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Rice Bran as a Novel Dietary Supplement Improves Lifespan, Antioxidant Defense, and Locomotor Performance in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae)

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ABSTRACT

Aging is characterized by declining physiological functions and weakened antioxidant defenses. Rice bran, a nutrient-dense byproduct rich in bioactive compounds, offers potential to modulate these systems, yet its in vivo effects on aging remain underexplored. We investigated the impact of dietary rice bran supplementation on lifespan, antioxidant enzyme activity, and functional capacity in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Adult male flies were fed control or rice bran-supplemented diets (0.5%, 1.0%, or 2.5% w/w). Survival, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities, and locomotor function were assessed. Optimal rice bran dosage significantly extended mean lifespan by 18–22% and median survival ($p < 0.01$), accompanied by increased antioxidant enzyme activities: SOD (25–30%), CAT (20–25%), and GPx (15–20%) ($p < 0.05$). Supplemented flies exhibited attenuated locomotor decline and preserved climbing ability compared to controls ($p < 0.05$). These findings demonstrate that dietary rice bran promotes longevity and functional maintenance in *D. melanogaster* through balanced antioxidant regulation, supporting optimized redox homeostasis as a critical determinant of healthspan.

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Pirinç Kepeği ile Desteklenmiş Diyetin *Drosophila melanogaster Meigen* (Diptera: Drosophilidae)'de Yaşam Süresi ve Fonksiyonel Yaşlanma Üzerindeki Etkileri

ÖZET

Yaşlanma, azalan fizyolojik fonksiyonlar ve zayıflayan antioksidan savunma sistemleri ile karakterizedir. Biyoaktif bileşikler açısından zengin, besin değeri yüksek bir yan ürün olan pirinç kepeği, bu sistemleri modüle etme potansiyeline sahiptir, ancak yaşlanma üzerindeki in vivo etkileri henüz yeterince araştırılmamıştır. Bu çalışmada, pirinç kepeği ile desteklenmiş diyetin *Drosophila melanogaster* Meigen'de yaşam süresi, antioksidan enzim aktivitesi ve fonksiyonel kapasite üzerindeki etkisi araştırılmıştır. Yetişkin erkek sinekler kontrol veya pirinç kepeği ile desteklenmiş diyetlerle (%0.5, %1.0 veya %2.5 a/a) beslenmiştir. Hayatta kalma, süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GPx) aktiviteleri ile lokomotor fonksiyon değerlendirilmiştir. Optimal pirinç kepeği dozajı, ortalama yaşam süresini %18-22 oranında ve median sağkalımı anlamlı şekilde uzatmıştır ($p < 0.01$); buna antioksidan enzim aktivitelerindeki artış eşlik etmiştir: SOD (%25-30), CAT (%20-25) ve GPx (%15-20) ($p < 0.05$). Desteklenmiş sinekler, kontrollere kıyasla azalmış lokomotor gerileme ve korunmuş tırmanma yeteneği sergilemişlerdir ($p < 0.05$). Bu bulgular, pirinç kepeği ile desteklenmiş diyetin, dengeli antioksidan regülasyonu aracılığıyla *D. melanogaster*'da ömür uzunluğunu ve fonksiyonel sürdürülebilirliği desteklediğini göstermekte ve optimize edilmiş redoks homeostazının sağlıklı yaşam süresinin kritik bir belirleyicisi olduğunu desteklemektedir.

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INTRODUCTION

Aging represents an intricate biological phenomenon defined by the progressive deterioration of physiological systems and a diminished ability to sustain cellular and metabolic homeostasis (Guo et al., 2022; Li et al., 2024). This process is not dictated by a solitary causal factor; instead, it arises from the cumulative disruption of integrated biological networks, such as stress signaling, energy metabolism, and endogenous antioxidant defense circuits (Khan et al., 2017; Li et al., 2024). Within this landscape, antioxidant enzymes are fundamental to maintaining cellular stability throughout the lifespan. Emerging research suggests that the hallmark of healthy aging is not peak antioxidant output per se, but the harmonious and synchronized regulation of these defense mechanisms across different life stages (Tan et al., 2018; Płóciniczak et al., 2025).

Consequently, dietary interventions employing food-derived bioactive molecules have emerged as viable strategies for the modulation of age-related biological pathways (Varesi et al., 2022; Varela-López et al., 2023). A broad spectrum of experimental evidence suggests that nutritional constituents can impact longevity and physiological health by refining antioxidant enzyme profiles, bolstering stress resilience, and sustaining functional capacity at the organismal level (Duangjan et al., 2019; Wang et al., 2021). In various model systems, life extension has been linked to the optimization of antioxidant defense networks rather than the non-selective overactivation of specific enzymes. This underscores the hypothesis that maintaining an equitable antioxidant equilibrium is a pivotal factor in determining aging trajectories (Varesi et al., 2022; Varela-López et al., 2023).

Within the realm of experimental biology, *D. melanogaster* serves as a formidable and universally recognized model for exploring the intersections of aging and nutritional intervention (Piper & Partridge, 2016; Staats et al., 2018). Its compressed life cycle, genetic versatility, and the profound evolutionary conservation of metabolic and stress-response pathways shared with higher organisms render *D. melanogaster an ideal candidate* for longevity and healthspan investigations (Piper & Partridge, 2016; Staats et al., 2018). Research utilizing the *D. melanogaster* model has repeatedly established that the integration of natural bioactive compounds into the diet can prolong lifespan, bolster the activity of antioxidant enzymes, and safeguard functional integrity during the aging process (Jaafaru et al., 2024; Hafeez et al., 2025). Previous studies have demonstrated that plant-derived nutritional supplements, including pumpkin seed membrane, can modulate survival and developmental parameters in *Drosophila*, suggesting the potential of agricultural by-products to influence aging-related outcomes (Ayar et al., 2021; Güneş & Biçer, 2021). Specifically, rice protein hydrolysates have been shown to increase survival and upregulate superoxide dismutase and catalase activities, illustrating the high sensitivity of antioxidant defenses to rice-based nutritional components (Yue et al., 2021). In a similar vein, supplementation with agar-derived oligosaccharides or fungal extracts has been documented to mitigate functional senescence while fine-tuning antioxidant enzyme profiles throughout the lifespan (Çolak et al., 2014; Ma et al., 2019; Dai et al., 2023).

