Theme: Advancing Animal Health and Productivity for A Sustainable One Health Ecosystem

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Effects of Raisin-supplemented Large-animal Feed on Hepatic, Renal and Reproductive Parameters in Male Wistar Rats

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ABSTRACT

Background: The high cost and limited availability of conventional laboratory animal diets have encouraged the search for affordable and nutritionally suitable alternatives. Raisins contain polyphenols and other bioactive compounds with antioxidant properties that may support physiological functions. This study aimed to evaluate the biochemical, histological and endocrine effects of feeding male Wistar rats a large-animal feed with or without raisin supplementation.

Methods: Eighteen adult male Wistar rats were randomly assigned to three groups (n = 6): a control group fed a standard laboratory diet, T1 fed large-animal feed and T2 fed large-animal feed supplemented with raisins. After four weeks, serum biochemical parameters, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), uric acid and creatinine, were measured. Serum testosterone concentrations were also determined. Organ weights were recorded and histopathological examinations of the liver, kidneys and testes were performed.

Result: ALT levels were significantly reduced in both T1 and T2 compared with the control group, whereas AST activity increased significantly in T2. BUN and uric acid levels were significantly lower in T2. Testosterone concentrations increased significantly in T1 but remained unchanged in T2. Histopathological examination revealed preserved tissue architecture without evident degeneration or inflammation in the liver, kidneys, or testes. Although reductions in the weights of some organs were observed in T2, no corresponding histopathological abnormalities were detected. The results indicate that raisin supplementation was associated with changes in selected biochemical parameters, particularly renal biomarkers, without evident histopathological damage during the four-week experimental period.

Key words: Alternative feed, Hepatic enzymes, Laboratory animal nutrition, Polyphenols, Renal biomarkers, Reproductive parameters.

INTRODUCTION

Laboratory animal nutrition is a fundamental determinant of experimental reliability, reproducibility and translational validity in biomedical research. Standardized rodent diets are specifically formulated to meet the physiological and metabolic requirements of laboratory animals, thereby minimizing variability and ensuring consistent growth, organ function and reproductive performance (Reeves *et al.*, 2020; Li *et al.*, 2024; Rinwa *et al.*, 2024). However, the rising cost and limited availability of commercial laboratory feeds in certain regions have highlighted the need for alternative, cost-effective feeding strategies that do not compromise animal welfare or experimental outcomes (Tobin and Schuhmacher, 2021; Ojediran *et al.*, 2024).

Large animal feed, commonly used in livestock production, has emerged as a potential alternative due to its wide availability and lower economic burden. These feeds are typically rich in energy and macronutrients; however, their composition differs significantly from conventional rodent diets, particularly in fiber content, micronutrient balance and digestibility (Acharya, 2025). Such differences may influence metabolic homeostasis, organ function and reproductive physiology in small laboratory animals. Therefore, a comprehensive evaluation of the physiological and biochemical impacts of these alternative feeds is essential prior to their adoption in laboratory settings.

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To overcome potential nutritional imbalances associated with unconventional diets, supplementation with natural bioactive compounds has gained increasing attention. Raisins (dried grapes) are rich in carbohydrates, dietary fiber, vitamins, minerals and polyphenolic compounds, including flavonoids and phenolic acids, which exhibit strong antioxidant and anti-inflammatory properties (Williamson and Carughi, 2010; Terao, 2023; Nale *et al.*, 2024). These compounds have been shown to mitigate oxidative stress and exert protective effects on vital organs such as the liver and kidneys (Surai *et al.*, 2021; Służały *et al.*, 2024). Given that oxidative stress plays a central role in the

pathophysiology of organ dysfunction and reproductive impairment, antioxidant-rich dietary interventions may provide significant physiological benefits (Averill-Bates, 2024; Pavuluri *et al.*, 2024).

Oxidative stress is also a key factor in male reproductive health, as excessive reactive oxygen species (ROS) can impair spermatogenesis, reduce sperm motility and disrupt endocrine function (Sengupta *et al.*, 2022; Pavuluri *et al.*, 2025). Testosterone, the principal androgen hormone, plays a critical role in regulating spermatogenesis and maintaining the structural and functional integrity of male reproductive organs (Schiesaro *et al.*, 2022; Ni, 2019). Consequently, dietary strategies that modulate oxidative stress and endocrine balance may directly influence male fertility outcomes.

Previous studies by our group have explored the feasibility of using large animal feed as an alternative to standard laboratory diets. In male Wistar rats, the inclusion of raisin supplementation was associated with improvements in sperm motility and specific kinematic parameters, despite variations in feed intake and body weight dynamics (Amran *et al.*, 2025). In a subsequent study on female rats, the same dietary strategy demonstrated no adverse effects on reproductive performance, maternal health, or offspring development, supporting its safety and applicability as a cost-effective feeding alternative (Amran *et al.*, 2026). Collectively, these findings suggest that raisin-supplemented alternative diets may support reproductive function while maintaining overall physiological stability.

