Effect of *Urtica Dioica* and *Camellia Sinensis* on Rooster Reproductive and Testicular Traits

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Article History:
Received: 20.08.2025 *Revised: 22.09.2025* *Accepted: 24.09.2025* *Published: 25.09.2025*

Abstract

This study aimed to investigate the effects of varying dosages of *Urtica dioica* (nettle) leaf powder and *Camellia sinensis* (white tea) powder on the reproductive and histological characteristics of rooster testes. Conducted at the College of Agriculture, Al-Qasim Green University, over a 42-day period, the experiment involved 21 roosters at 45 weeks of age, which were randomly assigned to seven treatment groups with three replicates each. The treatment groups included: a standard diet without any additives as the control; a standard diet supplemented with 100 mg/kg or 150 mg/kg of white tea powder; a standard diet supplemented with 100 mg/kg, 150 mg/kg, or 200 mg/kg of nettle leaf powder. The results demonstrated significant improvements in sperm concentration, osmotic resistance, ejaculate volume, and individual sperm motility in certain groups receiving white tea and nettle leaf powders.

Keywords: *Andrology, Antioxidants, Endocrinology, Testosterone, Fertility.*

Suggested citation: Ali, E.A., Al-Hassani, A.S., & Ali, N. A.-L. (2025). Effect of *Urtica Dioica* and *Camellia Sinensis* on Rooster Reproductive and Testicular Traits. E*uropean Journal of Theoretical and Applied Sciences, 3*(5), 128-137. https://doi.org/10.59324/ejtas.2025.3(5).14

Introduction

Histological changes during certain seasons cause many wild bird species to undergo testicular regression, leading to a complete cessation of sperm production (Abbas, et al., 2019). In contrast, domestic birds have been selectively bred for traits such as meat production, and their testicular development is limited primarily by factors such as age and illness, rather than environmental influences (Wang, & Ho, 2009). Since the number of fertilized eggs directly impacts the profitability of laying hens, the primary objective in poultry breeding is the production of fertilized eggs. Reduced fertility in broiler breeders, particularly after 50 weeks of age, results in significant economic losses in poultry production (Romero-Sánchez, et al., 2008). Studies on aging domestic male birds have revealed a substantial decline in gonadotropic secretions and a reduction in the anterior pituitary gland's production and release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Abed, & Abdul-Lateef Ali, 2022; Adnan, Ahmad, & Ahmed, 2013; Al-Hassani, & Al-Suhail, 2017; Koch, 2001; Rahim, et al., 2013).

The utilization of naturally occurring plants rich in bioactive compounds has garnered interest for

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improving reproductive traits in poultry. Among these, nettle (*Urtica dioica*), a member of the Urticaceae family, is particularly noteworthy due to its diverse phytochemical profile, including polysaccharides, terpenoids, coumarins, and phenylpropanoids (Adnan, Ahmad, & Ahmed, 2013; Yesilbag, & Colpan, 2006). Nettle has been shown to stimulate T lymphocytes, protect liver tissue from damage (Yener, et al., 2009), and act as a potent antioxidant (Costa, Gouveia, & Nobrega, 2002). Studies have also confirmed its ability to inhibit the conversion of testosterone into estrogen (Al-Rubaiey, & Al-Obaidi, 2010). Nettle and its extracts are considered among the safest natural remedies, with a wide range of therapeutic applications and no significant adverse effects (Cīrule, et al., 2017). Additionally, nettle roots contain bioactive compounds such as lectins (potent antivirals), scopoline, sterols, fatty acids, polysaccharides, and isolectins (H., & Al-Hassani, 2022).

White tea, derived from the youngest leaves of the *Camellia sinensis* plant before they fully mature, is a rare and valuable type of tea. The leaves are carefully harvested and allowed to dry naturally, preserving their bioactive compounds. White tea has been recognized for its ability to cool the body, stimulate mental alertness, and provide various health benefits due to its rich composition of flavonoids, particularly catechins. It also contains fluoride and several potent antioxidants, including polyphenols, caffeine, methylxanthine, theobromine, and tannic acid, along with vitamins E and C (Chrubasik, et al., 2007; Ekayanti, et al., 2017; Koch, 2001). Furthermore, white tea is a source of manganese, which plays a critical role in protein digestion (Beski, 2019; Hajhashemi, & Klooshani, 2013; Yener, et al., 2009).

