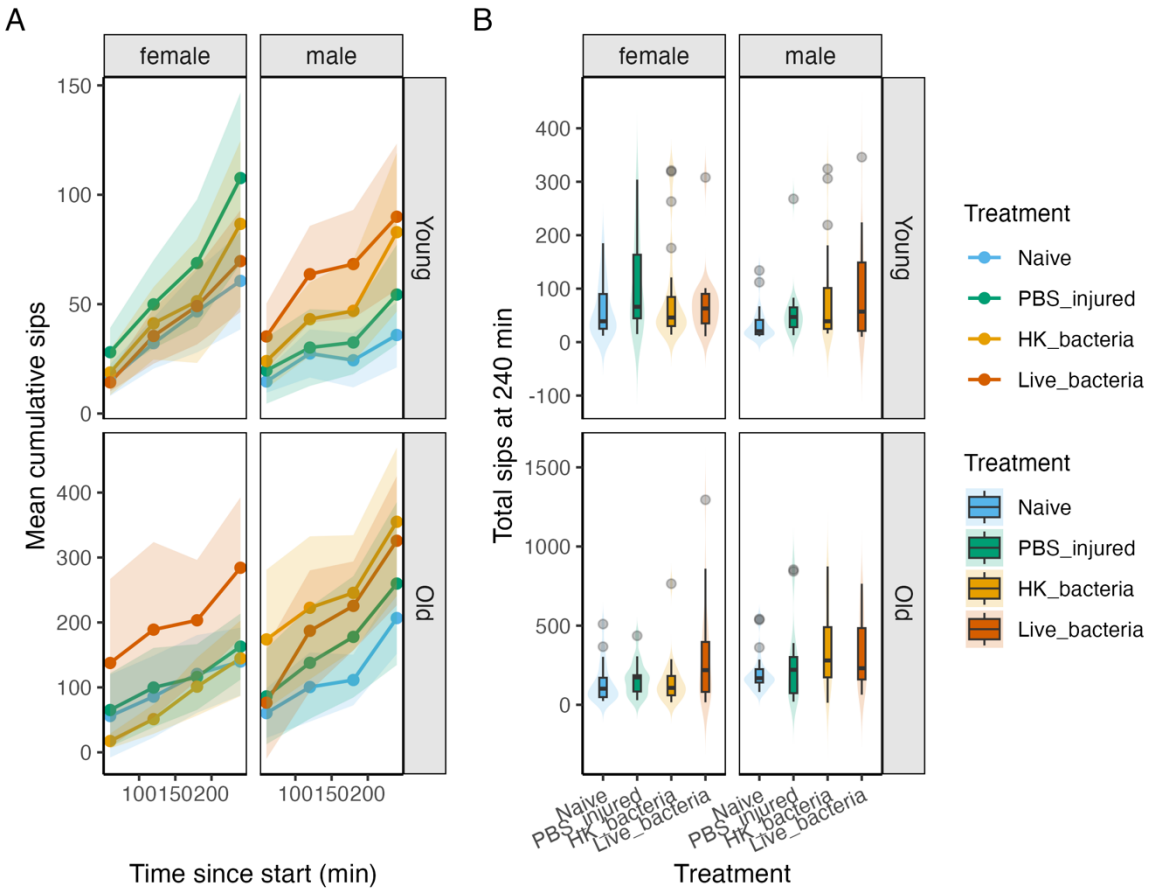

Figure 1. Total number of sips. A. Cumulative feeding over 4 h. Mean (lines) ± 95% CI (shaded ribbons) of cumulative sip counts across time, by treatment. B. Endpoint feeding (240 min). Distribution of total sips at 240 min shown as violins with inset boxplots by treatment, faceted by Age (rows) and Sex (columns). Boxes show medians and interquartile ranges; whiskers extend to 1.5 x Inter Quartile Range. Colours: Naive (blue), PBS-injured (green), HK bacteria (orange), Live bacteria (vermillion). Sample sizes are the number of flies per Sex × Age × Treatment (median n = 21, range 16-27).

Feeding increase rather than anorexia dominates age- and sex-specific responses to bacterial challenge

Changes in total food intake were not consistent with a single, generalised "sickness" response (Figure 1). Instead, effects depended on age, sex, and the nature of the challenge (Figure 1A and B). In young males, immune stimulation with heat-killed *P. entomophila* and infection with live *P. entomophila* showed increases in 240-min intake relative to Naive (ratios 2.31 and 2.47; both $p < 0.001$), with injury alone trending higher (ratio 1.58, $p = 0.064$; Table S3). This pattern is consistent with a host-driven increase in feeding after immune stimulation and a further increase with live infection. In young females, by contrast, intake was similar across injury, immune stimulation, and live infection (all vs Naive not significant; see Table S3 for pairwise contrasts), arguing against generalised anorexia over the first 4 h in this group.

