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Impact of *Moringa oleifera* leaf meal and leaf extract on semen quality of Nigerian indigenous normal-feathered chickens

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ABSTRACT. This study aimed to evaluate the effects of Moringa oleifera leaf meal and its aqueous extract on the semen quality of Nigerian Indigenous Normal Feathered Cocks (NINFC). A total of 375 birds were divided into five treatments, each receiving different levels of Moringa oleifera supplementation in their diets and extenders. Semen quality parameters such as sperm concentration, motility, viability, and lipid peroxidation were assessed. The results showed that Moringa oleifera supplementation improved sperm motility and viability, with the 15% inclusion level yielding the highest sperm viability and motility during cold storage (5°C). Additionally, the antioxidant properties of Moringa significantly reduced lipid peroxidation, especially at the 10% and 25% inclusion levels. The findings suggest that Moringa oleifera leaf meal, due to its rich nutritional composition and antioxidant benefits, holds promise as a dietary supplement for enhancing reproductive efficiency and semen preservation in indigenous poultry breeds. This has implications for improving the productivity of indigenous chickens in developing regions.

Keywords: Moringa oleifera; semen quality; sperm motility; sperm viability; antioxidant properties; cold storage.

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Introduction

Poultry production plays a crucial role in developing countries, significantly contributing to food security, economic empowerment, and poverty alleviation (Wong et al., 2017). Indigenous chicken breeds are particularly important in rural communities due to their adaptability, ability to scavenge for feed, and resistance to diseases (Mengesha, 2012). These birds thrive in environments where exotic breeds struggle, making them vital to local food systems (Wong et al., 2017). They provide meat and eggs, which are important sources of protein, and they serve as a financial resource when sold in markets (Mengesha, 2012; Food and Agriculture Organization [FAO], 2020). Despite their importance, the productivity of indigenous chickens remains limited, largely due to factors such as poor genetic potential, inadequate feed, and frequent disease outbreaks (Kariuki et al., 2022). Improving the reproductive efficiency of these chickens could greatly enhance their contribution to rural livelihoods and food security.

A critical aspect of poultry reproduction is the quality of semen produced by cocks, which directly influences fertility rates and the hatchability of eggs (King'ori, 2011; Abioja et al., 2022). Therefore, evaluating and improving semen quality is essential for breeding programs aimed at enhancing the productivity of indigenous poultry (Abioja et al., 2022). However, research on improving the reproductive potential of indigenous chickens, particularly through nutritional interventions, has been limited (Tesfay et al., 2020). Addressing this gap could help increase the overall productivity of these birds, which are economically, nutritionally, and culturally significant in many regions.

However, Moringa oleifera which is often referred to as the 'miracle tree,' has attracted considerable attention due to its rich nutritional profile and medicinal properties (Gopalakrishnan et al., 2016). Moringa leaves are particularly noted for their high content of essential nutrients such as vitamins, minerals, and antioxidants (Islam et al., 2021; Peñalver et al., 2022). These nutrients not only benefit overall health but also play a role in reproductive processes. Antioxidants, in particular, help maintain sperm quality by protecting sperm cells from oxidative damage, which can impair reproductive efficiency (Srivastava et al., 2023). Despite its well-documented benefits, the potential of Moringa to improve semen quality in poultry remains largely unexplored, particularly in Nigerian indigenous chicken breeds.

Given the increasing focus on sustainable agricultural practices, using natural plant like Moringa as supplement could offer a promising solution for improving poultry productivity. This study aims to assess the impact of Moringa oleifera leaf meal and its aqueous extract on the semen quality of Nigerian indigenous normal-feathered chickens. Through the exploration Moringa's potential as a dietary supplement and as an antioxidant component in semen extenders, this research seeks to provide valuable insights into improving the reproductive efficiency of indigenous poultry, ultimately contributing to enhanced food security and rural development.

Materials and methods

Description of the environment

This study was carried out at the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. It is located within the rainforest zone of Southwestern Nigeria at latitude 7^0 13', 49º 46'N, longitude 3º 26', 11º 98'E and altitude 76mm above sea. The climate was humid with a mean annual rainfall of 1037mm. The annual mean temperature and humidity were 34ºC and 82%, respectively (www.nimet.gov.ng).

Experimental birds and management

A total number of 375 Nigerian Indigenous Normal feathered chicks (NINFC) were brooded for twenty one (21) days. The NINFC birds were randomly allotted to five (5) treatments of seventy five (75) birds per treatment. Each treatment was replicated three times with twenty five birds per replicate. Each of the treatment was fed with one of the experimental diets. The birds were raised under an adequately ventilated deep litter system up to sixteen (16) weeks of age using wood shavings as bedding material. The birds were transferred into battery cage at 16 weeks of age until the end of the experiment.

