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# Acidic Food pH Increases Palatability and Consumption and Extends *Drosophila* Lifespan[1,2]

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## Abstract

**Background:** Despite the prevalent use of *Drosophila* as a model in studies of nutrition, the effects of fundamental food properties, such as pH, on animal health and behavior are not well known.

**Objectives:** We examined the effect of food pH on adult *Drosophila* lifespan, feeding behavior, and microbiota composition and tested the hypothesis that pH-mediated changes in palatability and total consumption are required for modulating longevity.

**Methods:** We measured the effect of buffered food (pH 5, 7, or 9) on male gustatory responses (proboscis extension), total food intake, and male and female lifespan. The effect of food pH on germfree male lifespan was also assessed. Changes in fly-associated microbial composition as a result of food pH were determined by 16S ribosomal RNA gene sequencing. Male gustatory responses, total consumption, and male and female longevity were additionally measured in the taste-defective *Pox neuro* (*Poxn*) mutant and its transgenic rescue control.

**Results:** An acidic diet increased *Drosophila* gustatory responses (40–230%) and food intake (5–50%) and extended survival (10–160% longer median lifespan) compared with flies on either neutral or alkaline pH food. Alkaline food pH shifted the composition of fly-associated bacteria and resulted in greater lifespan extension (260% longer median survival) after microbes were eliminated compared with flies on an acidic (50%) or neutral (130%) diet. However, germfree flies lived longer on an acidic diet (5–20% longer median lifespan) compared with those on either neutral or alkaline pH food. Gustatory responses, total consumption, and longevity were unaffected by food pH in *Poxn* mutant flies.

**Conclusions:** Food pH can directly influence palatability and feeding behavior and affect parameters such as microbial growth to ultimately affect *Drosophila* lifespan. Fundamental food properties altered by dietary or drug interventions may therefore contribute to changes in animal physiology, metabolism, and survival. *J Nutr* 2015;145:2789–96.

**Keywords:** aging, dietary restriction, feeding behavior, food intake, food pH, metabolism, microbiota, nutrition, palatability, physiology

## Introduction

*We tried it with and without pH—and it made no difference.*

This quote, attributed by the late Seymour Benzer to the prominent geneticist Theodosius Dobzhansky, is likely an urban legend. Nonetheless, its moral is still relevant—that many studies of biological phenomena fail to consider fundamental chemical processes.

Food properties play a large role in determining palatability and nutrient assimilation (1–4). Although quantitative studies of nutrition have been greatly facilitated by the use of *Drosophila* because of the ease in which their diet can be manipulated and the large number of animals that can be quickly and inexpensively generated (5–9), systematic studies on how fundamental chemical properties of food substrates, such as pH, affect fly health and physiology are lacking. Food pH potentially influences a number of sensitive biological parameters—such as microbial growth on the food substrate, gut homeostasis, and feeding behavior—and can be perturbed by changes in food ingredients or concentrations or by adding drugs to the diet.

Previous studies on flies have hypothesized that food pH and/or buffering capacity might affect internal acid-base equilibrium (10) or the solubility and digestion of dietary protein (11). Like

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the mammalian gut, the fly digestive tract has distinct regions where pH, acidic or alkaline, is tightly regulated (12). The energetic cost of maintaining these zones in response to ingesting foods of different pH and/or buffering capacity is unknown. How food pH affects nutrient assimilation is also poorly understood.

The sensory perception of food greatly affects palatability and consumption in humans (1–4). Food acidity or environmental pH is often recognized by gustatory or olfactory chemosensation (13–15). Recent studies in flies have shown that acids can perturb the neuronal response to sweet and bitter compounds (16, 17). Whether food pH can ultimately affect food intake and health has not yet been investigated.

## Methods

*Flies and dietary conditions.* All fly stocks were maintained as described (18). The Canton-S and Dahomey lines have been maintained in our laboratory for >5 y. The *Pox neuro* (*Poxn*)[5] mutant $Poxn^{\Delta M22\text{-}B5}$ and its transgenic rescue control $Poxn^{\Delta M22\text{-}B5}$ *SuperA-158* were validated by identifying morphological differences in labellar bristles (19). For behavioral assays, flies were typically aged 5–7 d at the start of the Expt.

All buffer salts for the food pH studies were obtained from VWR or Thermo Fisher Scientific. The yeast extract (YE), sucrose-only, and agar-only diets were composed of sucrose (VWR), Bacto YE and agar (BD Diagnostic Systems), yellow cornmeal (LabScientific), and deionized water ($diH_2O$) and prepared as described (20). **Table 1** shows the complete composition of the 3 diets. Diets were typically buffered to pH 5, 7, or 9 by diluting 1 mol/L stock solutions (composed of sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate) to a final concentration of 60 mM. However, in Expt. 1 (see "Experimental design"), different salt combinations were used to reach the indicated pH and approximately match $K^+$ concentrations (pH 1–2, hydrochloric acid/potassium chloride; pH 5.5, acetic acid/potassium acetate; pH 8, Tris/hydrochloric acid/potassium chloride; and pH 10–11, potassium carbonate/potassium bicarbonate). Final $K^+$ concentrations were as follows: pH 1–2, 60 mM; pH 5.5, 42 mM; pH 8, 30 mM; and pH 10–11, 47 mM.