Previous studies have investigated the biochemical, histological and reproductive effects of plant-derived extracts in experimental animals. For example, Alhimaidi *et al.* (2024) evaluated the biochemical and histological effects of a Ziziphus species extract on ovarian function in female rats. Similarly, Ammari *et al.* (2024) investigated the effects of Launaea angustifolia extract on female reproductive function. These studies highlight the relevance of assessing the physiological and reproductive effects of natural dietary constituents in animal models.

Despite these promising observations, the underlying mechanisms responsible for these effects remain unclear. In particular, there is a lack of comprehensive data on the impact of such dietary interventions on liver and kidney function, endocrine regulation and tissue integrity. Biochemical markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and uric acid are widely used indicators of hepatic and renal function, while histopathological evaluation provides direct insights into tissue-level alterations and cellular integrity. Additionally, assessing the relative weights of reproductive organs alongside testosterone levels offers valuable information on functional and structural reproductive changes.

The present study aims to provide a comprehensive evaluation of the biochemical, histological and endocrine effects of large animal feed with and without raisin supplementation in male Wistar rats.

MATERIALS AND METHODS

Experimental animals

A total of eighteen male Wistar rats (Rattus norvegicus), approximately 8-10 weeks old and weighing 200-220 g, were included in the study. The animals were obtained from the Animal Care Facility of the Department of Zoology, College of Science, King Saud University. The rats were housed in polypropylene cages under standardized environmental conditions, including a temperature of 25± 2°C, relative humidity of 50-60%, controlled 12 h light/ 12 h dark cycle. and access to feed and water throughout the study period. All experimental procedures were conducted in accordance with institutional regulations for laboratory animal welfare and were approved by the Institutional Animal Care and Use Committee (IACUC) of King Saud University (Approval No. KSU-SE-23-06).

Experimental design

The rats were randomly distributed into three experimental groups, with six animals assigned to each group:

The standard laboratory diet group (C) served as the reference group for assessing the effects of the alternative large-animal feed. Group T1 was included to evaluate the effects of the alternative feed without raisin supplementation, whereas group T2 was used to investigate the additional effects of incorporating raisins into the same feed. This experimental design enabled comparisons between the standard diet and the alternative feed (C vs. T1), as well as between the supplemented and raisin-supplemented alternative feeds (T1 vs. T2).

The dietary intervention was maintained continuously for four weeks.

Diet composition

Animals in the control group received a conventional rodent diet containing approximately 20% crude protein, 4% crude fat and 3.5% crude fiber, with an estimated metabolizable energy content of 2850 kcal/kg. The diet provided to the T1 group consisted mainly of yellow corn, barley, soybean meal, wheat bran and a vitamin-mineral premix, providing approximately 14.5% crude protein and approximately 2800 kcal/kg metabolizable energy.

For group T2, the basal large-animal feed was supplemented with 200 g of dried raisins per group per week. The six rats in this group shared the supplemented feed, which was provided twice daily in equal portions throughout the four-week experimental period.

Sample collection and organ weights

At the end of the experimental period, the rats were fasted overnight and exposed to carbon dioxide (CO_2) by inhalation. Following loss of consciousness and before death was confirmed, blood samples were collected by cardiac puncture. The blood samples were centrifuged at 3000 rpm for 10 min to separate the serum, which was stored at -20°C until biochemical and hormonal analyses were performed.

Following blood collection, death was confirmed in accordance with the approved institutional animal care and use protocol. The liver, kidneys, testes, epididymis, vas deferens, prostate gland, seminal vesicles and spleen were carefully excised. The organs were rinsed with physiological saline to remove residual blood and tissue debris, gently blotted dry and weighed using a calibrated analytical balance.

Biochemical analysis

Serum biochemical parameters were determined using commercially available diagnostic reagent kits supplied by BioSystems S.A. (Spain), according to the manufacturers' instructions. Hepatic biochemical assessment included alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities. Renal biochemical assessment included blood urea nitrogen (BUN), uric acid and creatinine concentrations.

Hormonal assay

Serum testosterone concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits obtained from Abcam (USA) and Cayman Chemical, according to the manufacturers' protocols. Absorbance was measured at 450 nm using a microplate spectrophotometric reader (BioTek ELx800, USA / Thermo Scientific Multiskan™ GO, USA).

Histological examination

Liver, kidney and testicular specimens were collected immediately after sacrifice and fixed in 10% neutral buffered formalin. The tissues were processed routinely by dehydration through ascending concentrations of ethanol, clearing with xylene and embedding in paraffin wax. Paraffin blocks were sectioned at approximately 4-5 µm thickness and stained with hematoxylin and eosin (H and E). The stained sections were examined under a light microscope at ×100 and ×400 magnifications to assess tissue architecture and identify potential morphological abnormalities, including degenerative changes, necrosis, inflammatory infiltration and other treatment-related alterations.

