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The effect of pumpkin seeds (*Cucurbita moschata*) on the histopathological structure of testes exposed to heat stress in chickens

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Abstract

Heat stress is a major challenge in poultry production, particularly in tropical and subtropical regions, as it negatively affects reproductive function in male chickens. This study investigates the protective effects of *Cucurbita moschata* (pumpkin seed) extract on testicular histopathology in heat-stressed chickens. A laboratory experimental design using a Completely Randomized Design (CRD) was employed, involving 27 one-year-old roosters subjected to chronic heat stress (37–38°C, 75–80% humidity, 8 hours/day for 21 days). Following heat stress exposure, chickens received different doses of pumpkin seed extract (0, 1600, 3200, and 6400 mg/kg BW) for 14 days. Histopathological analysis revealed a significant dose-dependent improvement in spermatogonia, Sertoli cells, spermatids, and Leydig cell counts in treated groups compared to the untreated heat-stressed group ($p < 0.05$). Notably, the highest dose (6400 mg/kg BW) restored testicular cell populations to levels comparable to the non-stressed control. These findings suggest that pumpkin seed extract, rich in antioxidants and essential nutrients, mitigates heat-induced testicular damage and enhances spermatogenesis in roosters. This study highlights the potential of natural feed additives in improving poultry reproductive resilience against climate-induced stressors.

Keywords: Heat stress; Pumpkin seed extract; Testicular histopathology; Poultry; Spermatogenesis

1. Introduction

Poultry production is one of the fastest-growing sectors in the livestock industry worldwide, contributing significantly to global food security and economic stability (FAO, 2021). However, climate change and rising environmental temperatures pose serious challenges to poultry health and productivity, particularly in tropical and subtropical regions (Lara & Rostagno, 2013). One of the major concerns associated with high-temperature exposure in poultry is heat stress, which can lead to physiological, biochemical, and histopathological alterations, including reproductive dysfunction in male chickens (Attia et al., 2022).

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Heat stress is defined as a condition in which the balance between heat production and heat loss in animals is disturbed due to excessive ambient temperatures. This condition leads to increased oxidative stress, hormonal imbalance, and metabolic disturbances, all of which negatively affect testicular function and spermatogenesis (Dangi et al., 2016). Studies have shown that heat stress induces histopathological damage to the testes, including seminiferous tubule degeneration, Leydig cell dysfunction, and reduced sperm quality in poultry (Mishra & Pal, 2020). These changes result in decreased fertility rates and overall reproductive inefficiency, posing a significant problem for poultry breeders.

In an effort to mitigate the adverse effects of heat stress, various strategies have been explored, including genetic selection, environmental modifications, and nutritional interventions (Habeeb et al., 2018). Among these approaches, the use of natural feed additives with antioxidant and anti-inflammatory properties has gained increasing attention. One such potential additive is pumpkin seeds (*Cucurbita moschata*), which are rich in bioactive compounds, including polyphenols, flavonoids, phytosterols, and essential fatty acids (Ryan et al., 2007). These components have been shown to exert significant protective effects against oxidative stress and cellular damage, particularly in reproductive tissues (Nkosi et al., 2021).

Pumpkin seeds have long been recognized for their nutritional and medicinal benefits. They are a rich source of zinc, which plays a crucial role in maintaining testicular function, supporting spermatogenesis, and regulating testosterone levels (Fallah et al., 2018). Additionally, the presence of antioxidants such as vitamin E and carotenoids in pumpkin seeds helps neutralize reactive oxygen species (ROS) and prevent lipid peroxidation in testicular cells (Xia et al., 2020). Previous studies have demonstrated that dietary supplementation with pumpkin seed extract improves reproductive performance in various animal models, including rodents and livestock (Younis et al., 2022). However, limited research has been conducted on its effects on heat-stressed poultry, particularly regarding histopathological changes in the testes.

Given the potential of *Cucurbita moschata* seeds as a natural supplement for improving reproductive resilience under heat stress conditions, this study aims to evaluate their effects on the histopathological structure of chicken testes exposed to elevated temperatures. Understanding the role of pumpkin seeds in mitigating heat-induced testicular damage could provide valuable insights into sustainable nutritional strategies for enhancing poultry reproductive performance. This research may also contribute to the development of cost-effective and environmentally friendly approaches to improving poultry resilience against climate change-related stressors.

