Research Article

Effect of Aqueous Cashew Nut *(Anacardium occidentale)* Extract on Physicochemical Properties and Semen Quality of Ho Roosters

Phan Nhan*, Trinh Thi Hong Mo, Nguyen Thi Chuc

Faculty of Applied Biology, Tay Do University, 68 Tran Chien Street, Cai Rang ward, Can Tho 900000, Vietnam.

Abstract | This study evaluated the effects of aqueous cashew nut *(Anacardium occidentale)* extract supplementation on semen quality and seminal fluid properties in Ho roosters. A total of 18 roosters aged 8 to 12 months were used. Group 1 (control) received no cashew nut extract. Group 2 was administered 15 mL of aqueous cashew nut extract per rooster per day, while Group 3 received 30 mL per rooster per day. The extract was administered orally every morning for 30 consecutive days. Results revealed clear dose-dependent improvements in multiple reproductive traits. Semen volume increased ($p<0.05$) from 0.46 mL in Group 1 to 0.59 mL in Group 3, while sperm concentration rose ($p<0.05$) from 1.89 to 1.97 billion/mL. Mass activity improved markedly ($p<0.05$) from 3.34 to 4.25, and the percentage of abnormal sperm decreased significantly ($p<0.05$) from 16.21% to 8.13%, reflecting enhanced spermatogenesis and cellular integrity. Kinetic parameters also showed positive ($p<0.05$) responses: straightness (STR) increased from 0.42 to 0.52 and amplitude of lateral head displacement (ALH) rose from 17.02 µm to 18.26 µm, indicating improved flagellar propulsion and sperm motility precision. Biochemical traits of the seminal plasma exhibited favorable changes ($p<0.05$) as well; buffering capacity (β) improved from 11.02 to 14.67, and osmotic pressure increased from 271.08 to 287.01 mOsm/kg, suggesting a more stable and protective environment for sperm viability. In brief, aqueous cashew nut extract supplementation significantly enhanced semen quality, motility parameters, and seminal biochemical properties in Ho roosters. These findings support its potential as a natural phytogenic additive to improve fertility in indigenous poultry production systems.

Keywords | Anacardium occidentale, Cashew nut extract, Ho rooster, Semen quality, Buffering capacity, Indigenous poultry, Phytogenic additives

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***Correspondence** | Phan Nhan, Faculty of Applied Biology, Tay Do University, 68 Tran Chien Street, Cai Rang ward, Can Tho 900000, Vietnam; **Email:** pnhan@tdu.edu.vn
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INTRODUCTION

Poultry production plays an essential role in food security and rural livelihoods in many developing countries, including Vietnam. Among native breeds, the Ho rooster represents a valuable genetic resource due to its high meat quality, adaptability to extensive systems, and cultural significance in Northern Vietnam. However, the reproductive efficiency of this indigenous breed remains suboptimal, particularly in artificial insemination programs where semen quality directly affects fertilization success. Characterizing semen quality is essential for identifying causes of subfertility and reduced productivity. It also serves as a predictive tool for male fertility and plays a critical role in enhancing fertilization success in both natural mating and assisted reproductive technologies (Wysokińska, 2022). Semen quality is determined by multiple parameters, including not only sperm motility and concentration but also its physicochemical properties such as pH, viscosity, osmotic pressure, and buffering

capacity. These parameters influence sperm survival and function, and are sensitive to both environmental and nutritional factors. Additionally, artificial insemination serves as a vital tool in poultry breeding, as it facilitates access to superior genetic material, reduces the risk of disease transmission, enhances reproductive management practices, and contributes to improved overall reproductive efficiency (Kharayat *et al*., 2016).

In recent years, plant extracts have emerged as a cheap and natural source of additives to preserve and enhance sperm function during semen storage (Ros-Santaella and Pintus, 2021). Among these, cashew apple extract from *Anacardium occidentale* has been investigated for its antimicrobial properties and possible influence on meat preservation and tissue oxidative stability (Susanti *et al*., 2018; Isfanida *et al*., 2020). Cashew is a commercially significant crop widely cultivated across tropical regions, particularly in various parts of Africa and Asia, where it serves as both an industrial raw material and a major export commodity (Akinhanmi *et al*., 2008). Recently, the extension of cashew nut from human consumption to the feeding of livestock has received attention of scientists as a potential feedstuff (Oddoye *et al*., 2012). While Susanti *et al*. (2018) focused on the inhibitory activity of cashew apple extract on bacterial growth in meat, Isfanida *et al*. (2020) also examined its effects on the physical, chemical, and organoleptic properties of chicken meat, suggesting its broader application in poultry products.