The aim of this study was to evaluate the effects of administering nettle (*Urtica dioica*) and white tea (*Camellia sinensis*) leaves on specific reproductive and histological characteristics of rooster testes. These plants were utilized to enhance the reproductive traits of roosters and broiler breeders, particularly to improve fertility (Duncan, 1955; Ezzat, 2023).

Materials and Methods

This study was conducted at the College of Agriculture, Al-Qasim Green University, from April 10 to May 22, 2018, for a duration of 42 days. The experiment aimed to evaluate the effects of *Camellia sinensis* (white tea) powder and *Urtica dioica* (nettle) leaf powder on specific reproductive and histological characteristics of the testes in 21 roosters aged 45 weeks. The birds were randomly assigned to seven treatments, with each treatment consisting of three replicates, and one rooster per replicate. The treatment groups were as follows:

the control group received a standard diet without any supplementation, while Treatment 2 was supplemented with 100 mg of white tea powder per kilogram of feed, Treatment 3 with 150 mg of white tea powder per kilogram of feed, and Treatment 4 with 200 mg of white tea powder per kilogram of feed. Similarly, Treatment 5 was supplemented with 100 mg of nettle leaf powder per kilogram of feed, Treatment 6 with 150 mg of nettle leaf powder per kilogram of feed, and Treatment 7 with 200 mg of nettle leaf powder per kilogram of feed. The feed composition was formulated according to the nutritional guidelines provided in the broiler breeder manual (Khishtan, & Beski, 2020).

The study focused on several reproductive and testicular characteristics, including ejaculate volume, sperm concentration, sperm motility, sperm resistance to salt, the percentage of dead sperm, and histological traits of the testes. Tables 1 and 2 show detailed information on the active compounds found in white tea and nettle. Throughout the study, the birds were exposed to a photoperiod of 14 hours of light per day.

Semen Collection and Analysis

Semen was collected from the roosters weekly over six weeks using the method described by Yener, et al. (2009). Microtubes were used for semen collection. Sperm motility was assessed by diluting a 10-microliter semen sample in sodium citrate solution (1:20 dilution). The sample was incubated at 37 °C, placed on a glass slide, covered with a slide cover, and examined under a light microscope equipped with a

camera. Individual sperm motility was evaluated at 40× magnification, while collective sperm motility was assessed at 10× magnification (Al-Salhi, 2020; Ezzat, et al., 2023).

Dead Sperm Estimation

A 10-microliter drop of eosin dye was mixed with an equal volume of semen. The mixture was smeared on a glass slide using another slide at a 45-degree angle. Dead and live sperm were differentiated under a microscope, and 200 sperm were counted per sample in duplicate (Jagadeeswaran Appusamy, 2014).

Sperm Concentration

Sperm concentration was determined using a hemocytometer according to standard procedures.

Histological Examination

Testicular tissues were processed and sectioned for histological examination following the methods described in Ekayanti, et al. (2017), Kuhn, et al. (2004) and Türkdoğan, et al. (2003).

Table 1. Assessment of Biochemical Components of White Tea

Biochemical Components	White tea (Methanolic)	White tea (Aqueous)
Total Polyphenols (%)	35±.01	29±.01
Catechins (%)	19±.01	13±.01
Total Flavonoids (gm/100gm)	5±.001	1±.001
Caffeine (gm/100gm)	4.8±.01	3.9±.01
Tannins (%w/w)	11±.001	9±.001

Source: Chrubasik, et al. (2007)

Table 2. Assessment of Biochemical Components of *Urtica dioica*

Biochemical Components	ml/Mg
Flavonoids	0.2993
Saponins	3.826
Phenols	.8160
Tannins	0.773
Steroids	0.5415
Turbines	0.7891

Source: SPSS (2008)

The effect of various treatments on the traits under investigation was analyzed using a completely randomized design (CRD). Significant differences among the means were determined using Duncan's multiple range test (Vizcarra, Kirby, & Kreider, 2010). All experimental data were statistically analyzed using the SPSS software package, as outlined in (Unachukwu, et al., 2010).

