ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 2, December 2025
Pages: 598-606

E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g090226

Dietary lotus seed powder enhances semen quality and storage stability in Mia roosters

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Manuscript received: 31 August 2025. Revision accepted: 5 October 2025.

Abstract. *Nhan P, Tri NM. 2025. Dietary lotus seed powder enhances semen quality and storage stability in Mia roosters. Asian J Agric 9: 598-606.* Semen quality, a key determinant of reproductive efficiency in poultry, plays a crucial role in breeding programs and in the conservation of indigenous genetic resources. Natural dietary supplements with antioxidant activity may improve sperm function and preservation. Lotus seed powder (*Nelumbinis semen*) is rich in bioactive compounds with antioxidant and membrane-stabilizing properties, but its effects on avian reproduction remain unexplored. This study represents the first report in avian species evaluating the impact of dietary lotus seed powder supplementation on semen quality and storage stability in Mia roosters, a Vietnamese indigenous breed. Thirty healthy roosters (10-11 months) were fed either a control diet or diets supplemented with 2% or 4% lotus seed powder for 60 days. Semen was collected every three days and assessed for volume, concentration, motility parameters, morphology, viability, membrane integrity, and chilled storage stability (0-24 h at 4°C). Compared with the control (C), T2 showed higher semen volume (0.48±0.05 mL vs 0.42±0.05 mL), sperm concentration (3.47±0.27×10^9/mL vs 3.21±0.26×10^9/mL), VSL (46.25±3.15 µm/s vs 42.35±3.12 µm/s), and viability (91.03±2.68% vs 87.46±2.85%). Abnormal morphology decreased from 9.84±1.21% in C to 8.15±1.12% in T2. Motility after 24 h storage remained higher in T2 (63.20±4.15%) compared with C (51.81±4.91%). Dietary lotus seed powder, particularly at 4% inclusion, significantly improves semen quality by enhancing sperm motility, viability, and membrane integrity, while also prolonging chilled storage stability in Mia roosters. These outcomes underscore its potential as a natural reproductive enhancer with applications in Artificial Insemination (AI) and the conservation of indigenous poultry genetic resources.

Keywords: Lotus seed powder, Mia rooster, *Nelumbinis semen*, semen quality, sperm motility

Abbreviations: AI: Artificial insemination, ALH: Amplitude of Lateral Head Displacement, BCF: Beat cross frequency, CASA: Computer-Assisted Sperm Analysis, STR: Straightness, VCL: Curvilinear Velocity, VSL: Straight-Line Velocity

INTRODUCTION

The Mia rooster is an indigenous Vietnamese breed highly valued for its meat quality, adaptability to local farming systems, and cultural importance. Despite these advantages, its breeding efficiency is often limited by suboptimal semen quality, particularly under smallholder production conditions. Enhancing reproductive performance in Mia roosters is therefore essential not only to improve productivity but also to safeguard the genetic resources of this native breed. In poultry breeding farms, Artificial Insemination (AI) is commonly applied to produce the next generation and enhance meat and egg yields. However, the fertility rate of cryopreserved avian semen remains low, which limits the widespread use of AI in birds (Ushiyama et al. 2016). Advances in poultry biotechnology, especially Artificial Insemination (AI), offer powerful tools to support genetic conservation and reproductive management (Nahak et al. 2015). Semen quality is widely recognized as a key determinant of fertility and hatchability, and in indigenous breeds such as the Mia rooster, improving sperm traits is particularly important for genetic preservation and sustainable poultry production. According to Hidayat et al. (2020), the quality of spermatozoa determines the success of artificial insemination; therefore, maintaining semen quality from the time of collection is essential. In line with this, Nhan (2024) emphasized that spermatozoa directly influence fertilization efficiency and progeny quality, underscoring that maintaining high semen quality is essential for reproductive progress through the effective use of genetically superior roosters. The relationship between sperm quality and fertility potential in avian species has been well documented, indicating that poor sperm quality leads to reduced fertility and higher embryo mortality rates (Feyisa et al. 2018).