Statistical analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed using GraphPad Prism software (version 8). Differences among the experimental groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effects of dietary treatments on liver function markers

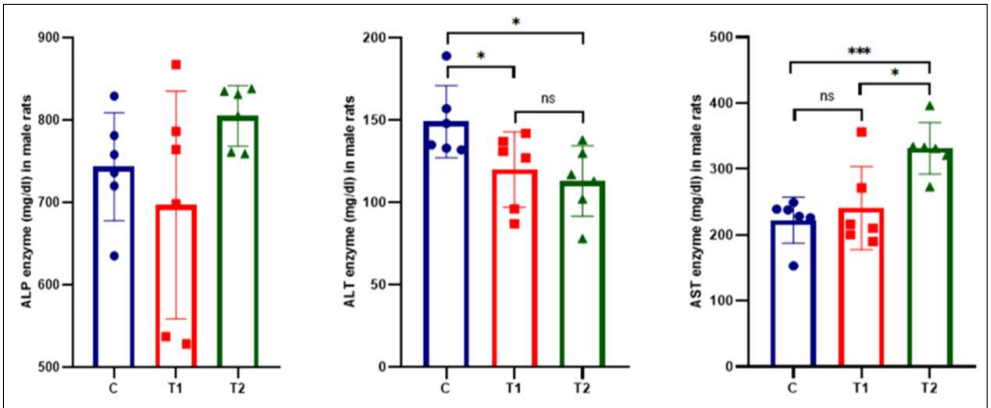
The activities of serum liver enzymes, namely alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are illustrated in Fig 1.

Alkaline phosphatase (ALP)

Analysis of ALP activity demonstrated no statistically relevant differences among the studied groups. The recorded mean values were 743 U/L for the control group (C), 697 U/L for treatment group 1 (T1) and 805 U/L for treatment group 2 (T2). Comparative statistical analysis showed that the differences between C and T1 ($p = 0.47$), C and T2 ($p = 0.07$) and T1 and T2 ($p = 0.09$) were not significant, indicating that the dietary regimens had minimal influence on ALP activity.

Alanine aminotransferase (ALT)

Serum ALT concentrations were noticeably lower in both treatment groups compared with the control animals. Mean ALT activities reached 149 U/L in the control group, whereas values declined to 120 U/L in T1 and 113 U/L in T2.



Serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in control (C), large animal feed (T1) and raisin-supplemented diet (T2) groups. Data are expressed as mean ± SEM (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. *p<0.05, ***p<0.001 and ns = Not significant.

Fig 1: Effects of dietary treatments on liver enzyme activities (ALP, ALT and AST) in male Wistar rats.

Statistical testing confirmed significant reductions in ALT activity in both T1 (p = 0.05) and T2 (p = 0.02) relative to the control group. Nevertheless, comparison between the two treatment groups revealed no statistically significant difference (p = 0.60).

Aspartate aminotransferase (AST)

Animals assigned to treatment group 2 (T2) exhibited a pronounced increase in serum AST activity. The mean AST values were 222.17 U/L in the control group (C), 240.50 U/L in treatment group 1 (T1) and 331.33 U/L in T2. Statistical analysis demonstrated that AST activity in T1 was not significantly different from the control animals (p = 0.50). In contrast, rats in T2 showed significantly elevated AST levels compared with both the control group (p = 0.001) and T1 (p = 0.01), indicating a clear treatment-associated effect on hepatic enzyme response.

Present results indicated that both treatment groups were associated with reduced ALT activity relative to the control group, whereas increased AST activity was mainly confined to T2. Meanwhile, ALP values remained generally unchanged, as no statistically significant variation was detected among the experimental groups.

Effects of dietary treatments on kidney function markers

Serum biochemical indicators associated with renal function, including uric acid, blood urea nitrogen (BUN) and creatinine, are presented in Fig 2.

Uric acid

A reduction in serum uric acid concentration was observed in the treatment groups, with the strongest decline detected in treatment group 2 (T2). Mean uric acid values were recorded as 35.62 mg/dL in the control group (C), 29.97 mg/dL in treatment group 1 (T1) and 13.85 mg/dL in T2. Statistical comparisons showed no significant difference between C and T1 (p = 0.30). However, T2 animals exhibited markedly lower uric acid levels than both the control group (p = 0.001) and T1 (p = 0.001).

Blood urea nitrogen (BUN)

Serum BUN values decreased considerably in both treatment groups relative to the control animals. The average concentrations were 61.00 mg/dL in the control group, 40.50 mg/dL in T1 and 38.00 mg/dL in T2. Statistical analysis demonstrated highly significant reductions in BUN levels in both T1 (C vs T1: p<0.0001) and T2 (C vs T2: p = 0.001) compared with the control group. Nevertheless, the difference between T1 and T2 was not statistically significant (p = 0.60).