For the germfree studies (Expt. 3), axenic flies were generated by treating embryos with bleach and maintaining them on sterile food as described (21). Antibiotic-treated flies were developed on stock food and maintained on experimental diets supplemented with 500 μg/mL ampicillin, 200 μg/mL rifamycin, and 50 μg/mL tetracycline (22). Sterile food was prepared by autoclaving media before adding sterile-filtered stock buffer and dispensing to autoclaved vials. All food preparation and fly transfers were performed in a laminar flow hood using aseptic technique.

*Experimental design.* This study was divided into 5 Expts. In Expt. 1, we measured the survival of Canton-S males maintained on the YE diet using a variety of buffer salts to modulate food pH. In Expt. 2, we used only phosphate buffer to determine the effect of food pH on the survival of Canton-S males maintained on the YE, sucrose-only, or agar-only diet. We also assessed the effect of food pH on the survival of Canton-S females and Dahomey males on the sucrose-only diet. In Expt. 3, we assessed the effect of food pH on the survival of axenic or antibiotic-treated Canton-S males maintained on the sterile YE diet. We also identified bacterial species associated with Canton-S males on the YE diet under conventional (nongermfree) conditions. In Expt. 4, we measured the proboscis extension of Canton-S males in response to a buffered sucrose solution (5 g in 100 mL of $diH_2O$ supplemented with 60 mM phosphate buffer at pH 5, 7, or 9), buffer alone, unbuffered sucrose supplemented with 50 or 100 mM sodium chloride, or unbuffered sucrose supplemented with 10 mM sodium acetate. We also measured the effect of pH on 24-h food intake of Canton-S males on the YE or sucrose-only diet or of Dahomey males on the sucrose-only diet. In Expt. 5, we measured the effect of pH on proboscis extension and 24-h food intake of *Poxn* mutant males and their control on sucrose-only diet. We also assessed the effect of food pH on the survival of *Poxn* mutant males and females, and their controls, on the sucrose-only diet.

**TABLE 1** Composition of experimental diets[1]

| | YE | Sucrose-only | Agar-only |
|---|---|---|---|
| Sucrose, g | 5 | 5 | — |
| Bacto YE, g | 0.25 | — | — |
| Yellow cornmeal, g | 8.6 | — | — |
| Bacto agar, g | 0.5 | 1 | 1 |
| $diH_2O$ (final volume), mL | 100 | 100 | 100 |

[1] $diH_2O$, deionized water; YE, yeast extract.

*Survival.* All lifespan studies were performed with single-sex cohorts as previously described (20). For survival on the agar-only diet, dead flies were scored every 4–6 h. In axenic studies, food was periodically tested for microbe contamination by culturing swabs from spent vials (21). Food pH was measured using 1–2 drops of universal liquid pH indicator per vial (RICCA Chemical Company).

*Proboscis extension response.* Proboscis extension response (PER) assays were performed as described (23), with minor modifications. Briefly, nonstarved flies were assessed 3–4 times each with alternating solutions touched to the labellum. Experimenters were blinded to the conditions being tested. Responses were recorded as follows: 1, full extension; 0.5, half extension; and 0, no extension. The average response to each condition for each fly was considered as 1 data point.

*Food intake.* Total consumption using the capillary feeder (CAFE) assay or radioisotope labeling was measured as described (18). The CAFE assay was performed with 4 flies per chamber for more than 24 h. For the radioisotope labeling assay, ~10 flies per vial were habituated on the experimental diet overnight and then transferred to the same food supplemented with 1 mCi/mL [$\alpha$-$^{32}$P]dCTP (PerkinElmer). Tracer accumulation in flies was assessed after 24 h. Results from each chamber or vial was considered as 1 data point.

*Buffering capacity.* Individual ingredients [5 g of sucrose, 8.6 g of cornmeal, 0.25 (low) or 5 (high) g of YE, or 5 g of Brewer's yeast (MP Biomedicals)] were prepared in 200 mL of $diH_2O$ and heated to boiling. After simmering for 5 min and cooling to room temperature, each ingredient was titrated with aliquots of glacial acetic acid (Thermo Fisher Scientific) under constant stirring until the final pH was <4.5 (Sper Scientific Benchtop pH meter).

*Bacterial identification.* Bacterial species associated with conventionally raised flies were identified as described (21, 22). Briefly, bacterial 16S rRNA gene sequences were PCR-amplified from whole-fly homogenate DNA and cloned (TOPO TA Cloning, Life Technologies). Random clones were then cultured and DNA-sequenced (Genewiz). Bacterial species were identified using the Ribosomal Database Project (24).