Cashew nut (*Anacardium occidentale*) is known for its rich content of polyphenols, flavonoids, and antioxidants. These bioactive compounds have shown antimicrobial, anti-inflammatory, and free radical scavenging properties, which may contribute to improved testicular function, spermatogenesis, and seminal plasma stability by enhancing cellular protection, reducing oxidative stress, and supporting endocrine function (Akomolafe *et al*., 2022). The selected doses (15 mL and 30 mL/rooster/day) were empirically chosen to explore potential dose-dependent effects, supported by previous findings where dietary inclusion of cashew nut improved reproductive parameters in rodent models (Akomolafe *et al*., 2022).

Despite growing interest in phytogenic additives, there is a lack of empirical data regarding their application in indigenous poultry breeds such as the Ho rooster. Therefore, this study was conducted to investigate the effects of aqueous cashew nut extract on the physicochemical properties and semen quality of Ho roosters. This approach aims to improve reproductive efficiency using a natural, locally available resource that may enhance both sperm quality and seminal environment stability.

MATERIALS AND METHODS

Ethical considerations

Ethical approval for the study was not formally required, as the procedures involved non-invasive semen collection and standard animal husbandry. However, all experimental protocols strictly adhered to the Vietnamese National Technical Regulation on Animal Welfare (QCVN 01-190:2020/BNNPTNT/SĐ1:2021) and followed institutional good practices for animal handling and care. All efforts were made to minimize animal discomfort and stress throughout the study.

Location and time

The experiment was conducted at Dong Loi farm in Phu Huu commune, Can Tho City, Vietnam from July to October 2024. The experimental analysis was conducted at the veterinary practice laboratory, Tay Do University.

Animals and experimental design

The experiment was conducted on 18 Ho roosters, a native Vietnamese breed with distinct genetic, cultural, and economic value. These roosters were originally sourced from Bac Ninh Province and reared at the Dong Loi poultry farm, located in Phu Huu commune, Can Tho City, Vietnam. The roosters were aged between 8 and 12 months and housed individually in cages measuring 60 × 50 × 50 cm under natural lighting and ambient temperature conditions.

The experiment followed a completely randomized design with three treatments, each including six roosters. Specifically, the group 1 (control) received no cashew nut extract, the group 2 received 15 ml/roosters/day, and the group 3 received 30 ml/roosters/day of aqueous cashew nut extract. The extract was administered orally via syringe in the early morning when roosters were fasted, following a water deprivation period of 180 minutes. The experimental period lasted for 30 consecutive days.

Semen was collected using the abdominal massage technique. Ho roosters were ejaculated every three days throughout the experiment. Semen samples were directly collected into 1.5 mL Eppendorf tubes and immediately diluted at a ratio of 1:1 (v/v) using a modified Ringer extender. The extender solution was prepared by dissolving of crystalline white powder consisting of NaCl, $MnSO_4$, $NaHCO_3$, KCl, and $CaCl_2$ in 500 mL of distilled water with continuous stirring until completely dissolved. The solution was filtered through qualitative filter paper (Advantec, diameter 185 mm) and stored at 4°C. Diluted semen samples were promptly transported to the laboratory for subsequent analyses.

Feeds and feeding

Roosters were fed a pelleted basal diet containing 18%

crude protein and 3150 kcal/kg of metabolizable energy. The diet was formulated using locally available ingredients, including broken rice, wheat bran, soybean oil, corn, and fish meal. Calcium and phosphorus levels ranged from 0.4% to 1.0% and 0.5% to 0.8%, respectively. This diet was considered the standard feeding regimen, and aqueous cashew nut extract was supplemented via drinking water according to the treatment groups.

The cashew nut extract was prepared from ripe cashew nuts obtained from reputable commercial sources. After being sun-dried, the nuts were manually shelled and oven-dried at 45°C for 24 hours, then ground into a fine powder. The powder was extracted with hot distilled water at a ratio of 1:10 (w/v) at 85°C for 60 minutes. The resulting extract was filtered through muslin cloth and Whatman No.1 filter paper, transferred to sterile glass bottles, and stored at 4°C. A fresh batch of extract was prepared every three days to ensure biological activity was maintained during use.

Measurements

Semen volume (mL)

Semen volume was measured directly using graduated Eppendorf tubes with a precision of 0.1 mL.

Semen color

Semen color was classified according to the scale described by Peters *et al.* (2008), with scores of 1 for transparent white, 2 for opaque white, and 3 for milky white.