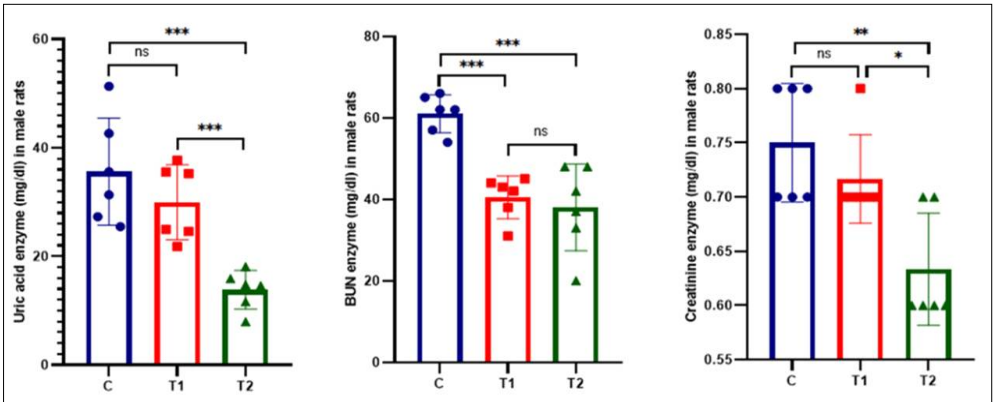
Creatinine

Creatinine concentration showed a moderate but significant decline in treatment group 2. Mean creatinine values were 0.75 mg/dL for the control group, 0.72 mg/dL for T1 and 0.63 mg/dL for T2. No significant difference was detected between C and T1 (p = 0.30). Conversely, creatinine levels in T2 were significantly lower than those measured in the control group (p = 0.004) and T1 (p = 0.01).

Overall, renal biomarkers showed lower serum concentrations in the treated animals, particularly in T2, where substantial reductions were observed in uric acid, BUN and creatinine levels, particularly in T2, where substantial reductions were observed in uric acid, BUN and creatinine levels. By comparison, T1 produced less pronounced changes that were not consistently statistically significant.

Effects of dietary treatments on serum testosterone levels

Serum testosterone measurements obtained from the experimental groups are illustrated in Fig 3. The results demonstrated that animals in treatment group 1 (T1) exhibited higher testosterone concentrations compared with the control group (C). Mean testosterone values were



Serum levels of uric acid, blood urea nitrogen (BUN) and creatinine in control (C), large animal feed (T1) and raisin-supplemented diet (T2) groups. Data are expressed as mean ± SEM (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. *p<0.05, **p<0.01, ***p<0.001 and ns = Not significant.

Fig 2: Effects of dietary treatments on kidney function markers (uric acid, BUN and creatinine) in male Wistar rats.

1.67 ng/mL in the control animals, 2.33 ng/mL in T1 and 1.79 ng/mL in treatment group 2 (T2).

Statistical evaluation confirmed a significant increase in testosterone levels in T1 relative to the control group ($p = 0.05$). In contrast, testosterone concentrations in T2 did not differ significantly from those of the control animals ($p = 0.62$). Likewise, no statistically meaningful variation was detected between T1 and T2 ($p = 0.12$).

These findings suggest that administration of the large-animal feed formulation may enhance serum testosterone levels, whereas supplementation with raisins did not result in an additional statistically significant hormonal effect. Despite the significant increase in serum testosterone in T1, no corresponding histopathological alterations were observed in the testes.

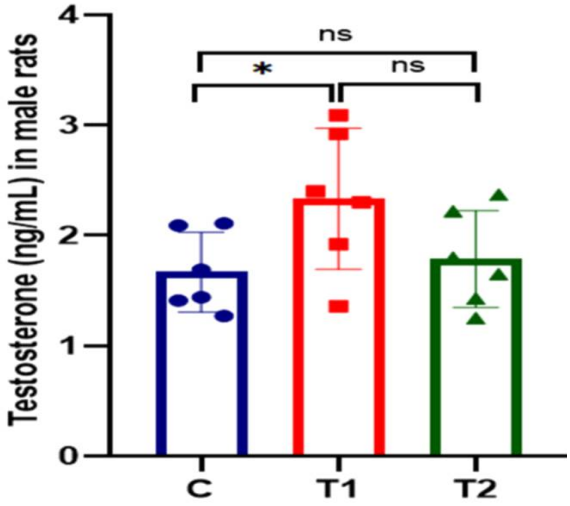
Effects of dietary treatments on vital organ weights

The relative masses of major vital organs, including the spleen, liver and kidneys, are illustrated in Fig 4. Noticeable differences were detected among the experimental groups.

Spleen weight increased significantly in treatment group 1 (T1), reaching 1.03 g compared with 0.67 g in the control animals (C) ($p = 0.001$). Conversely, animals in treatment group 2 (T2) exhibited a marked reduction in spleen weight to 0.47 g, which was significantly lower than both the control group ($p = 0.01$) and T1 ($p < 0.0001$).