In recent years, natural dietary supplements have attracted attention as safer alternatives to synthetic hormones and pharmaceuticals in improving reproductive traits (Izanloo et al. 2022). Recent studies on aquatic ecosystems, which are essential for maintaining biodiversity, have highlighted *Nelumbo nucifera* Gaertn. as a species of both cultural significance and scientific interest (Zhao et al. 2023). *Nelumbo nucifera*, belonging to the monogeneric family Nymphaeaceae, is an aquatic perennial herb with a long history of nutritional, medicinal, and cultural importance (Yu et al. 2013; Li et al. 2020). Lotus has been cultivated for nearly 4,000 years in Asia, Iran, and Egypt, where it has served both cultural and symbolic roles (Kandeler and Ullrich 2009). Archaeological evidence

shows its seeds were used as food and medicine more than 7,000 years ago (Ming et al. 2013), and today it remains an economically important crop in Asia, with large-scale seed production in India, Japan, and China (Zhang et al. 2012). Owing to their extraordinary capacity for long-term survival and successful germination, lotus seeds have become a focal point of scientific inquiry, with promising applications in seed conservation and modern agriculture (Shen-Miller et al. 2013; Salaemae et al. 2018).

Lotus seeds (*Nelumbinis semen*) have long been used in traditional medicine for their high nutritional and therapeutic value (Asma et al. 2022). They are rich in bioactive compounds including alkaloids, flavonoids, polysaccharides, essential oils, glycosides, polyphenols, and triterpenoids, which together contribute to diverse pharmacological activities (Huang et al. 2009; Zhenjia et al. 2010; Chen et al. 2012). The bioactive compounds of *N. nucifera* contribute significantly to its use in traditional remedies as well as in contemporary medical practices (Ming et al. 2013). These bioactives may reduce oxidative stress, protect cell membranes, and support spermatogenesis, thereby improving semen quality (Soleimanzadeh et al. 2018a). Studies in rodents and other mammals have shown beneficial reproductive effects of lotus seed extracts under stress conditions (Mireille et al. 2017), but there is little evidence of their application in avian species. Commercially, dried lotus seeds are marketed in two forms, white and brown-coated seeds (Acharya and Srikanth 2014).

Recently, no published study has specifically investigated the dietary use of lotus seed powder in roosters. This knowledge gap is particularly relevant for native poultry breeds that play an important role in smallholder farming systems. Therefore, the present study was conducted to evaluate the effects of dietary supplementation with *Nelumbinis semen* on semen characteristics such as ejaculate volume, sperm concentration, motility, and viability in Mia roosters. The findings aim to provide a scientific basis for lotus seed as a functional feed additive in poultry reproduction, with potential applications in improving AI efficiency and conserving indigenous genetic resources. Based on this rationale, we hypothesize that dietary lotus seed powder supplementation improves semen quality and extends storage stability in Mia roosters.

MATERIALS AND METHODS

Location and time

Mia roosters were obtained from Dong Loi chicken farm in Phu Huu commune, Can Tho City, Vietnam. Semen collection and quality evaluation were conducted at the Veterinary Laboratory of Tay Do University between January and April 2025.

Animals and experimental design

The experiment was conducted on thirty healthy Mia roosters, uniform in body weight and aged between 10 and 11 months. All roosters were fully vaccinated according to standard veterinary protocols and underwent health checks prior to the start of the trial. The roosters were housed individually under controlled environmental conditions of temperature, humidity, and lighting. During the first two weeks, the birds were fed a basal diet and trained to adapt to semen collection procedures at a frequency of once every three days at 06:30 h. Dietary supplementation with lotus seed powder was introduced only after the birds had consumed it for approximately one week to ensure feed acceptance and safety before the commencement of the main experimental period.

Thirty roosters were randomly allocated into three groups, each consisting of ten birds. The control group (C) was fed a basal diet without lotus seed powder supplementation. The basal diet was primarily composed of corn, broken rice, wheat bran, soybean oil, and fish meal, formulated to provide approximately 18% crude protein and 3150 kcal/kg metabolizable energy (see Feed and Feeding subsection for details). Treatment group T1 received the basal diet supplemented with lotus seed powder at 2% of daily feed weight, and treatment group T2 received 4%. The main experimental period lasted 60 days, starting from the initiation of the experimental diets. Semen was collected at 06:30 h every three days throughout the experimental period, following protocols similar to those reported in studies on donkey semen quality and oxidative stress (Baqerkhani et al. 2024). Ejaculates were collected directly into 1.5 mL sterile Eppendorf tubes and immediately diluted at a 1:1 volume-to-volume ratio with a modified Ringer's solution, then stored at 4°C. The diluted semen samples were promptly transported to the laboratory for subsequent analysis of ejaculate volume, sperm concentration, total and progressive motility, live sperm percentage, and the proportion of morphologically normal spermatozoa.

Feed and feeding

Throughout the study period, the roosters were fed a pelleted basal diet formulated to provide 18 percent crude protein and 3,150 kilocalories per kilogram of metabolizable energy. The diet was composed of locally available ingredients such as broken rice, wheat bran, soybean oil, corn, and fish meal. Calcium content ranged from 0.4 to 1.0%, while phosphorus levels varied from 0.5 to 0.8%.