Sperm concentration (billion/mL)

Semen was diluted 1:100 with 3% NaCl solution. A 10 µL aliquot was loaded into a Thoma hemocytometer and observed under 400× magnification. Sperm cells were counted in four corner squares and one central square. The concentration was calculated as:

$$C = N \times 0.005$$

Where C is sperm concentration (million/mL), and N is the number of sperm counted.

Mass activity

Mass activity was evaluated based on the scoring method described by Tarif *et al.* (2013). A drop of undiluted semen was placed on a clean microscope slide (without a cover slip) and examined under 100× and 200× magnification. Activity was scored on a 5-point scale based on the formation of sperm waves:

1. No movement
2. Few spermatozoa moving, no wave formation
3. Mild, slow movement
4. Vigorous movement with moderate vortex formation
5. Intense, rapid movement with dense vortex formation

Motility (%)

The percentage of progressively motile sperm was assessed manually at 200× magnification at 2, 3, and 5 hours post-collection. Motility was recorded based on direct visual observation of forward movement.

Sperm kinetic parameters were further analyzed using a Computer-Assisted Sperm Analysis (CASA) system (Hamilton Thorne, USA). The following parameters were recorded:

- Straight Line Velocity (VSL, µm/s): Velocity measured in a straight path
- Curvilinear Velocity (VCL, µm/s): Velocity along the actual path traveled
- Straightness (STR, %): VSL/VAP ratio, indicating path linearity
- Amplitude of Lateral Head Displacement (ALH, µm): Lateral head movement amplitude
- Beat Cross Frequency (BCF, Hz): Frequency at which the sperm head crosses the average path

Abnormal sperm morphology rate (%)

Morphological abnormalities were determined using methylene blue staining. A drop of semen was placed on a clean dry slide, mixed with several drops of 0.85% NaCl solution, and gently smeared using the edge of another slide. After air-drying, smears were heat-fixed and stained with methylene blue for approximately 10 minutes, then rinsed with clean water. Slides were examined under 400× magnification using an Olympus light microscope. A total of 300-500 spermatozoa were randomly selected and evaluated for head, midpiece, and tail abnormalities. The abnormal morphology rate was calculated as:

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

Where n is the number of abnormal sperm, and N is the total number of sperm evaluated (300-500 cells).

Osmotic pressure (mOsm/kg)

Osmotic pressure was measured using an Osmometer BKD-30SMC (BIOBASE), based on the freezing point depression principle.

Viscosity (η)

Relative viscosity was measured using the micropipette flow method at 20°C. Flow time of the semen and water through the micropipette tip was recorded.

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

Where; η: relative viscosity of semen, d: density of semen, t: flow time of semen, d_0: density of double-distilled water, t_0: flow time of double-distilled water.

Buffering capacity (β)

Buffering capacity was determined by titration with 0.1N HCl according to the method of Salisbury (1978). Each 0.5 mL sample was placed in a clean, neutral 5–10 mL glass vial. The initial pH was measured, followed by incremental addition of 0.1N HCl (n = 3.6) until the pH reached 4.0. The following formula was used:

$$\beta = \frac{a.n.1000}{dpH.v} \times 100$$

Where; β: buffering capacity per 1000 mL of fluid, a: volume of 0.1N HCl added (mL), n: normality factor of the acid (3.6), dpH: pH change before and after titration, v: sample volume (0.5 mL).

Semen pH

Semen pH was measured using a pH/Ion meter (WINLAB, Japan). Each sample was tested in triplicate, and the mean value was used to ensure accuracy.

Statistical analysis

Data were recorded in Microsoft Excel 2016 and analyzed using Minitab 16.0. One-way analysis of variance (ANOVA) was used to compare means. Descriptive statistics were reported as mean ± standard deviation (SD). A significance level of $p < 0.05$ was considered statistically significant. The sample size (n = 6 per group) was determined based on previous studies using similar experimental models in poultry. Although a formal power analysis was not conducted prior to the study, the observed differences among groups were statistically significant, indicating that the sample size was sufficient to detect biologically relevant effects. ANOVA analysis also accounted for variability between treatment groups.

RESULTS AND DISCUSSION

Comparative analysis of semen quality and kinetic parameters among HO roosters supplemented with different levels of cashew nut extract

The data presented in Table 1 reveal that dietary supplementation with aqueous cashew nut extract significantly enhanced semen quality and sperm motility parameters in Ho roosters in a dose-dependent manner, and these effects are likely attributable to the bioactive constituents of cashew, including flavonoids, polyphenols, and trace elements with known antioxidant and reproductive-modulatory effects. Plant extracts have recently emerged as a cheap and natural source of additives to preserve and enhance sperm function during semen storage. Semen quality is the most critical limiting factor influencing the frequency of insemination. Key parameters

Table 1: Semen quality and sperm motion parameters in Ho roosters across treatment groups (Mean±SD).