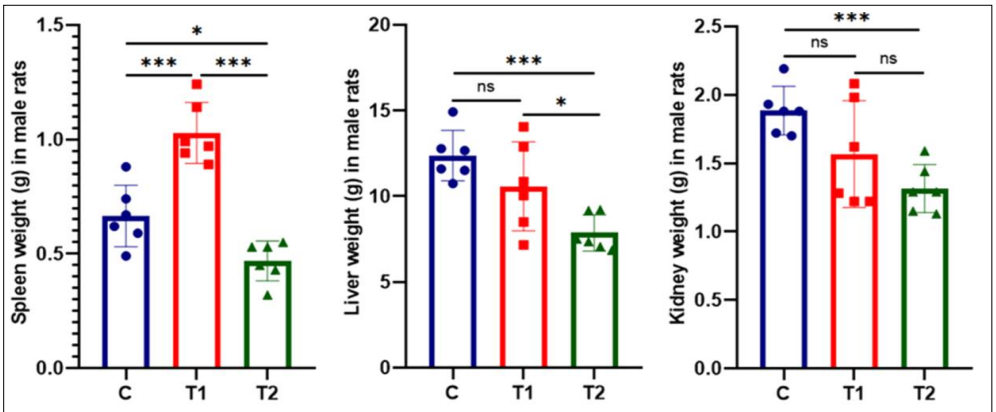
A significant decline in liver weight was also observed in T2, where the mean value reached 7.86 g compared with 12.37 g in the control group ($p = 0.0001$) and 10.58 g in T1 ($p = 0.04$). However, liver weights did not differ significantly between the control and T1 groups ($p = 0.20$). Kidney weight showed a similar decreasing tendency in the treated animals. Rats in T2 displayed significantly lower kidney weight (1.32 g) than the control group (1.88 g; $p = 0.0002$). In contrast, no statistically significant differences were identified between C and T1 ($p = 0.10$) or between T1 and T2 ($p = 0.20$).

Collectively, the results suggest that T1 treatment primarily affected spleen weight, whereas T2 treatment produced more pronounced reductions in spleen, liver and kidney weights. Meanwhile, liver and kidney measurements in T1 remained generally comparable to those recorded in the control animals.



Serum testosterone levels were evaluated in control (C), large-animal feed (T1) and raisin-supplemented diet (T2) groups. Data are presented as mean ± SEM ($n = 6$). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ was considered statistically significant, whereas ns indicates non-significant differences.

Fig 3: Effects of dietary treatments on serum testosterone concentrations in male Wistar rats.



Relative organ weights of spleen, liver and kidneys in control (C), large-animal feed (T1) and raisin-supplemented diet (T2) groups. Data are presented as mean ± SEM ($n = 6$). Statistical comparisons were analyzed using one-way ANOVA followed by Tukey's post hoc test. $p < 0.05$ and $^{**}p < 0.001$ were considered statistically significant, whereas ns indicates non-significant differences.

Fig 4: Effects of dietary treatments on vital organ weights (spleen, liver and kidneys) in male Wistar rats.

Effects of dietary treatments on reproductive organ weights

The relative weights of reproductive organs, including testes, epididymis, vas deferens, prostate gland and seminal vesicles, are presented in Fig 5.

Testicular weight showed a moderate decline in treatment group 2 (T2), with a mean value of 2.56 g compared with 2.84 g in the control group (C). Statistical analysis demonstrated a significant difference between the control and T2 groups ($p = 0.02$), whereas the remaining group comparisons did not reveal statistically significant variation.

No significant differences were detected in epididymis weight among the experimental groups. In contrast, vas deferens weight was significantly lower in both treatment groups compared with the control animals (C vs T1: $p = 0.0001$; C vs T2: $p = 0.004$). However, no significant difference was observed between T1 and T2.

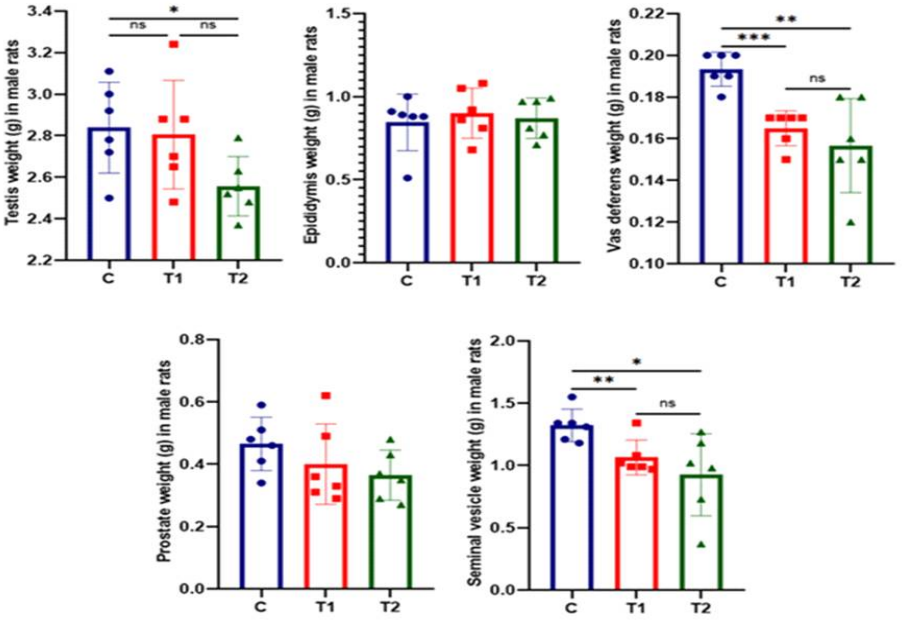
Prostate gland weight remained relatively constant across all experimental groups, with no statistically meaningful differences detected. Conversely, seminal vesicle weight was significantly reduced in both T1 ($p = 0.01$) and T2 ($p = 0.02$) compared with the control group. Comparisons between the two treatment groups did not demonstrate statistical significance.