Experimental treatment

The two groups of treatments Moringa leave Meal (MLM) and Moringa olifera leave extract (MOLE) used are explained further below.

Moringa leave meal (MLM)

Moringa oleifera leaves were oven dried at 40℃ to produce Moringa leave meal (MLM) and the MLM were included in the feed at one gram per kilogram of feed at starter and grower phases.

Moringa olifera leave extract (MOLE)

Preparation of aqueous extract was done by extracting the *Moringa oleifera* leaves in distilled water (500 g 1000 mL^{-1}), centrifuged at 120 rpm for 30 minutes to get rid of unwanted debris. The supernatant was collected as the extract and stored in a refrigerator (5℃) before use. *Moringa oleifera* leaf extract (MOLE) were the experimental treatments that were in the water at the following level:

- Treatment one (G1/T1); 0 g kg^{-1} MLM, and 0 mL 100 L^{-1} MOLE.
- Treatment two (G2); 200 mg kg^{-1} MLM
- Treatment Three (G3); 400 mg kg^{-1} MLM
- Treatment four (T2); 20 mL 100 L^{-1} MOLE
- Treatment five (T3); 40 mL 100 L^{-1} MOLE

Chicks in G2 and G3 were fed with two levels of *Moringa* leaves meal (200 mg kg^{-1} and 400 mg kg^{-1}) respectively which was included in the experimental diet. Chicks in T2 and T3 were given (orally) with aqueous *Moringa* extract 20 mL and 40 mL extract per 100 liters of water respectively, while chicks in G1 were given water without moringa extract inclusion (control group). Table 1 shows the feed composition of the starter phase while Table 2 shows grower phases.

Semen quality

Cocks of NINFC in treatment G1, G2, G3, T2 and T3 were trained for artificial semen collection for three weeks starting from 20 weeks old. Semen collection from NINFC cocks was accomplished by abdominal

massage technique as described by Adenaike et al. (2016). Blood stained and contaminated semen was discarded. The semen quality was determined on the semen volume, sperm concentration, sperm motility and sperm viability.

Table 1. Composition (%) of the experimental Ration for starter.

Ingredients	Percentage
Maize	40.00
Fish meal	2.00
Soyabean meal	18.00
Palmkernel	10.00
Wheatoffals	25.00
Bone meal	2.00
Oyster shell	2.00
Lysine	0.25
Methionine	0.25
*Vit/mineral premix	0.25
Salt	0.25
Total	100
Calculated analysis (%)	
Crude protein	18.71
Ether Extract	5.09
Crude fibre	4.56
Ash	3.58
Calcium	1.62
Phosphorus	0.93
Lysine	0.73
Methionine	0.28
Energy (MJ/Kg)	10.32

*Vit. / Min. Premix contains B_1, 1 g; B_2, 6 g; B_{12}, 0.02 g; K_3, 3 g; E; 30 g; biotin, 0.05 g; folic acid, 1.5; choline chloride, 250 g; nicotinic acid, 30 g; Ca-Pantothenate, 15 g; Co, 0.4; Cu, 8 g; Fe, 32 g; I, 0.8 g; Zn, 40 g; Mn, 64 g; Se, 0.16 g, BHT, 5 g.

Table 2. Composition (%) of the experimental Ration for grower.

Ingredients	Percentage
Maize	50.00
Soybean meal	12.00
Wheat offal	33.00
Bone meal	2.00
Oyster shell	2.00
Methionine	0.25
*Vit/mineral premix	0.25
Salt	0.25
Total	100.00
Calculated analysis (%)	
Crude protein	15.09
Ether Extract	4.95
Crude fibre	4.22
Ash	3.60
Calcium	1.87
Phosphorus	0.97
Lysine	0.62
Methionine	0.24
Energy (MJ/Kg)	10.17

*Vit. / Min. Premix contains Vit. A, 10 000 000iu; D_3 2 000 000iu ; E, 12 500iu ; K, 1.30 g; B_2 4.00 g; D Calcium-Pantothenate, 1.30 g; B6, 0.01 g; nicotinic acid, 15.00 g; folic acid, 0.05 g; biotin, 0.02 g; Cu, 5.00 g; Fe, 25.00 g; I, 0.06 g; Mn, 48.00 g; Se, 0.10 g; Zn, 45.00 g; choline chloride, 200.00 g; BHT , 50.00 g.

Sperm concentration

This was evaluated using photometer. Two mL of the semen is placed in a cuvette and inserted into the photometer. The photometer measures absorbance (optical density) at a 546 nm wavelength.

Sperm motility

Sperm motility was accessed using digital microscope (Celestron Penta® LCD) viewed at X400 magnification, with a warm stage maintained at 37ºC. Semen was thawed at 37ºC for 3 minutes. A semen mount was made using 5µl semen and the semen was placed directly on a microscope slide and it was covered

using cover slide. For each sample, five microscopic fields were examined two times to observe sperm cells that are progressively moving in a straight line.

