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Semen Quality, Fertility and Egg Hatchability in Local Turkey Toms Fed Moringa and Gongronema Leaf Powder Diets

Research Article

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ABSTRACT

The study was conducted to determine the semen quality, fertility and egg hatchability in local Nigeria turkey toms fed Moringa (MO) and Gongronema leaf (GL) powder diets. A total of 36 (10 weeks) local turkey toms (1.2-1.3 kg) were randomly assigned to 9 dietary treatments with 4 replicates of 1 tom each in 3 × 3 factorial arrangement. Also, 72 turkey hens at point of first lay (26 weeks) weighing 3.4-3.5 kg were allotted to 9 dietary treatments with 4 replicates of 2 hens each. Treatments include: T1= basal diet (BD) only, T2= BD + 15 g MO + 0 g GL/kg, T3= BD + 30 g MO + 0 g GL/kg, T4= BD +15 g GL + 0 g MO/kg, T5= BD + 30 g GL + 0 g MO/kg, T6= BD + 15 g MO + 15 g GL/kg, T7= BD + 30 g MO +15 g GL/kg, T8= BD + 15 g MO + 30 g GL/kg and T9= BD + 30 g MO + 30 g GL/kg diet. In the main effect of Moringa and Gongronema leaf powder on semen quality, toms fed 30 g MO/kg had the highest (P<0.05) semen quality, while the toms fed 15 and 30 GL/kg had the least (P<0.05) semen quality. In the combined effect of Moringa and Gongronema, semen volume, sperm cell concentration and normal sperm cell values of toms on treatment C were the highest (P<0.05), while the toms on treatment C and E had the highest (P<0.05) value for progressive motility. Percentage of fertile and hatched eggs were higher (P<0.05) in 15 and 30 g MO/kg than in control, 15 and 30 g GL/kg. In combined effect of Moringa and Gongronema on % fertility and egg hatchability of tom's semen, % hatchability caused by semen from toms on treatment C and E were higher (P<0.05) than in the other treatments. It was concluded that improved semen quality in turkey can be achieved using 30 g MO/kg and combination 30 g MO kg + 15 g GL/kg diet.

KEY WORDS egg-hatchability, *Gongronema latifolium*, *Moringa oleifera*, toms, turkey.

INTRODUCTION

In several rural villages in Africa such as Nigeria, people depend on local birds for sources of quality protein of animal origin (Manyeula *et al.* 2019). According to Ben Larbi *et al.* (2013), there has been a reduction in the population of local birds such as turkeys, due to their very low reproductive and productive performance. It is well documented in the literature that poor fertility and egg hatchability of observed in local birds under natural mating conditions are as a result of low quality semen which is linked to poor nutrition (Bucak *et al.* 2010). There is a strong association between nutrition and overall fertility in farm animals (Hudson and Wilson, 2013). However, the use of plant-based products can be applied as a nutritional protocols to enhance semen quality in male poultry such as turkey toms