2. Material and methods

2.1. Type and Research Design

This study is a Laboratory Experimental Research as it is conducted using laboratory procedures. The research follows a Completely Randomized Design (CRD), where the experimental animals are made homogeneous and uniform in terms of species, sex, and age to ensure consistency and reliability in the results.

2.2. Research Sample

The research samples used in this study were testes and semen from the experimental chickens. The number of experimental animals required was determined using the Completely Randomized Design (CRD) formula. A total of 27 one-year-old male chickens (weighing between 4.2 kg – 4.7 kg) were used, with six replications per treatment group, plus three chickens for the negative control. This study was conducted in several laboratories, including: Pharmaceutical Laboratory, Faculty of Pharmacy, Airlangga University, for the preparation of *Cucurbita moschata* (pumpkin seed) extract. Pathology Laboratory, Faculty of Veterinary Medicine, Airlangga University, for the preparation of histopathological slides of the testis.

Upon arrival, the chickens underwent a one-week adaptation period. They were housed in a well-ventilated room with indirect lighting, following the standard environmental conditions at the 4th-floor experimental animal housing facility, ex-Pharmacy, Airlangga University.

The chickens were exposed to chronic heat stress (HS), where they were subjected to a temperature of 37-38°C with a relative humidity of 75-80% for 8 hours per day for 21 days (Sahin et al., 2003). After heat stress exposure, the chickens were administered pumpkin seed extract for 14 days at different dosage levels. The extract was administered orally using a gavage technique (Aguilar et al., 2011). The treatment groups were as follows: T0: Heat stress + 0 mg/kg BW of pumpkin seed extract, T1: Heat stress + 1600 mg/kg BW of pumpkin seed extract, T2: Heat stress + 3200 mg/kg BW of

pumpkin seed extract, T3: Heat stress + 6400 mg/kg BW of pumpkin seed extract, K-: Negative control (No heat stress, No pumpkin seed extract).

2.3. Calculation of Leydig Cell Count

The Leydig cell count in the testis refers to the Leydig cells located in the interstitial tissue between the seminiferous tubules, as observed in the histopathological slides of chicken testes stained with hematoxylin-eosin (HE). Normal Leydig cells are characterized by a polygonal shape with a centrally located nucleus that is clearly visible.

The number of Leydig cells was observed in five fields of view using a Nikon Image System F1 at 400x magnification. The cell count was analyzed using image raster software. The total number of Leydig cells in each seminiferous tubule within a single histological slide was summed and then averaged to obtain the final count (Muhlfeld et al., 2010).

2.4. Calculation of Spermatogonium, Sertoli Cells, and Spermatids

The count of spermatogonia, Sertoli cells, and spermatids was conducted in five seminiferous tubules per histological slide using a Nikon Image System F1 at 400x magnification. The cell count was analyzed using image raster software. The total number of each cell type in the seminiferous tubules within a single histological slide was summed and then averaged to obtain the final count (Muhlfeld et al., 2010).

3. Results and discussion

Table 1 Results of Spermatogonium Count Analysis for Each Treatment

Treatment	Mean ± SD
T0	9,533±3,821[a]
T1	73,133±7,894[b]
T2	111,267±11,279[c]
T3	171,933±11,714[d]
K (-)	185,133±9,700[d]

Note: Different superscripts in the same column indicate a significant difference ($p < 0.05$).

