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Research Article

Ashwagandha (*Withania somnifera*) as a Sustainable Natural Additive for Enhancing Physiological and Reproductive Performance in Quail

Eelaff Mishaal Mohammed[1], Bashar Ahmed M. Lehmood[2], Alaa Ahmed Assaf[3], Tahreer Mohammed Al-Thuwani[2]*, Hussam A. Fadhil[2], Dhurgam A. Jasim[2]

[1]Department of Animal Production, Al-Mussaib Technical College, Al-Furat Al-Awsat University, Iraq; [2]Department of Animal Production, College of Agriculture, Al-Qasim Green University, Babil, Iraq; [3]Department of Medical Biotechnology, College of Science, Al-Mustaqbal University, Babylon, Iraq.

Abstract | The experiment was conducted to evaluate the effects of supplementing drinking water with Ashwagandha (*Withania somnifera*) on the productive performance, sexual maturation, physiological, and reproductive parameters of Japanese quail. A total of 180 birds were randomly allocated to four treatment groups, each with three replicates. The treatments included: a control group (T1) with no supplementation; a group receiving Ashwagandha leaf extract (T2, 100 mg/L); a group receiving Ashwagandha root extract (T3, 100 mg/L); and a combination group (T4, 200 mg/L). Birds in the T4 group showed a significant increase ($P \leq 0.05$) in final body weight and total weight gain compared to the control. Supplementation with the combined Ashwagandha leaf and root extracts (T4) also accelerated sexual maturity, with both male and female birds reaching puberty earlier ($P \leq 0.05$). Furthermore, T4 birds exhibited significantly heavier reproductive organs, enhanced testicular and ovarian development, and histological improvements, including thicker germ cell layers and a higher number of primary follicles ($P \leq 0.05$). Hormonal analysis revealed that T4 birds had the highest levels of testosterone and estrogen ($P \leq 0.05$). These results suggest that the inclusion of Ashwagandha extract in drinking water can improve growth performance, reproductive traits, and hormonal profiles in Japanese quail, supporting its potential use as a natural, plant-based supplement to enhance poultry productivity.

Keywords | Ashwagandha, Japanese quail, Hormones, Sustainable nutrition, Reproductive health, Physiological performance

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***Correspondence** | Tahreer Mohammed Al-Thuwani, Department of Animal Production, College of Agriculture, Al-Qasim Green University, Babil, Iraq; **Email:** tahrearmohammed@agre.uoqasim.edu.iq
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INTRODUCTION

Animal protein plays a crucial role in global food production and human nutrition, with poultry being essential for providing high-quality eggs and meat (Abd El-Hack *et al.*, 2022b). The increasing consumption of poultry-based protein sources has driven intensive production, transforming backyard farming into organized commercial operations (El-Saadony *et al.*, 2022). However, the industry faces challenges related to sustainability, food safety, and antibiotic-free production (Montesqrit *et al.*, 2025). To address these concerns, natural feed additives are widely used to enhance growth, feed conversion, disease resistance, and overall poultry health (Salem *et al.*, 2022). A feed additive must ensure safety and affordability while remaining eco-friendly and biodegradable. It must be non-toxic, help overcome drug resistance issues, enhance productivity, and ensure ecological safety without compromising public health, positioning it as a viable tool for sustainable growth in livestock production (Abd El-Hack *et al*., 2022a). Traditional medicinal herbs are widely used as therapeutic agents and have demonstrated significant efficacy in mitigating the detrimental effects of heat stress on the developmental performance of broilers (Owen, 2011). Their primary role in avian diets involves enhancing metabolism by reducing anxiety and regulating hormonal balance (Arif *et al*., 2022). Extensive field studies worldwide have highlighted the remarkable benefits of medicinal herbs in poultry nutrition, demonstrating their effectiveness in improving growth rates, feed conversion, survival, and overall vitality, while contributing to lower mortality rates among treated flocks (Jogi *et al*., 2023).