Sperm viability

This was done by placing a drop of semen on a glass slide, mixed with one drop of eosin-nigrosin smears, and observed under light microscope. The unstained cells represent the live cells while the stained cells represented the dead cells.

Semen dilution and storage

Semen pooled from cocks of NINFC in treatment G1 only, was diluted using Tris-egg yolkbased extender consisting of Tris (3.61 g), citric acid (2.02 g), glucose (1.49 g), streptomycin (0.28 g), egg yolk (20 mL), and distilled water was added to make it up to 100 mL as control (0). The extender was supplemented each with Moringa leaf extract at 10, 15, 20, 25, 50% / 100 mL respectively. Only ejaculates of > 70% motility, > 0.2 mL volume was pooled. This was done for uniformity and to maximize individual semen differences. Following dilution, the semen was dispensed into 10 mL tubes sealed and gradually chilled to 5°C for 72 hours.

Semen Evaluation

Semen evaluation was carried out at 24, 48 and 72 hours on the sperm motility, sperm viability and lipid peroxidation of the sperm cells.

Statistical analysis for semen quality

Completely randomized design was used. Data for semen quality were subjected to analysis of variance using SAS 2000. Means that were significantly different were separated using least squares differences (lsd). The model is shown below:

$Y_{ij} = \mu + T_i + e_{ij}$

Y_{ij} = observed values measured
μ = population mean
T_i = effect of *moringa* as treatment on observed values measured.
e_{ijk} = residual error.

Statistical analyses for semen preservation and storage

Randomized complete block design was used. The data collected for semen preservation were subjected to repeated measurement analysis using storage as a subject. The model is shown below:

$Y_{ijk} = \mu + T_i + H_j + (TH)_{ij} + e_{ijk}$

Y_{ik} = observed values measured
μ = population mean
T_i = i^{th} effect of inclusion level of moringa extract as treatment on observed values measured.
H_{ij} = jth effect of storage duration on observed values measured.
$(TH)_{ij}$ = effects of interaction inclusion level of moringa leaf extract and storage duration.
e_{ijk}= residual error.

Results and discussion

Proximate analysis of moringa oleifera leaf meal

The result for the proximate analysis of Moringa oleifera leaf meal is shown on Table 3 below. The proximate composition of Moringa oleifera leaf meal (MLM) found in this study aligns with previous research that highlights its rich nutritional profile, particularly its high crude protein content of 30.63%. Moringa's protein content is often emphasized as one of its most significant advantages, supporting its use in animal nutrition. According to Abbas (2013), Moringa leaves contain substantial amounts of protein, making them suitable for improving the nutritional quality of poultry diets. The high protein content found in this study suggests that MLM can be a valuable protein supplement in poultry feeds, enhancing growth and reproductive performance which resonates the findings of Marappan et al. (2024).

Table 3. Proximate composition of *Moringa oleifera* leaf (% Dry matter basis).

Fraction	composition
Dry matter (%)	98.33
Ash (%)	6.67
Ether extract	3.00
Crude fibre (%)	36.67
Crude protein (%)	30.63
Neutral detergent fibre (%)	30.00
Acid detergent fibre (%)	11.33
ADL (%)	2.00
Hemicellulose	18.67
Cellulose	9.33

ADL: Average Droplet Length.

Furthermore, the ash content of 6.67% reflects the mineral composition of Moringa, which is consistent with findings from Gopalakrishnan et al. (2016) and Srivastava et al. (2023) that report Moringa leaves as rich sources of essential minerals like calcium, potassium, and magnesium. These minerals play a crucial role in maintaining poultry health and supporting reproductive functions. For instance, calcium is essential for eggshell formation and muscle function, while potassium is involved in maintaining electrolyte balance and enzyme activation.

The crude fibre content of 36.67% is relatively high, but this is not uncommon for plant-based feedstuffs. Although high fibre content may limit digestibility, the presence of 18.67% hemicellulose and 9.33% cellulose indicates that a portion of the fibre is relatively fermentable, potentially contributing to gut health and enhancing nutrient absorption. Study by Jha and Mishra (2021) have indicated that while high fibre diets may reduce energy availability, moderate fibre levels can improve gut health by promoting beneficial microbial activity, which is important for overall poultry performance.

However, the result showed that there is relatively low acid detergent fibre (ADF) content of 11.33% and acid detergent lignin (ADL) of 2% which indicated that the indigestible portions of the fibre are minimal, suggesting that Moringa leaves have a favourable balance of digestible and indigestible fibres. This could imply that Moringa leaf meal offers a good source of dietary fibre without severely affecting the digestibility of other nutrients. The low ADL content supports the use of MLM as a digestible fibre source, as high levels of lignin can impair nutrient availability by binding to other feed components (Popoola-Akinola et al., 2022).