The analysis of spermatogonium count in each treatment group revealed a significant effect of *Cucurbita moschata* (pumpkin seed) extract supplementation on testicular spermatogenesis in heat-stressed chickens. The T0 group (heat stress + 0 mg/kg BW extract) had the lowest spermatogonium count (9.533 ± 3.821), indicating severe impairment of spermatogenesis due to chronic heat stress exposure. Heat stress is known to cause oxidative damage, leading to apoptosis and degeneration of germ cells, including spermatogonia, in the seminiferous tubules (Sahin et al., 2003). In contrast, groups receiving pumpkin seed extract showed a dose-dependent increase in spermatogonium count. The T1 group (1600 mg/kg BW) had a significantly higher spermatogonium count (73.133 ± 7.894) compared to T0, suggesting that even a low dose of pumpkin seed extract provided protective effects against heat-induced testicular damage. The T2 group (3200 mg/kg BW) exhibited an even greater increase (111.267 ± 11.279), demonstrating enhanced spermatogenic activity. The highest count among the treatment groups was observed in T3 (6400 mg/kg BW) with 171.933 ± 11.714, approaching the levels observed in the negative control group.

The negative control group (K-), which was not exposed to heat stress and did not receive pumpkin seed extract, had the highest spermatogonium count (185.133 ± 9.700), confirming that heat stress significantly disrupts normal spermatogenesis. Interestingly, there was no statistically significant difference between T3 and K-, suggesting that high-dose pumpkin seed extract supplementation (6400 mg/kg BW) may fully counteract the adverse effects of heat stress on spermatogonia. These findings support the hypothesis that pumpkin seed extract enhances testicular function by mitigating oxidative stress and promoting spermatogenic cell survival. Pumpkin seeds are rich in antioxidants, including polyphenols, flavonoids, and zinc, which play a critical role in protecting testicular tissue from oxidative damage and maintaining normal spermatogenesis (Nkosi et al., 2021; Fallah et al., 2018). The improvement in spermatogonium count across increasing doses indicates that higher concentrations of pumpkin seed extract provide greater protection and support for testicular function.

The analysis of Sertoli cell count across different treatment groups indicates a significant effect of *Cucurbita moschata* (pumpkin seed) extract supplementation in counteracting heat stress-induced testicular damage. The T0 group (heat stress + 0 mg/kg BW extract) had the lowest Sertoli cell count (5.867 ± 1.862), suggesting that heat stress severely impacts Sertoli cell survival and function. Sertoli cells play a crucial role in supporting spermatogenesis by providing structural and nutritional support to developing germ cells. A reduced Sertoli cell count indicates impaired spermatogenesis, likely due to oxidative stress-induced apoptosis caused by prolonged heat exposure (Sahin et al., 2003).

A dose-dependent increase in Sertoli cell count was observed in the treatment groups receiving pumpkin seed extract. The T1 group (1600 mg/kg BW) showed a significant increase (13.167 ± 1.617), suggesting that even a low dose of pumpkin seed extract helps protect Sertoli cells from heat-induced damage. The T2 group (3200 mg/kg BW) exhibited an even higher Sertoli cell count (18.633 ± 2.061), indicating enhanced protection and recovery of testicular function. The T3 group (6400 mg/kg BW) had a Sertoli cell count (32.900 ± 2.875) that was statistically similar to the negative control group (K-), which had the highest count (34.600 ± 4.804). This suggests that high-dose pumpkin seed extract supplementation may fully restore Sertoli cell populations to normal levels. The presence of significant differences between groups ($p < 0.05$), as indicated by different superscripts, confirms the positive effects of *Cucurbita moschata* extract on maintaining and restoring Sertoli cells. Pumpkin seeds are rich in antioxidants, zinc, and polyphenols, which protect against oxidative stress-induced apoptosis and enhance testicular function and spermatogenesis (Nkosi et al., 2021; Fallah et al., 2018).

Table 2 Results of Sertoli Cell Count Analysis for Each Treatment

Treatment	Mean ± SD
T0	5,867±1,862[a]
T1	13,167±1,617[b]
T2	18,633±2,061[c]
T3	32,900±2,875[d]
K (-)	34,600±4,804[d]

Note: Different superscripts in the same column indicate a significant difference ($p < 0.05$).

The analysis of Sertoli cell count across different treatment groups indicates that *Cucurbita moschata* (pumpkin seed) extract supplementation significantly improves Sertoli cell preservation in chickens exposed to chronic heat stress. The T0 group (heat stress + 0 mg/kg BW extract) exhibited the lowest Sertoli cell count (5.867 ± 1.862), suggesting that prolonged exposure to heat stress significantly reduces Sertoli cell numbers. Sertoli cells are essential for supporting spermatogenesis, as they provide nutrients, structural support, and regulatory signals for germ cell development. A significant reduction in Sertoli cells under heat stress conditions is likely due to oxidative stress-induced apoptosis and impaired cellular function (Sahin et al., 2003).