*Statistical analysis.* For survival studies, significant differences were determined between survival curves by the log-rank test and between median lifespan values by Fisher's exact test, respectively (25). All other analyses were performed using Prism version 5.04 (GraphPad Software). For PER and food intake studies where diet or genotype was tested in addition to food pH, 2-factor ANOVA with Bonferroni post hoc tests were used to determine the effects of pH, genotype, and their interaction or the effects of pH, diet, and their interaction. The effect of sodium chloride concentration or sodium acetate on PER to unbuffered sucrose was analyzed by 1-factor ANOVA followed by a Tukey's post hoc test for multiple comparisons or an unpaired 2-tailed Student's *t* test, respectively. Other food intake data were analyzed by 1-factor ANOVA

[5] Abbreviations used: CAFE, capillary feeder; $diH_20$, deionized water; PER, proboscis extension response; *Poxn*, *Pox neuro*; YE, yeast extract.

followed by a Tukey's post hoc test for multiple comparisons. In all PER and food intake studies where the effect of food pH (5, 7, or 9) was examined, a linear-trend posttest was also used to compare the behavioral measurement to food pH and was reported as *P*-trend. Nonparametric data were analyzed using the Kruskal-Wallis test. All *P* values were corrected for multiple comparisons where appropriate. Differences were considered significant at $P < 0.05$, and tendencies were highlighted at $P < 0.10$.

## Results

*Acidic food pH extends fly lifespan.* We assessed the buffering capacity of popular fly food ingredients at commonly used concentrations (20, 26, 27). Whereas pH values for sucrose, cornmeal, and low-concentration YE were rapidly perturbed by acid, high-concentration YE showed considerable buffering capacity (**Figure 1**A). Brewer's yeast, another form of yeast commonly used in *Drosophila* studies (28), showed similar buffering capacity to YE (data not shown).

To investigate the role of food pH on survival, a low-concentration YE diet previously associated with dietary restriction-mediated longevity was buffered to different pH values using various salts (Expt. 1). Acidic conditions were optimal for fly lifespan (Figure 1B), with pH 5.5 food resulting in significantly longer median lifespan (36 d) compared with each of the other conditions (33, 19, and 19 d for pH 1–2, 8, and 10–11 food, respectively). To eliminate the confound of using different buffer salts for each pH, we repeated the lifespan study using phosphate buffer over a narrower pH range (Expt. 2). Longevity was again maximized under acidic conditions (Figure 1C), with pH 5 food resulting in significantly longer median lifespan (36 d) compared with pH 7 (20 d) or pH 9 (14 d) diets. All subsequent Expts. used only phosphate buffer to modulate pH.

On a sucrose-only diet lacking YE, acidic medium also resulted in a longer lifespan in flies compared with pH 7 or 9 food (Figure 1D). The effect of acidic medium on lifespan, compared with pH 7 or 9 diets, was reproducible in females (Figure 1E) and another control genotype, Dahomey (Figure 1F). In all cases, a pH 5 diet (20, 22, and 16 d for Canton-S males, Canton-S females, and Dahomey males, respectively) resulted in significantly longer lifespans compared with flies on pH 7 (18, 16, and 14 d, respectively) or 9 (all 14 d) food. In contrast, starvation resistance, tested by measuring survival on an agar-only diet, was insensitive to buffer pH (Figure 1G).

*Microbial growth and food pH interact to affect fly lifespan.* Under conventional YE diet conditions (Figure 1C), food pH in spent vials decreased regardless of the starting pH, resulting in a cycling of food pH every 2 d when flies were transferred to fresh medium (**Figure 2**A). In contrast, food pH of spent vials housing axenic or antibiotic-treated flies remained consistent. Conventionally raised flies were associated with a different microbial composition depending on the food pH, with Proteobacteria (primarily *Acetobacter*) dominating on the acidic diet and Firmicutes appearing more common on the pH 9 medium. Firmicutes present on the acidic diet included *Enterococcus* and *Lactobacillus*, whereas those identified on pH 7 or 9 food were predominantly *Bacillus*.

We next assessed the influence of food pH on survival in the absence of microbes (Expt. 3). Regardless of treatment, acidic medium extended survival compared with pH 7 or 9 food (Figure 2B, C), although the survival curves between axenic flies on pH 5 and 9 food were not significantly different ($P = 0.10$). The median lifespan for flies on the pH 5 diet was significantly greater than those on pH 7 or 9 food in all conditions except when comparing antibiotic-treated flies on pH 5 and 9 food (Figure 2D). In addition, removing microbes at each pH tested resulted in a significantly longer median lifespan compared with the conventional condition (Figure 2D).