Ether extract, which accounts for 3%, represents the fat content of MLM, providing essential fatty acids necessary for maintaining cellular integrity and supporting reproductive health in poultry. The dry matter content of 98.33% ensures that MLM is concentrated, minimizing water content and enhancing its storage potential.

Conclusively, the findings from this study suggest that MLM is a nutritionally rich feed component that can significantly contribute to the dietary needs of poultry, particularly in improving reproductive outcomes. The nutrient-rich composition of MLM justifies its use as both a feed supplement and an extender for poultry semen, as discussed by Al-kirshi et al. (2010), who highlighted the importance of natural antioxidants, such as those found in Moringa, in supporting reproductive performance.

Semen quality

The results presented in Table 4 reveal that while there were no significant differences in semen volume, concentration, or sperm viability among the different treatments, the progressive motility of the semen in Nigerian indigenous normal-feathered (NINF) cocks fed with Moringa oleifera leaf meal (MOLM) showed notable improvements. Specifically, the progressive motility percentages in G2 (200 mg kg^{-1} MOLM) and G3 (400 mg kg^{-1} MOLM) were significantly higher than those in the control group (G1), despite no significant differences between the two Moringa-supplemented groups.

The improved motility in the G2 and G3 groups aligns with the findings of previous studies that have demonstrated the positive effects of Moringa oleifera on reproductive health. Moringa is rich in essential nutrients and bioactive compounds, such as flavonoids and polyphenols, which possess strong antioxidant properties (Zeng et al., 2019). These antioxidants help to mitigate oxidative stress, a key factor that can negatively affect sperm motility by damaging the sperm membrane and impairing mitochondrial function (Kaltsas, 2023). By reducing oxidative damage, the inclusion of MOLM in the diet likely enhanced the sperm's ability to maintain motility, which is crucial for successful fertilization.

Table 4. The effect of level of Moringa oleifera leaf meal on NINF cocks semen quality.

Parameters	G1	G2	G3
Volume (mL)	1.00 ± 0.27	0.77 ± 0.15	0.77 ± 0.15
Motility (%)	50.00 ± 5.83[b]	70.25 ± 4.48[a]	77.75 ± 6.71 [a]
Concentration (x10^6 mL^{-1})	3905.0 ± 152.42	3668.3 ± 401.84	4189.7 ± 212.19
Sperm viability	85.00 ± 5.00	90.00 ± 0.00	70.00 ± 10.00

[ab]Means within rows with different superscripts are significantly different, *P < 0.001. G1: 0 mg kg^{-1} (MLM), G2: 200 mg Kg^{-1} (MLM), G3: 400 mg Kg^{-1} (MLM).

Moreover, the fact that both G2 and G3 exhibited similar improvements in progressive motility, despite the different concentrations of MOLM, suggests that even a moderate level of Moringa supplementation (200 mg kg^{-1}) can produce beneficial effects. This finding is consistent with research by Paul et al. (2013), which reported that Moringa oleifera leaves, due to their high nutrient content, can support reproductive performance at relatively low inclusion levels. It is also worth noting that progressive motility is an essential semen parameter directly linked to fertility potential in poultry, as sperm motility is necessary for successful migration through the female reproductive tract (Van et al., 2022).

The results in Table 5 demonstrate that while there were no significant differences in semen volume, concentration, or sperm viability among the NINF cocks given Moringa oleifera leaf extract (MOLE), the progressive motility percentage was notably affected by the treatments. Cocks in the T2 group (20 mL 100 L^{-1} MOLE) exhibited significantly higher progressive motility than those in the control group (T1) and T3 group (40 mL 100 L^{-1} MOLE), with the lowest motility observed in T1.

Table 5. The effect of level of *Moringa oleifera* leaf extract on NINF cocks semen quality.

Parameters	T1	T2	T3
Volume (mL)	1.00 ± 0.27	0.90 ± 0.26	1.37 ± 0.09
Motility (%)	50.00 ± 5.83[b]	81.25 ± 6.63 [a]	76.00 ± 5.07 [a]
Concentration (10^6 mL^{-1})	3905.0 ± 152.42	3854.7 ± 272.86	4048.7 ± 153.58
Sperm viability	85.00 ± 5.00	75.00 ± 5.00	82.50 ± 2.50

[ab]Means within rows with different superscripts are significantly different, *P < 0.001. T1: 0 mL 100 mL^{-1} (*Moringa oleifera* leaf extract), T2: 20 mL 100 L^{-1} (*Moringa oleifera* leaf extract), T3: 40 mL 100 L^{-1} (*Moringa oleifera* leaf extract).