Rice and its associated by-products constitute a prolific and nutritionally significant reservoir of bioactive molecules with prospective roles in the regulation of aging (Colombo et al., 2023). While polished white rice consists predominantly of carbohydrates, less-processed fractions of the grain harbor an extensive array of phytochemicals including phenolic acids (ferulic acid, caffeic acid, sinapic acid), tocopherols and tocotrienols (vitamin E family), γ-oryzanol, flavonoids, anthocyanins, polysaccharide fractions, and bioactive proteins, many of which exhibit potent antioxidant, anti-inflammatory, and stress-mitigating properties (Goufo & Trindade, 2014; Sen et al., 2020; Sohail et al., 2017; Colombo et al., 2023). Nutritional and nutrigenomic assessments in *Drosophila melanogaster* indicate that the consumption of whole or minimally refined rice fractions, as opposed to polished varieties, optimizes metabolic signatures and strengthens stress resilience by modulating insulin signaling and energy metabolism-related networks (Saka et al., 2023). Such evidence reinforces the premise that rice-derived bioactive constituents can provide systemic protective advantages over the course of biological aging (Ren et al., 2021).

Among the various fractions derived from rice, rice bran stands out as a primary focus of study because it retains a dense concentration of bioactive constituents that are typically discarded during the milling process (Gong et al., 2021; Zaky et al., 2021). This byproduct harbors an intricate assembly of phenolic molecules, dietary fibers, lipid-soluble antioxidants, and diverse phytochemicals that synergistically underpin its robust antioxidant capacity observed in vitro (Wattanasiritham et al., 2016; Phongthai et al., 2018; Zaky et al., 2019; Xiao et al., 2020). Notwithstanding this well-defined chemical profile and the potent bioactivity of its protein hydrolysates in

laboratory assays, in vivo data concerning the direct impact of whole rice bran on organismal aging remains sparse (Gong et al., 2021). Current *Drosophila* research has leaned heavily toward investigating isolated rice protein hydrolysates or specific peptides, often overlooking the potential of whole rice bran as a comprehensive dietary intervention (Ren et al., 2021). Consequently, the physiological significance of whole rice bran supplementation in modulating lifespan and functional aging trajectories has yet to be comprehensively addressed (Gong et al., 2021; Yue et al., 2021).

It is critical to recognize that a mere extension of lifespan does not inherently equate to improved physiological vitality, as certain interventions can prolong survival while simultaneously impairing motor or other functional attributes (Rana et al., 2017; Shaposhnikov et al., 2022). As a result, the concept of healthspan the duration of life spent in a state of high functional capacity has gained prominence in gerontology and is typically evaluated through metrics such as locomotor activity, stress resilience, and metabolic stability (Bak et al., 2023; Li et al., 2023). In *D. melanogaster*, the age-associated deterioration of locomotor performance, often measured via standardized negative geotaxis or rapid iterative negative geotaxis (RING) assays, serves as a precise indicator of neuromuscular and physiological robustness (Gargano et al., 2005; Rhodenizer et al., 2008; Jones & Grotewiel, 2011). Geroprotective strategies, including dietary nitrite supplementation, mitochondrial uncoupling, amino acid restriction, and upregulated autophagy, often maintain climbing proficiency in advanced age, effectively connecting superior locomotor function to strengthened antioxidant defenses, mitochondrial health, and stress endurance (Moretti et al., 2020; Li et al., 2023; Taylor et al., 2024). Consequently, merging functional negative geotaxis evaluations with survival data is essential for distinguishing between treatments that truly enhance healthspan and those that simply increase chronological longevity (Gargano et al., 2005; Rhodenizer et al., 2008; Jones & Grotewiel, 2011).

In this study, we evaluated the geroprotective influence of rice bran (RB) on the longevity and physiological aging of *D. melanogaster*. Our data establish that RB supplementation, with a specific focus on the 1.0% concentration, substantially prolongs lifespan, strengthens endogenous antioxidant defense systems, and safeguards locomotor performance throughout the aging process. These findings suggest that rice bran serves as a potent dietary modulator capable of enhancing both chronological survival and functional vitality at the organismal level.

MATERIAL and METHOD

Experimental design overview

We investigated the effects of dietary rice bran supplementation on lifespan, antioxidant defense systems, and age-related functional performance in *D. melanogaster*. All experiments were conducted using male flies only, following established practices in lifespan and dietary intervention studies (Partridge et al., 2005; Piper & Partridge, 2007). Five independent biological replicates (n = 5) were used for all experiments, with each replicate consisting of 20 male flies maintained in separate culture vials prepared at different times to ensure true biological replication (Linford et al., 2013). Experiments were initiated using 3-5 day old adult flies to eliminate confounding effects of larval nutrition (Piper & Partridge, 2007). Lifespan was monitored continuously, while antioxidant enzyme activities and locomotor performance were evaluated at Day 10, Day 30, and Day 45, representing young adult, middle-aged, and aged stages, respectively (Grotewiel et al., 2005). All subsequent sections refer to this general experimental design unless otherwise stated.