*Acidic food pH increases palatability and total consumption.* We next determined whether food pH affects gustatory responses and food intake (Expt. 4). Robust PER is elicited when positive tastants such as sucrose are touched to the fly labellum,

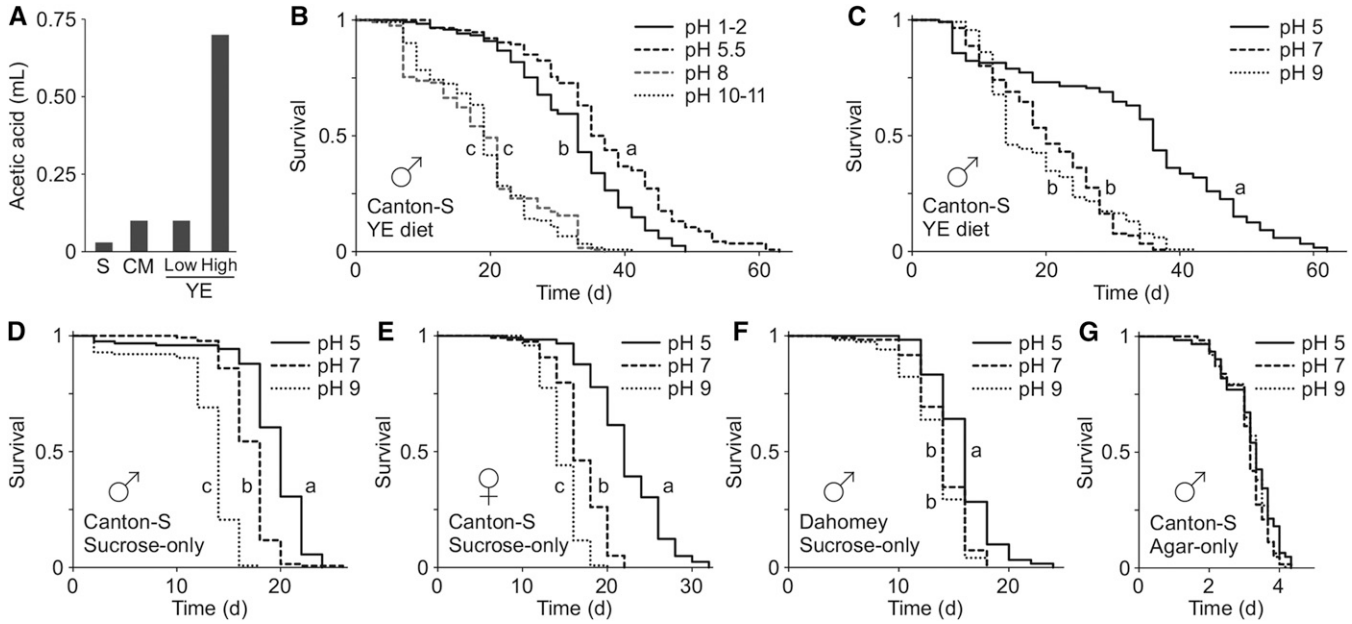


**FIGURE 1** Effect of food pH on *Drosophila* adult survival. (A) Buffering capacity—volume of acid required to change pH of indicated dietary component by 1 unit. (B) Lifespan on YE diet buffered to indicated pH (Expt. 1; $n$ = 114–122 flies per group). (C–G) Lifespan on phosphate-buffered YE (C), sucrose-only (D–F), or agar-only (G) diet [Expt. 2; $n$ = 115–136 (C–F) or 61–63 flies per group (G)]. Labeled curves without a common letter differ, $P < 0.05$. CM, cornmeal; S, sucrose; YE, yeast extract.