These findings support the role of Moringa oleifera as a supplement that enhances sperm motility, even when administered as an aqueous extract. The improved motility in the T2 group aligns with the bioactive properties of Moringa oleifera, particularly its rich composition of vitamins, minerals, and antioxidants, which can improve semen quality by protecting sperm cells from oxidative stress (Ghadimi et al., 2024). The antioxidants in Moringa, such as vitamin C and flavonoids, are known to counteract free radical damage, which is a common factor affecting sperm motility and overall reproductive performance (Mohlala et al., 2023). By mitigating oxidative damage to sperm membranes and mitochondria, Moringa may contribute to improved sperm function and progressive motility, essential for fertilization (Ghadimi et al., 2024).

Interestingly, the progressive motility in T2 (20 mL 100 L^{-1}) was significantly higher than in T3 (40 mL 100 L^{-1}), suggesting that the 20 mL 100 L^{-1} MOLE concentration may represent an optimal level of supplementation. This is consistent with the idea that excessive doses of certain bioactive compounds can sometimes have diminishing returns or even adverse effects. Study by Gümüş et al. (2024) on plant extracts in animal feed have showed that higher concentrations do not always lead to linear improvements in reproductive performance and may even interfere with metabolic processes.

The lowest progressive motility observed in the control group (T1) further reinforces the positive impact of Moringa supplementation. Without the antioxidant support provided by MOLE, sperm in the T1 group may have been more susceptible to oxidative stress, resulting in reduced motility. These findings suggest that MOLE, particularly at the moderate concentration of 20 mL 100 L^{-1}, can significantly enhance progressive sperm motility, potentially leading to improved fertility outcomes in poultry breeding.

Semen Preservation

The results in Table 6 show that Moringa oleifera leaf extract (MOLE) significantly enhances sperm quality when included in a Tris-egg yolk extender. The highest progressive motility (48.94 ± 7.87%) was recorded at a 25% inclusion level of MOLE, which was notably higher than the control and other inclusion levels. The antioxidant properties of Moringa, including flavonoids and phenolic compounds, may contribute to improved sperm motility by reducing oxidative stress (Carrera-Chávez et al., 2020). This finding aligns with

findings by Dimitriadis et al. (2023) that report the efficacy of plant-based antioxidants in enhancing sperm motility and overall fertility potential (Agarwal et al., 2008).

Sperm viability was highest at a 15% inclusion level, suggesting that moderate levels of MOLE support cell survival during cold storage. The reduction in lipid peroxidation at 10% MOLE inclusion, with the lowest malondialdehyde (MDA) concentration (1.24 ± 1.98), further emphasizes Moringa's protective role in preserving sperm membrane integrity (Ghadimi et al., 2024). Lipid peroxidation is a marker of oxidative damage, and the results suggest that Moringa's antioxidant properties mitigate this, particularly at lower inclusion levels. These findings support the potential use of Moringa extract as an effective natural supplement in semen extenders to enhance sperm viability and reduce oxidative stress during storage (Iqbal et al., 2021).

Table 6. The effect of level of inclusion of *Moringa oleifera* leaf extract on spermatozoa viability and lipid peroxidation of cold stored NINF cocks semen.

	Inclusion levels (%)					
Parameter	0	10	15	20	25	50
Motility (%)	24.69 ±7.04^{b}	33.13 ± 9.63^{b}	45.00 ± 7.75^{a}	48.94 ± 7.87^{a}	49.56 ± 3.37^{a}	8.31± 4.56^{c}
Sperm viability	75.00 ± 3.27^{b}	81.25 ± 0.82^{ab}	85.63 ± 2.90^{a}	59.38 ± 4.48^{c}	76.88 ± 4.32^{b}	78.75 ± 4.30^{ab}
L.P.O. (x 10^{-6})	2.8 ± 1.40^{a}	1.24 ± 0.20^{d}	2.00 ± 0.80^{c}	2.14 ± 1.00^{b}	1.92 ± 0.60^{c}	0.72 ± 0.20^{e}

^{abcd} Means within rows with different superscripts are significantly different, P < 0.001, L.P.O: Lipid Peroxidation.

The results in Table 7 illustrate the impact of storage duration on spermatozoa viability and lipid peroxidation of cold-stored NINF cock semen. As storage time increased, there was a noticeable decline in progressive motility, with a significant drop observed after 72 hours. This decrease is likely due to prolonged exposure to oxidative stress and metabolic changes during storage, which negatively affect sperm function (Carrera-Chávez et al., 2020). The decrease in motility aligns with the general understanding that sperm quality deteriorates over time in cold storage, especially without adequate antioxidant protection (Silvestre et al., 2021).