Lotus seeds (*Nelumbinis semen*) were sourced from a local agricultural market and manually inspected to remove damaged or moldy seeds. The seeds were then washed thoroughly with clean water to remove surface impurities and air-dried for 1-2 hours. To facilitate dehusking, the seeds were lightly roasted over low heat for 3-5 minutes until the outer skin loosened. The seed coats were removed manually, and the green embryos inside each seed were separated to reduce bitterness. The kernels were subsequently dried in a hot-air oven at 50-55°C for 24 hours to achieve a moisture content below 10%. This drying condition was selected to preserve the stability of bioactive compounds, as similar temperature-controlled methods have been reported to maintain functional integrity of plant-derived materials (Kabirian et al. 2018).

Once dried, the kernels were ground using a high-speed grinder to obtain a fine powder, which was then sieved through a 0.5 mm mesh to ensure uniform particle size. The lotus seed powder was stored in airtight, opaque containers at room temperature and protected from moisture until incorporation into the experimental diets.

Ethics statement

All procedures involving roosters were conducted in accordance with institutional policies and Vietnamese regulations on animal welfare. The study complied with Decision No. 24/2010/QĐ-UBND issued by the People's Committee of Can Tho City, which governs livestock management and use in agricultural research.

Measurements of semen quality assessment in Mia roosters fed diets supplemented with lotus seed powder (*Nelumbinis semen*)

Semen volume

Ejaculate volume was measured immediately after collection using a graduated glass pipette. Only complete ejaculates free from contamination were included in the analysis.

Motility (A: 0%<A≤100%)

Progressive motility was assessed under 200× magnification at 2, 3, and 5 h post-collection. These time points were selected to capture the dynamics of motility decline during liquid storage, following approaches reported in turkey semen studies (Izanloo et al. 2021). The percentage of motile spermatozoa was estimated based on forward movement. In addition, sperm motility and kinematic parameters were analyzed using a Computer-Assisted Sperm Analysis (CASA) system (Hamilton Thorne, USA), which provided objective measurements of total motility, progressive motility, straight-line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), straightness (STR), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF).

Sperm concentration

Sperm concentration was measured using an SDM1 sperm densimeter (Minitube, Germany). Each sample was gently mixed and analyzed in triplicate, and the mean value ($\times 10^9$/mL) was used for data analysis.

Abnormal sperm morphology rate

The proportion of abnormal spermatozoa was determined by methylene blue staining. Smears were prepared, fixed by gentle heat, and stained with methylene blue. Slides were examined under 400× magnification (Olympus microscope), and 300-500 spermatozoa were evaluated per sample. The percentage of abnormal morphology was calculated as:

$$K\ (\%) = \frac{n}{N}\ x\ 100$$

Where:

n : Number of abnormal sperm

N : Total sperm counted

Sperm resistance

Sperm resistance (R) was assessed using the chain dilution method described by Milovanov (1962). Briefly, semen samples were serially diluted with a 1% NaCl solution until progressive motility was no longer observed. The resistance value was calculated as:

$$R = r_o + r \times n$$

Where:

R : Sperm resistance

r_o : Initial dilution level

r : Dilution factor for each subsequent addition (r=200)

n : Number of subsequent dilutions with 1% NaCl solution at which sperm maintained forward motility

Sperm agglutination test

Sperm agglutination was evaluated microscopically by mixing semen 1:1 (v/v) with modified Ringer's solution and examining under a phase-contrast microscope at 400× magnification. Spermatozoa adhering by their heads, midpieces, or tails were recorded as agglutinated. For each sample, at least 200 spermatozoa were assessed, and the proportion of agglutinated spermatozoa was calculated as:

$$\text{Agglutination (\%)} = \frac{\text{Number of agglutinated spermatozoa}}{\text{Total spermatozoa counted}}\ x100$$

pH value

Semen pH: Determined using a pH/Ion meter (WINLAB, Japan). Each sample is measured three times, and the average value of the three measurements is recorded.

Osmotic pressure

Osmotic pressure (mOsm/kg) was measured using an osmometer (BKD-30SMC, BIOBASE).