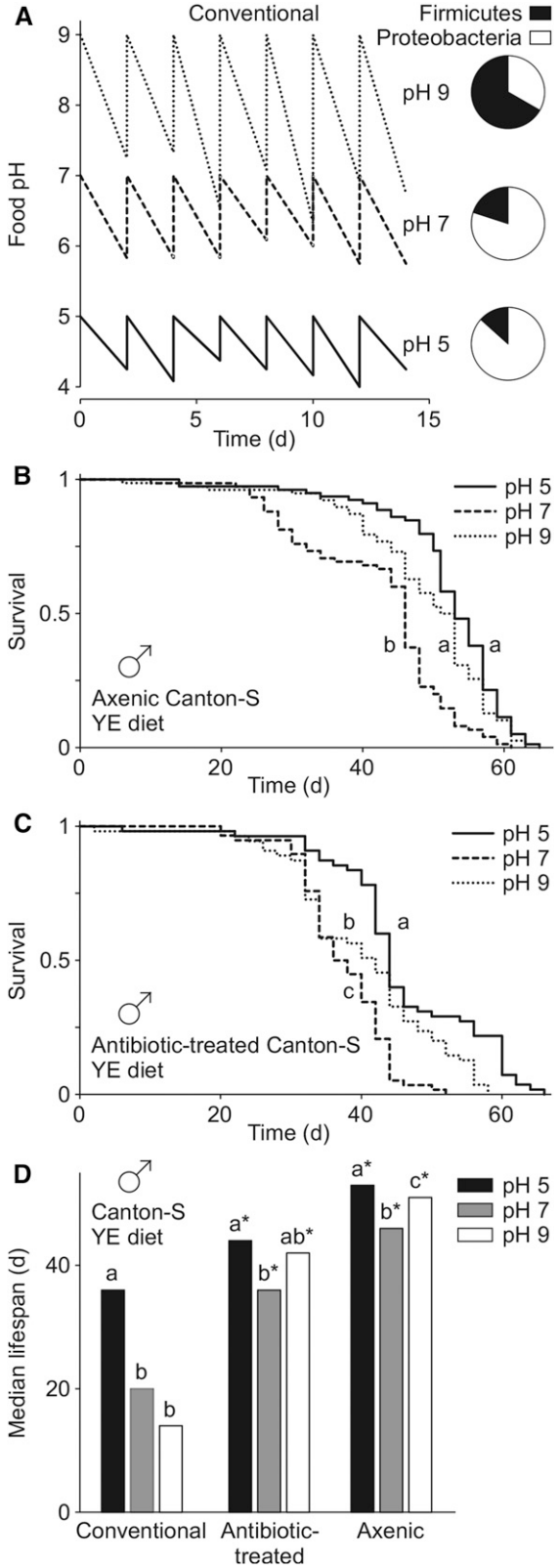


**FIGURE 2** Relation between food pH, lifespan, and fly-associated microbes in *Drosophila* males (Expt. 3). (A) Food pH during conventional lifespan study (left) and associated bacterial composition at the phylum level (right). For clarity, pH values are means only ($n$ = 6 vials per group). (B) Effect of food pH on axenic fly lifespan ($n$ = 75–79 flies per group). (C) Effect of food pH on antibiotic-treated fly lifespan ($n$ = 55–58 flies per group). Curves without a common letter differ, $P < 0.05$ (B–C). (D) Effect of food pH on median lifespan of conventional, axenic, or antibiotic-treated flies. Labeled values within each condition without a common letter differ, $P < 0.05$. *Different from corresponding conventional condition, $P < 0.0001$. YE, yeast extract.

and this measure of palatability was greater with acidic-buffered sucrose compared with pH 7 or 9 solutions (**Figure 3**A). The pH of buffer alone did not affect PER in any comparison (Figure 3A). Sodium chloride supplementation at the levels equivalent in phosphate buffer did not affect PER to unbuffered sucrose (Figure 3B). In contrast, the addition of sodium acetate raised the pH of unbuffered sucrose to 8 and significantly reduced gustatory responses compared with sucrose alone (Figure 3B).

Compared with the intake of pH 7 or 9 food, acidic pH resulted in an increased total consumption of sucrose-only diet, and this result was reproduced in 2 genotypes (Figure 3C). The CAFE assay, which is an alternative method for assessing food intake, also resulted in increased feeding with decreasing food pH (Figure 3D), although of the pairwise comparisons only the difference in consumption between pH 5 and 9 diets tended toward significance ($P$ = 0.06). Increased feeding with decreasing food pH was similarly observed for flies on the YE diet (Figure 3E).

*Taste is required for the effects of food pH on palatability, consumption, and survival.* We used the taste-defective *Poxn* mutant (19) to assess the importance of taste on proboscis extension, feeding, and survival in response to food pH (Expt. 5). Control flies showed a significantly higher PER to acid-buffered sucrose compared with pH 7 or 9 solutions, whereas the *Poxn* mutant was insensitive to pH (**Figure 4**A). Similarly, control flies consumed significantly more pH 5 food compared with pH 9 medium and showed increased intake with decreasing pH (Figure 4B). In contrast, food intake of the *Poxn* mutant was insensitive to food pH (Figure 4B).

Control males and females each showed greater survival on pH 5 food compared with those on pH 7 or 9 diets (Figure 4C), and median lifespan was also significantly longer in those on acidic medium (males, 19 d; females, 18 d) compared with pH 7 (males, 16 d; females, 16 d) or pH 9 (males, 14 d; females, 13 d) food. In contrast, the survival and median lifespans of *Poxn* males were insensitive to food pH. Female *Poxn* mutants showed significant differences in survival, with a median pH 7 diet lifespan (18 d) being greater than those on acidic or alkaline food (each 16 d).

## Discussion

Food composition strongly affects *Drosophila* behavior, metabolism, and survival (5, 26, 29–32). Nonetheless, fundamental properties that might be affected by food composition, such as acidity and buffering capacity, are not routinely considered for their impact on fly health and physiology. YE and Brewer's yeast have greater capacity to buffer media than the other common fly food ingredients that we tested. Because yeast is the main source of protein in fly food and its concentration is typically manipulated in dietary restriction studies (27, 33, 34), our results suggest that, in addition to modulating nutrition, changing dietary yeast concentration might substantially affect buffering capacity and food pH.