Additionally, while sperm viability remained stable within the first 48 hours, a significant decline was observed at 72 hours. This indicates that sperm cells can maintain their structural integrity for up to two days but begin to lose viability thereafter. The lipid peroxidation data, with a peak between 24 and 48 hours and a subsequent reduction at 72 hours, suggests oxidative damage is initially high but may decrease as non-viable sperm degrade. Lipid peroxidation is a key marker of oxidative stress, and its rise during storage is consistent with the degradation of sperm membrane lipids (Evans et al., 2021). These findings underscore the need for antioxidants in extenders to protect sperm quality during extended storage.

Table 7. The effect of storage duration (*Moringa oleifera* leaf extract) on spermatozoa viability and lipid peroxidation of cold stored NINF cocks semen.

	Duration (hours			
Parameter	0	24	48	72
Motility (%)	66.58 ± 4.91^{a}	40.29 ± 5.12^{b}	22.08 ± 5.17^{c}	10.79 ± 3.62^{d}
Sperm viability	81.25 ± 2.89^{a}	81.25 ± 3.80^{a}	75.41 ± 3.45^{a}	66.67 ± 3.10^{b}
L.P.O.	0.00 ± 0.00	1.00 ± 0.06 ^{b}	3.83 ± 0.64^{a}	0.65 ± 0.09^{c}

^{abcd} Means within rows with different superscripts are significantly different, P < 0.001 ·L.P.O: Lipid peroxidation.

Figure 1 illustrates the interaction effect of Moringa oleifera leaf extract inclusion levels in tris egg yolk extender and storage duration on the motility percentage of Nigerian indigenous normal feathered cock spermatozoa. The interaction was significant (P < 0.001) throughout the study, with notable trends observed across different inclusion levels and storage durations. At 0 hours, the 15% inclusion level exhibited the highest motility (87%), followed by the 10% inclusion (85%) and 20% inclusion (83%). This suggests that Moringa leaf extract has a potent initial effect on maintaining sperm motility, particularly at moderate inclusion levels. Studies have shown that natural antioxidants like Moringa can enhance sperm motility by scavenging free radicals and reducing oxidative stress, which aligns with these findings (Carrera-Chávez et al., 2020; Mohlala et al., 2023; Ghadimi et al., 2024). In contrast, the 50% inclusion level showed the lowest motility at 0 hours, possibly indicating that excessive inclusion of Moringa might have detrimental effects on sperm motility, a phenomenon supported by Ghadimi et al. (2024) indicating the potential toxicity of high concentrations of plant extracts.

At 24 hours of storage, the highest motility percentage (65%) was observed in the 20% inclusion group, followed by 10%, 15%, and the control (0%) group, while the lowest motility was again seen in the 50% inclusion group. Interestingly, by 48 and 72 hours, the 25% inclusion level demonstrated the highest motility

percentages (64% and 60%, respectively), suggesting that Moringa's antioxidant effects may become more pronounced over longer storage periods. This delayed enhancement of motility at higher inclusion levels could be due to the gradual release of bioactive compounds from Moringa that stabilize sperm membranes against oxidative stress during storage (El-Seadawy et al., 2022). The 50% inclusion consistently exhibited the lowest motility percentages across all storage durations, reaffirming the hypothesis that over-inclusion of Moringa may impair sperm function rather than improve it (Silvestre et al., 2021).