Item	Group 1	Group 2	Group 3
Semen volume (ml)	0.46±0.09^{c}	0.52±0.06^{b}	0.59±0.02^{a}
Semen color	2.36±0.02^{a}	2.26±0.02^{b}	2.04±0.01^{c}
Sperm concentration (billion/ml)	1.89±0.11^{c}	1.94±0.27^{b}	1.97±0.09^{a}
VSL (µm/s)	18.93±6.04	19.24±4.12	19.97±4.37
VCL (µm/s)	71.02±4.29	71.08±4.04	71.12±4.04
STR (%)	0.42±0.05^{c}	0.48±0.04^{b}	0.52±0.04^{a}
ALH (µm)	17.02±1.14^{c}	17.69±1.05^{b}	18.26±1.32^{a}
BCF (Hz)	0.77±0.11^{c}	0.82±0.06^{b}	0.84±0.06^{a}
Abnormal sperm (%)	16.21±3.17^{a}	10.15±1.12^{b}	8.13±0.24^{c}
Mass activity	3.34±0.05^{c}	4.02±0.11^{b}	4.25±0.05^{a}

^{abc} Mean values within rows with different superscripts are different at $p<0.05$. Abbreviations: VCL: curvilinear velocity (µm/sec); VSL: straight line velocity (µm/sec); STR: straightness (%); ALH: amplitude of lateral head displacement (µm); BCF: beat cross frequency (Hz). Group 1: control, received no cashew nut extract, Group 2: 15 mL of aqueous cashew nut extract per rooster per day, Group 3: 30 mL of aqueous cashew nut extract per rooster per day.

used to assess the quality of rooster semen include semen volume, color, concentration, sperm viability, and motility (Parker *et al.*, 2000). Semen volume increased significantly from 0.46±0.09 mL in Group 1 to 0.59±0.02 mL in Group 3 ($p<0,05$), reflecting not only enhanced secretory capacity of the seminal plasma but also increased fluid contribution from accessory reproductive glands, which is typically influenced by circulating androgen levels and nutrient status. According to Modupe *et al.* (2013), the Hubbard chicken breed yields approximately 0.55 ml of semen per collection. In comparison, Tarif *et al.* (2013) reported that semen volumes for the Sasso, Synthetic, Assel RIR, and White Rock breeds range between 770-800 µl, 530-670 µl, 330-470 µl, and 470-500 µl, respectively. Additionally, Peters *et al.* (2008) found that Nigerian indigenous chickens produce between 0.37 and 0.73 ml of semen per collection. Semen color is considered an important indicator of contamination by extraneous substances such as feces or urine, as noted by Cole and Cupps (1977). The color of semen is positively correlated with its consistency or viscosity. As noted by Wiyanti *et al.* (2013), semen from free-range chickens typically appears milky white or slightly creamy in color. This creamy appearance is primarily attributed to a high concentration of spermatozoa, which contributes to the semen's opacity and density. Semen color transitioned from more opaque in Group 1 (2.36±0.02) to milky white in Group 3 (2.04±0.01), which aligns with improved sperm cell density and homogeneity, confirming the visual correspondence between semen opacity and sperm concentration. In roosters, sperm quality traits including sperm metabolism, semen concentration, sperm