Density

Density (d) was determined using a pycnometer according to standard procedures, calculated as:

$$d = \frac{M}{Mo}$$

Where:

M : Mass of double-distilled water

M_o : Mass of the liquid sample of the same volume

Viscosity

Relative viscosity (η) was measured at 20°C using a micropipette, with calculation based on density and flow time:

$$\eta = \frac{d.t}{do.to}$$

Where:

d and t : The density and flow time of the test liquid

d_o and t_o : The corresponding values for double-distilled water

Buffering capacity

Buffering capacity (β) was determined following Salisbury et al. (1978) using 0.1 N HCl. Briefly, 0.5 mL of the sample was titrated until the pH reached 4.0, and the buffering capacity was calculated as:

$$\beta = \frac{a.n.1000}{dpH.v} x100$$

Where:

a : Volume of acid used (amount of 0.1N HCl)
n : Equivalent weight of the acid
dpH : pH deviation before and after treatment
v : Volume of liquid used

Sperm viability

Sperm viability was determined using the eosin–nigrosin staining technique. Briefly, semen was mixed with eosin–nigrosin solution (1-2%, w/v), smeared, air-dried, and examined under oil immersion at 1000× magnification. For each sample, at least 200 spermatozoa were counted from duplicate or triplicate smears. Live spermatozoa remained unstained, whereas dead spermatozoa showed pink or red staining. Viability (%) was calculated as:

$$\text{Viability (\%)} = \frac{\text{Number of live spermatozoa}}{\text{Total spermatozoa counted}} x100$$

Hypo-osmotic swelling test (HOST)

Plasma membrane integrity was assessed using the Hypo-Osmotic Swelling Test (HOST) with a solution adjusted to 100 mOsm/kg, prepared from sodium citrate and fructose in distilled water. An aliquot of semen was incubated in HOST solution at 37 °C for 30 min, then examined under a phase-contrast microscope (400-1000× magnification). For each sample, at least 200 spermatozoa were evaluated in 2-3 replicates. Spermatozoa exhibiting tail swelling or coiling were classified as HOST-positive, while those with straight tails were HOST-negative. The percentage of HOST-positive spermatozoa was calculated as:

$$\text{HOST-positive (\%)} = \frac{\text{Number of HOST}-\text{positive spermatozoa}}{\text{Total spermatozoa counted}} x100$$

Semen longevity during chilled storage

Semen longevity was evaluated to assess the effect of lotus seed powder supplementation on sperm quality during short-term chilled storage. Immediately after collection, ejaculates were diluted 1:1 (v/v) with modified Ringer's solution and stored at 4°C. Progressive motility was assessed at 0, 6, 12, and 24 h of storage under light microscopy (200× magnification, 37°C stage). For each time point, multiple microscopic fields were examined in duplicate or triplicate, and mean values were used for analysis.

From the motility–time data, longevity indices were derived, including the change in motility from 0-24 h (ΔMotility), average hourly motility decline rate, time to 50% motility (t_{50}), and the area under the curve (AUC, 0-24 h) calculated by the trapezoidal rule.

Statistical analysis

All data were expressed as mean ± Standard Deviation (SD). Parameters measured once (e.g., semen volume, concentration, physicochemical properties, viability, HOST-positive rate) were analyzed using One-Way Analysis of Variance (ANOVA) with dietary treatment as the fixed factor. Progressive motility during chilled storage was analyzed using repeated measures ANOVA, with treatment as the between-subject factor and storage time as the within-subject factor. The motility decline rate (0-24 h) and time to 50% motility (t_{50}) were calculated from motility–time data and compared among treatments using one-way ANOVA. When significant differences were detected, Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparisons. Differences were considered statistically significant at $P<0.05$. Statistical analyses were performed using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). This software version has been widely applied for repeated measures ANOVA in semen quality studies (Sheikholeslami et al. 2020).

RESULTS AND DISCUSSION

Semen quality assessment in Mia roosters fed diets supplemented with lotus seed powder *(Nelumbinis semen)*

Table 1 presents the effects of dietary supplementation with lotus seed powder (*Nelumbinis semen*) on semen quality parameters in Mia roosters. Clear differences were observed among treatments, with most indicators showing significant improvement in the supplemented groups compared with the control ($P<0.05$). Roosters receiving lotus seed powder had higher ejaculate volume, sperm concentration, and kinematic parameters, including VSL, VCL, and STR, compared with controls ($P<0.05$). The 4% supplementation group (T2) consistently showed the best performance. In contrast, ALH and BCF did not differ among treatments, indicating that supplementation mainly enhanced linear motility traits rather than head displacement or beat frequency.

Morphological and functional sperm parameters were also affected by dietary treatment. The proportion of abnormal spermatozoa and the incidence of agglutination decreased significantly in supplemented groups, with the lowest values observed at 4% inclusion. At the same time, sperm resistance was higher in T1 and T2 than in controls, suggesting improved tolerance to osmotic stress. These findings indicate an overall enhancement of structural integrity and functional stability of spermatozoa in roosters receiving lotus seed powder.