and thus, increasing their productivity. In animal nutrition, plants based materials have been prepared and used as additives as a result of their nutritional quality, low cost, availability and medicinal benefits. One of the plants whose parts has been prepared and used as phytogenic feed additive in animal nutrition is *Moringa oleifera* plant. Literature has shown that *Moringa oleifera* has long been known to have high nutritional contents (Mune *et al*. 2016) such as antimicrobial properties (Al husnan and Alkahtani, 2016). According to Sebola *et al.* (2015), *Moringa oleifera* leaf is most recognized for its high concentration of protein (27%) and appropriate amino acid profile, as well as its high levels of vitamins A and E, low levels of anti-nutritional chemicals, and fatty acids. Moringa plant contains 1.108 mg β-carotene, 25.02% proteins, 10.42% fat, 15.2 mg/100 g vitamin C, 11.83% dietary fiber, 326.4 µg/100 g vitamin B1, and 28.50% carbs (González-Burgos *et al*. 2021). Regular consumption of *Moringa* fruit, increases the number of sperm cells in male birds and thus, increasing the probability of fertilizing an egg. The process of spermatogenesis to create semen of a phenomenal quality requires amino acids such as methionine, cysteine (Fouad *et al.* 2020) and high concentrations of these amino acid are found in Moringa leaves. These amino acids are critical for the growth of germ cells and spermatogenesis, which produces produces spermidine and putrescine precursors (Ebisch *et al*. 2006). Moringa leaves contain poly unsaturated fatty acids which functions to maintain sperm cell viability, sperm maturation and fertility (Conquer *et al.* 2000). Moreover, another plant that can be used in animal nutrition as a phytogenic feed additive is *Gongronema latifolium.* Literature shows that *Gongronema latifolium* is highly nutritive with high amounts of proteins and carbohydrates and has antibacterial properties (Afolabi *et al.* 2008). It is a member of the Asclepiadaceae family. This plant found in tropical rainforests is mostly used as a spice. The Igbo tribe in South Eastern Nigeria refers to it as Utazi. The phytochemical analysis of according to Antai *et al.* (2009) revealed that the ethanolic extract of Gongronema root contains modest amounts of reducing sugars, glycosides, polyphenols, and a large number of alkaloids. Gongronema preparations were found to contain other compounds, including lupenylester, B-sitosterol, essential oil, and pregnane ester (Ekundayo, 1980). Similarly, according to Agbo *et al.* (2005), *Gongronema latifolium* has been identified to be nutritionally high in iron, zinc, vitamins, protein and amino acids and thus could complement the inadequacies of these substances in feed. The findings of Nwanjo *et al.* (2006) supported the findings of Atawodi (2005) about the antioxidant potentials of Gongronema. The leaf extracts of Gongronema have been shown to have anti-oxidative properties and are being utilized in management of diabetes mellitus and other tropical diseases (Agbo *et al.* 2005). Farm animals' systems getting rid of reactive oxygen species by Gongronema. Gongronema ethanolic and aqueous extracts have been shown by Ugochukwu and Babady (2002). With the knowledge of the numerous benefits of *Moringa oleifera* and *Gongronema latifolium* in humans, their availability and chemical contents as reported in numerous research materials, it is expected that they will have positive effects on semen quality of turkey toms when used as feed additives. The study was therefore designed to determine the semen quality, fertility and egg hatchability in local turkey toms fed Moringa and Gongronema leaf powder diets.

MATERIALS AND METHODS

Ethical consideration

The study was carried out in accordance with the guidelines set by the University of Nigeria, Nsukka's Ethical Committee on the use of farm animals for research (M330AGRIC08).

Study site

The study was carried out at the Poultry Units of the Department of Animal Science Teaching and Research Farm, University of Nigeria. Nsukka lies within longitude 6˚ 45′E and 7˚ E and latitude 7˚ 12.5 ′N and on the altitude of 447m above sea level. The climate of the study area is typically tropical, with relative humidity ranging from 65 to 80% and mean daily temperature of 26.8 ˚C (Okonkwo and Akubuo, 2007) According to the Metrological Center, Crop Science Department, University of Nigeria, Nsukka Enugu State, the yearly rainfall of the study area ranges from 1567.05 mm-1846.98 mm. The feeding trial lasted for 21 weeks.

Experimental materials

The test plants utilized were *Moringa oleifera* and *Gongronema latifolium.* The leaves of *Gongronema latifolium* and *Moringa oleifera* were harvested from a private farm in Billiri Gombe state, Northern Nigeria. The leaves were then allowed to air dry for a period of seven days. Following chemical analysis, of the dried leaves of both plants, they were ground into powder and stored in a clean jute bag.