Results and Discussion

Table 3 presents the effects of varying dosages of white tea powder and nettle leaves on selected reproductive traits of broiler breeder roosters. The findings indicate that the fourth, fifth, sixth, and seventh treatments significantly outperformed the other experimental groups in terms of individual sperm motility, with highly significant differences observed ($p \leq 0.01$). However, there was no significant difference in individual sperm motility between the sixth treatment and the fourth, fifth, or seventh treatments. Additionally, no discernible difference was observed between the third and sixth treatments (SPSS, 2008).

Data in Table 3 shows that there was no significant difference in collective sperm motility between the fourth and fifth treatments or between the sixth and seventh treatments. Moreover, the third treatment did not differ significantly from the other experimental treatments. Notably, the fourth and fifth treatments demonstrated significant superiority ($p \leq 0.01$) over the other treatments in terms of

collective sperm motility. Regarding sperm mass migration, the sixth and seventh treatments were particularly noteworthy, demonstrating significantly better results compared to the control group and other treatments. In contrast, the control and second treatments showed significantly higher percentages of sperm death compared to the other experimental treatments ($p \leq 0.01$). However, all experimental treatments exhibited significant improvements in reducing sperm death rates compared to the control group ($p \leq 0.01$). For sperm resistance to salt, the third, fourth, fifth, and seventh treatments significantly outperformed the control group ($p \leq 0.05$). Meanwhile, the second and sixth treatments did not exhibit significant differences from each other. Regarding sperm concentration, the fourth treatment demonstrated significant superiority over the control group ($p \leq 0.05$), while it did not differ significantly from the second, fifth, sixth, or seventh treatments (Saffari, & Sadrzadeh, 2004; Spinaci, et al., 2018). The superiority of nettle leaf and white tea treatments in most semen characteristics can be attributed to their biologically active compounds with potent antioxidant properties, particularly flavonoids. These compounds prevent the formation of free radicals, thereby preserving the properties of semen and protecting sperm from damage and death. Consequently, these treatments significantly reduce the percentage of dead sperm and enhance overall semen quality (Mostafa, et al., 2019; Spinaci, et al., 2018; SPSS, 2008). In the second treatment, only 25% of the seminiferous tubules reached the stage of completion in the sperm biosynthesis process, as observed in the histological section presented in the figure.

The diameter of the seminiferous tubules was relatively smaller compared to the other treatments. The tubule walls exhibited normal integrity, without any signs of thickening or damage, and both Sertoli cells and spermatogenic cells appeared normal.

The process of spermatogenesis, which involves the formation of primary and secondary sperm cells, proceeded without abnormalities in this treatment (Gargi, et al., 2017; Giannenas, et al., 2003).

Table 3. The Effect of Adding Different Levels of White Tea Powder and Nettle Leaf on the Semen Characteristics of Broiler Mother's Roosters

Treat-ments	Sperms Conc. ($\times 10^9$)	Salt of Resistance (%)	Live Sperms (%)	Death Sperms (%)	Collective Movement (%)	Individual Movement (%)
t1	3.050 ± 0.39 B	73.025 ± 1.80 B	81.615 ± 1.29 C	18.380 ± 1.28 A	77.180 ± 0.52 C	74.970 ± 0.47 C
t2	3.435 ± 0.15 AB	76.915 ± 0.47 AB	85.220 ± 0.78 B	14.780 ± 0.77 B	78.165 ± 0.28 C	76.770 ± 1.10 C
t3	3.720 ± 0.39 AB	78.780 ± 0.46 A	90.210 ± 1.67 A	9.785 ± 1.66 C	80.045 ± 0.28 BC	77.045 ± 0.71 BC
t4	4.215 ± 0.22 A	80.075 ± 0.42 A	91.720 ± 0.62 A	8.280 ± 0.62 C	88.320 ± 0.44 A	81.655 ± 0.89 A
t5	3.550 ± 0.19 AB	79.020 ± 1.31 A	90.910 ± 1.02 A	9.090 ± 1.02 C	87.315 ± 0.32 A	80.270 ± 0.29 A
t6	2.945 ± 0.06 B	76.335 ± 1.78 AB	89.540 ± 0.90 A	10.460 ± 0.90 C	84.570 ± 3.83 AB	79.940 ± 1.61 AB
t7	3.530 ± 0.03 AB	79.275 ± 0.94 A	90.175 ± 0.62 A	9.825 ± 0.62 C	85.220 ± 0.34 AB	81.270 ± 0.40 A