Withania somnifera (L.) Dunal, commonly known as Ashwagandha, is widely recognized for its adaptogenic properties. A resilient plant belonging to the Solanaceae family, Ashwagandha is renowned in traditional medicine for its therapeutic effects, which are often compared to those of Panax ginseng. It naturally thrives in arid regions of India, North Africa, and the Middle East (Sprengel *et al*., 2025). It is also widely acknowledged for its healing properties (Singh and Sharma, 2018). Incorporating Ashwagandha into poultry diets may help improve metabolism and reduce stress, thereby supporting growth and enhancing immunity (Punetha *et al*., 2010). Ashwagandha root extract is rich in active phytochemicals, such as flavonoids and phenolic compounds, which aid in stress management and inflammation reduction. Its effects are primarily attributed to withanolides, a group of naturally occurring steroidal lactones known for their adaptogenic and therapeutic potential (Shinde *et al*., 2023). Studies have shown that *Withania somnifera* improves hematological parameters by increasing hemoglobin levels, erythrocyte count, and white blood cell concentration (Gómez Afonso *et al*., 2023). Additionally, several of its phytochemicals exhibit muscle-supporting and mood-stabilizing effects, making it beneficial for conditions such as arthritis, chronic stress, and age-related health issues (Bashir *et al*., 2023). Research indicates that *Withania somnifera* (Ashwagandha) may help regulate cortisol levels, reduce fatigue, and enhance resilience to stress. In livestock exposed to environmental or physiological stressors, Ashwagandha has demonstrated potential in promoting homeostasis and improving overall health. Similarly, studies in avian species suggest that Ashwagandha contributes to maintaining physiological stability and resilience during periods of stress (Priyanka *et al*., 2020). Given the limited research on the use of Ashwagandha in quail, this study aimed to evaluate its effects when administered through drinking water, with a focus on growth performance, physiological indicators, and reproductive traits.

MATERIALS AND METHODS

Birds and grouping

The experiment was conducted at the Quail Research Facility at Al-Mussaib Technical College, Iraq, from March 29, 2025, to May 4, 2025. The Ethics Committee of Al-Mussaib Technical College (Agri. No. 3314, September 20, 2024) approved the broiler chicken management guidelines. A total of 180 birds were divided into four distinct treatment groups, each containing three replicates. The initial mean live weight, measured on the first day of the experiment, was 7.45 g. The birds were obtained from a private hatchery located in the Babylon Governorate. Treatment T1 served as the control group and received no supplementation. In treatments T2 and T3, 100 mg of Ashwagandha leaf extract and root extract, respectively, were added per liter of drinking water. In treatment T4, a combination of 100 mg each of leaf and root extracts (totaling 200 mg) was added per liter of drinking water. All birds were housed in quail cages under uniform environmental and management conditions. During the starter phase (up to three weeks of age), the birds were fed a diet containing 24% crude protein and 2,900 kcal/kg of metabolizable energy. Afterward, they were provided a commercially available quail ration with 20% crude protein and 2900 kcal/kg of metabolizable energy, following the guidelines of the NRC (1994) and previous studies by Sultan *et al*. (2019). Feed and water were offered ad libitum throughout the experiment.

Preparation of ashwagandha extract

Ashwagandha was locally sourced from marketplaces in Babil, Iraq. The samples were examined to verify their morphological characteristics and ensure their authenticity and quality. The collected plant parts, including leaves and roots, were washed to remove dirt and then air-dried at room temperature. After drying, the plant materials were ground using a laboratory blender into a coarse powder. The resulting powder was stored in sterile, opaque glass bottles under controlled conditions at 21°C to preserve its stability and potency for future use.

To prepare the extract, 50 g of the ground sample and 250 mL of ethanol were added to a closed glass container, which was then kept in warm water at 37°C for 24 hours. This was followed by continuous stirring for one hour using a magnetic stirrer to ensure maximum dissolution of bioactive compounds. After simple straining through a piece of thin cloth, the liquid was transferred into centrifuge tubes and subjected to centrifugation for 15 minutes at 3000 rpm. The clear supernatant was carefully collected, while the sediment was discarded. The collected liquid was poured into Petri dishes and placed inside a drying oven at 37°C. After dehydration, the final product was gently gathered and stored in a refrigerator to maintain stability and potency. This process was repeated to obtain sufficient quantities for experimentation (Altemimi *et al*., 2017).

Performance analysis

The study monitored various growth and efficiency parameters throughout the experimental period, following the methodology outlined by Al-Jebory *et al.* (2023). Data were recorded weekly to ensure precise tracking of performance indicators. Sexual maturity in male quails was assessed by the presence of cloacal foam, observed from the cloacal gland beginning on day 21. In females, sexual maturity was determined by the laying of the first egg and further confirmed when 50% of the flock had commenced egg production. Body weights at maturity and at the onset of sexual maturity were measured using a precision balance to ensure accurate and reliable data collection (Al-Salhie *et al*., 2017).