Flies lived longer on acidic food compared with neutral or alkaline diets, and this effect was conserved in different genetic backgrounds and diets and in both sexes. Our findings differ from previous studies that reported that neutral pH medium

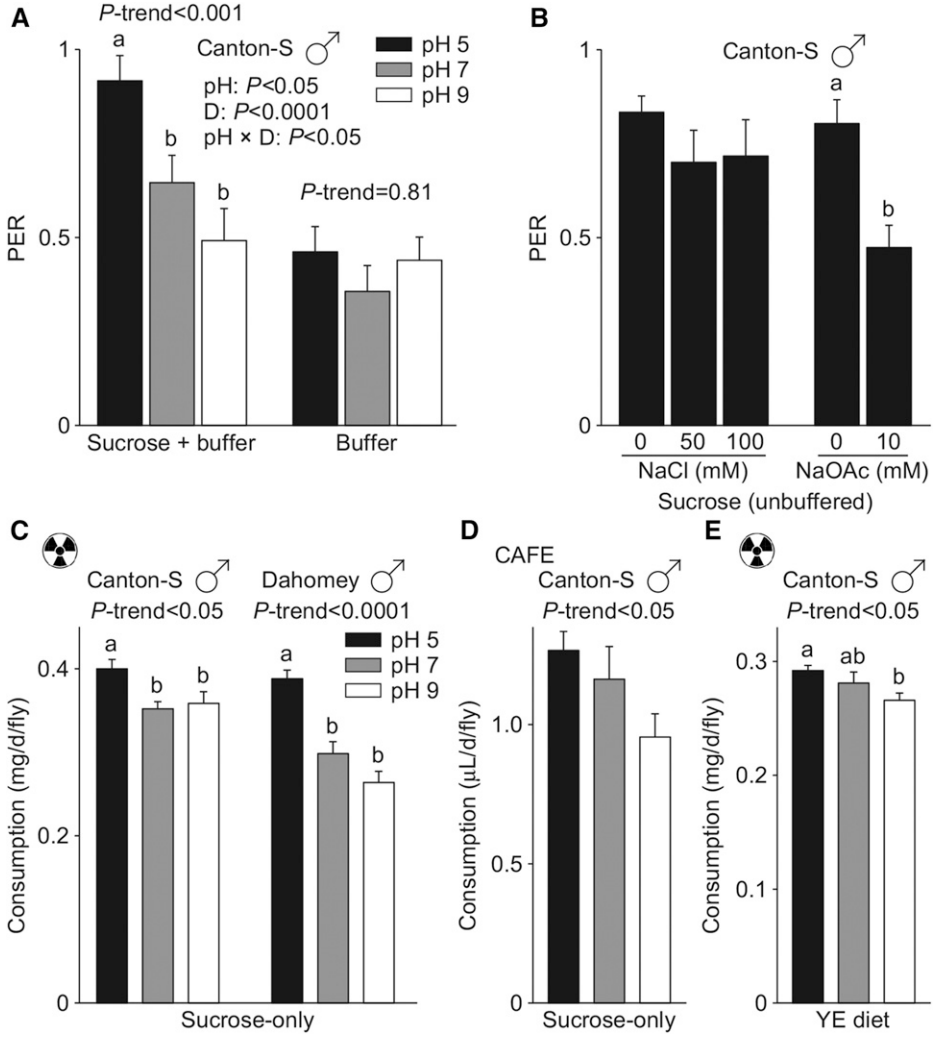


**FIGURE 3** Effect of food pH on palatability and total consumption in *Drosophila* males (Expt. 4). (A) Effect of food pH and diet on PER [$n$ = 10 (sucrose + buffer) or 22 (buffer) flies per group]. (B) Effect of sodium chloride or sodium acetate on PER ($n$ = 10 flies per group). (C) Effect of food pH on total consumption as measured by radioisotope labeling [$n$ = 8 (Canton-S) or 4 (Dahomey) vials per group]. (D) Effect of food pH on total consumption as measured by CAFE assay ($n$ = 4 chambers per group). (E) Effect of food pH on total consumption of YE diet as measured by radioisotope labeling ($n$ = 12–14 vials per group). Values are mean ± SEM. Labeled means within each series without a common letter differ, $P < 0.05$. CAFE, capillary feeder; D, diet; NaCl, sodium chloride; NaOAc, sodium acetate; PER, proboscis extension response; YE, yeast extract.

resulted in longer lives compared with acidic or alkaline food (10, 11). These studies were possibly confounded by the comparison of different buffer salts (10), which we ruled out as an explanation by using only phosphate buffer, or the poor solubility of casein in acid (11, 35), which we ruled out by testing a sucrose-only diet that did not contain any protein. Different fly lines might also show different sensitivity to food pH, although we showed a consistent effect on lifespan in 3 distinct genetic backgrounds. Although dietary protein is not necessary for the longevity mediated by acid, some nutrition is required to observe a pH effect on survival because starvation resistance—measured in buffered agar with no other nutrients—was insensitive to pH. These results suggest that acid does not directly affect fly metabolism or physiology in extending life.