Fly maintenance and standard rearing conditions

Experiments were conducted using male Oregon-R wild-type *D. melanogaster*, a reference strain commonly used in aging studies (Ayar et al., 2021; Yue et al., 2021). Flies were maintained under standard conditions: 25 ± 1°C, 60–70% relative humidity, and a 12h:12h light:dark photoperiod (Fidan, 2025). All flies were reared on a standard cornmeal-based diet and transferred to fresh food every 2–3 days to maintain nutritional stability and minimize microbial contamination (Ayar et al., 2021). Experiments were initiated using 3–5-day-old adult male flies to eliminate confounding effects of larval nutrition and ensure physiological stability at the onset of dietary intervention (Piper & Partridge, 2007; Yue et al., 2021). Male flies were used exclusively to minimize variability associated with sex-specific reproductive.

Preparation of rice bran

Rice bran was obtained as a by-product during milling of newly harvested rice from Osmancık district, Çorum, Turkey. Rice bran was selected based on its retention of bioactive compounds largely removed during rice polishing (Yue et al., 2021; Tan et al., 2023). Freshly collected rice bran was separated from the polished grain immediately after milling and stored in airtight containers protected from light until use (Das et al., 2024). Prior to incorporation into experimental diets, rice bran was visually inspected, homogenized to ensure uniform particle size, and

incorporated into the standard diet without chemical extraction or fractionation to preserve its natural composition (Sunthonkun et al., 2019; Spaggiari et al., 2021; Tan et al., 2023). All preparation steps were performed uniformly across experimental batches to maintain reproducibility (Yue et al., 2021; Das et al., 2024).

Diet preparation and supplementation

Dietary supplementation was applied using rice bran incorporated into the standard *D. melanogaster* diet. Based on preliminary dose-selection experiments- and previous rice-based dietary intervention studies-, three supplementation levels were selected to evaluate dose-dependent effects- while avoiding toxicity or feeding aversion (Zambrana et al., 2019; Yue et al., 2021). Rice bran was added to the standard diet at final concentrations of 0.5%, 1.0%, and 2.5% (w/w). These concentrations were chosen to represent low, moderate, and high levels of dietary supplementation within ranges previously applied in rice-based nutritional studies- using *D. melanogaster* and other model systems (Pfluger et al., 2022). The selected doses were confirmed in preliminary experiments to support normal survival, feeding behavior, and development during short-term exposure- prior to initiation of the main experiments. Four experimental dietary groups were established:

- Control group: standard diet without rice bran
- Low-dose group: standard diet supplemented with 0.5% (w/w) rice bran
- Medium-dose group: standard diet supplemented with 1.0% (w/w) rice bran
- High-dose group: standard diet supplemented with 2.5% (w/w) rice bran

All diets were prepared fresh by thoroughly mixing rice bran into the molten standard diet to ensure homogeneous distribution of the supplement. Diets were allowed to cool and solidify at room temperature before flies were transferred. Dietary exposure was applied exclusively during the adult stage, and flies were transferred to freshly prepared food every 2–3 days throughout the experimental period, consistent with standard feeding protocols (Ayar et al., 2021; Fidan, 2025).

Each experimental group consisted of five independent biological replicates (n = 5). Each biological replicate comprised 20 male flies housed in a separate culture vial, resulting in a total of 100 flies per dietary group. All dietary treatments were initiated using 3–5-day-old adult male flies, and experimental conditions were maintained consistently across all groups to ensure reproducibility and accurate comparison of dietary effects.

Lifespan assay

Lifespan analysis was conducted to evaluate the effects of dietary rice bran supplementation on survival in *D. melanogaster*, following standard procedures widely used in dietary intervention and lifespan studies (Beghelli et al., 2024). Flies were randomly assigned to either control or rice bran–supplemented dietary groups and maintained under standard laboratory conditions throughout the experimental period (Staats et al., 2018). All experimental groups were established as described in the experimental design. Survival was monitored daily until all flies had died. During each observation, dead flies were counted and removed from the vials. Mean lifespan, median lifespan (T50), and maximum lifespan were calculated for each experimental group, and Kaplan–Meier survival curves were generated to visualize longevity differences between control and rice bran–supplemented groups (Beghelli et al., 2024). All lifespan experiments were conducted concurrently across all dietary groups to minimize environmental variability, and handling procedures were applied consistently to ensure the reproducibility of survival outcomes (Landis et al., 2020; Staats et al., 2018).

Sex selection rationale and sampling strategy

Male flies were collected at Day 10, Day 30, and Day 45 (young adult, middle-aged, and aged stages) from five independent biological replicates (n = 5), with each replicate consisting of 20 male flies maintained in separate culture vials.

Antioxidant enzyme activity assays

Antioxidant enzyme activities were determined using classical and widely accepted spectrophotometric methods that have been extensively applied in biochemical studies of endogenous antioxidant systems. All analyses were conducted as described in the experimental design. Flies were homogenized three times in ice-cold 0.05 M potassium phosphate buffer (pH 7.2) using a homogenizer (Güneş, 2016), and the homogenates were centrifuged at 20,000 × g for 30 min at 4°C to obtain clear supernatants. Supernatants were stored at −18°C until biochemical analyses were performed. Total protein content was determined spectrophotometrically at 750 nm using the Lowry et al., (1951) method, with bovine serum albumin (BSA) as the standard (Güneş & Nizamlıoğlu, 2023). All enzyme activities were normalized to total protein content to allow accurate comparison among experimental groups.