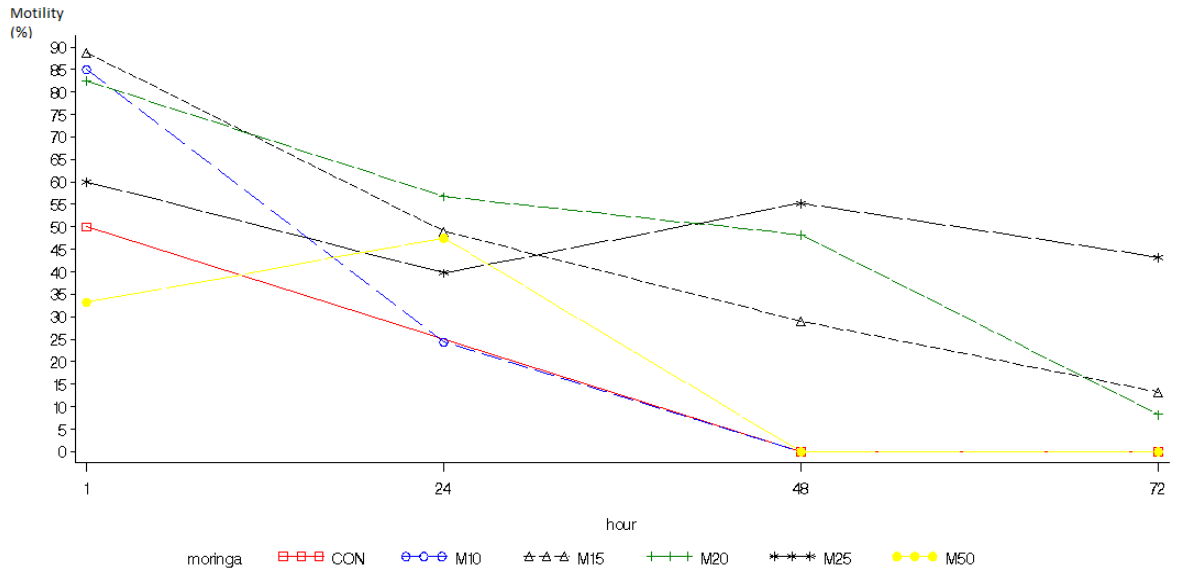

Figure 1. Interaction effect of level of inclusion of *Moringa oleifera* leaf extract and storage duration on progressive motility (%) of cold stored NINF cocks spermatozoa. CON: control, M10: 10% inclusion, M15: 15% inclusion M20: 20% inclusion, M25: 25% inclusion and M50: 50% inclusion of *Moringa oleifera* leaf extract.

The interaction effect between Moringa oleifera leaf extract inclusion level and storage duration on sperm viability, as presented in Figure 2, highlights a significant interaction ($P < 0.001$). The inclusion level of 15% showed the most promising results, maintaining a high sperm viability of 87.5% from 0 to 48 hours before a moderate decline to 77.5% at 72 hours. This suggests that moderate inclusion of Moringa extract provides optimal antioxidant protection during cold storage, effectively stabilizing sperm membrane integrity up to 48 hours, consistent with findings on plant-derived antioxidants enhancing sperm preservation (El-Seadawy et al., 2022). The lower viability observed at 72 hours is likely due to oxidative stress surpassing the antioxidant capacity of the extender.

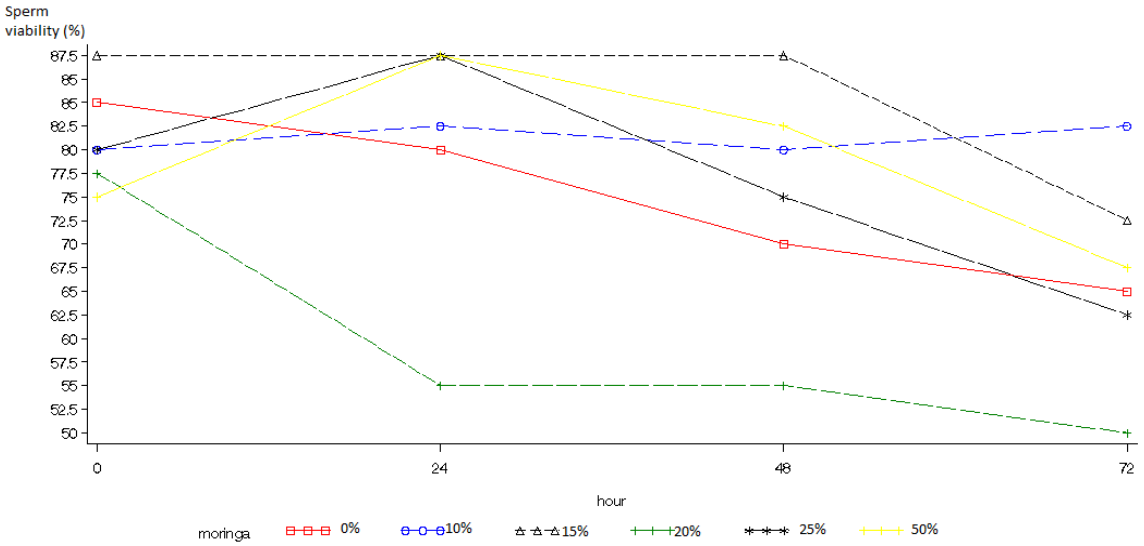

Figure 2. Interaction effect level of inclusion of *Moringa oleifera* leaf extract and storage duration on sperm viability of cold stored NINF cocks spermatozoa. 0%: control, 10%: 10% inclusion, 15: 15% inclusion 20: 20% inclusion, 25: 25% inclusion and 50%: 50% inclusion of *Moringa oleifera* leaf extract.

In contrast, the 20% inclusion level exhibited a sharp decline in sperm viability from 77.5% at 0 hours to 57.5% at 24 hours, maintaining this level until 48 hours before further reduction to 50% at 72 hours. This rapid decline suggests that higher inclusion levels might introduce an imbalance in the extender's osmotic properties or cause oxidative stress beyond the optimal antioxidant benefit (Tan et al., 2018). The control group (0% inclusion) showed a steady decline in sperm viability over the storage period, from 85% at 0 hours to 72.5% at 72 hours, highlighting the natural reduction of sperm quality in the absence of antioxidant supplementation. These findings emphasize the importance of optimizing Moringa extract levels in extenders to preserve sperm viability during prolonged cold storage.