Chemical analysis of dried leaves of *Moringa oleifera* and *Gongronema latifolium*

The analytical data for dried leaves of *G. latifolium* and *M. oleifera* are displayed in Table 3-4. Atomic Absorption Spectroscopy (AAS) (Perkin Elmer Analyst 200, USA) was used to determine the concentrations of calcium, potassium, magnesium, phosphorus, copper, zinc, and iron in the dried *Moringa leaves.* Utilizing colorimetric approach, vitamin A was ascertained. For the analysis of thiamin and riboflavin,

a gram of the representative part of the test material was measured into a conical flask and mixed in 100 mL of de-ionized water, agitated vigorously, heated for 5 minutes, cooled and filtered. The filtrate was emptied into a cuvette, and the respective wavelengths were set at 242 nm for the thiamin and riboflavin to read their absorbance using a spectrophotometer. Concentration of vitamin C was done by the titrimetric method, as reported by Kirk and Sawyer (1991). Concentration of vitamin E was done by the use of Futter – Mayer colorimetric methods according to (AOAC, 2006). A simple UV-Vis spectrophotometric technique was applied to determine β-carotene content of Gongronema, while, solvent extraction technique applying petrochemical solvents was used to determine the carotene contents of Moringa.

Experimental diets

Basal diets for the turkey toms and diet for the hens are shown in Tables 1-2. The chemical compositions of diets were analyzed according to AOAC (2012) methods.

Table 1 Ingredient (%) and chemical compositions (g/kg DM) of basal diets for the turkey toms

Ingredients (%)	Percentage
Maize	48.00
Wheat middling	14.74
Soybean meal	18.00
Palm kernel cake	13.00
Fish meal	3.00
Lime stone	2.00
Lysine	0.25
Methionine	0.38
Vitamin and mineral premix	0.38
Salt	0.25
Calculated compositions (%)	
Crude protein	18.60
Metabolizable energy (kcal/kg)	2897.03
Crude fiber	4.20
Calcium	1.10
Phosphorous	0.80
Chemical compositions (%)	
Crude matter	89.20
Crude protein	18.56
Crude fiber	3.50
Crude fat	3.80
Nitrogen free extract	63.68

Vitamins and minerals premix compositions: vitamin A: 10000000 IU; vitamin D3: 2000000 IU; vitamin E: 10000 mg; vitamin K3: 1000 mg; vitamin B1: 1000 mg; vitamin B2: 5000 mg; vitamin B6: 1500 mg; vitamin B12: 10 mg; Pantothenic acid: 10000 mg; Niacin: 30000 mg; Biotin: 50 mg; Folic acid: 1000 mg; Choline: 250 mg; Selenium: 100 mg; Copper: 4000 mg; Iron: 30000 mg; Manganese: 60000 mg; Zinc: 50000 mg; Iodine: 1000 mg; Cobalt: 100 mg and $CaCO_3$: 3000 g.

Table 2 Ingredient (%) and chemical compositions (g/kg DM) of diets for the turkey hens

Ingredients (%)	Percentage
Maize	44.00
Wheat middling	22.00
Palm kernel cake	12.00
Soybean meal	14.00
Oyster shell	2.00
Pail oil	2.00
Bone meal	3.00
Lysine	0.25
Methionine	0.25
Salt	0.25
Vitamin and mineral premix	0.25
Total	100
Calculated composition	
Crude protein	16.50
Metabolizable energy (kcal/kg)	2590.00
Calcium	3.50
Phosphorous	0.50
Chemical compositions (%)	
Crude fiber	6.45
Ether extract	3.50
Crude matter	91.00
Crude protein	16.45
Nitrogen free extract	64.95

Vitamins and minerals premix compositions: vitamin A: 10000000 IU; vitamin D3: 2000000 IU; vitamin E: 10000 mg; vitamin K3: 1000 mg; vitamin B1: 1000 mg; vitamin B2: 5000 mg; vitamin B6: 1500 mg; vitamin B12: 10 mg; Pantothenic acid: 10000 mg; Niacin: 30000 mg; Biotin: 50 mg; Folic acid: 1000 mg; Choline: 250 mg; Selenium: 100 mg; Copper: 4000 mg; Iron: 30000 mg; Manganese: 60000 mg; Zinc: 50000 mg; Iodine: 1000 mg; Cobalt: 100 mg and $CaCO_3$: 3000 g.