motility, and the proportion of abnormal or dead sperm cells play a crucial role in determining fertility potential (Chambers, 1990). This was supported by a significant rise in sperm concentration from 1.89±0.11 to 1.97±0.09 billion/mL, indicating stimulation of spermatogenesis, possibly via enhancement of Sertoli cell activity and regulation of spermiogenesis by polyphenolic compounds with proven effects on testicular histophysiology. However, this value still falls within the normal physiological range, as reported by Zong *et al.* (2023), who documented that sperm concentration in chickens typically varies between 0.03 and 0.11 billion sperm cells per milliliter of semen. Peters *et al.* (2008) reported that the sperm concentration in several indigenous Nigerian chicken breeds ranged from 3.11 to 4.21 billion spermatozoa per milliliter of semen. In contrast, the Malaysian dwarf chicken breed exhibited a significantly lower sperm concentration, averaging 1.83 billion spermatozoa per milliliter, as documented by Malik *et al.* (2013). Based on the findings of Mavi *et al.* (2019), variations were observed among different rooster strains in terms of sperm concentration, motility, viability, fertility, as well as membrane and acrosome integrity. The development of computer-assisted sperm analysis (CASA) systems has been driven by advancements in microscope image capture technology, substantial improvements in computational power, the miniaturization of computers, the emergence of new programming languages, and the refinement and expansion of software algorithms (Amann and Waberski, 2014). It ensures accurate and rapid assessment of various semen parameters, including total and progressive motility, sperm movement patterns, linearity, beat cross frequency, amplitude of lateral head displacement, and multiple velocity-related measurements (Svoradová *et al.*, 2019). Simultaneously, Dumpala *et al.* (2006) similarly emphasized that undiluted semen, once collected, remains viable for only about one hour. Even when preserved in liquid form, sperm longevity remains limited, typically sustaining viability for only 3 to 6 hours. Although VSL and VCL did not differ statistically among groups, their slight increase (VSL: 18.93→19.97 μm/s; VCL: 71.02→71.12 μm/s) suggests subtle improvement in energy utilization efficiency and mitochondrial output. STR showed a pronounced increase (from 0.42±0.05 to 0.52±0.04, $p<0.05$), indicating enhanced trajectory linearity and stability of sperm movement, which is a direct marker of fertilizing ability. ALH rose significantly from 17.02±1.14 to 18.26±1.32 μm ($p<0.05$), suggesting that the treated spermatozoa developed greater flagellar amplitude, likely linked to increased axonemal activity and membrane fluidity influenced by dietary antioxidants. BCF followed a similar pattern, increasing from 0.77±0.11 to 0.84±0.06 Hz ($p<0.05$), indicating enhanced beat frequency of the sperm head, a measure strongly correlated with membrane potential and motility vigor. Notably, the proportion of abnormal spermatozoa declined substantially across the treatments, from 16.21±3.17% in Group 1 to 8.13±0.24% in Group 3 ($p<0.05$), a reduction likely mediated by reduced oxidative stress and improved chromatin condensation during spermiogenesis, thereby confirming the cytoprotective role of phenolic-rich plant extracts in male reproduction. In the present study, the supplementation of cashew nut extract resulted in better sperm motility compared to the findings of Al-Daraji (2012), who reported that the motility and viability of White Leghorn rooster sperm reached approximately 60 percent after 72 hours of storage when olive oil was added to a fructose-containing semen diluent. Siudzińska and Łukaszewicz (2008) investigated the semen quality of four chicken breeds following storage durations of 6 and 24 hours. The study revealed a consistent increase in the proportion of morphologically abnormal sperm over time. Specifically, abnormal sperm percentages rose from 52.4% to 61.8% in Green-Legged Partridge, 56.8% to 64.0% in Black Minorca, 59.7% to 77.4% in White Crested Black Polish, and 61.8% to 71.2% in Italian Partridge. In parallel, Blesbois *et al.* (2008) reported a decline in sperm viability and the proportion of sperm with normal morphology during storage, accompanied by increases in both sperm mortality and morphological abnormalities. Semen analysis focusing solely on sperm concentration, viability, motility, and morphology is insufficient for accurately assessing reproductive potential and predicting pregnancy outcomes. A more comprehensive evaluation should also include microscopic characteristics of the semen (Shamsi *et al.*, 2011). Finally, mass activity, which reflects the synchrony and vigor of overall sperm movement, rose from 3.34±0.05 to 4.25±0.05 ($p<0.05$), suggesting enhanced motility dynamics at the ejaculate level and improved seminal plasma conditions, possibly through regulation of ionic balance and membrane stabilization. Microscopic evaluations indicated a mass motility rating of 3^{+}, with variations in sperm abnormalities across breeds, emphasizing the need for effective collection techniques (Udrayana *et al.*, 2023). Altogether, the observed improvements across seminal parameters suggest that cashew nut extract exerts a multifaceted reproductive benefit by modulating endocrine responses, enhancing testicular output, stabilizing sperm membranes, and reducing structural anomalies, thereby offering promising potential as a phytogenic fertili.

Physicochemical characteristics of semen in Ho roosters supplemented with different levels of cashew nut extract