Histological examination

At the end of the experiment, a total of 24 birds were slaughtered for organ evaluation comprising one male and one female from each replicate, across four treatment groups with three replicates per group (4 treatments × 3 replicates × 2 birds = 24 birds). The testes and ovaries were carefully dissected and weighed separately to the nearest gram. Organ weights were expressed as a percentage of body weight to assess reproductive system development. Histological analyses were performed at a local laboratory. Tissue samples from the testes and ovaries were collected from the same birds at 35 days of age and processed according to the procedure described by Humason (1962).

Following tissue section preparation, the samples were subjected to microscopic examination. Imaging was performed using a digital microscope at 40× magnification to document histological features. Histological measurements in the testes included the diameter of spermatogenic tubules, the thickness of the germ cell layer, and the diameter of the spermatogenic tubule lumen. For the ovaries, the primary ovarian follicle diameter and the number of primordial ovarian follicles were assessed to evaluate reproductive tissue development. The analysis was carried out at the Biotechnology Laboratory, College of Science, Future University.

Hormonal assays

Blood samples were collected 35 days after the start of the study from the same birds that were sacrificed for reproductive organ examination. Three males and three females were selected from each treatment group. Blood was drawn into clean tubes without anticoagulants (EDTA) to allow serum separation. The samples were centrifuged at 4,000 rpm for 20 minutes, after which the serum was separated and stored at −18°C for subsequent hormone analysis. Concentrations of testosterone, estrogen, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured using a commercial ELISA test kit (Bioactive Diagnostics, Germany). The analysis employed ELISA technology based on the Direct Sandwich principle to ensure accurate hormone quantification.

Statistical analysis

Data analysis was performed using SPSS (2011) following a completely randomized design (CRD). Significant differences between treatments were evaluated using Duncan's multiple range test (Duncan, 1955). The statistical model applied was:

Yij=μ+Ti+eiY_{ij} = \mu + T_i + e_iYij=μ+Ti+ei

Where; YijY_{ij}Yij is the observed value, μ\muμ is the overall mean of the trait studied, TiT_iTi represents the treatment effect, and eie_iei denotes the experimental error.

RESULTS AND DISCUSSION

Productive traits

Table 1 illustrates the impact of supplementing drinking water with varying concentrations of Ashwagandha leaf and root extracts on the cumulative productive traits of Japanese quail over 35 days. The monitored traits include final live body weight, cumulative weight gain, cumulative

Table 1: Influence of Ashwagandha extract on selected cumulative productive traits in Japanese quail (Days 1–35) (mean ± standard error).

Cumulative feed intake (g/bird)	Cumulative feed conversion ratio	Cumulative weight gain (g/bird)	Final weight (g/bird)	Treatments
340.025±0.267	2.298±0.010^{a}	147.903±0.267^{c}	154.608±0.267^{c}	T1
338.624±0.195	2.255±0.006^{ab}	150.153±0.195^{bc}	156.858±0.312^{bc}	T2
339.732±0.369	2.221±0.013^{bc}	152.934±0.369^{b}	159.639±0.369^{b}	T3
336.878±0.639	2.162±0.003^{c}	155.794±0.639^{a}	162.499±0.639^{a}	T4
N.S.	*	*	*	Significant

*Values in a column not sharing a common letter are significantly different at the 0.05 level. N.S., Not significant.

Table 2: Influence of Ashwagandha extract on age and weight of male and female Japanese quail at sexual puberty (Mean ± Standard Error).

Weight at sexual puberty (g)		Age at sexual puberty (days)		Treatments
Females	Males	Females	Males	
158.649±1.779	120.015±0.557[a]	33.599±0.299[a]	30.360±0.179[a]	T1
157.149±1.829	116.730±0.945[b]	32.699±0.299[b]	29.920±0.146[b]	T2
160.749±1.035	118.710±1.131[ab]	32.400±0.363[b]	29.920±0.146[b]	T3
157.459±0.622	116.145±0.738[b]	32.400±0.259[b]	29.810±0.179[b]	T4
*	*	*	*	Significant

*Values in a column not sharing a common letter are significantly different at the 0.05 level.