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was measured according to the classical method described by Beauchamp and Fridovich (1971). This method is based on the capacity of the enzyme to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) and represents a widely used and validated procedure for SOD determination in biological samples (Beauchamp & Fridovich, 1971; Beyer & Fridovich, 1987). SOD activity was expressed as units per milligram of protein (U/mg protein), where one unit of enzyme activity is defined as the amount of enzyme required to inhibit the rate of NBT reduction by superoxide by 50%.

Catalase (CAT) activity

Catalase (CAT) activity was determined following the classical method described by Aebi (1984), which measures the decomposition rate of hydrogen peroxide spectrophotometrically. This method is internationally accepted as a standard procedure for CAT activity determination in biological tissues, including insect models. CAT activity was calculated based on the decrease in absorbance associated with hydrogen peroxide decomposition and expressed relative to protein content.

Glutathione peroxidase (GPx) activity

Glutathione peroxidase (GPx) activity was measured using the established method of Paglia & Valentine, (1967), which monitors the oxidation of reduced glutathione coupled to the reduction of NADPH. This assay is widely employed as a reference method for GPx activity determination across a broad range of biological systems. GPx activity was expressed as units per milligram of protein.

Negative geotaxis assay

Age-related locomotor performance was assessed using the Rapid Iterative Negative Geotaxis (RING) assay, a widely used behavioral test that quantifies the innate escape response and climbing ability of *D. melanogaster* as a measure of neuromuscular function and physiological integrity (Gargano et al., 2005; Rhodenizer et al., 2008; Nichols et al., 2012). This assay provides a sensitive functional readout of healthspan by evaluating the preservation of locomotor capacity during aging (Jones & Grotewiel, 2011). Assays were performed as described in the experimental design. Flies were briefly anesthetized on ice and transferred to empty polystyrene vials (25 mm diameter) marked with three horizontal lines at 1-inch (approximately 2.54 cm) intervals from the bottom, denoting bottom, middle, and top compartments (Sekiya & Iijima, 2021). Flies were allowed to recover from ice anesthesia for at least 60 minutes before testing to ensure full recovery of normal locomotor function. Following recovery, flies were allowed to acclimate to the experimental vial for 3 minutes. After acclimation, each vial was gently tapped 3–5 times against a layered towel with minimal force to displace all flies to the bottom of the vial. The distribution of flies at the bottom, middle, and top compartments was recorded 10 seconds after tapping by capturing a digital image of the vial. This procedure was repeated five times for each replicate, with a 3-minute rest period between consecutive trials to prevent fatigue. All assays were performed at the same time of day under standard laboratory conditions (25 ± 1°C) to minimize circadian and environmental variability. Climbing success rate was calculated as the percentage of flies reaching the top compartment, and mean values across five trials were used for statistical comparison among dietary groups.

Statistical analysis

All statistical analyses were performed using the R statistical computing environment (R Core Team, 2023). Data processing, statistical testing, and graphical visualization were conducted entirely within R to ensure transparency, reproducibility, and consistency across all analyses. All statistical evaluations were based on five independent biological replicates (n = 5) per dietary group, with each biological replicate consisting of 20 male *D. melanogaster* maintained in a separate culture vial. Analyses were performed at the biological replicate level to preserve statistical independence and to avoid pseudoreplication.

Survival data were analyzed using Kaplan–Meier survival analysis, and differences among dietary groups were evaluated using the log-rank test. Mean lifespan, median lifespan (T50), and maximum lifespan were calculated for each experimental group. Survival analyses and curve visualization were implemented using the *survival* and *survminer* packages in R.

Antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), measured at Day 10, Day 30, and Day 45, were analyzed using analysis of variance (ANOVA)-based approaches. Prior to inferential testing, data were evaluated for normality and homogeneity of variance. Comparisons among dietary groups and age points were performed using one-way or two-way ANOVA, as appropriate, followed by Tukey's honestly significant difference (HSD) post hoc test when significant effects were

detected. Enzyme activity data are presented as mean ± standard deviation (SD).

Negative geotaxis (climbing) performance data obtained at Day 10, Day 30, and Day 45 were analyzed using ANOVA-based methods to assess age-dependent functional decline and dietary effects. For each biological replicate, the mean climbing success rate calculated from five consecutive trials was used for statistical comparison among experimental groups. All graphical representations, including survival curves and enzyme activity plots, were generated directly from analyzed datasets using the *ggplot2* package to ensure accurate correspondence between statistical results and visual presentation. A p-value < 0.05 was considered statistically significant for all analyses.

RESULTS

To evaluate the effects of dietary rice bran supplementation on aging in *D. melanogaster*, we examined lifespan, antioxidant enzyme activities, and locomotor performance in male flies maintained on diets containing 0.5%, 1.0%, or 2.5% (w/w) rice bran. Rice bran supplementation significantly extended mean lifespan across all concentrations, with 1.0% exhibiting optimal efficacy (Table 1, Figure 1). Analysis of antioxidant enzyme activities revealed dose-dependent enhancement of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities at all assessed timepoints (Days 10, 30, and 45), with 1.0% rice bran demonstrating the greatest increases (Table 2, Figure 2). Assessment of age-related locomotor decline via negative geotaxis assay showed that rice bran supplementation significantly preserved climbing ability at middle-aged and aged stages compared to controls (Table 3, Figure 3). These findings indicate that rice bran supplementation, particularly at 1.0% concentration, promotes longevity and healthspan extension through enhancement of antioxidant defense and preservation of motor function during aging.