Note: NS: Nonsignificant; *P≤0.05; ** P≤0.01

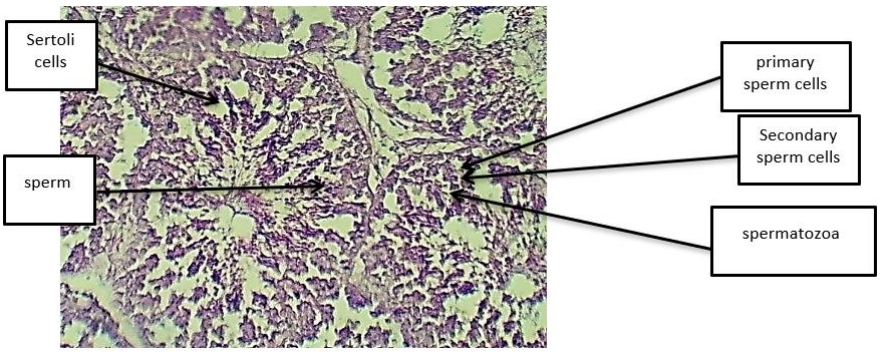

Figure 1. Shows a Histological Slice of the Testis Treated with T2

The slides reveals that 60% of the seminiferous tubules reached an advanced stage in the sperm synthesis process. The tubule diameter was larger compared to the second treatment, and the tubule walls remained intact, showing no signs of thickening or damage. Additionally, both Sertoli cells and Leydig cells appeared normal, indicating proper functioning and structural integrity (Jaiswal, & Lee, 2022; NRC, 1994).

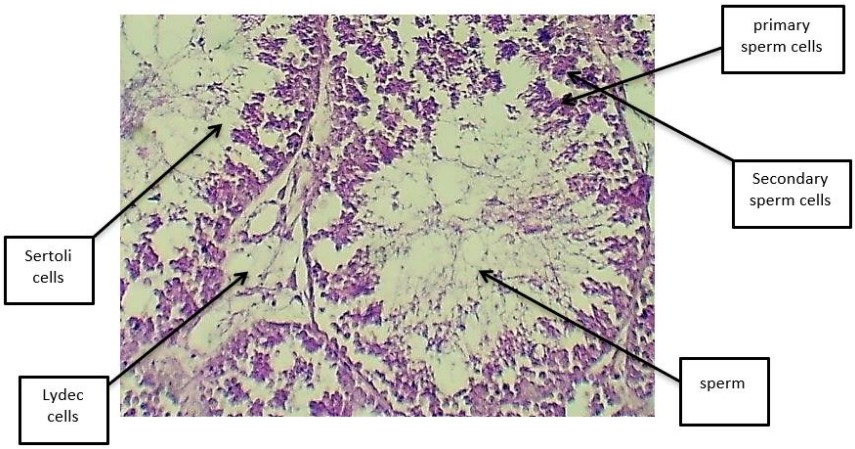

Figure 2. Histological Section of the T3-Treated Testicle

The histological section demonstrates that 60% of the seminiferous tubules have progressed to the stages of sperm production, with their diameters matching those observed in the control treatment (Chen, H., Yu, C., Wu, H., Li, G., Li, C., Hong, W., Yang, X., Wang, H., & You, X. (2022; NRC). (1994).