feed conversion ratio, and cumulative feed intake. Statistical analysis revealed significant differences ($P \leq 0.05$) in final live body weight, with treatment T4 outperforming all others, reaching an average of 162.499 g, while the control group (T1) recorded the lowest average at 154.608 g. The trend in cumulative weight gain mirrored that of final live body weight, with T4 significantly ($P \leq 0.05$) surpassing all treatments at an average of 155.794 g, and T1 recording the lowest average at 147.903 g. The cumulative feed conversion ratio also showed significant differences among treatments, with T4 demonstrating a notable improvement ($P \leq 0.05$) compared to T1. Specifically, T4 recorded a feed conversion ratio of 2.162 g feed/g weight gain, whereas the control treatment (T1) had the highest ratio at 2.298 g feed/g weight gain. However, the comparative assessment of feed intake indicated no significant variation among the treatment groups. The superior growth metrics observed may be attributed to the bioactive compounds present in the ashwagandha extract, which contains a diverse array of biochemical and nutritional substances, including minerals, various carbohydrates, organic acids, alkaloids, fats, withanolides, free amino acids, vitamins, saponins, sitoindosides, and numerous enzymes and cofactors (Sprengel *et al*., 2025). The improvement in feed conversion ratio (FCR) upon supplementation with ashwagandha extract may result from the presence of bioactive phenolic and flavonoid complexes that enhance tissue resistance to toxic substances and harmful pathogens. Additionally, these compounds promote an increase in the number of goblet cells in the small intestine, which serve as a defensive barrier against chemical and mechanical damage and trap invasive pathogens, potentially improving metabolic conversion rates (Senthil *et al*., 2015). Furthermore, the beneficial effects may also be linked to the vitamin E content in ashwagandha, which stimulates the pituitary gland to release TSH. This hormone increases the secretion of thyroid hormones thyroxine and triiodothyronine, which play a critical role in elevating the metabolic rate in body tissues and organs such as the liver and skeletal muscles (Wiciński *et al*., 2023).

Age and weight of males and females at sexual puberty

Table 2 illustrates the effect of Ashwagandha extract added to drinking water on the age of sexual puberty in male and female Japanese quail. The findings showed statistically significant differences, particularly in males, where the supplemented treatments at the 0.5% level reached puberty earlier than the control group. The treatment involving a mixture of Ashwagandha leaves and root extract (T4) resulted in the earliest sexual puberty, with the lowest average age of 30.36 days. In contrast, the control treatment exhibited the longest puberty period at 27.6 days. Regarding the age of females at sexual puberty, the supplementation treatments followed a similar pattern to that of males. Treatments T2, T3, and T4 reached sexual puberty earlier, averaging 32.699, 32.400, and 32.400 days, respectively. In contrast, the control treatment (T1) showed delayed sexual puberty, recording the longest period at 33.599 days.

As shown in Table 2, male quail in treatments T2 and T4 exhibited a significant reduction in average body weight at the onset of sexual maturity ($P \leq 0.05$), recording 116.730 g and 116.145 g, respectively. In contrast, males in the control group (T1) reached the highest average weight of 120.015 g. However, no significant differences were observed in the average body weight of females at sexual maturity across the experimental treatments. The earlier onset of sexual maturity in birds following the addition of Ashwagandha extract may be linked to elevated concentrations of sex hormones (testosterone and estrogen) in the blood serum of Japanese quail. These hormones are crucial for the development of reproductive organs, leading to earlier sexual maturity. Some studies have indicated that testosterone accelerates testicular growth, and well-developed testes contribute to early sexual maturity due to the positive correlation between testicular development and early sexual maturation in males (Gupta and Srivastava, 2025). The earlier onset of sexual maturity in male quail was also associated with an increase in testicular weight, accompanied by elevated serum concentrations of LH and testosterone (Khan *et al*., 2024). Just before ovulation begins, increasing estrogen levels stimulate the secretion of LH, which in turn makes follicles more responsive. This hormonal interplay underscores the key role played by estrogen in triggering ovulation and accelerating sexual development in females (Derese *et al*., 2024). The reduced body weight observed in quail experiencing early sexual maturity may reflect a clear negative relationship between the timing of puberty onset and body weight. Birds that mature earlier have a shorter window for somatic growth and muscle development, which can result in reduced final body weight. This association appears to be shaped by underlying hormonal and physiological mechanisms, especially those governing testicular development and metabolic regulation. These findings align with the hypothesis that early-maturing individuals tend to attain lower mature body weights compared to their later-maturing counterparts (Chimezie *et al*., 2017). Reason: The text was revised to correct minor grammatical errors, improve sentence structure, and enhance clarity and readability. Vocabulary was refined for precision and flow, and technical terms were clarified to ensure accuracy. The overall meaning and scientific content were preserved while making the passage more coherent and professional.