In older flies, responses also differed by sex. Old males showed broadly similar cumulative intake across challenges at 240 min (Figure 1B) (all vs Naive not significant; Table S3). Old females, however, increased intake specifically with live infection relative to Naive (ratio 2.20, $p = 0.001$), whereas injury and sterile immune stimulation did not alter intake (Table S3). This selective increase points to pathology-linked changes during live infection rather than a uniform response to injury or immune stimulation.

Macronutrient choice shows selective age- and sex-specific shifts rather than a uniform "sickness diet"

Protein-carbohydrate choice changed over the 4-h assay, sowing a general decrease in protein preference (or rather, an increase in the preference for the carbohydrate-rich substrate) (Figure 2A). The magnitude of this change in preference depended strongly on age and time since challenge (age and hour, both $p < 0.001$; treatment × hour, $p < 0.001$; Table S4). We focus on the 240-min endpoint while noting these dynamics (Figure 2B).

In young flies, female protein intake remained low (5-8% of sips) and did not differ detectably among challenges (Live vs Naive trended higher, $p = 0.068$; Table S5). In young males, injury increased the protein fraction compared to Naive (PBS vs Naive OR =1.81, $p = 0.002$), whereas heat-killed and live bacteria were similar to Naive, suggesting injury-driven changes in macronutrient preference rather than pathogen exposure in this short window (Figure 2B).

Older flies consumed a higher protein fraction than young flies overall at the endpoint, (spanning 20–29%) and much lower fractions with PBS or HK (7-12%). Consistent with this, all challenges reduced protein preference versus Naïve controls (Figure 2B), most strongly after injury and sterile immune stimulation (PBS OR 0.32; HK OR 0.17; both $p < 0.001$), with live infection also lower than Naive (OR 0.63, $p < 0.001$; Table S5). Old males showed a complementary pattern: injury and sterile immune stimulation increased protein preference relative to Naive (PBS OR 1.96; HK OR 2.32; both $p < 0.001$), whereas live infection was similar to Naive (OR 0.97, $p = 0.68$). Together, these endpoint patterns indicate selective, age- and sex-specific macronutrient shifts, rather than a uniform "sickness diet."