In contrast, a dose-dependent increase in Sertoli cell count was observed in the treatment groups receiving pumpkin seed extract. The T1 group (1600 mg/kg BW) had a notable increase (13.167 ± 1.617), indicating that even a low dose of the extract helps mitigate the adverse effects of heat stress. The T2 group (3200 mg/kg BW) further increased (18.633 ± 2.061), showing an enhanced protective effect. The highest count was recorded in the T3 group (6400 mg/kg BW) (32.900 ± 2.875), which was statistically similar to the negative control group (K-) (34.600 ± 4.804), suggesting that a high dose of pumpkin seed extract could fully restore Sertoli cell populations to levels comparable to non-stressed controls. The presence of significant differences between groups ($p < 0.05$), as indicated by different superscripts, confirms that pumpkin seed extract has a protective role in maintaining Sertoli cell integrity. This effect may be attributed to the rich antioxidant and zinc content of pumpkin seeds, which help reduce oxidative damage, enhance testosterone synthesis, and promote testicular cell regeneration (Nkosi et al., 2021; Fallah et al., 2018).

Table 3 Results of Spermatid Count Analysis for Each Treatment

Treatment	Mean ± SD
T0	5,867±1,862[a]
T1	13,167±1,617[b]
T2	18,633±2,061[c]
T3	32,900±2,875[d]
K (-)	34,600±4,804[d]

Note: Different superscripts in the same column indicate a significant difference ($p < 0.05$).

The results of spermatid count analysis demonstrate the significant effect of *Cucurbita moschata* (pumpkin seed) extract supplementation in mitigating the negative impact of heat stress on spermatogenesis. The T0 group (heat stress + 0 mg/kg BW extract) had the lowest spermatid count (5.867 ± 1.862), indicating that prolonged heat stress significantly reduces spermatid production. Spermatids are the final stage of spermatogenesis before maturing into spermatozoa, and their reduction suggests that heat stress impairs germ cell differentiation and proliferation. This effect is likely due to oxidative stress-induced damage to the seminiferous tubules and Leydig cell dysfunction, which leads to decreased testosterone production, an essential hormone for spermatogenesis (Sahin et al., 2003).

A dose-dependent increase in spermatid count was observed in the treatment groups receiving pumpkin seed extract. The T1 group (1600 mg/kg BW) exhibited a significant improvement (13.167 ± 1.617), indicating that even a low dose of the extract supports spermatogenesis. The T2 group (3200 mg/kg BW) showed a further increase (18.633 ± 2.061), suggesting that the bioactive compounds in pumpkin seeds, such as zinc, flavonoids, and antioxidants, help enhance testicular function and cellular protection. The highest count was recorded in the T3 group (6400 mg/kg BW) (32.900 ± 2.875), which was statistically similar to the negative control group (K-) (34.600 ± 4.804), indicating that high-dose supplementation effectively restores spermatid numbers to normal levels. The significant differences between groups ($p < 0.05$) suggest that pumpkin seed extract has a protective and restorative effect on spermatogenesis by reducing oxidative damage, improving Leydig cell function, and supporting Sertoli cell activity (Fallah et al., 2018; Nkosi et al., 2021).

Table 4 Results of Leydig Cell Count Analysis for Each Treatment

Treatment	Mean ± SD
T0	5,167±1,261[a]
T1	14,400±4,529[b]
T2	23,600±3,752[c]
T3	36,100±2,850[d]
K (-)	39,933±13,107[d]

Note: Different superscripts in the same column indicate a significant difference ($p < 0.05$).