Age and weight of females at sexual maturity, and the weight of the first egg

Table 3 summarizes the impact of Ashwagandha extract supplementation on the age at sexual maturity in female quail, as well as the mass of the first egg. While no statistically significant differences were observed in the mean weight of the first-laid egg among the treatments, notable variations emerged in the age at sexual maturity. Females in treatments T3 and T4 reached sexual maturity earlier, at approximately 33.433 days, whereas the control group (T1) exhibited the longest age at 35.416 days. No significant differences were found among treatments in the average weight of females at sexual maturity. The earlier sexual maturity observed in female quail following Ashwagandha extract supplementation may be linked to elevated estrogen concentrations in the blood serum of birds treated with date palm pollen (Al-Salhie *et al*., 2017). Estrogen plays a crucial role in increasing the number of FSH receptors in target tissues, thereby enhancing follicular maturation and ultimately leading to earlier sexual maturity in females. Additionally, studies suggest that active compounds in Ashwagandha, such as withanolides, may interact with the hypothalamic-pituitary-adrenal (HPA) axis, resulting in hormonal modulation that accelerates sexual maturity in birds (Guyton, 2006). The early sexual maturity of Japanese quail after Ashwagandha extract supplementation may also be attributed to its high vitamin E content. Vitamin E has stimulatory effects on the gonads, enhancing the secretion of FSH and LH, both of which are essential for reproductive development and gamete maturation (Al-Salhie *et al*., 2017).

Table 3: Influence of Ashwagandha extract on age and weight of female Japanese quail at sexual maturity and weight of the first egg (Mean ± Standard Error).

Sexual maturity			
Weight of female (g)	Age of female (g)	Weight of first egg (g)	Treatments
167.292±1.035	35.416±0.283[a]	7.077±0.255	T1
166.734±1.829	34.850±0.490[a]	6.551±0.374	T2
166.767±1.035	33.433±0.056[b]	6.526±0.475	T3
166.103±0.622	33.433±0.141[b]	7.197±0.110	T4
N.S.	*	N.S.	Significant

*Values in a column not sharing a common letter are significantly different at the 0.05 level. N.S., Not significant.

Relative weights of testes, ovaries, and the oviduct

Table 4 illustrates the impact of Ashwagandha on the relative weights of the testes, ovaries, and oviducts at 35 days from the start of the study. The results showed that males and females in the T4 treatment group significantly outperformed ($P \leq 0.05$) the other treatments in terms of average weight. As shown in Table 4, treatments T3 and T4 also led to a significant increase ($P \leq 0.05$) in the relative weight of the testes compared to T1. Regarding the relative weight of the ovary and oviduct at 35 days, treatment T4 outperformed all other treatments, while the T1 treatment recorded the lowest means. The significant increase in relative testicular weight following the addition of Ashwagandha extract to drinking water may be attributed to elevated testosterone levels. This suggests that Ashwagandha enhances the number of testosterone receptors, thereby increasing testicular responsiveness and leading to greater testicular mass. Our findings further support Ashwagandha's role in stimulating LH secretion, which in turn activates Leydig cells, enhances testosterone production, and contributes to improved testicular function and efficiency (Wiciński *et al*., 2023). The recorded increase in testis weight following Ashwagandha extract supplementation may be due to the direct correlation between serum testosterone levels and testis size. Elevated testosterone concentrations likely stimulate testicular growth, potentially by increasing testosterone receptor density and enhancing tissue responsiveness. These findings are consistent with earlier research highlighting testosterone's essential role in testicular growth and reproductive performance in avian species (Goretskaia and Beme, 2021). The marked increase in relative ovarian weight observed in female quail can be attributed to the influence of Ashwagandha extract in enhancing physiological growth. This effect is likely mediated by increased plasma

Table 4: Influence of Ashwagandha extract on the relative weight of the testes, ovaries, and oviduct in Japanese quails at 35 days of age (mean ± standard error).