We thus hypothesized that food pH might affect palatability, which we assessed quantitatively by measuring PER. Although low pH buffer alone did not elicit a response, suggesting that acid is not a positive tastant on its own, acid-buffered sucrose induced higher PER than either neutral- or alkaline-buffered sucrose. Salt (sodium chloride) content was ruled out as a confound in our studies because salt concentration equivalent to that of phosphate buffer had no effect on PER to unbuffered sucrose. Using sodium acetate to raise the pH of unbuffered sucrose also significantly reduced PER. Collectively, our results suggest that acidic pH enhances or maintains the palatability of food, whereas alkaline pH reduces it. These findings are consistent with, and extend upon, previous studies that showed that strong acids (pH <3) inhibit sweet sensing (16), whereas milder conditions (pH 3–6) seem to have no effect unless bitter compounds are present (17). Given the abundance of acids in the natural food substrate for *D. melanogaster* (36, 37) and the potential benefit of acid-producing microbes to fly health and development (21, 38, 39), it is perhaps not surprising to find that flies prefer a mildly acidic diet. Toxic alkaloids, phenols, and terpenoids—often alkaline and/or bitter tasting—may also drive avoidance and reduced feeding. Further studies will be needed to fully delineate the effect of broad pH ranges, buffer capacity, and different buffer salts on fly chemosensation.

To our knowledge, palatability and its relation to actual food intake have never been studied in *Drosophila*. To determine whether food pH-mediated differences in gustatory responses affect total consumption, we used 2 independent methods to measure fly food intake over 24 h (18) and observed a significant systematic increase in consumption as food pH was reduced on both sucrose-only and YE diets. Pairwise comparisons between the ingestion of pH 5, 7, and 9 food also revealed significantly greater feeding ($P < 0.05$) or the tendency for greater feeding ($P < 0.10$) on acidic food compared with pH 7 and/or 9 diets. Although flies seemed to overcome differences in palatability to consume food to nearly meet nutritional requirements, careful measurements of total consumption revealed consistent, small changes in intake that correlated with gustatory responses.

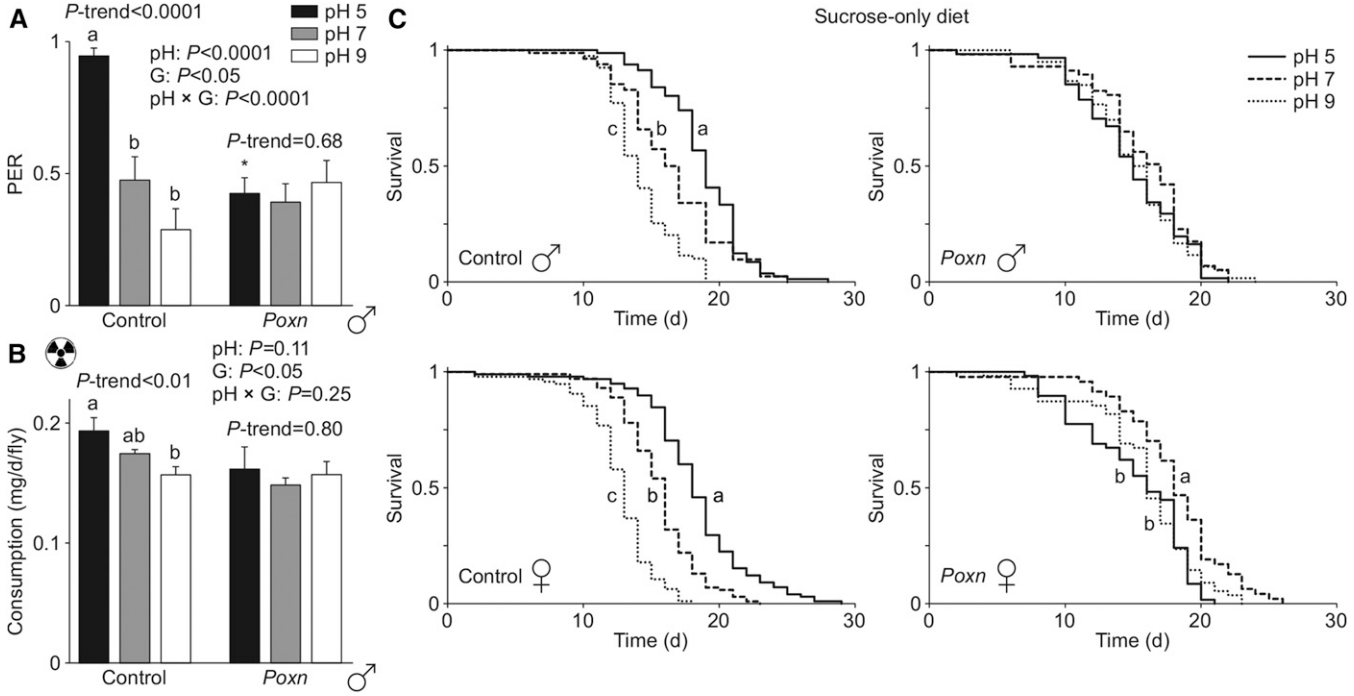


**FIGURE 4** Effect of food pH on palatability, total consumption, and survival in a taste-defective *Poxn* mutant and its rescue control (Expt. 5). (A) Effect of food pH and genotype on PER in males ($n$ = 10 flies per group). (B) Effect of food pH and genotype on total consumption in males as measured by radioisotope labeling ($n$ = 6–8 vials per group). Values are mean ± SEM. Labeled means within each series without a common letter differ, $P < 0.05$. *Different from corresponding control, $P < 0.05$. (C) Effect of food pH on *Poxn* mutant lifespan and its control [males, $n$ = 79–82 (control) or 57–61 (*Poxn*) flies per group; females, $n$ = 95–100 (control) or 47–58 (*Poxn*) flies per group]. Labeled curves without a common letter differ, $P < 0.05$. G, genotype; PER, proboscis extension response; *Poxn*, *Pox neuro*.