Table 3 Analytical results of *Moringa oleifera* dried leaves

Vitamins/minerals	Dried leaves
Carotene	18.30
Thiamin	3.01
Riboflavin	19.76
Vitamin C	18.02
Calcium	2000
Copper	0.49
Iron	27.54
Magnesium	338
Phosphorus	197.67
Potassium	1.64
Zinc	3.01

Table 4 Analytical results of *G. latifolium*

Vitamins/phytochemicals	Composition (mg/100 g)
β-carotene	5.60
Vitamin E	4.12
Vitamin C	14.34
Vitamin A	42.09

Experimental birds and management

A total of 36 (10 weeks) Nigerian local turkey toms weighing 1.2-1.3 kg were randomly assigned to 9 dietary treatments with 4 replicates of 1 tom each in 3 × 3 factorial arrangement in completely randomized design. Also, 72 turkey hens at point of their first lay (26 weeks) weighing 3.4-3.5 kg were allotted to 9 dietary treatments with 4 replicates of 2 hens each. Treatment for the toms were as follows: T1= Basal diet only, T2= 15 g MO + O g GL/kg, T3= 30 g MO + 0 g GL/kg, T4= 15 g GL + 0 g MO/kg, T5= 30 g GL + 0 g MO/kg, T6= 15 g MO + 15 g GL/kg, T7= 30 g MO + 15 g GL/kg, T8= 15 g MO + 30 g GL/kg and T9= 30 g MO + 30 g GL/kg diet. *Moringa oleifera* leaf powder was included in the diet at 3 levels of 0, 15 and 30 g/kg diet, while *G. latifolium* powder was added to the diet at 3 levels of 0, 15 and 30 g/kg diet. They toms and hens were bought from Ekwulobia, Anambra state Nigeria and were quarantined and stabilized for two weeks before allotting them into treatments. A stress pack was given to the birds via drinking water following the producer's recommendation in order to boost hunger and energy supply immediately their arrival at the farm. Dietary treatments and clean water were provided *ad libitum* during the entire periods of feeding trial. The birds were housed in a deed litter system and were looked after by a veterinarian throughout the experimental periods. General flock prophylactic management and routine vaccination was administered accordingly.

The temperature of the experimental house was monitored using thermometer. Lighting was provided using a 200 v watt white bulbs.

Data collection

Semen collection

At the age of twenty-six weeks, all the toms from each treatment group were trained for semen collection and to check for each treatment age at puberty. By 29 weeks, the entire toms were ready for semen collection. Semen was collected using abdominal massage technique as described by Bakst and Long, (2010), which involves massaging the cloacal area to trigger phallic tumescence. This was followed by a 'cloacal stroke', a squeezing of the region surrounding the sides of the cloaca to express the semen as described by Kalamah *et al*. (2002). Semen was collected into a microhematocrit tube. Immediately after each collection, the semen examination took place (Figure 1).

Artificial insemination and fertility test

Hens were inseminated with semen according to their proposed treatment. For fertility test, this was carried out using 72 hens, divided into 9 treatments, each containing 8 hens. The pooled semen from T1 toms was used to inseminate treatment 2 hens, the pooled semen from T2 toms was used to inseminate treatment 2 females, and so on for each of the 9 treatments.

During the insemination process, each inseminated hen was kept upright while the abdomen surrounding the vent, especially on the left side, was gently compressed. The oviduct protruded and the cloaca everted as a result. Now, the second operator put a 2.5 cm plastic straw into the oviduct, depositing the right amount of semen at the vaginal-uterine junction. Throughout the insemination periods, semen from donor males were pooled in 10 mL beakers and kept warm using stage warmer. Every syringe used was stored at the exact same temperature. Freshly collected, undiluted pooled semen was inserted into the female's vagina of each inseminated hen at a depth of two centimeters using a one milliliter syringe within five minutes of the semen collection process. According to Macpherson *et al*. (1977), rough handling of the hens was avoided during capture prior to insemination, and each hen was gently released following insemination to prevent semen from regurgitating from the vagina, which may lead to diminished fertility. As the semen was expelled into the vagina, pressure around the vent was released, which aided the hen in retaining sperm in the vagina or the oviduct. The hens were inseminated twice a week with 0.25 mL of undiluted semen for optimal fertility.