Relative weights at 35 days of age					
Weight of ovary (g)	**Weight of oviduct (g)**	**Female weight (g)**	**Weight of testicles (g)**	**Male weight (g)**	**Treatment**
0.0005±0.001^{c}	0.0009±0.002^{b}	142.725±3.016^{b}	0.0040±0.002^{c}	140.982±6.431^{b}	T1
0.0006±0.001^{c}	0.0011±0.001^{b}	145.475±4.603^{b}	0.0057±0.001^{b}	142.706±3.016^{b}	T2
0.0012±0.001^{b}	0.0012±0.001^{b}	146.575±2.381^{b}	0.0066±0.001^{a}	146.575±2.381^{b}	T3
0.0015±0.001^{a}	0.0018±0.001^{a}	153.725±6.191^{a}	0.0069±0.001^{a}	153.706±6.191^{a}	T4
*	*	*	*	*	Significant

*Values in a column not sharing a common letter are significantly different at the 0.05 level.

Table 5: Influence of Ashwagandha extract on some histological parameters of the testes and ovaries in Japanese quails (mean ± standard error).

Diameter of primary follicles (µm)	**Number of primary follicles (N)**	**Diameter of lumen of seminiferous tubule (µm)**	**Thickness of germinal cell layer (µm)**	**Diameter of seminiferous tubule (µm)**	**Treatment**
438.28±33.63^{c}	7.33±0.18^{c}	131.17±2.66^{a}	96.25±4.67^{c}	326.97±10.76^{c}	T1
534.53±57.02^{b}	8.43±0.18^{b}	127.32±2.88^{ab}	108.62±5.45^{bc}	349.25±10.19^{b}	T2
673.75±27.61^{a}	10.26±0.36^{a}	121.82±3.09^{b}	115.50±5.84^{b}	356.125±9.24^{b}	T3
677.18±18.25^{a}	10.63±0.18^{a}	119.07±3.22^{b}	129.25±2.89^{a}	380.87±5.73^{a}	T4
*	*	*	*	*	Significant

*Values in a column not sharing a common letter are significantly different at the 0.05 level.

Table 6: Influence of Ashwagandha extract on the concentration of sex hormones in the serum of male and female Japanese quails at 35 days of age

Female sex hormones			Male sex hormones			
LH (IU/L)	**FSH (IU/L)**	**Estrogen (pg/ml)**	**LH (IU/L)**	**FSH (IU/L)**	**Testosterone (ng/L)**	**Treatment**
1.320±0.275^{c}	2.211±0.126^{b}	135.025±0.170^{c}	1.122±0.062^{c}	1.925±0.126^{c}	2.456±0.040^{d}	T1
1.859±0.044^{b}	2.258±0.034^{b}	140.305±0.083^{b}	1.337±0.003^{bc}	2.192±0.034^{b}	2.907±0.072^{c}	T2
1.903±0.082^{b}	2.365±0.056^{ab}	150.775±0.227^{a}	1.587±0.212^{b}	2.211±0.056^{b}	3.325±0.059^{b}	T3
2.206±0.036^{a}	2.764±0.119^{a}	151.913±0.066^{a}	2.090±0.012^{a}	2.530±0.119^{a}	3.666±0.079^{a}	T4
*	*	*	*	*	*	Significant

*Values in a column not sharing a common letter are significantly different at the 0.05 level.

concentrations of reproductive hormones, as observed in our study. Estrogen is known for its key role in stimulating the growth and maturation of the female reproductive tract, leading to an earlier onset of sexual maturity (Gupta and Srivastava, 2025).

Histological study of testes and ovaries

Table 5 and Figure 1 present the impact of Ashwagandha supplementation on the testes and ovaries of male and female quails at 35 days from the start of the study. In males, treatment T4 resulted in a significant increase ($P \leq 0.05$) in seminiferous tubule diameter (STD) and germinal layer thickness (GLT) compared to the other treatments, indicating enhanced spermatogenic activity. Conversely, the tubular lumen diameter was significantly greater in the T1 treatment than in T4, with no significant differences observed between T4, T2, and T3 at 35 days of age. The results in the same Table 5 indicate a significant increase ($P \leq 0.05$) in both the number and diameter of primary ovarian follicles in birds from the T3 and T4 treatments compared to the control group. The rise in FSH and testosterone levels following the administration of Ashwagandha extract (Table 6) may contribute to the enhancement of testicular weight and size. Consequently, this hormonal stimulation increases the diameter of seminiferous tubules, supporting improved spermatogenesis and reproductive efficiency (Ruthig and Lamb, 2022). The significant increase in seminiferous tubule diameter (STD) and germinal layer thickness (GLT), along with the observed reduction in tubular lumen diameter, appears to reflect strong positive correlations with testicular weight. These morphological changes indicate heightened activity, likely driven by increased proliferation of spermatogonial stem cells. The thickening of the germinal layer suggests intensified sperm production, which overall improves testicular function and enhances reproductive efficiency (Gupta and Srivastava, 2025). The rise in FSH levels following Ashwagandha extract supplementation may stimulate the growth and maturation of Sertoli cells, which are essential for supporting spermatogenesis. Enhanced activity of these cells likely promotes an increase in germinal layer thickness. This improved cellular environment facilitates more efficient sperm production and contributes to better testicular function and reproductive performance (Baines *et al*., 2008).