Our collective results are consistent with the idea that pH is an important modulator of the gustatory response to food and that longevity on acidic diets results from increased palatability and food intake. To further support this idea, we measured PER, food intake, and lifespan of taste-defective *Poxn* mutant flies (19), which we hypothesized would be insensitive to food pH. Whereas control flies showed higher PER and greater food intake on acidic medium compared with those on pH 7 or 9 food, the *Poxn* mutant showed gustatory responses and consumption that were insensitive to food pH. As with Canton-S and Dahomey flies, the *Poxn* rescue control lived longer on acid-buffered sucrose than on pH 7 or 9 food, and this was consistent in both sexes. In stark contrast, the lifespan of *Poxn* mutant males was insensitive to food pH. Female *Poxn* mutants showed statistically significant differences in survival, but these results were clearly different from the effects observed in control flies. Overall, our studies strongly support the idea that food pH affects palatability and total consumption to influence survival because the elimination of external taste sensation abolishes these phenotypes. In addition to the tarsi and labellum, pharyngeal sweet sensing, which lies at the interface of external and internal nutrient-sensing mechanisms, is required for the sustained feeding of sugar (40). Interestingly, the *Poxn* mutant has intact pharyngeal sweet taste (40), suggesting that the effect of food pH on total consumption is driven only by labellar and/or tarsal chemosensation. Given the limited number of *Drosophila* studies that incorporate food intake measurements, much is still unknown about how information from the various sense organs is integrated to both initiate and sustain feeding.

Another consideration of changing food properties is their effect on microbial growth. Previous studies on the impact of microbes on fly lifespan have been inconsistent (21, 22, 41, 42), suggesting that microbial influences on fly health and physiology are highly dependent on environmental factors. Although we observed minimal microbial growth on sucrose-only food surfaces, microbes were clearly associated with flies on a YE diet. Removing fly-associated microbes extended lifespan on the YE diet regardless of pH, which is consistent with studies that have reported that infection might be a primary cause of mortality in older flies (22) and that germfree flies show enhanced intestinal homeostasis during aging (43–45)—a critical determinant of fly lifespan (46, 47). Regardless, axenic and antibiotic-treated flies continued to show greater longevity on the acidic YE diet compared with pH 7 or 9 food. We also observed an increase in the ratio of Firmicutes to Proteobacteria, the predominant phylum present under standard conditions in our laboratory flies, with increased food pH. At the genus level, *Bacillus* was present only on neutral or alkaline food, whereas more common fly-associated microorganisms such as *Acetobacter*, *Enterococcus*, and *Lactobacillus* (48, 49) were prevalent under acidic conditions. We hypothesize that high food pH might contribute to dysbiosis, increasing the presence of pathogenic compared with commensal bacteria, which ultimately alters intestinal homeostasis and impairs health (50). The shift toward Firmicutes on high pH food is reminiscent of the shift in the dominant phyla or ratio of phyla seen in aging mammals. In mammals, these changes can be associated with detrimental effects on health (51, 52). Future work using more comprehensive approaches to assess microbial species diversity might better reveal how changes in microbiota composition and microbial load are associated with survival in different environments (50, 53, 54).

Our results demonstrate that although acidic food can independently affect fly feeding behavior and extend life, the presence of microbes is detrimental and a shift in the microbes

present in alkaline pH diets might exacerbate this deleterious effect. In addition, many microbes are known to acidify the substrates upon which they grow (55). Consistent with this finding, we observed that flies were exposed to a lifelong cycling of food pH as a result of medium acidification from microbial growth and subsequent, periodic transfers to fresh food. How the additional complexity of a food pH-microbiota interaction affects fly health and physiology will require further study. Although the *Poxn* mutants we used in this study were on a sucrose-only diet, which minimizes microbial growth, they may be valuable in future work for independently assessing the impact of different microbes on health and survival while eliminating differences in nutrient intake as a potential confound.

Our results emphasize not only the importance of considering fundamental food properties in *Drosophila* studies but also the value of food intake measurements. We hypothesize that small differences in nutrient intake over a lifetime can profoundly affect health and survival. This is evident not only in our studies on sucrose-only diets where flies were short-lived but also on low-protein diets typically associated with dietary restriction-mediated longevity (5, 26, 27, 33), where reduced feeding might lead to undernutrition. Food additives, including acids or drugs, are often supplemented to fly media without considering the effect they have on pH. In particular, many of these studies use diets containing only sucrose, which has negligible buffering capacity, perhaps confounding results. Given the fundamental nature of feeding behavior to animal health, we suggest that studies of *Drosophila* physiology and metabolism should include rigorous assessments of nutrient ingestion that are comparable to the total consumption and body weight measurements in mammalian model studies.


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