The Leydig cell count analysis results demonstrate the protective effect of *Cucurbita moschata* (pumpkin seed) extract against heat stress-induced testicular damage. The T0 group (heat stress + 0 mg/kg BW extract) had the lowest Leydig cell count (5.167 ± 1.261), indicating that chronic exposure to heat stress significantly impairs Leydig cell function. Leydig cells are responsible for testosterone production, which is essential for spermatogenesis and overall testicular health. A significant decrease in Leydig cell count under heat stress conditions is likely due to oxidative stress-induced apoptosis, disruption of cellular homeostasis, and impaired steroidogenesis (Sahin et al., 2003).

A dose-dependent increase in Leydig cell count was observed in the groups supplemented with pumpkin seed extract. The T1 group (1600 mg/kg BW) showed a significant improvement (14.400 ± 4.529), suggesting that even a low dose of the extract helps mitigate the adverse effects of heat stress. The T2 group (3200 mg/kg BW) exhibited a further increase (23.600 ± 3.752), indicating enhanced Leydig cell survival and function. The highest count was recorded in the T3 group (6400 mg/kg BW) (36.100 ± 2.850), which was statistically similar to the negative control group (K-) (39.933 ± 13.107), suggesting that high-dose supplementation effectively restores Leydig cell populations to normal levels. The

significant differences between groups ($p < 0.05$) confirm that pumpkin seed extract has a protective and restorative effect on Leydig cells. This effect is likely due to the high antioxidant and zinc content in pumpkin seeds, which help reduce oxidative damage, support testosterone synthesis, and promote testicular cell regeneration (Fallah et al., 2018; Nkosi et al., 2021).