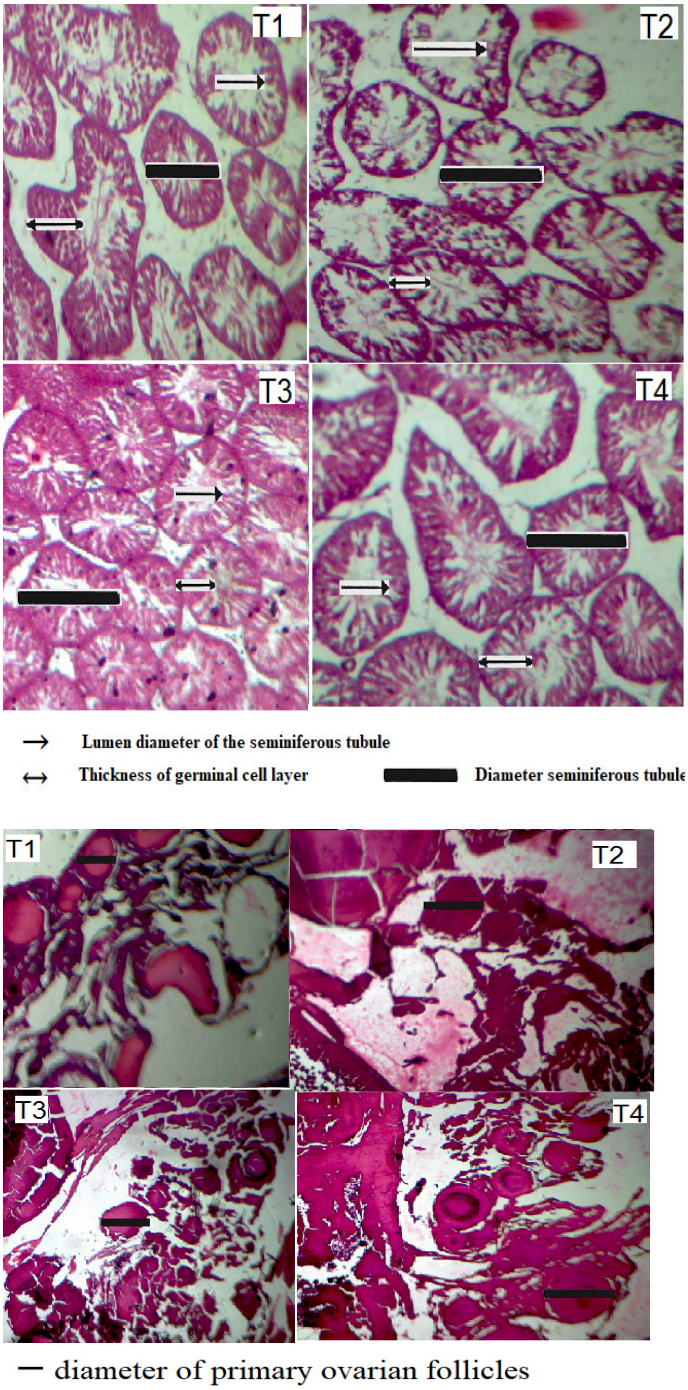

Figure 1: The impact of Ashwagandha supplementation on the testes and ovaries of male and female quails at 35 days. T1, control with no supplementation, Treatment receiving Ashwagandha leaves extract (T2, 100 mg/L through drinking water), a treatment receiving root extract (T3, 100 mg/L through drinking water), and a combination treatment (T4, 200 mg/L through drinking water).