The physicochemical properties of semen are shown in Table 2. Osmotic pressure and buffering capacity were significantly increased by lotus seed supplementation, whereas density, viscosity, and pH remained unchanged ($P>0.05$). These results suggest that lotus seed powder improved the extracellular environment of semen without altering its basic physical characteristics.

Functional assessments of semen quality further confirmed the positive effects of supplementation. Sperm viability and membrane integrity, as determined by the HOST, were both significantly higher in T1 and T2 compared with controls, with the greatest improvement again recorded in the 4% group (Table 3).

Table 1. Indicators of semen quality in Mia roosters fed diets supplemented with lotus seed powder (*Nelumbinis semen*) (Mean ± SD)

Parameter	C (0%)	T1 (2%)	T2 (4%)
Volume (V, mL)	0.42±0.05^{c}	0.45±0.06^{b}	0.48±0.05^{a}
VSL (μm/s)	42.35±3.12^{c}	44.87±3.08^{b}	46.25±3.15^{a}
VCL (μm/s)	82.54±4.20^{c}	86.73±4.15^{b}	88.96±4.10^{a}
STR (%)	78.65±2.95^{c}	80.42±3.02^{b}	82.10±2.88^{a}
ALH (μm)	2.42±0.21	2.41±0.19	2.42±0.21
BCF (Hz)	27.04±1.85	27.04±1.81	27.05±1.76
Concentration (C, ×10^{9}/mL)	3.21±0.26^{c}	3.39±0.25^{b}	3.47±0.27^{a}
Abnormal sperm morphology (K, %)	9.84±1.21^{a}	8.92±1.15^{b}	8.15±1.12^{c}
Sperm resistance (R)	2400.74±120.08^{c}	2520.96±115.26^{b}	2580.18±110.15^{a}
Sperm agglutination test (%)	4.62±0.65^{a}	4.08±0.64^{b}	3.75±0.55^{c}

Note: Means with different superscripts in the same row differ significantly (P<0.05). C: Basal diet without lotus seed powder supplementation, T1: Basal diet + 2% lotus seed powder, T2: Basal diet + 4% lotus seed powder

Semen longevity during chilled storage

During chilled storage at 4°C, progressive motility gradually declined in all groups, but the rate of decline was significantly slower in supplemented birds (Table 4). After 24 h of storage, semen from the 4% group maintained the highest motility, while the control group declined most sharply. Derived indices of longevity supported these findings: decline rate was lowest and t_{50} was longest in the T2 group, indicating improved preservation of sperm function (Figures 1 and 2).

Discussion

Dietary supplementation with lotus seed powder improved semen output and concentration in Mia roosters, placing the results in context with earlier findings on indigenous breeds. The volumes recorded in the supplemented groups (0.45-0.48 mL) were above the 0.25 mL average reported by Gordon (2005) for abdominal massage collection and the 0.28 mL observed by Bah et al. (2001) in Nigerian local cocks. These figures also fall within the general range of 0.1-1.5 mL described by Cupps (1977) and the 0.2-0.5 mL reported for White Leghorns (Hafez and Hafez 2000), but they lean toward the higher end. By contrast, smaller ejaculate volumes have been reported in Indonesian local chickens (0.20 mL; Asmarawati et al. 2019) and Naked Neck chickens (0.14-0.22 mL; Abbass et al. 2017). Larger volumes were associated with better sperm quality in Thai native chickens (>0.3 mL; Mussa et al. 2023). Comparable ranges were reported for PB2 × Indigenous and Dahlem Red chickens (0.27-0.29 mL; Kalita et al. 2018) and in the Horasi ecotype (0.52-0.68 mL; Burilo and Kashoma 2023). In a similar study, Nhan et al. (2025) reported that the semen volume in Ho roosters reached 0.59 mL when each bird was supplemented with 30 mL of aqueous cashew nut extract per day. These comparisons suggest that lotus seed supplementation can elevate Mia rooster semen production to levels competitive with other native breeds, which is valuable for maintaining breeding efficiency. Improvements were also evident in sperm motility and kinematic parameters. VSL, VCL, and STR increased significantly in supplemented birds, reflecting more efficient and linear movement of spermatozoa, while ALH and BCF were unaffected. This suggests that lotus seed powder enhances mitochondrial activity and flagellar propulsion, leading to more directed progression without altering the amplitude or frequency of head displacement. CASA-derived motility traits are closely linked to fertilization potential (Ugur et al. 2019), and the improvements observed here hold considerable importance for fertility outcomes. Sperm concentration also increased, which may indicate enhanced spermatogenesis or improved sperm release efficiency. Peters et al. (2008) reported sperm concentrations ranging from 3.11 to 4.21×10^{9}/mL in Nigerian indigenous chickens, values that align with those seen in the present trial. For applied breeding purposes, maintaining adequate sperm density is crucial: Zaniboni et al. (2006) identified ~1.5×10^{9} cells/mL as optimal for cryopreservation, while Ruiz et al. (2024) found that diluted samples with 250×10^{6} spermatozoa per straw preserved motility and viability better than those stored at 500×10^{6}. Nutritional interventions are a key factor in achieving such improvements; for example, Hayanti et al. (2022) demonstrated that vitamin E supplementation enhances sperm counts and semen quality in chickens. The present results suggest that lotus seed powder acts in a comparable way, but with the advantage of being a natural feed additive (Ramazani et al. 2023a).