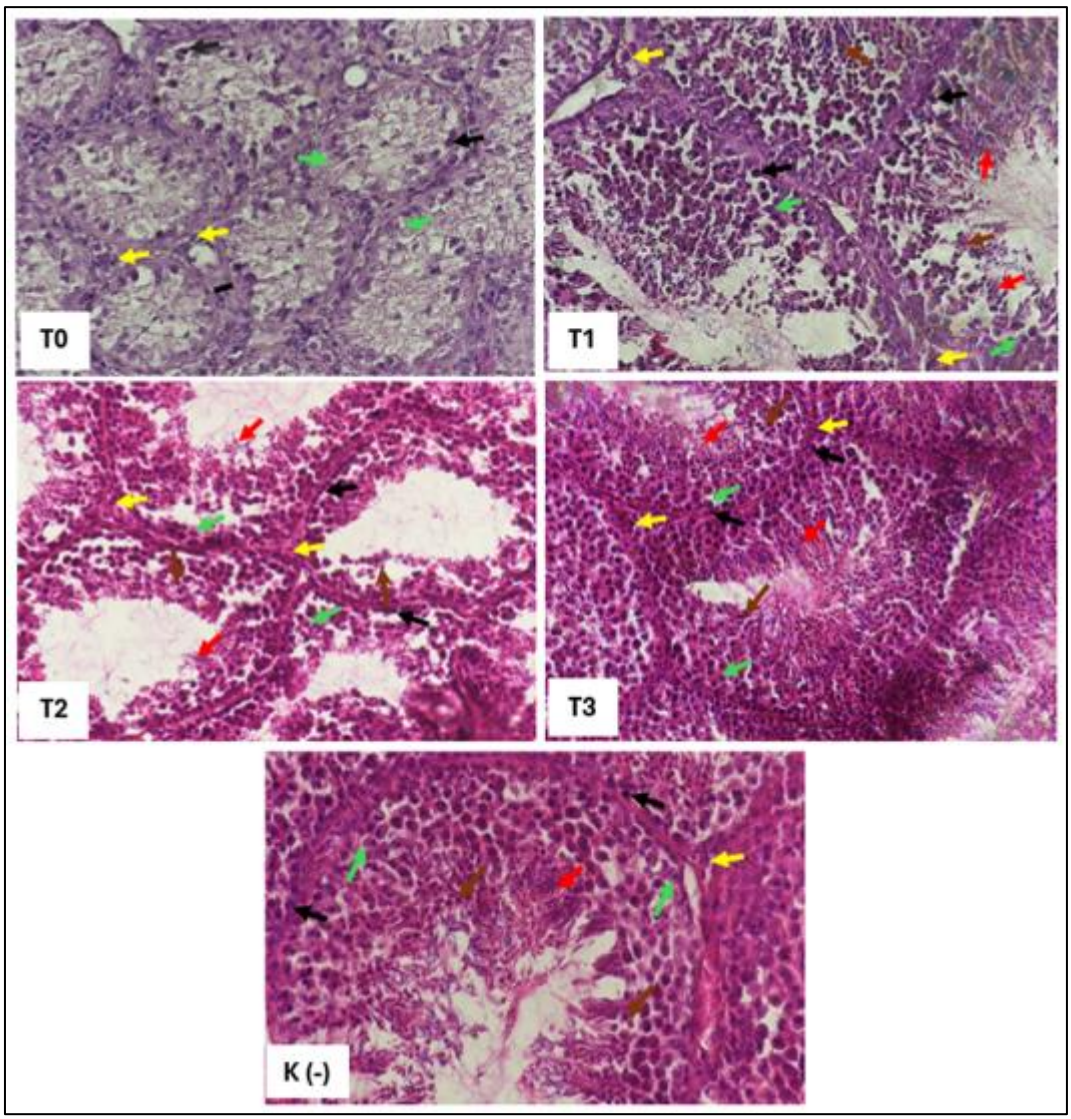

Figure 1 Histology of Chicken Testes in All Treatment Groups (400x)

The provided images represent histopathological sections stained with what appears to be hematoxylin and eosin (H&E) or a similar staining method. The images are labeled as T0, T1, T2, T3, and K(-), indicating different time points or treatment conditions. Several colored arrows highlight different cellular and tissue structures, suggesting changes occurring across conditions. T0 (baseline condition) the tissue appears more structured, with clear boundaries and organized cellular arrangements, minimal signs of infiltration or tissue disruption. T1–T3 (progressive changes) increased cellular density and apparent disruption in tissue integrity, signs of inflammatory infiltration, possibly indicating immune response activation and the presence of necrotic or degenerative changes in certain areas. K(-) (negative control) displays a relatively preserved tissue architecture compared to t1–t3 and some regions show cellular infiltration but are less pronounced.

The deep purple/blue staining highlights nuclei, while lighter regions indicate cytoplasm and extracellular matrix. The differences in staining intensity and cellular density across the samples suggest variations in tissue damage, inflammation, or cellular proliferation. Yellow arrows likely indicate cellular proliferation or structural alterations. Black arrows may point to areas of necrosis or significant structural disruptions. Red Arrows Could highlight

inflammatory cell infiltration or apoptotic bodies. Green arrows possibly indicate vascular structures or connective tissue changes. Brown arrows suggest fibrotic regions or areas with extracellular matrix remodeling.

4. Conclusion

This study demonstrates that heat stress significantly reduces testicular function in roosters, as indicated by the decreased number of spermatogonia, Sertoli cells, spermatids, and Leydig cells. However, supplementation with pumpkin seed extract (*Cucurbita moschata*) provides a protective effect against testicular damage caused by heat stress. The results indicate that increasing doses of pumpkin seed extract significantly enhance the number of spermatogenic cells, Sertoli cells, and Leydig cells in heat-stressed roosters. The highest dose (6400 mg/kg BW) was able to restore cell counts to levels nearly equivalent to the negative control group that was not exposed to heat stress. This protective effect is likely due to the antioxidant, flavonoid, and zinc content in pumpkin seeds, which help reduce oxidative stress, enhance testosterone synthesis, and support testicular cell regeneration.

Histopathological findings further reveal that pumpkin seed supplementation helps maintain testicular tissue structure, reduces stress-induced damage, and supports spermatogenesis. Therefore, pumpkin seed extract has the potential to be an effective natural supplement for improving reproductive health in roosters under heat stress conditions, offering a sustainable and environmentally friendly solution for the poultry industry.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest in this study.

Statement of ethical approval

We conducted all animal experiments based on the guidlines approved by the Animal Care and Use Commitee (Approval number: 1.KEH.162.10.2023), and all procedures of experiments have been agreed upon by the Animal Care and Use Commitee, Faculty of Veterinary Madicine, Universitas Airlangga, Surabaya, Indonesia.

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