Sex hormones in blood serum

The results presented in Table 6 demonstrate the impact of Ashwagandha extract supplementation on the concentrations of certain sex hormones (testosterone, estrogen) and gonadotropic hormones (FSH and LH) in the blood serum of male and female Japanese quails at 35 days of age from the start of the study. The results in Table 6show a significant increase ($P \leq 0.05$) in the concentration of testosterone in the blood serum of males in the fourth treatment group compared to the other treatments at 35 days of age, with an average concentration of 3.666 ng/mL. Table 6 also indicates significant differences among treatments in the concentration of FSH in the blood serum of males. The fourth treatment group showed superiority at 35 days of age compared to all other treatments, recording an average of 2.530 IU/L, while the lowest average was observed in the control group (T1), which reached 1.925 IU/L. Regarding the concentration of FSH in the blood serum of females, the results in Table 6 indicate significant differences among treatments. The fourth treatment group recorded the highest significant value compared to the other treatments at 35 days of age.

The results in Table 6 show significant differences among the treatments regarding LH hormone concentration in the blood serum of males at 35 days of age. The fourth treatment outperformed the others, recording the highest average of 2.090 IU/L, while the control treatment had a concentration of 1.122 IU/L. Similarly, the results indicate that the fourth treatment demonstrated significant superiority ($P \leq 0.05$) in LH hormone concentration in the blood serum of females compared to the other treatments at 35 days of age. It recorded the highest average of 2.206 IU/L, whereas the control treatment had the lowest average of 1.320 IU/L.

The results presented in Table 6 indicate a significant increase ($P \leq 0.05$) in the concentration of estrogen hormone in the blood serum of females in the second, third, and fourth treatment groups compared to the control group. The treatment groups recorded estrogen concentrations of 140.305, 150.775, and 151.913 pg/mL, respectively, while the control group had the lowest concentration of 135.025 pg/mL at 35 days of age. Ashwagandha's ability to alleviate stress, inflammation, and oxidative damage plays a central role in supporting hormonal balance. Its active constituents particularly withanolides are believed to modulate pituitary function by stimulating the release of LH and FSH, both of which are key regulators of reproductive hormones such as testosterone and estrogen. Additionally, Ashwagandha's antioxidant properties help protect reproductive cells from oxidative damage and improve blood flow to endocrine glands, thereby fostering a more favorable hormonal environment. These combined effects contribute to elevated estrogen levels in females and increased testosterone concentrations in males, ultimately enhancing reproductive health and functionality (Wiciński *et al*., 2023). The upregulation of estrogen expression following Ashwagandha supplementation is likely attributed to its antioxidant and anti-stress properties, which enhance the functionality of reproductive cells and promote hormonal secretion. Ashwagandha may also modulate pituitary gland activity, resulting in increased secretion of LH and FSH both pivotal for regulating testosterone and estrogen synthesis. Collectively, these effects support hormonal balance, improve fertility, increase sperm count, and enhance overall reproductive function (Gupta and Srivastava, 2025).

CONCLUSION

The study shows that supplementing drinking water with Ashwagandha extract can positively influence quail growth, physiology, and reproductive traits. The results indicate that birds receiving a combination of leaf and root extracts (T4, 200 mg/L in drinking water) gained more body weight, reached sexual maturity earlier, exhibited increased reproductive organ weights, and showed improved histological characteristics. Additionally, higher levels of sex hormones, including testosterone and estrogen, were recorded, indicating enhanced reproductive activity.

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NOVELTY STATEMENT

This study introduces a novel approach to improving poultry productivity through the incorporation of Ashwagandha extract derived from both leaves and roots into the drinking water of Japanese quail. The findings reveal marked enhancements in growth performance, physiological stability, and reproductive health. Ashwagandha's bioactive compounds appear to accelerate sexual maturation, regulate the secretion of key reproductive hormones (testosterone, estrogen, LH, and FSH), and support the development of reproductive organs. As a natural feed additive, Ashwagandha offers a promising, sustainable alternative to synthetic growth enhancers, promoting animal well-being, alleviating stress, and improving metabolic efficiency factors that collectively benefit both productivity and animal welfare in the poultry sector.

AUTHOR'S CONTRIBUTION

EMM: Conceptualization, investigation, supervision, and validation.
BAML: Formal analysis, methodology, investigation, writing original draft.
AAA, HAF, DAJ: Data curation and investigation.
TMA-T: Conceptualization and investigation.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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