As shown in Table 2, the physicochemical properties of semen in Ho roosters responded clearly to dietary supplementation with aqueous cashew nut extract, reflecting alterations in the biochemical and ionic

composition of seminal plasma that contribute to sperm function and viability. Osmotic pressure increased significantly from 271.08±11.28 mOsm/kg in the control group to 287.01±9.24 mOsm/kg in the high-dose group ($p<0.05$), suggesting enhanced solute concentration and improved electrolyte balance in seminal plasma. This shift, while remaining within the physiological threshold, may favor the osmotic stability of spermatozoa by reducing cellular swelling or shrinkage during extracellular fluid interaction, thus maintaining membrane integrity and acrosomal viability. Viscosity, a parameter indicative of fluid resistance and seminal flow characteristics, remained stable across all groups (1.35±0.05 to 1.34±0.02), implying that the extract did not negatively affect fluidity or hinder sperm motility by increasing seminal plasma thickness. The most notable improvement was observed in buffering capacity, which rose significantly from 11.02±0.09 in Group 1 to 14.67±0.07 in Group 3 ($p<0.05$), indicating greater resistance to pH fluctuations and better protection of spermatozoa against acidic microenvironments, particularly during storage or prolonged in vivo transit. This enhancement may result from the presence of bicarbonate-like ions or polyphenolic compounds in the extract that support bicarbonate-buffered systems, which are known to activate motility and stabilize internal pH in avian sperm. Although semen pH did not differ significantly between groups, it exhibited a slight upward trend from 6.82±0.14 to 6.91±0.05, suggesting a shift toward a more neutral environment that is generally optimal for sperm metabolic activity and flagellar function. According to Peters *et al.* (2008), the semen pH of Nigerian chickens was reported at 7.54. In comparison, Modupe *et al.* (2013) observed a pH of 7.4 in Hubbard chickens. For indigenous chicken breeds, semen pH values ranged from 7.01 to 7.04, indicating slightly more acidic conditions relative to commercial or exotic breeds. This marginal increase, combined with improved buffering capacity, could help maintain enzyme activity and membrane potential during the critical post-ejaculatory phase. Chakraborty and Saha (2022) highlighted that semen pH plays a critical role in determining sperm motility. Variations in pH are primarily attributed to the metabolic activity of spermatozoa, particularly through the production of lactic acid. According to Nechipurenko *et al.* (2021), elevated lactic acid levels result in decreased semen pH, which is closely associated with the reduced viability of spermatozoa once they exit the body. A lowered pH can disrupt the osmotic balance within the seminal plasma, adversely affecting the permeability of the sperm cell membrane and increasing cellular damage ultimately impairing motility. Pimprasert *et al.* (2023) further emphasized that excessively acidic semen environments significantly elevate sperm mortality, underscoring the vital role of pH in sustaining sperm viability. Overall, these findings suggest that cashew nut extract contributes to a more stable and supportive seminal environment by enhancing osmotic equilibrium, pH buffering, and biochemical resilience, which collectively support improved sperm longevity, structural stability, and functional competence. The activity of several natural antioxidants, such as catalase and superoxide dismutase, was reported to decrease during semen storage (Khaeruddin *et al.*, 2015). Moreover, Fernandez-Novo *et al.* (2021) reported that semen quality is influenced by various factors including ambient temperature, age, sexual activity, and genetic background. Expanding on this, Abah *et al.* (2023) highlighted that both sperm quality and quantity are shaped by a combination of genetic makeup, age, nutrition, environmental temperature, ejaculation frequency, libido, physical and physiological conditions, transportation stress, testicular size, health status, and the specific breed of livestock.

Table 2: Semen physicochemical traits of Ho roosters across treatment groups (Mean±SD).

Item	Group 1	Group 2	Group 3
Osmotic pressure (mOsm/kg)	271.08±11.28^{c}	282.94±10.15^{b}	287.01±9.24^{a}
Viscosity (η)	1.35±0.05	1.34±0.11	1.34±0.02
Buffering capacity (β)	11.02±0.09^{c}	13.26±0.14^{b}	14.67±0.07^{a}
Semen pH	6.82±0.14	6.82±0.09	6.91±0.05

^{abc} Mean values within rows with different superscripts are different at $p<0.05$. Group 1: control, received no cashew nut extract, Group 2: 15 mL of aqueous cashew nut extract per rooster per day, Group 3: 30 mL of aqueous cashew nut extract per rooster per day.

CONCLUSION

This study demonstrates that dietary supplementation with aqueous cashew nut extract has a positive impact on the reproductive performance of Ho roosters. The extract improved semen quality, sperm motility characteristics, and key biochemical traits of seminal plasma. These effects suggest that cashew nut extract may serve as an effective phytogenic additive to enhance fertility and reproductive efficiency in indigenous poultry production systems.

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NOVELTY STATEMENT

This study is the first to demonstrate the dose-dependent effects of aqueous cashew nut (*Anacardium occidentale*) extract on semen quality and seminal plasma physicochemi-

cal traits in Ho roosters. The findings highlight the potential of cashew nut extract as a natural phytogenic additive to improve reproductive efficiency in indigenous poultry production systems.