Table 1. Effect of rice bran supplementation on lifespan
Tablo 1. Pirinç kepeği takviyesinin yaşam süresi üzerindeki etkisi

Group	n	Mean ± SD (days)	Median (days)	Maximum (days)	% Increase	Significance
Control	100	43.05 ± 3.27	43.0	50	-	-
0.5% RB	100	47.30 ± 3.38	47.0	54	+9.9%	***
1.0% RB	100	53.55 ± 3.70	53.0	61	+24.4%	***
2.5% RB	100	50.30 ± 3.38	50.0	57	+16.8%	***

Data are presented as mean ± SD from five biological replicates (n = 5, 20 flies per replicate, 100 flies total per group). Statistical analysis: one-way ANOVA followed by Tukey HSD post-hoc test. *** p < 0.001 compared to control. RB: rice bran; SD: standard deviation.

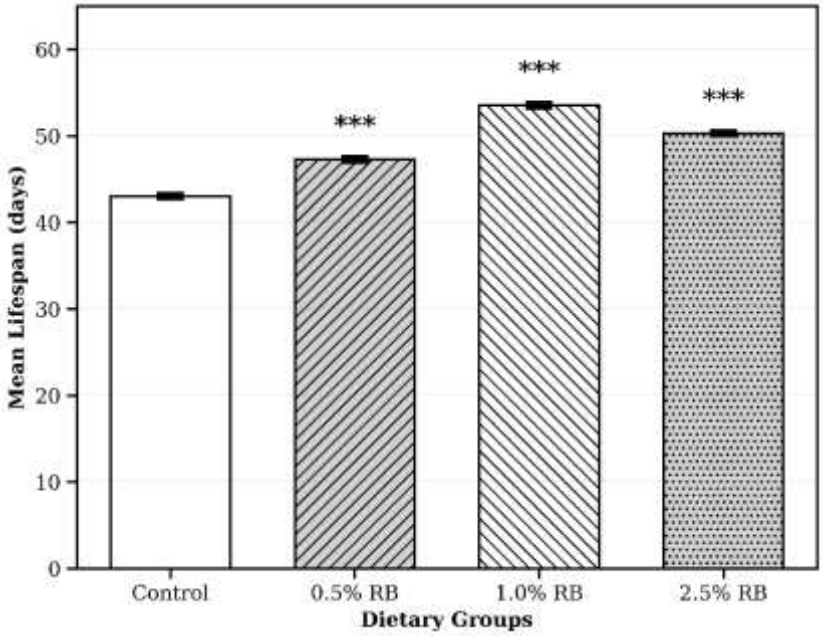

Figure 1. Effect of dietary rice bran supplementation on mean lifespan in *Drosophila melanogaster*.
Şekil 1. Diyete pirinç kepeği takviyesinin Drosophila melanogaster'de ortalama yaşam süresi üzerindeki etkisi.
Male flies were maintained on control diet or diets supplemented with 0.5%, 1.0%, or 2.5% (w/w) rice bran throughout their adult life. Bars represent mean lifespan ± SEM from five independent biological replicates (n = 5, 20 flies per replicate, 100 flies total per group). Different bar patterns distinguish dietary groups. Statistical analysis was performed using one-way ANOVA followed by Tukey HSD post-hoc test. *** p < 0.001 compared to control group. RB: rice bran; SEM: standard error of the mean.

Table 2. Antioxidant enzyme activities at day 10.
Tablo 2. 10. gündeki antioksidan enzim aktiviteleri

Group	SOD (U/mg)	% Inc	Sig	CAT (U/mg)	% Inc	Sig	GPx (U/mg)	% Inc	Sig
Control	18.20 ± 0.45	-	-	32.66 ± 0.82	-	-	10.40 ± 0.35	-	-
0.5% RB	21.40 ± 0.38	+17.6%	***	37.30 ± 0.75	+14.2%	***	11.60 ± 0.32	+11.5%	***
1.0% RB	23.90 ± 0.52	+31.3%	***	40.60 ± 0.95	+24.3%	***	12.40 ± 0.41	+19.2%	***
2.5% RB	22.60 ± 0.48	+24.2%	***	38.90 ± 0.88	+19.1%	***	12.00 ± 0.39	+15.4%	***

Enzyme activities measured at Day 10. Data are presented as mean ± SEM from five biological replicates (n = 5). Statistical analysis: one-way ANOVA followed by Tukey HSD post-hoc test. *** $p < 0.001$ compared to control. RB: rice bran; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; SEM: standard error of the mean.