Sperm morphology has been suggested as one of the most important qualitative parameters for evaluating semen quality in poultry (Ntemka et al. 2016). Morphological traits and functional stability were also positively influenced. The proportion of abnormal sperm decreased in supplemented groups, and sperm agglutination was lower, indicating healthier membranes and reduced cell-to-cell adhesion. These values remain below the 10-15% abnormality threshold generally regarded as acceptable in poultry semen (Garner and Hafez 2008). Comparable observations were made by Hossain et al. (2024), who reported rising head, midpiece, and tail defects in Aseel chicken semen during storage at 4°C, and by Solihati et al. (2006), who noted that prolonged storage increased plasma membrane damage, thereby raising abnormality rates. Tail deformation is recognized as the most frequent morphological abnormality that negatively affects post-thaw fertility in chickens (Feyisa et al. 2018). A low incidence of structural damage and cell death in sperm is essential for maintaining the overall quality and viability of

healthy chicken semen (Gonçalves et al. 2015). The higher sperm resistance values in the supplemented groups provide further evidence of improved tolerance to osmotic stress, suggesting greater resilience of the plasma membrane. According to Marks (1978), packed sperm volume correlates with daily egg mass in hens, linking male reproductive performance with female productivity; thus, enhancements in semen quality may translate into broader benefits for flock fertility.

Table 2. Physicochemical properties of semen in Mia roosters fed diets supplemented with lotus seed powder (*Nelumbinis semen*) (Mean ± SD)

Parameter (unit)	C	T1	T2
Osmotic pressure (mOsm/kg)	326.45±5.82^{c}	329.87±5.21^{b}	332.14±5.55^{a}
Buffering capacity (β)	28.42±1.65^{c}	29.36±1.58^{b}	30.12±1.54^{a}
Density (d, g/mL)	1.03±0.04	1.03±0.04	1.03±0.03
Viscosity (η, relative to H_2O)	1.81±0.06	1.82±0.03	1.81±0.05
pH	7.02±0.05	7.04±0.06	7.04±0.05

Note: Means with different superscripts in the same row differ significantly (P<0.05). C: Basal diet without lotus seed powder supplementation, T1: Basal diet + 2% lotus seed powder, T2: Basal diet + 4% lotus seed powder

Table 3. Functional sperm quality parameters in Mia roosters fed diets supplemented with lotus seed powder (*Nelumbinis semen*) (Mean ± SD)

Parameter	C	T1	T2
Viability (%)	87.46±2.85^{c}	89.62±2.75^{b}	91.03±2.68^{a}
HOST-positive (%)	83.15±3.12^{c}	85.28±2.98^{b}	86.94±2.85^{a}

Note: Means with different superscripts in the same row differ significantly (P<0.05). C: Basal diet without lotus seed powder supplementation, T1: Basal diet + 2% lotus seed powder, T2: Basal diet + 4% lotus seed powder

Table 4. Progressive motility (%) of semen from Mia roosters fed diets supplemented with lotus seed powder (*Nelumbinis semen*) during storage at 4°C (Mean ± SD)

Storage time (h)	C	T1	T2
0	82.32±3.56^{c}	84.70±3.02^{b}	86.20±3.18^{a}
6	75.11±4.29^{c}	79.80±3.52^{b}	81.40±3.77^{a}
12	66.54±5.12^{c}	72.90±4.21^{b}	76.30±4.03^{a}
24	51.81±4.91^{c}	59.60±4.43^{b}	63.20±4.15^{a}

Note: Means with different superscripts in the same row differ significantly (P<0.05). C: Basal diet without lotus seed powder supplementation, T1: Basal diet + 2% lotus seed powder, T2: Basal diet + 4% lotus seed powder