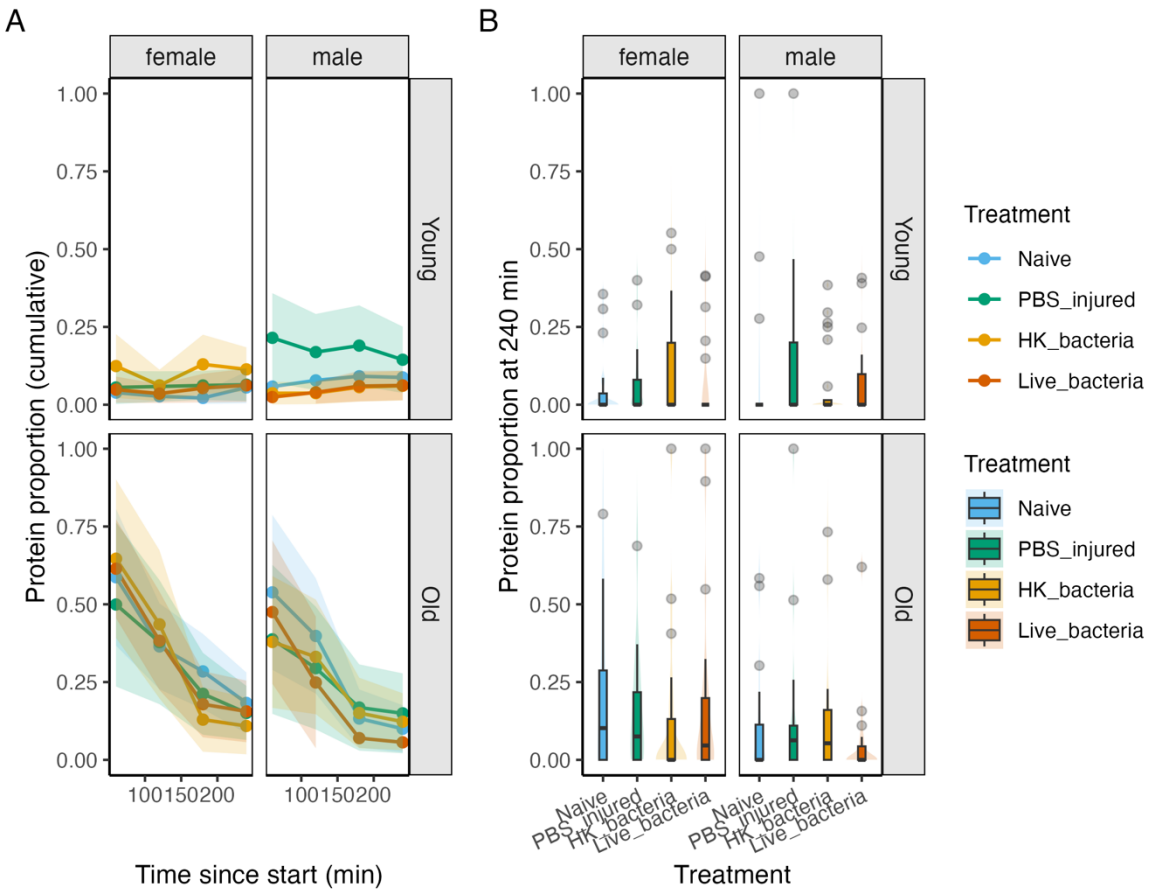

Figure 2. Changes in macronutrient preference. A. Macronutrient preference over time. Mean (lines) ± 95% CI (shaded ribbons) of the protein proportion (protein sips / total sips) across time by treatment, faceted by Age and Sex. The y-axis is bounded within [0, 1]. **B.** End-point macronutrient preference at 240-min shown as violins with inset boxplots by treatment, faceted by Age (rows) and Sex (columns). Boxes show medians and interquartile ranges; whiskers extend to 1.5 x Inter Quartile Range. Colours: Naive (blue), PBS-injured (green), HK bacteria (orange), Live bacteria (vermillion). Sample sizes are the number of flies per Sex × Age × Treatment (median n = 21, range 16-27).

Discussion

We set out to test whether infection-related shifts in food intake and macronutrient preference were consistent with an adaptive, host-driven anorexia-like sickness behaviour, or if these responses might be more parsimoniously ascribed to physiological responses to injury and/or pathogen-induced pathology. Total food intake changed in an age- and sex-specific manner, with a brief increase in young males early after challenge, and a sustained increase in old females specifically under live infection. In this acute window, feeding in Drosophila did not manifest as anorexia, as total intake was maintained or increased under both sterile immune stimulation and live infection. We also did not find strong evidence for a uniform macronutrient reallocation. The clearest shift was a higher protein preference after injury in young males. In old females, all challenges reduced the protein preference despite the general increase in feeding during live infection, indicating that total intake and macronutrient balance can be regulated independently in the early hours after challenge.

Elevated intake under live infection (notably in old females) contrasts with classic reports of "sickness anorexia" in vertebrates and some insects (e.g. Manduca sexta and honey bees following immune challenge; (Adamo et al. 2007; Kazlauskas et al. 2016), but aligns with a growing body of work showing that anorexia is not universal. In orthopterans, immune challenge often leaves activity and feeding unchanged (Sullivan et al. 2016; Kelly and Mc Cabe Leroux 2020), and in *Tenebrio molitor* repeated assays have revealed little consistent suppression of activity or exploration after LPS (Bour and Kelly 2024). It is also echoed in social insects, such as *Bombis terrestris* where immune stimulation has been associated with higher food consumption (Tyler et al. 2006). Across insects, sickness feeding is therefore variable in magnitude and in direction. One explanation for the increase in feeding we observe here is compensatory feeding: live *P. entomophila* is known to damage the gut and to provoke strong immune activation (Opota et al. 2011; Prakash et al. 2022, 2023), and even at low inoculation doses these processes are energetically and biosynthetically demanding (Kutzer et al. 2024). Elevated intake in old females may therefore reflect the combined needs of defence and repair in a physiology that is concurrently navigating immunosenescence and (potentially) reproductive investment.