Egg collection, storage and hatchability

After the hens started to lay, egg collection began and lasted for one week. To identify each treatment, daily collected eggs were marked with an indelible marker at the time of collection. This was to allow proper identification of egg accordingly. Eggs were kept at room temperature in egg crates. After being collected for seven days, eggs were sorted to get rid of extra-small and huge cracks. Out of the remaining clean eggs in each treatment, 25 eggs weighing average of 56-60 g were randomly selected from each treatment for hatching.

The eggs after being taken to the commercial incubator (Infitek ICB-E Series) were placed on 130 egg capacity setting tray and incubated at 58-60% relative humidity and 37 $^{\circ}$C. Turning frequency of the eggs in the incubator was done 2 times per hour to improve quality of poults. On the 14th day of incubation, candling took place. On the 27th day of incubation, the eggs finally began to hatch. Unhatched eggs were opened, the reasons of which were determined to be embryonic mortality, and the embryos were categorized as dead-in-shell.

Fertility was calculated using the following expressions:

% Fertility= (number of fertile eggs / number of incubated eggs) × 100

Hatchability and embryonic mortality were obtained as follows:

% Hatchability= (number of poult hatched / number of eggs set) × 100

% Dead in shell embryos= ((number of dead germs + dead in shell poult)/(number of fertile eggs set)) × 100

Semen color

This was assessed visually on collection and score as outlined by Bearden *et al*. (2004), 1 (good quality semen)= viscous and creamy-white. 2 (samples with low concentration)= watery or less opaque and 3= Pink/yellow appearance. Records were taken within 30 minutes of collection.

Semen volume

Semen volume from each of the treated toms were evaluated using a calibrated collection test tube graduated in ml. and then the volume was read off and recorded in mL

Sperm cell concentration

The sperm concentration was determined by applying direct cell count method. Here, hemocytometer (Improved Neubauer counting chamber) was used for counting sperm cells described by Baker *et al.* (1985). For the final result, the concentration of sperm per was found using the formula:

Sperm cell concentration= $(N \times DF \times 10^6) / (A \times D)$
Where:
N: number of cells.
A: area of chamber counted which is 0.0002 mm^2
DF the dilution factor: 1/0.05
D: depth of chamber which is 0.1 mm (Baker *et al*. 1985)

Percentage live/dead spermatozoa and percentage normal/abnormal sperm cells

Percentage live/dead and percentage normal/abnormal sperm cell were determined using staining technique of Bakst and Cecil, (1997). The histological smears were made within 20 min after semen collection. The stains were prepared exactly according to the methods described by Bakst and Cecil, (1997). A mixture of Eosin/nigrosine was used as dye/stain, a drop at warm temperature (38 ˚C) was placed near one end of a glass slide on slide warmer (38 ˚C). Using a plastic dropper, a small amount (1 drop) of semen was dropped, with a glass rod it was stirred in 2 or 3 circular movements of the rod. With a second slide, the dye-semen drop was spread in a thin layer. A smear was made by placing a warm slide over the first and spreading the mixture evenly between the two slides. The two slides were separated by pulling the ends in. Thereafter, on microscopic examination, the slides were respectively placed on the stage of a phase contrast microscope and observed at 100 magnifications. Normal and abnormal sperm cell morphology was observed. Stained spermatozoa were considered dead and damage, while live and normal spermato-unstained ones were considered alive for every 100 counted sperm per slide and the average count of each type determined. The live, dead, abnormal and normal sperm cells were recorded in percentage.

Sperm motility

A small drop of normal saline solution was placed with the aid of a micropipette on clean glass slide warmed at 38 ˚C. With a clean glass rod, a very small dab of whole semen was drop on the buffer, a coverslip was placed upon the drop, allowing it to occupy all the space under the coverslip, but not flood out beyond it. It was then placed on a microscope for examination with low magnification (microscope). A magnification of × 400 was used. Several fields were examined and an estimate to the nearest 0 to 20% of motile sperm was made.