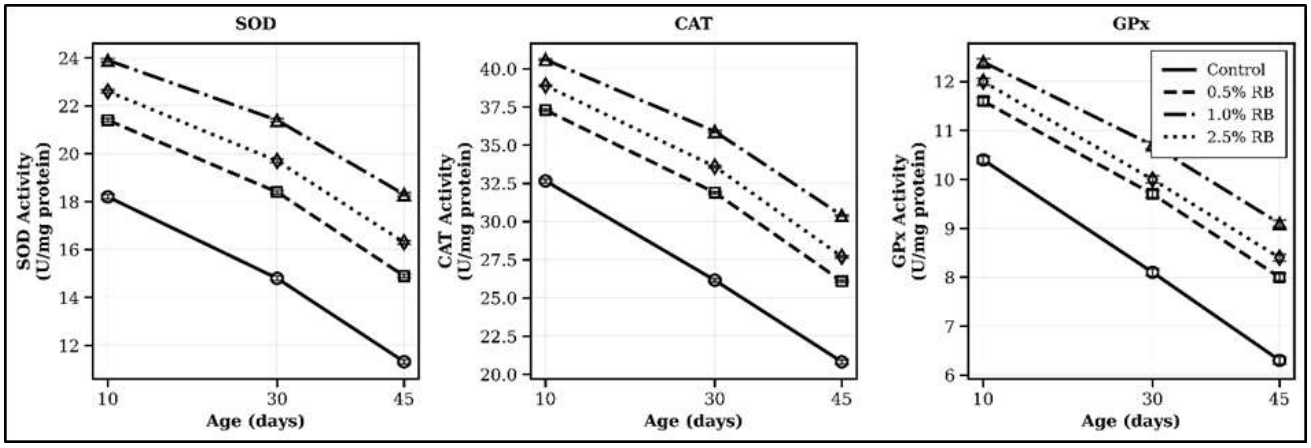

Figure 2. Temporal changes in antioxidant enzyme activities during aging in rice bran-supplemented flies
Şekil 2. Pirinç kepeği takviyeli sineklerde yaşlanma sırasında antioksidan enzim aktivitelerindeki zamansal değişimler

Activities of (A) superoxide dismutase (SOD), (B) catalase (CAT), and (C) glutathione peroxidase (GPx) were measured at Days 10, 30, and 45 post-eclosion. Data are presented as mean ± SEM from five independent biological replicates (n = 5). Different line styles and markers distinguish dietary groups: solid line with circles (Control), dashed line with squares (0.5% RB), dash-dot line with triangles (1.0% RB), dotted line with diamonds (2.5% RB). Error bars represent SEM. Statistical analysis was performed using two-way ANOVA (group × age) followed by Tukey HSD post-hoc test ($p < 0.001$ for main effects of group and age, and their interaction). RB: rice bran; SEM: standard error of the mean.

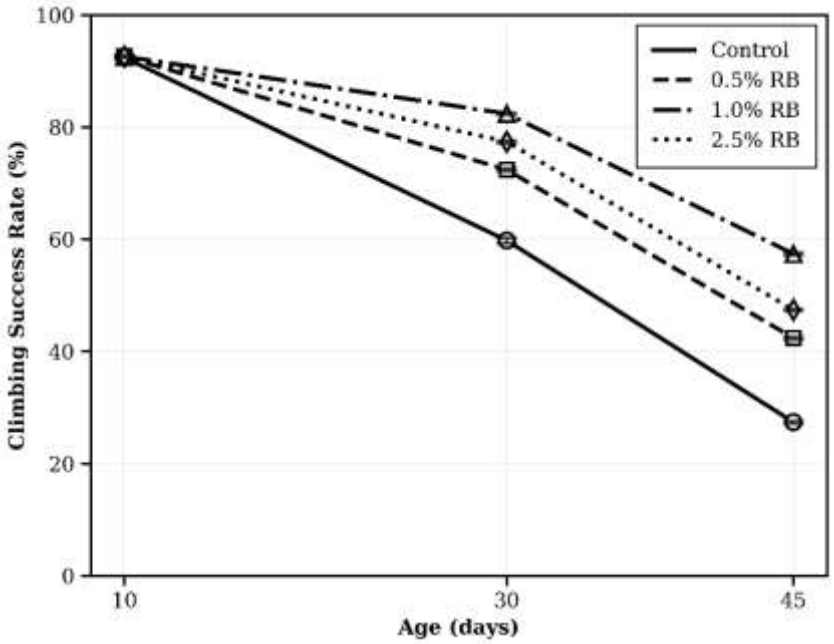

Figure 3. Rice bran supplementation attenuates age-related decline in locomotor performance
Şekil 3. Pirinç kepeği takviyesi lokomotor performanstaki yaşa bağlı düşüşü azaltır

Climbing success rate (percentage of flies reaching the top within 10 seconds) was assessed via negative geotaxis assay at Days 10, 30, and 45. Data are presented as mean ± SEM from five independent biological replicates (n = 5, 20 flies per replicate, 5 consecutive trials per timepoint). Line styles and markers as in Figure 2. Error bars represent SEM. Statistical analysis was performed using two-way ANOVA (group × age) followed by Tukey HSD post-hoc test ($p < 0.001$ for main effects and interaction). RB: rice bran; SEM: standard error of the mean.

Table 3. Age-related climbing performance
Tablo 3. Yaşa bağlı tırmanma performansı

Group	Day 10 (%)	Sig	Day 30 (%)	Sig	Day 45 (%)	Sig
Control	92.4 ± 1.2	-	59.8 ± 2.4	-	27.4 ± 3.1	-
0.5% RB	92.6 ± 1.1	ns	72.4 ± 2.1	***	42.4 ± 2.8	***
1.0% RB	92.6 ± 0.9	ns	82.4 ± 1.8	***	57.4 ± 2.5	***
2.5% RB	92.6 ± 1.3	ns	77.4 ± 2.2	***	47.4 ± 2.9	***

Climbing success rate (%) assessed via negative geotaxis assay at Days 10, 30, and 45. Data are presented as mean ± SEM from five biological replicates (n = 5, 20 flies per replicate, 5 trials per timepoint). Statistical analysis: two-way ANOVA (group × age) followed by Tukey HSD post-hoc test. *** $p < 0.001$; ns: not significant ($p > 0.05$) compared to control at the same age. RB: rice bran.

Rice bran supplementation extends lifespan

Rice bran supplementation significantly extended mean lifespan in a dose-dependent manner (Table 1, Figure 1). All three tested concentrations (0.5%, 1.0%, and 2.5% w/w) increased survival compared to controls, with the 1.0% dose exhibiting optimal efficacy. The dose-response relationship displayed an inverted U-shaped curve, as the higher 2.5% concentration showed reduced effectiveness compared to 1.0%, suggesting potential adverse effects of excessive supplementation. Statistical analysis confirmed highly significant differences among groups (one-way ANOVA, $p < 0.001$).