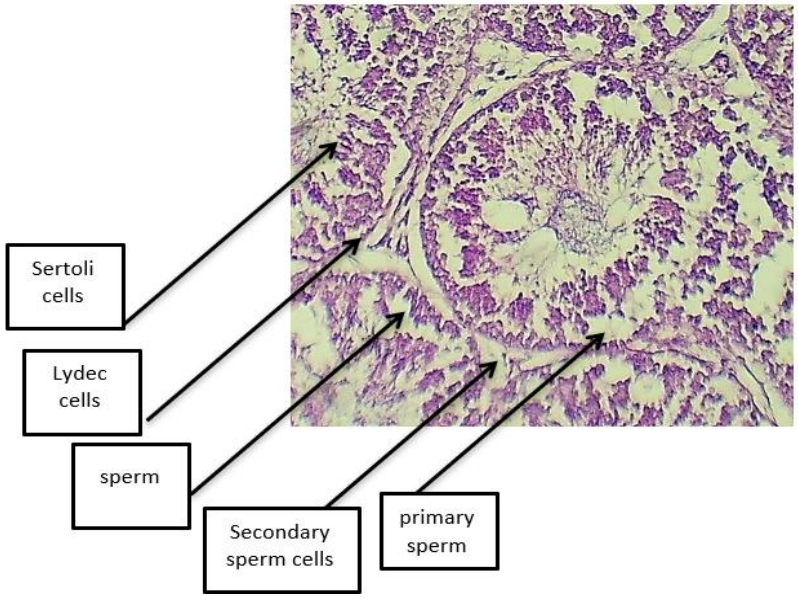

Figure 3. Histological Analysis of the Testicle Treated with T4

The histological section shows that approximately 65% of the seminiferous tubules have reached the stage of complete sperm synthesis. The tubule diameter is comparable to

that observed in the fourth treatment and the control group. The tubule walls exhibit no thickening or damage, and both Sertoli cells and Leydig cells appear normal (Cabrera, Reyes, & Rafael, 2006; Scanes, Butler, & Kidd, 2020).

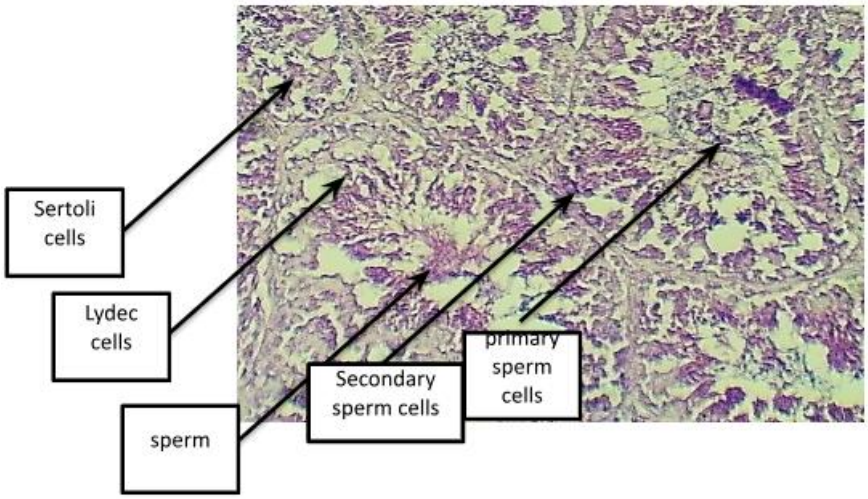

Figure 4. Histological Section of the T5-Treated Testicle

The histological section indicates that approximately 75% of the seminiferous tubules have reached the stage of complete sperm synthesis. The diameter of the seminiferous tubules is larger in the fourth and fifth treatments compared to the control group. Additionally, both Sertoli cells and Leydig cells appear normal (Cabrera, Reyes, & Rafael, 2006; Capcarová, et al., 2014; Friedman, 2007).