The interaction between the inclusion level of Moringa oleifera leaf extract and storage duration on lipid peroxidation in cold-stored NINF cock semen, as depicted in Figure 3, reveals significant changes ($P < 0.001$). A rapid increase in lipid peroxidation was observed for the 15%, 20%, and 25% inclusion levels between 24 to 48 hours, followed by a drastic reduction from 48 to 72 hours. This trend suggests that the antioxidant properties of Moringa leaf extract, while initially effective, may reach a threshold where oxidative stress increases before antioxidant defenses are reactivated or lipid breakdown decreases, aligning with findings that plant antioxidants like Moringa can delay but not fully prevent lipid oxidation in extended semen (Silvestre et al., 2021). The lipid peroxidation peak around 48 hours reflects the susceptibility of spermatozoa to oxidative damage during intermediate storage times, a phenomenon well-documented in cryopreservation studies where reactive oxygen species (ROS) generation increases after freezing and thawing, exacerbating lipid peroxidation (Evans et al., 2021; Ghadimi et al., 2024).

Interestingly, for the 25% and control (0%) inclusion levels, a gradual decrease in lipid peroxidation was observed from 24 to 72 hours, suggesting that these levels may have conferred some stabilizing effects on the sperm membrane over longer storage durations. This protective effect of Moringa extract on lipid peroxidation aligns with earlier studies, where natural antioxidants like flavonoids and phenolics present in Moringa were shown to reduce lipid peroxidation and maintain sperm integrity during cold storage (Jahan et al., 2018; Ragab et al., 2024). Furthermore, the lowest lipid peroxidation concentration (0.0003) recorded at 48 hours for the 25% inclusion level suggests that Moringa, when used at appropriate levels, provides significant protection against oxidative stress, preventing extensive lipid breakdown and thus supporting sperm motility and viability over time. This further underscores the role of Moringa as a potent antioxidant that can effectively mitigate the effects of ROS in sperm storage (Jahan et al., 2018).

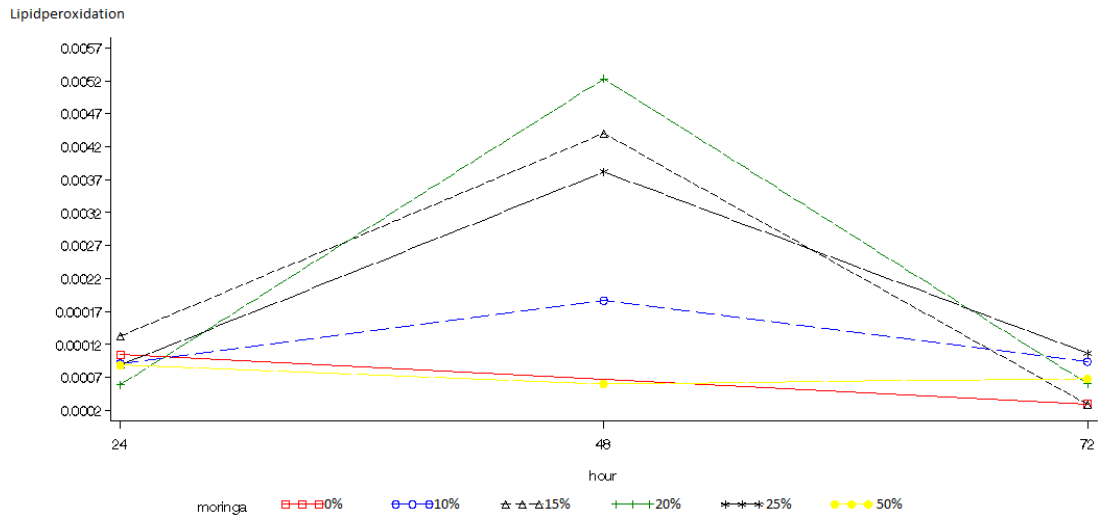

Figure 3. Interaction effect level of inclusion of *Moringa oleifera* leaf extract and storage duration on lipid peroxidation of cold stored NINF cocks semen. 0%: control, 10%: 10% inclusion, 15: 15% inclusion 20: 20% inclusion, 25: 25% inclusion and 50%: 50% inclusion of *Moringa oleifera* leaf extract.

Conclusion

Moringa oleifera leaf meal, with its rich nutritional profile, including 30.63% crude protein, 36.67% crude fiber, and notable levels of dry matter, ash, and fiber fractions like NDF (30%) and ADF (11.33%), demonstrates significant potential as a feed supplement for poultry. Its antioxidant properties have been shown to positively impact semen quality, improving sperm motility, viability, and concentration in cocks. These

findings highlight the value of incorporating Moringa oleifera leaf meal into poultry diets to enhance reproductive performance and overall health in Nigerian indigenous normal feathered cocks.

Data availability

The data is available in www.drive.google.com/drive/u/0/my-drive

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