Liver histology

Histological examination of liver sections from all experimental groups (C, T1 and T2) revealed generally preserved hepatic architecture (Fig 6). In the control group, the liver exhibited normal structural organization, with well-arranged hepatocytes radiating from the central vein, intact sinusoidal spaces and normal vascular structures. Similarly, the T1 group showed a comparable histological appearance, with preserved hepatic cords, normal hepatocellular morphology and no evident signs of necrosis or cellular degeneration. Mild vascular congestion was occasionally observed but without structural disruption. In the T2 group, liver sections also demonstrated intact hepatic architecture, with well-defined hepatocytes and maintained sinusoidal arrangement. No obvious pathological alterations such as necrosis, inflammatory infiltration, or severe cellular degeneration were detected.

Kidney histology

Histological examination of kidney sections from all experimental groups (C, T1 and T2) demonstrated generally preserved renal architecture (Fig 7). In the control group, normal renal histology was observed, with well-defined glomeruli and intact tubular structures. The T1 group exhibited a similar appearance, with preserved renal corpuscles and tubular organization, although mild vascular congestion was occasionally noted. In the T2 group, kidney sections also showed intact structural integrity, with normal glomerular morphology and well-arranged renal tubules. Mild tubular dilation and slight vascular congestion were observed in some areas, but no evidence of necrosis,



Relative weights of testes, epididymis, vas deferens, prostate gland and seminal vesicles in control (C), large-animal feed (T1) and raisin-supplemented diet (T2) groups. Data are presented as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc multiple comparison test. Statistical significance was considered at $p<0.05$, *$p<0.01$ and **$p<0.001$, whereas ns indicates non-significant differences.

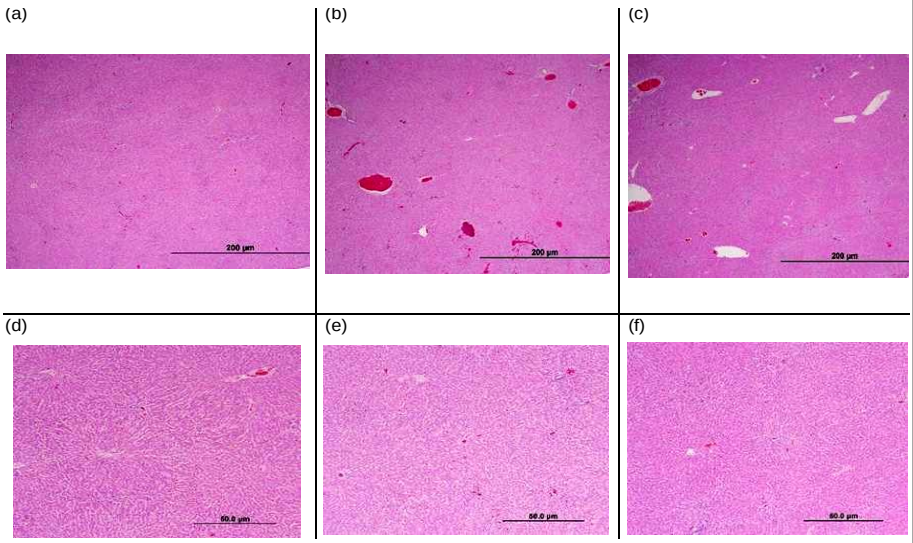
Fig 5: Effects of dietary treatments on reproductive organ weights in male Wistar rats.

inflammatory infiltration, or severe pathological alterations was detected.

Histopathological examination of testicular tissue

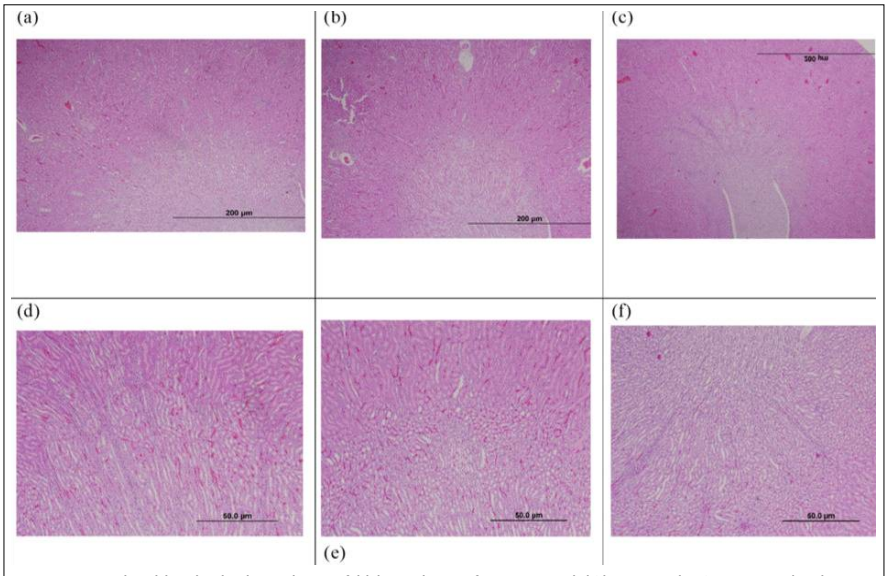
Histological examination of testicular sections revealed normal architecture in all experimental groups. The seminiferous tubules were well-organized with intact basement membranes and normal interstitial spaces. At higher magnification, all groups exhibited complete spermatogenic series, including spermatogonia, spermatocytes, spermatids and spermatozoa within the tubular lumen. No evidence of degeneration, necrosis, or disruption of spermatogenesis was observed in either treated groups compared to the control (Fig 8).