With respect to macronutrient choice, we did not observe a uniform "sickness diet." Instead, sterile cues alone were sufficient to shift protein preference in aged flies (down in old females; up in old males) and in young males (up after injury), with live infection often counteracting these sterile-induced shifts in a sex- and age-specific manner. There is abundant evidence across host taxa that the dietary composition can have profound effects on an individuals' response to infection (Ponton et al. 2013). Through experiments manipulating specific macronutrient intake, P:C ratio has been identified as key in the infection response (reviewed in Ponton et al. 2011, 2013). In several lepidopterans, infected caterpillars increase protein intake and do better on protein-rich diets, consistent with the protein costs of resistance (Lee et al. 2006; Povey et al. 2014). Together with evidence that carbohydrate-biased diets can improve survival in Drosophila bacterial infections (Ponton et al. 2020) and that high protein exacerbates mortality during septic *P. entomophila* (Savola et al. 2021), our early

post-challenge data points away from a universal protein-seeking 'immune' appetite and toward context- and state-dependent macronutrient preferences.

Age modulated both how much flies ate and what they chose to eat in the first hours after challenge. Most strikingly, old females (22-24 d) increased total intake under live *P. entomophila* relative to other treatments, whereas young males (2-4 d) showed only a transient increase in the amount eaten. For macronutrient choice, sterile cues alone were sufficient to shift preference in aged flies but in opposite directions by sex. These age-by-sex-by-treatment interactions are consistent with prior work from nutrition and ageing in flies. Classic work shows that older Drosophila compensate for declining metabolic efficiency by eating more and relying more on carbohydrate as an energy source (Driver and Lamb 1980), which aligns with the elevated intake we observed in old females and their injury-induced shift away from protein. Choice-fed flies tend to regulate toward a protein-rich intake target that prioritises reproduction at the expense of lifespan, but this targeting disperses by Day 30 (Strilbytska et al. 2024). Our aged flies similarly did not converge on a single 'sickness diet'; instead, preferences were plastic and cue-dependent over the first 4 h.

A complementary immune-centric mechanism may contribute to such plastic dietary choices. Ageing flies mount broader, less specific AMP repertoires across IMD and Toll pathways and show signs of immunopathology (Shit et al. 2022). This deregulation likely raises near-term energy needs (favouring carbohydrate to fuel immune activation and repair) and amino-acid demand (for AMP synthesis and tissue maintenance), providing a mechanistic rationale for both the increased total intake we detect in old females and the sex- and context-dependent shifts in protein preference. Live infection's tendency to counteract injury-induced changes in aged flies suggests that pathogen-specific processes (e.g., gut pathology, damage signalling) can re-prioritise these age-dependent nutritional demands in the acute post-challenge window. This predicts that experimentally amplifying late-life immune signalling (e.g., via reduced IMD negative regulation) should bias early post-challenge intake upward and tilt P:C toward carbohydrate, whereas constraining AMP synthesis capacity should dampen any protein-seeking.

In sum, during the first hours after challenge, Drosophila did not exhibit anorexia; if anything, live bacterial infection sometimes increased intake, most clearly in old females. Macronutrient choice did not converge on a single 'sickness diet.' Instead, wounding and immune stimulation alone shifted protein preference in age- and sex-specific ways, and live infection often countered these shifts. These patterns align with ageing-related compensatory feeding and with emerging evidence that late-life immune responses are broader and costlier, together implying that early post-challenge feeding is shaped by the joint demands of damage control and deregulated immunity rather than a single, host-driven anorexia response. Designs that explicitly partition injury, immune stimulation, and live infection will be essential for dissecting both host- and pathogen-driven behavioural responses to infection.

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Supplementary material

Table S1. Anova output for Negative binomial GLMM of total number of sips

model term	df	F.ratio	Chisq	p.value
sex	1	1.31	1.31	0.2518
age	1	187.10	187.10	0.0000
treatment	3	2.80	8.40	0.0385
hour	3	5.79	17.38	0.0006
sex:age	1	11.14	11.14	0.0008
sex:treatment	3	1.45	4.34	0.2270
sex:hour	3	0.15	0.45	0.9297
age:treatment	3	0.53	1.60	0.6606
age:hour	3	4.79	14.36	0.0025
treatment:hour	9	1.80	16.16	0.0635
sex:age:treatment	3	1.61	4.84	0.1843
sex:age:hour	3	0.33	0.98	0.8063
sex:treatment:hour	9	1.62	14.54	0.1043
age:treatment:hour	9	1.27	11.39	0.2500
sex:age:treatment:hour	9	1.44	12.92	0.1662

Table S2. Mean ± SE number of cumulative sips measured at 240 min for each age-, sex-, and treatment combination. P-values for these contrasts were adjusted by Holm's method.