Effects of rice bran supplementation on endogenous antioxidant defense systems

To evaluate the impact of rice bran supplementation on antioxidant capacity, the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were measured at Day 10, 30, and 45, representing early, middle-aged, and aged stages, respectively (Table 2, Figure 2). Rice bran supplementation at all tested concentrations significantly enhanced antioxidant enzyme activities compared to the control group across all timepoints ($p < 0.001$). The enzyme activity enhancements followed a clear dose-dependent pattern, with the 1.0% concentration demonstrating optimal efficacy. At Day 10, the 1.0% rice bran group exhibited the greatest increases: 31.3% for SOD, 24.3% for CAT, and 19.2% for GPx relative to controls (Table 2).

This superior antioxidant enhancement was maintained throughout aging, as evidenced by sustained elevation of all three enzyme activities at Days 30 and 45 (Figure 2). Notably, the 2.5% rice bran group showed lower enzyme activity levels compared to the 1.0% group across all measured timepoints, despite remaining significantly elevated relative to controls. This non-monotonic dose-response relationship where intermediate concentrations provide maximal benefits suggests that rice bran exerts hormetic effects on antioxidant defense systems, with excessive supplementation potentially diminishing efficacy. These findings indicate that the lifespan extending effects of rice bran are strongly associated with coordinated upregulation of key antioxidant enzymes, enhancing cellular resistance to oxidative stress in a dose-optimized manner.

Rice bran supplementation attenuates age-related locomotor decline

To assess whether lifespan extension was accompanied by preservation of functional capacity, locomotor performance was evaluated using the negative geotaxis assay at Days 10, 30, and 45 (Table 3, Figure 3). As expected, all experimental groups exhibited progressive, age-dependent decline in climbing success rate over the 45-day period. At Day 10, representing the young adult stage, no significant differences were observed among dietary groups (all >92%, $p > 0.05$), indicating that rice bran supplementation does not alter baseline neuromuscular function in early adulthood (Table 3).

However, rice bran supplementation significantly attenuated the decline in locomotor capacity at both middle-aged (Day 30) and aged (Day 45) stages compared to controls ($p < 0.001$). Consistent with the lifespan and antioxidant enzyme findings, the 1.0% dose provided the most substantial functional protection, with rice bran-fed flies maintaining markedly higher climbing success rates than controls at both timepoints (Figure 3). While the 0.5% and 2.5% doses also improved locomotor performance relative to controls, their protective effects were less pronounced than those observed with the 1.0% dose, further supporting a hormetic dose-response relationship. These results demonstrate that rice bran supplementation, particularly at the 1.0% concentration, not only extends lifespan but also preserves physiological robustness and neuromuscular integrity during aging, thereby enhancing overall healthspan. The parallel improvements in survival, antioxidant capacity, and functional performance suggest that rice bran's anti-aging effects are mediated through comprehensive enhancement of stress resistance and preservation of age-related physiological decline.

DISCUSSION

We investigated the geroprotective potential of rice bran (RB) on the lifespan and functional aging of *D. melanogaster*. Our results clearly demonstrate that RB supplementation, particularly at a 1.0% concentration, significantly enhances longevity, boosts endogenous antioxidant defense mechanisms, and preserves locomotor functions during the aging process.

Effects of rice bran on lifespan and the hormetic response

The survival analysis revealed that RB supplementation significantly extended both the mean and maximum lifespan of male flies compared to the control group (Table 1). The most pronounced effect was observed in the 1.0% RB group, which showed a 24.4% increase in mean lifespan. This finding is consistent with studies showing that rice-derived protein/peptides- or pigmented rice bran prolong lifespan in model organisms by modulating longevity-related signaling pathways- and oxidative stress defenses (Sunthonkun et al., 2019; Yue et al., 2021).

However, we observed a non-monotonic dose-response-, where the 2.5% RB dose was less effective than the 1.0% dose. This suggests a hormetic effect, in line with reports that low or moderate doses of dietary bioactives often maximize lifespan benefits, whereas higher doses lose efficacy or become detrimental (Kim et al., 2019; Yue et al., 2021; Lee et al., 2025). The magnitude of the lifespan extension we observed also falls within the typical 30–60% upper limit described for hormetic dose–response relationships in longevity studies (Calabrese et al., 2023). While moderate doses of RB bioactive compounds trigger beneficial stress-response mechanisms-, excessive concentrations may lead to metabolic saturation or pro-oxidant/toxic effects-, thereby limiting the anti-aging benefits, a pattern broadly documented for polyphenol and antioxidant-rich interventions- in insects and nematodes (Berry & López-Martínez-, 2020).

Modulation of antioxidant enzymes and oxidative stress

Aging is intrinsically linked to the accumulation of reactive oxygen species (ROS) and the subsequent decline in antioxidant enzymes, particularly SOD, CAT, and GPx, which constitute the first-line enzymatic defense against oxidative stress (Vincent et al., 2021; Jena et al., 2023; Jomová et al., 2024). Our biochemical analyses (Table 2) showed a significant upregulation of SOD, CAT, and GPx activities in RB-supplemented groups. By day 45, the 1.0% RB group maintained 31.3% higher SOD activity compared to age-matched controls, consistent with reports that food-derived nutraceuticals can enhance redox enzyme activities in vivo (Ramírez-Mendoza- et al., 2022; Obeme-Nmom- et al., 2024; Mahfudh et al., 2025).