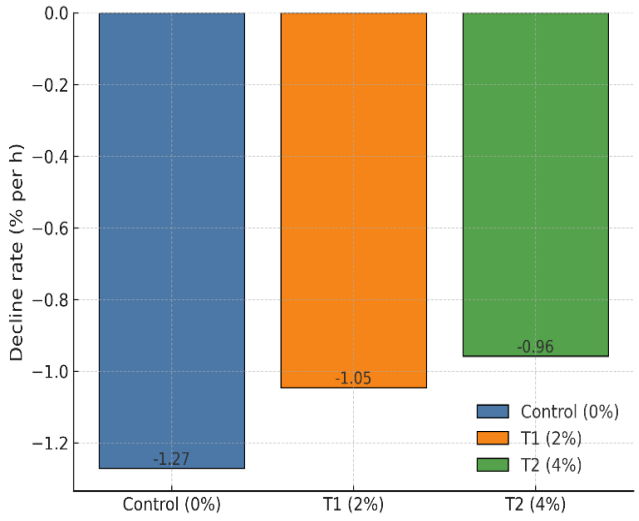

Figure 1. Decline rate of progressive motility during chilled storage at 4°C in semen from Mia roosters fed diets with or without lotus seed powder (*Nelumbinis semen*)

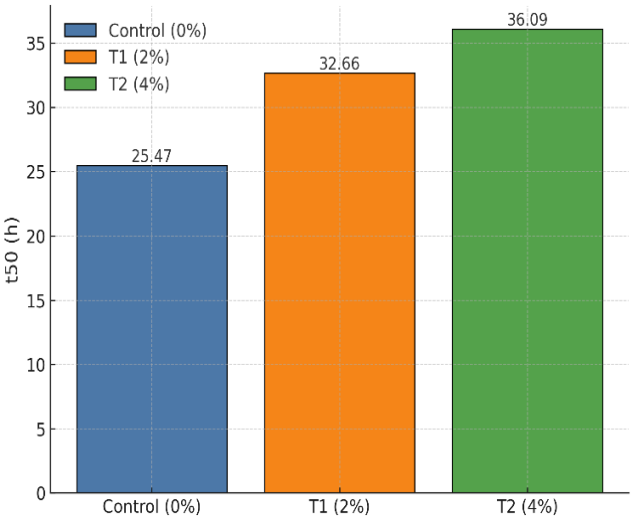

Figure 2. Time to 50% progressive motility (t_{50}) estimated from motility time data using a linear method

The biochemical properties of semen were also altered in favorable ways. Osmotic pressure increased modestly but significantly from 326.45 mOsm/kg in the control to 332.14 mOsm/kg in T2, suggesting a more stable osmotic environment for spermatozoa. Proper osmotic regulation is critical to prevent swelling or shrinkage during handling, which can compromise motility (Ramazani et al. 2023b). Buffering capacity also rose from 28.42 in the control to over 30.0 in T2, counteracting pH fluctuations and protecting enzyme systems that sustain sperm activity. In contrast, density (≈1.03 g/mL), viscosity, and pH (≈7.0) remained unchanged, indicating that lotus seed powder supplementation enhanced biochemical stability without disrupting fundamental physical characteristics of seminal plasma. Functional assessments of viability and membrane integrity provided further support for the beneficial effects of lotus seed supplementation. Viability increased from 87.46% in the control to 91.03% in T2, while HOST-positive sperm rose from 83.15% to 86.94%, consistent with reports that antioxidant supplementation improves sperm viability and membrane integrity (Shakouri et al. 2021). These improvements are of practical significance, as values above 60% are generally required for fertile semen (Lukman et al. 2014). Similar reproductive benefits of lotus have been reported in mammalian studies: Mireille et al. (2017) found that Nymphaea lotus extracts enhanced sperm count, motility, viability, and reproductive organ weights in rats, supporting its role as a natural fertility enhancer. The upward trends observed here indicate a dose-dependent effect, with 4% inclusion providing the greatest benefit.

Finally, semen longevity during chilled storage was enhanced in supplemented groups. Progressive motility declined in all treatments over 24 h, but the rate of decline was slower and t_{50} was longer in birds fed lotus seed powder. T2 maintained the highest motility values after 24 h, showing clear advantages for short-term preservation. Such improvements are crucial in AI programs, where semen is often stored before insemination, and they directly enhance reproductive efficiency.