The findings of the present study are consistent with our previous investigations on both male and female rats.



Representative histological sections of liver tissue from control, T1 and T2 groups. (a-c) Low magnification (×100; scale bar = 200 µm). (d-f) High magnification (×400; scale bar = 50 µm). (a, d) Control group; (b, e) T1 group; (c, f) T2 group. All groups showed preserved hepatic architecture with normal hepatocyte arrangement and intact sinusoidal spaces.

Fig 6: Representative histological sections of liver tissue in control and treated groups.

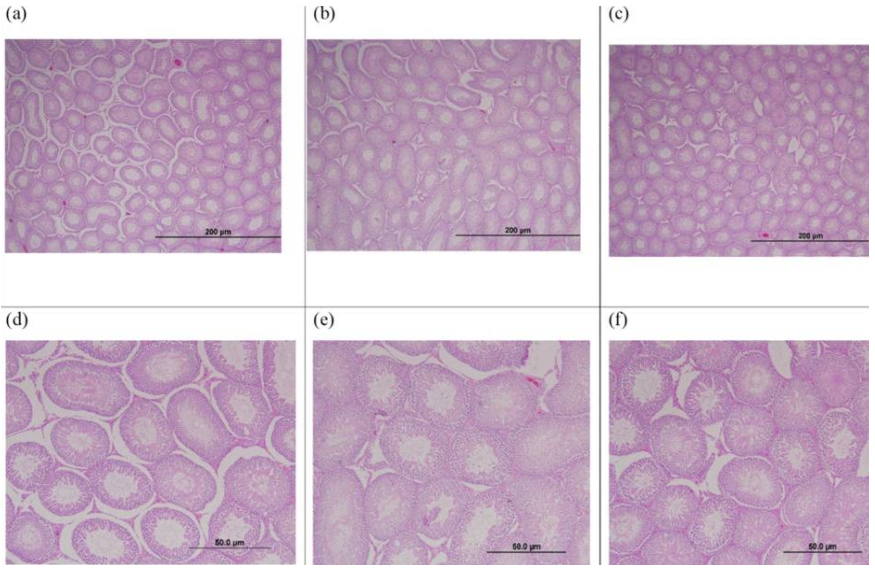


Representative histological sections of kidney tissue from control (C), T1 and T2 groups. (a-c) Low magnification (×100; scale bar = 200 µm). (d-f) High magnification (×400; scale bar = 50 µm). All groups exhibited preserved renal architecture with normal glomeruli and tubular structures. Mild vascular congestion and slight tubular dilation were observed in treated groups.

Fig 7: Representative histological sections of Kidney tissue in control and treated groups.

In our earlier work (Amran *e al.*, 2025), the use of large animal feed supplemented with raisins was associated with maintained growth performance and improved reproductive parameters in male rats, without evidence of physiological impairment. Likewise, our subsequent study (Amran *e al.*, 2026) demonstrated that raisin-supplemented diets did not adversely affect reproductive outcomes or offspring development in female rats, further supporting the safety of this feeding strategy.

In agreement with these results, our study provides additional evidence supporting the safety and physiological compatibility of the dietary intervention under the experimental conditions used in our study. The absence of significant histopathological alterations in the liver, kidneys and testes, together with the maintenance of normal organ architecture and physiological parameters, reinforces the conclusion that raisin supplementation, when combined with large animal feed, represents a safe and biologically compatible dietary alternative. Although AST activity was significantly elevated in the T2 group, this isolated finding should be interpreted in the context of the overall dataset. ALT activity was significantly reduced, ALP remained unchanged and histopathological examination revealed preserved hepatic architecture without necrosis or inflammatory infiltration. Collectively, these results suggest that the observed AST elevation is unlikely to reflect clinically relevant hepatocellular injury and may instead represent a non-specific physiological response. Although significant reductions in liver and kidney weights were observed in the T2 group, these findings should be interpreted alongside the biochemical and histopathological results. The absence of structural abnormalities in both organs, together with preserved biochemical profiles, suggests that the observed reductions in organ weight are unlikely to reflect tissue damage or toxicity. These changes may reflect physiological adaptation or differences in nutrient intake associated with the dietary intervention. However, the possibility that differences in hydration status contributed to the observed changes cannot be excluded. In addition, the significant reduction in serum creatinine observed in the T2 group was consistent with the preserved renal histological architecture observed in the treated animals. These findings may be related to the combined action of several bioactive constituents naturally present in raisins, including polyphenols, flavonoids, vitamins, minerals and dietary fiber, which have been reported to contribute to antioxidant activity and physiological homeostasis. Although previous studies have demonstrated that raisin polyphenols may activate antioxidant pathways such as Sirt1-Nrf2 signaling, these mechanisms were not directly investigated in the present study. Therefore, the proposed antioxidant effects should be interpreted as plausible explanations based on existing evidence rather than experimentally confirmed mechanisms. Our results are further supported by Ali *et al.* (2019), who demonstrated that dark raisins exert renoprotective effects by preserving renal tubular structure and reducing histopathological alterations, consistent with the improved kidney function observed in our study. Importantly, clinical evidence also supports the antioxidant efficacy of raisin-based interventions. Shishehbor *et al.* (2022) reported that raisin supplementation significantly reduced serum malondialdehyde