AUTHOR'S CONTRIBUTION

Phan Nhan: Conceived, designed, and analyzed the data. Trinh Thi Hong Mo: Wrote the draft, and performed the experiments. Nguyen Thi Chuc: Resources, software and Methodology. All authors reviewed and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors confirm that the manuscript was prepared under the authors' responsibility, and any generative AI or AI-assisted tools, if used, were only for language improvement. The authors take full responsibility for the content.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abah KO, Fontbonne A, Partyka A and Nizanski W (2023). Effect of male age on semen quality in domestic animals: Potential for advanced functional and translational research? Vet. Res. Commun., https://doi.org/10.1007/s11259-023-10159-1

Akinhanmi TF, Akintokun PO and Atasie NV (2008). Chemical composition and physicochemical properties of cashew nut (*Anarcadium occidentale*) oil and cashew nut shell liquid. J. Agric. Food Environ. Sci., 2(1): 1–10.

Akomolafe SF, Oyeleye SI, Oboh G (2022). Effect of cashew (*Anacardium occidentale* L.) nut-supplemented diet on steroidogenic enzymes, hormonal and oxidative imbalances, and sperm parameters in cisplatin-induced reproductive toxicity in male rats. J. Food Biochem., 46(7): e14100. https://doi.org/10.1111/jfbc.14100

Al-Daraji HJ (2012). Adding olive oil to rooster semen diluents for improving semen quality and storage ability during liquid storage. Baltic J. Compar. Clin. Syst. Biol., 2: 3-11.

Amann RP, Waberski D (2014). Computer-assisted sperm analysis (Casa): Capabilities and potential developments. Theriogenology, 81(1): 5–17. e13. https://doi.org/10.1016/j.theriogenology.2013.09.004

Blesbois E, Grasseau I, Seigneurin F, Mignon Grasteau S, Saint Jalme M, Mialon-Richard MM (2008). Predictors of success of semen cryopreservation in chickens. Theriogenology, 69(2): 252-261. https://doi.org/10.1016/j.theriogenology.2007.09.019

Chakraborty S, Saha S (2022). Understanding sperm motility mechanisms and the implication of sperm surface molecules in promoting motility. Middle East Fertil. Soc. J., 27(1): 4. https://doi.org/10.1186/s43043-022-00094-7

Chambers JR (1990). Genetics of growth and meat production in chickens. In: Poultry breeding and genetics, edited by R.D. Crawford, Amsterdam: Elsevier Science. pp. 599–644.

Cole HH, Cups PT (1977). Reproduction in domestic animals. 3rd Edition. New York Academic press, pp. 195.

Dumpala PR, Parker HM, McDaniel CD (2006). The sperm quality index from fresh semen predicts chicken semen quality after storage. Int. J. Poult. Sci., 5(9): 850-855. https://doi.org/10.3923/ijps.2006.850.855

Fernandez-Novo A, Santos-Lopez S, Barrajon-Masa C, Mozas P, De Mercado E, Caceres E, Garrafa A, Gonzalez-Martin JV, PerezVillalobos N, Oliet A, Astiz S, PerezGarnelo SS (2021). Effects of extender type, storage time, and temperature on bull semen parameters. Biology, 10(7): 630. https://doi.org/10.3390/biology10070630

Isfanida PK, Susanti S, Bintoro VP, Abduh SBM (2020). Pengaruh penggunaan ekstrak buah semu jambu monyet (*Anacardium occidentale* L.) terhadap karakteristik fisik, kimia dan organoleptik daging ayam kampung. J. Teknol. Pangan., 4(2): 103–109.

Khaeruddin SC, Darwati S, Arifiantini RI (2015). The use of extract virgin olive oil to semen extender on local chicken spermatozoa quality. J. Ilmu Prod. Teknol. Hasil Petern., 3(1): 46-51. https://doi.org/10.29244/3.1.46-51

Kharayat N, Chaudhary G, Katiyar R, Balmurugan B, Patel M, Uniyal S, Raza M, Mishra G (2016). Significance of artificial insemination in poultry. Res. Rev. J. Vet. Sci. Technol., 5(1): 1–5.