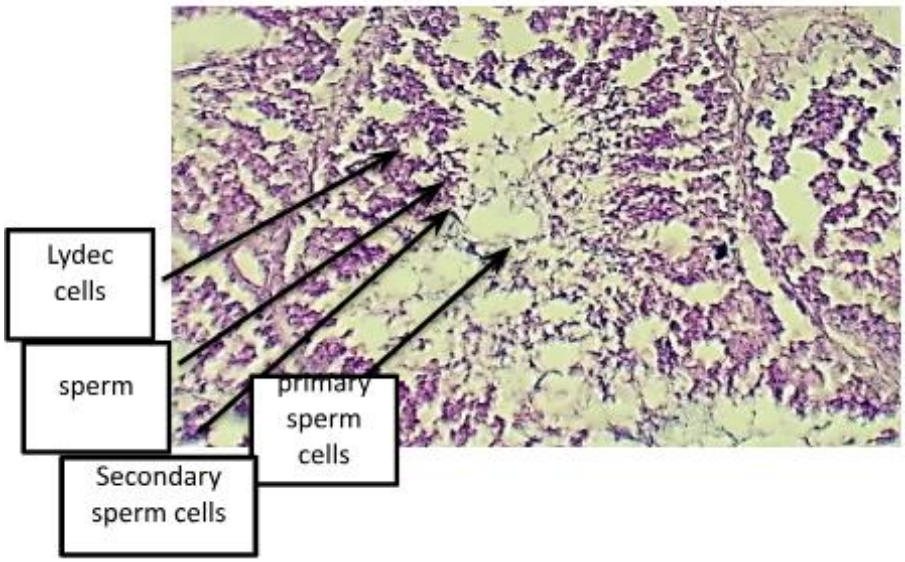

Figure 5. Histological Section of the T6 Treated Testicle

The histological section reveals that approximately 20% of the seminiferous tubules have reached the stage of complete sperm synthesis. The diameter of the seminiferous tubules is smaller compared to the other treatments (Capcarová, et al., 2014; Gargi, et al., 2017; Gülçin, et al., 2004).

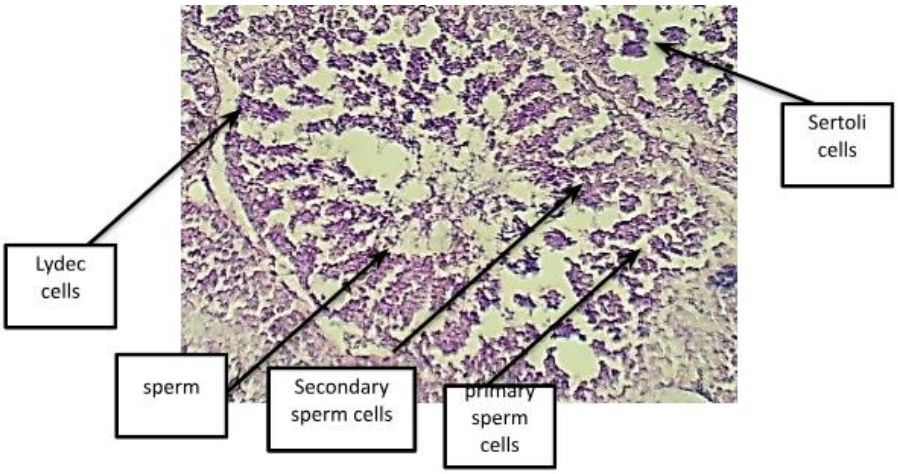

Figure 6. Histological Section of the Testis Treated as Control

Conclusion

The addition of nettle leaves and white tea, which contain active ingredients that enhance sperm production, resulted in significant improvements in testicular tissue in the treated groups (NRC, 1994; Rahim, et al., 2013; Romero-Sánchez, et al., 2008).

The findings further demonstrated that the observed enhancements in seminiferous tubule tissue characteristics and daily sperm production can be attributed to elevated testosterone levels and improved efficiency of Sertoli cells (Adnan, Ahmad, & Ahmed, 2013; Al-Ghurairi, Al-Hayani, & Maaeni, 2023; Al-Hassani, et al., 2022).

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