Taken together, the evidence shows that lotus seed powder supplementation improves semen output, motility, morphology, biochemical stability, and storage capacity in Mia roosters. The likely mechanisms are the antioxidant, anti-inflammatory, and membrane-stabilizing actions of bioactive compounds in lotus seed (Soleimanzadeh et al. 2020), including alkaloids, flavonoids, polysaccharides, glycosides, polyphenols, and triterpenoids (Huang et al. 2009; Zhenjia et al. 2010; Chen et al. 2012). These compounds can reduce oxidative stress, protect membrane phospholipids from peroxidation, and sustain mitochondrial energy production, ultimately preserving sperm quality. The consistency between current findings and previous work in both poultry and mammalian systems (Mireille et al. 2017; Ugur et al. 2019; Hayanti et al. 2022) strengthens the biological plausibility of this interpretation. From a practical perspective, the use of lotus seed powder, particularly at 4% inclusion, offers a natural and sustainable option to improve artificial insemination outcomes and to support conservation of valuable indigenous genetic resources such as the Mia rooster.

Sperm motility is considered a key factor determining male fertility, as it directly influences the ability of sperm to reach and fertilize the ovum (Mohan et al. 2018; Bondarenko and Cosson 2019). Sperm motility is widely recognized as a key index of semen quality (Liu et al. 2023). In this study, the motility of Mia roosters, particularly in the supplemented groups, was consistently high during chilled storage, exceeding several values reported for other breeds. Tesfay et al. (2020) recorded average motility rates of 67.44% in Rhode Island Red and 49.66% in White Leghorn roosters, while Peters et al. (2008) reported a range of 62.55-87.35% in Nigerian indigenous chickens. The higher motility values sustained by supplemented Mia roosters suggest that lotus seed powder helped preserve sperm function more effectively than in many previously studied lines. Blesbois (2018) stated that high sperm motility is essential for achieving high fertility rates in poultry.

The analysis of decline rates further confirmed this benefit. The control group lost motility at an average rate of 1.27% per hour, while T1 and T2 declined at slower rates of 1.05% and 0.96% per hour, respectively. The estimated time to reach 50% motility (t_{50}) was extended from 25.47 h in the control to 32.66 h in T1 and 36.09 h in T2. This prolongation indicates a dose-dependent protective effect of lotus seed supplementation. Such effects are consistent with the antioxidative potential of *Nelumbinis semen*, which may stabilize plasma membranes, protect against lipid peroxidation, and maintain mitochondrial activity essential for sperm motility.

These findings align with previous work highlighting the challenges of semen preservation. Han et al. (2005) reported that freezing and thawing can cause profound changes in sperm motility and morphology, while Sood et al. (2012) emphasized that successful cryopreservation requires optimization of collection, dilution, equilibration, freezing, storage, and thawing steps. Several studies have indicated that the sperm membrane plays a crucial role in determining resistance to cold shock. This resistance is affected by both the chemical composition and temperature variations of the extender, factors that can be useful indicators for predicting the fertility of frozen–thawed sperm (Sun et al. 2021). Similarly, Ehling et al. (2012) noted the importance of semen storage for ex-situ conservation of poultry genetic resources. By extending motility longevity under chilled storage, lotus seed supplementation may provide breeders with greater flexibility in semen handling and transportation, thereby improving the practical use of artificial insemination.

Overall, the data demonstrate that lotus seed powder supplementation slows the decline of progressive motility during cold storage, reduces the rate of motility loss, and extends the functional lifespan of spermatozoa. The improvements were more pronounced at 4% inclusion, suggesting a clear dose-response effect. Similar dose-dependent antioxidant responses have been reported with plant-derived bioactives, such as the protective effects of *Quercus brantii* Lindl. extract against lead-induced oxidative stress in male reproductive systems

(Soleimanzadeh et al. 2018b). These results reinforce the potential role of lotus seed powder as a natural dietary additive to enhance semen preservation, fertility outcomes, and the conservation of valuable genetic resources such as the Mia rooster.

In conclusion, dietary supplementation with lotus seed powder (*Nelumbinis semen*) improved semen volume, motility, concentration, and membrane integrity in Mia roosters. Physicochemical traits such as osmotic pressure and buffering capacity were also enhanced, supporting better sperm preservation. Supplementation slowed motility decline during chilled storage, with the greatest benefits observed at the 4% inclusion level, suggesting a dose-response effect. Future studies should include fertility trials in hens and large-scale artificial insemination applications to validate the long-term reproductive benefits of lotus seed powder supplementation.

ACKNOWLEDGEMENTS

We acknowledge the support of time and facilities from Tay Do University (TDU), Vietnam, for this study.

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