treatment	sex	age	Number of sips	SE
Naive	female	Young	58.87	11.44
PBS_injured	female	Young	99.69	19.45
HK_bacteria	female	Young	77.69	14.06
Live_bacteria	female	Young	66.76	11.74
Naive	male	Young	34.48	6.61
PBS_injured	male	Young	54.62	10.20
HK_bacteria	male	Young	79.69	14.01
Live_bacteria	male	Young	85.01	14.68
Naive	female	Old	138.80	25.18
PBS_injured	female	Old	175.23	35.73
HK_bacteria	female	Old	149.61	25.89
Live_bacteria	female	Old	305.18	51.33
Naive	male	Old	208.63	38.36
PBS_injured	male	Old	257.08	53.45
HK_bacteria	male	Old	329.87	63.53
Live_bacteria	male	Old	315.57	60.90

Table S3. Pairwise contrasts calculates using Estimated Marginal Means for the NB GLMM in Table S1.

contrast	sex	age	ratio	SE	z.ratio	p.value
PBS_injured_vs_Naive	female	Young	1.69	0.43	2.05	0.1197
HK_bacteria_vs_Naive	female	Young	1.32	0.32	1.14	0.5100
Live_bacteria_vs_Naive	female	Young	1.13	0.27	0.52	0.6012
PBS_injured_vs_Naive	male	Young	1.58	0.39	1.85	0.0639
HK_bacteria_vs_Naive	male	Young	2.31	0.55	3.52	0.0009
Live_bacteria_vs_Naive	male	Young	2.47	0.58	3.82	0.0004
PBS_injured_vs_Naive	female	Old	1.26	0.32	0.92	0.7138
HK_bacteria_vs_Naive	female	Old	1.08	0.25	0.33	0.7443
Live_bacteria_vs_Naive	female	Old	2.20	0.50	3.49	0.0014
PBS_injured_vs_Naive	male	Old	1.23	0.32	0.81	0.4181
HK_bacteria_vs_Naive	male	Old	1.58	0.39	1.86	0.1884
Live_bacteria_vs_Naive	male	Old	1.51	0.37	1.68	0.1884

Table S4. Anova output for Binomial GLMM for Proportion of sips taken on protein vs carbohydrate rich substrates.

model term	df1	F.ratio	Chisq	p.value
sex	1	0.94	0.94	0.33170
age	1	15.76	15.76	0.00007
treatment	3	2.21	6.64	0.08443
hour	3	85.04	255.12	0.00000
sex:age	1	0.28	0.28	0.59415
sex:treatment	3	0.49	1.48	0.68611
sex:hour	3	2.12	6.37	0.09482
age:treatment	3	0.52	1.55	0.67017
age:hour	3	139.25	417.75	0.00000
treatment:hour	9	9.77	87.93	0.00000
sex:age:treatment	3	1.55	4.64	0.20044
sex:age:hour	3	5.21	15.62	0.00135
sex:treatment:hour	9	5.19	46.70	0.00000
age:treatment:hour	9	13.77	123.90	0.00000
sex:age:treatment:hour	9	1.68	15.09	0.08830

Table S5. Pairwise contrasts calculated using Estimated Marginal Means for the Binomial GLMM in Table S4. P-values for these contrasts were adjusted by Holm's method.

contrast	sex	age	odds.ratio	SE	z.ratio	p.value
PBS_injured_vs_Naive	female	Young	1.32	0.20	1.82	0.1365
HK_bacteria_vs_Naive	female	Young	1.08	0.17	0.49	0.6267
Live_bacteria_vs_Naive	female	Young	1.43	0.23	2.28	0.0682
PBS_injured_vs_Naive	male	Young	1.81	0.32	3.37	0.0022
HK_bacteria_vs_Naive	male	Young	0.77	0.14	-1.41	0.3167
Live_bacteria_vs_Naive	male	Young	0.89	0.15	-0.71	0.4753
PBS_injured_vs_Naive	female	Old	0.32	0.02	-16.26	0.0000
HK_bacteria_vs_Naive	female	Old	0.17	0.01	-21.83	0.0000
Live_bacteria_vs_Naive	female	Old	0.63	0.03	-9.04	0.0000
PBS_injured_vs_Naive	male	Old	1.96	0.14	9.44	0.0000
HK_bacteria_vs_Naive	male	Old	2.32	0.15	12.92	0.0000
Live_bacteria_vs_Naive	male	Old	0.97	0.07	-0.41	0.6829