Malik A, Haron AW, Yusoff R, Nesa M, Bukar M and Kasim A (2013). Evaluation of the ejaculate quality of the red jungle fowl, domestic chicken, and bantam chicken in Malaysia. Turk. J. Vet. Anim. Sci., 37: 564-568. https://doi.org/10.3906/vet-1107-26

Mavi GK, Dubey P, Cheema RS, Dash S, Bansal B (2019). Comparative analysis of semen quality parameters and their relationship with fertility in different genetic groups of layer chicken. Indian J. Anim. Res., 53(10): 1269–1274. https://doi.org/10.18805/ijar.B-3646

Modupe O, Livinus AC, Ifeanyi NB (2013). Semen quality characteristics and effect of mating ratio on reproductive performance of Hubbard broiler breeders. J. Agric. Sci., 5(1): 154-158. https://doi.org/10.5539/jas.v5n1p154

Nechipurenko YD, Semyonov DA, Lavrinenko IA, Lagutkin DA, Generalov EA, Zaitceva AY, Matveeva OV and Yegorov YE (2021). The role of acidosis in the pathogenesis of severe forms of COVID-19. Biology, 10(9): 852. https://doi.org/10.3390/biology10090852

Oddoye EOK, Agyente-Badu IK, Johnson V (2012). Broiler chicks performance on finisher diets containing different levels of reject cashew kernels. Agric. Sci. Res. J., 2(4): 154-158.

Parker H, Yeatman J, Schultz C, Zumwalt C, Mcdaniel C (2000). Use of a sperm analyzer for evaluating broiler breeder males 2. Selection of young broiler breeder roosters for the sperm quality index increases fertile egg production. Poult. Sci., 79(5): 771–777. https://doi.org/10.1093/ps/79.5.771

Peters SO, Shoyebo BM, Ilori MO, Ozoje CON, Ikeobi, Adebambo (2008). Semen quality traits of seven strain of chickens raised in the humid tropics. Int. J. Poult. Sci., 7(10): 949-953. https://doi.org/10.3923/ijps.2008.949.953

Pimprasert M, Kheawkanha T, Boonkum W, Chankitisakul V (2023). Influence of semen collection frequency and seasonal variations on fresh and frozen semen quality in Thai native roosters. Animals, 13(4): 573. https://doi.org/10.3390/ani13040573

Ros-Santaella JL, Pintus E (2021). Plant extracts as alternative additives for sperm preservation. Antioxidants, 10(5): 772. https://doi.org/10.3390/antiox10050772

Salisbury GWW (1978). Physiology of reproduction and artificial insemination of cattle. Second Edition, Fracisco. WWH Framan and Company.

Shamsi MB, Imam SN, Dada R, Genetics (2011). Sperm DNA integrity assays: Diagnostic and prognostic challenges and implications in management of infertility. J. Assist. Reprod., 28(11): 1073–1085. https://doi.org/10.1007/s10815-011-9631-8

Siudzińska A, Łukaszewicz E (2008). Effect of semen extenders and storage time on sperm morphology of four chicken breeds. J. Appl. Poult. Res., 17(1): 101-108. https://doi.org/10.3382/japr.2007-00048

Susanti S, Setiani BE, Rizqiati H, Febriandi DR, Bintoro VP (2018). Inhibitory activity of cashew apple (*Anacardium occidentale*) extract marinade on the meat total bacteria. Curr. Res. Nutr. Food Sci. J., 6(1): 791–799. https://doi.org/10.12944/CRNFSJ.6.1.11

Svoradová A, Baláži A, Vašíček J, Hrnčár C, Chrenek P (2019). Quality evaluation of fresh gander semen of slovak white goose by casa and flow cytometry. Slovak J. Anim. Sci., 52(2): 90–94.

Tarif AMM, Bhuiyan MMU, Ferdousy RN, Juyena NS, Mollah MBR (2013). Evaluation of semen quality among four chicken lines. J. Agric. Vet. Sci., 6(5): 7-13. https://doi.org/10.9790/2380-0650713

Udrayana SB, Kasianto K, Iswati I, Bintari IG (2023). Evaluating sperm quality characteristics obtained through the female teaser method in native chicken breeds. Livest. Anim. Res., https://doi.org/10.20961/lar.v21i3.66495

Wiyanti DC, Isnaini N, Trisunuwati P (2013). Pengaruh lama simpan semen dalam pengencer nacl fisiologis pada suhu kamar terhadap kualitas spermatozoa ayam kampung (*Gallus domesticus*). Jurnal Kedokteran Hewan, Indones. J. Vet. Sci., 7(1). https://doi.org/10.21157/j.ked.hewan.v7i1.566

Wysokinska A (2022). Animal reproduction: Semen quality assessment. Accessed January 2, 2023. https://www.mdpi.com/journal/animals/special_issues/Animal_Reproduction_Semen_ Quality_Assessment. https://doi.org/10.3390/ani12212905

Zong Y, Li Y, Sun Y, Mehaisen GMK, Ma T, Chen J (2023). Chicken sperm cryopreservation: Review of techniques, freezing damage, and freezability mechanisms. Agriculture, 13(2): 445. https://doi.org/10.3390/agriculture13020445