This enzymatic boost can be explained by the activation of the Nrf2/Keap1 signaling pathway, which is a conserved master regulator of antioxidant responses, driving ARE-dependent expression of cytoprotective genes including SOD, CAT, and GPx (Raghunath et al., 2018; Thiruvengadam et al., 2021; Sykiotis & Bohmann, 2008; Liu et al., 2022). Rice bran harbors a diverse array of bioactive constituents with established antioxidant properties, including phenolic compounds (ferulic acid, caffeic acid, sinapic acid), tocopherols and tocotrienols (vitamin E family), γ-oryzanol, flavonoids, anthocyanins, polysaccharide fractions, and bioactive proteins (Sen et al., 2020; Sohail et al., 2017; Park et al., 2017), all of which fit within the broader class of natural Nrf2 activators that promote nuclear translocation of Nrf2 and upregulation of ARE-driven antioxidant enzymes (Zhou et al., 2019; Rungratanawanich et al., 2018; Culletta et al., 2024). Our data suggest that RB does not merely act as a direct scavenger of ROS but rather "reprograms" the fly's internal defense system by boosting endogenous antioxidant enzyme expression, a mechanism widely recognized for dietary polyphenols and bioactive peptides (Rungratanawanich et al., 2018; Thiruvengadam et al., 2021; Obeme-Nmom et al., 2024).

Preservation of locomotor integrity (healthspan)

A critical hallmark of aging is the progressive loss of motor coordination. The negative geotaxis assay results (Table 3) demonstrated that RB supplementation significantly delayed the decline in climbing ability. While all groups showed a decrease in performance over time, the 1.0% RB group exhibited superior robustness at day 45, maintaining a much higher success rate than the control. Comparable preservation of negative geotaxis performance has been reported in other interventions that target neuromuscular aging in Drosophila (Moretti et al., 2020).

This preservation of "healthspan" is likely due to the neuroprotective effects of RB. According to Araujo et al. (2015), rice bran components protect dopaminergic neurons and prevent neuromotor deficits by mitigating mitochondrial dysfunction. Consistent with this, modulation of mitochondrial function and redox homeostasis has been shown to delay age-related locomotor decline and neuromuscular junction deterioration in flies (Spiers et al., 2019; Weinrich et al., 2017). Our findings suggest that the increased antioxidant enzyme levels in the RB groups (Table 2) protect the neuromuscular junctions and the central nervous system from oxidative damage,

thereby maintaining functional mobility well into old age (Spiers et al., 2019; Yusuf et al., 2024). Taken together, these results support the potential of rice bran as a practical and biologically relevant dietary supplement for model organism-based aging research, consistent with established approaches to nutritional supplementation in Drosophila culture (Uysal et al., 2002).

Study Limitations

While this study provides valuable insights into the geroprotective effects of rice bran supplementation in Drosophila melanogaster, several limitations should be acknowledged. First, consistent with widely established practices in Drosophila aging research (Partridge et al., 2005; Piper & Partridge, 2007), we used exclusively male flies to eliminate confounding effects of reproductive physiology and egg production. While this approach minimizes metabolic variability and ensures experimental consistency, it limits the generalizability of our findings to female cohorts, which exhibit distinct metabolic demands and stress responses. Future studies should include female flies to determine whether rice bran supplementation exerts sex-specific or universal effects on aging parameters. Second, our study employed whole rice bran without fractionation or isolation of individual bioactive constituents, an approach designed to preserve the natural synergistic interactions among its complex mixture of phenolic compounds (γ-oryzanol, ferulic acid), tocopherols, dietary fiber, and bioactive peptides (Goufo & Trindade, 2014; Tan et al., 2023). While this whole-food strategy reflects real-world nutritional applications and may capture combinatorial effects that isolated compounds cannot replicate, it precludes definitive attribution of observed effects to specific molecular actors. Future studies employing systematic fractionation, chemical characterization, and targeted supplementation with purified compounds would clarify which bioactive classes drive lifespan extension and whether their effects are additive, synergistic, or redundant. Third, we observed a clear hormetic dose-response pattern wherein the intermediate 1.0% dose outperformed the higher 2.5% dose in extending lifespan and enhancing antioxidant capacity. While our findings are consistent with established hormetic frameworks in aging research (Berry & López-Martínez, 2020; Calabrese et al., 2023), and we have proposed potential mechanisms including metabolic saturation, pro-oxidant effects at excessive doses, or nutrient balance disruption, we did not perform transcriptomic, proteomic, or metabolomic analyses to definitively establish the molecular basis of this non-monotonic response. Advanced omics approaches, combined with dose-titration studies and genetic pathway manipulations, would be valuable to resolve why moderate doses maximize healthspan while higher doses exhibit diminished efficacy. Despite these limitations, our findings establish rice bran as a promising dietary intervention for promoting healthy aging and provide a foundation for future mechanistic and translational investigations.

CONCLUSION

In conclusion, dietary supplementation with rice bran (RB) effectively promotes longevity and functional robustness in *D. melanogaster*. The most significant improvements in lifespan, antioxidant enzyme profiles (SOD, CAT, GPx), and locomotor activity were achieved with a 1.0% RB inclusion rate. These effects are mediated through a combination of direct antioxidant action and the potential upregulation of cytoprotective pathways. Future research should focus on the transcriptomic changes induced by RB to further elucidate the molecular intersections between rice bran bioactive compounds and the aging transcriptome.

Conflict of Interest

The author declares that there are no conflicts of interest to be disclosed.

Author Contributions

Conceptualization and design of the study: MF; Data collection: MF; Analysis and interpretation of results: MF; Preparation of the manuscript draft: MF. The author has reviewed and approved the final version of the manuscript.

Ethics Committee Approval

This study used *Drosophila melanogaster*, an invertebrate model organism that does not require ethics committee approval.

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