Representative histological sections of testicular tissue from control (C), T1 and T2 groups. (a-c) Low magnification (×100; scale bar = 200 µm) showing well-organized seminiferous tubules with regular distribution. (d-f) High magnification (×400; scale bar = 50 µm) demonstrating intact seminiferous tubules with complete spermatogenic series and normal interstitial tissue in all groups.

Fig 8: Histopathological assessment of testicular tissue in control, T1 and T2 groups.

levels, a key marker of oxidative stress, while enhancing total antioxidant capacity in hyperlipidemic patients. Together, these previously published results provide biological context for the observations reported in our study and support the plausibility of antioxidant-mediated protective effects, although such mechanisms were not directly evaluated in the current investigation. This reduction in oxidative stress may explain the preservation of tissue structure and the improvement in biochemical parameters observed in our study. Furthermore, oxidative stress is a well-established contributor to male reproductive dysfunction, affecting spermatogenesis and hormonal regulation. In this context, Bouhadana *et al.* (2025) highlighted the critical role of antioxidants in improving male fertility by reducing oxidative damage, enhancing sperm quality and supporting endocrine function. The preserved testicular architecture and normal spermatogenic progression observed in our study are in line with these results, suggesting that the antioxidant components of raisins may contribute to maintaining reproductive tissue integrity. Although testosterone levels showed variable responses among treatment groups. Although reductions in the weights of selected reproductive organs were observed in the T2 group, these changes were not accompanied by histopathological abnormalities or disruption of spermatogenesis. Furthermore, serum testosterone concentrations in T2 remained comparable with those of the control group. Collectively, our results suggest that the observed reductions in organ weights are unlikely to indicate impaired reproductive function but may instead reflect physiological adaptation to the dietary intervention. Nevertheless, further studies investigating reproductive endocrine regulation at the molecular level would help clarify the underlying mechanisms.This study has several limitations that should be acknowledged. The relatively small sample size and the four-week experimental period may limit the generalizability of the findings. In addition, oxidative stress biomarkers, inflammatory cytokines and molecular pathways were not directly assessed, limiting the ability to establish the mechanisms underlying the observed biochemical and histological findings. Future studies involving larger sample sizes, longer experimental periods and the assessment of oxidative stress, inflammatory and molecular markers would provide further insight into the biological effects and potential mechanisms of raisin-supplemented large-animal feed.

CONCLUSION

Our study demonstrates that feeding male Wistar rats with large-animal feed, with or without raisin supplementation, was not associated with evident histopathological damage in the examined tissues under the experimental conditions of this study. Raisin supplementation was associated with lower serum concentrations of blood urea nitrogen, uric acid and creatinine. Although AST activity increased in the T2 group, ALT activity decreased, ALP remained unchanged and histopathological examination revealed preserved liver architecture without evidence of tissue injury. In addition, normal testicular structure and spermatogenesis were maintained, indicating no histological evidence of adverse effects on male reproductive function despite variations in testosterone levels and reproductive organ weights. The findings suggest that raisin supplementation may contribute to maintaining physiological homeostasis, although the underlying molecular mechanisms remain unclear and require further investigation. Our study has several limitations that should be acknowledged. The relatively small sample size and the four-week experimental period may limit the generalizability of the findings. In addition, oxidative stress biomarkers, inflammatory cytokines and molecular pathways were not directly assessed. Therefore, larger studies with longer treatment durations and additional biochemical and molecular analyses are warranted to further clarify the biological effects and underlying mechanisms of raisin-supplemented large-animal feed.

Ethics

All experimental procedures involving animals were conducted in accordance with the ethical and biosafety regulations of the King Saud University Ethics Committee and were performed in compliance with the relevant institutional guidelines. The study was approved by the King Saud University Ethics Committee under approval No. KSU-SE-25-45.

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Conflict of interest

The authors confirm that no conflicts of interest exist in relation to this study or its publication. The research was conducted independently without any financial or commercial influence affecting the experimental design, data acquisition, interpretation of results, or manuscript preparation.

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