Statistical analysis

Data collected were subjected to way Analysis of Variance (ANOVA) and significant differences among the treatment means were separated using Duncan's New Multiple Range Test (DNMRT) with the aid of IBM SPSS Statistics version 28 (SPSS, 2021) and accepted at 5% ($P<0.05$) level of probability.
The statistical model used to test the effect of treatments on the entire parameters determined was stated below:

$$X_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \Sigma_{ijk}$$

Where:
X_{ijk}: overall observation on effects of the treatments.
μ: overall mean.
A_i: effects of *Moringa oleifera*.
B_j: effects of *Gongronema latifolium*.
$(AB)_{ij}$: interaction of the *M. oleifera* and *G. latifolium*.
$\sum_{ijk}$: experimental error.

RESULTS AND DISCUSSION

The results of the main effect of *Moringa oleifera* on semen quality traits of turkey toms are shown in Table 5. Values of semen color was not significant ($P>0.05$), while the values for semen volume (SV), progressive motility (PM), sperm cell concentration (SC), live sperm cells (LS), dead sperm cell (DS), normal sperm cell (NS) and abnormal sperm cells (ABS) were significant ($P<0.05$).

Semen volume, % values of normal sperm and sperm cell concentration were higher in turkey toms on 15 g Moringa/kg and 30 g Moringa/kg than those on 0 g MO/kg diet. Percentage values of live sperm cells and progressive motility were higher (P<0.05) in turkey toms on 30 g Moringa/kg compared to those on 15 g Moringa/kg and 0 g Moringa/kg diet. Percentage values for abnormal sperm and dead sperm cells were highest (P<0.05) in turkey toms on 0 g Moringa/kg diet.

The results of the main effect of *Gongronema latifolium* on semen quality traits of turkey toms are shown in Table 6. Values of semen color, % of semen volume (SV), progressive motility (PM), sperm cell concentration (SC), live sperm cells (LS), dead sperm cell (DS), normal sperm cell (NS) and abnormal sperm cells (ABS) were significant (P<0.05). Values of semen color, % of dead and abnormal sperm cells were similar (P>0.05) in turkey toms on 15 g GL/kg and 30 g GL/kg, but significantly higher (P<0.05) than those on 0 g GL/kg diet. Percentage values for progressive motility, sperm cell concentration, live and normal sperm cell were similar in turkey toms on 15 g GL/kg and 30 g GL/kg, but lower (P<0.05) than the values observed for toms on 0 g GL/kg diet. Percentage of semen volume observed for the toms on 0 g GL/kg diet and 15 GL/kg were similar (P>0.05), but higher than the value recorded for those on 30g GL/kg.

The results of the combined effect of *M. oleifera* and *G. latifolium* on semen quality traits of turkey toms are presented on Table 7 and Figure 2. Values of semen volume of toms on treatment C was higher than the values observed for those on A, B, D and E respectively. Percentage value for progressive motility of toms on treatment A (control) did not differ significantly (P>0.05) from those on B, but lower than the values observed for those on C and E and higher than the value recorded in D. Sperm cell concentration (%) of toms on treatment C was higher (P>0.05) than the values recorded in A, B, C and E. % of live sperm cells values were not significant (P>0.05) among the treatments. Percentage values of dead sperm cells of toms on treatment A, C and E are the same (P>0.05), but significantly lower than the values observed for those on treatment B and D. Normal sperm cell values (%) of toms on treatment A, B and E were similar (P>0.05), but significantly, lower than the value recorded in C and higher than those on treatment D. Abnormal sperm values of toms on A, B and E were the same (P>0.05), but significantly higher than value recorded in C and lower than the value obtained for those on D.

The results of the main effect of *M. oleifera* and *G. latifolium* inclusion on fertility and hatchability of turkey eggs are presented on Table 8. Percentage value of infertile eggs in 15 g MO/kg and 15 g MO/kg were similar (P>0.05), but significantly lower than the values recorded in control group, 15 g GL/kg and 30 g GL/kg/diet. Percentage value of fertile eggs in 30 g GL was lower compared to values recorded 15 g GL/kg, 30 g MO/kg, 15 g MO/kg and control treatment. Percentage value of dead-in-shell embryos in 15 g MO g/kg did not differ (P>0.05) from the values observed in 30 g MO/kg and 15 g GL/k, but significantly lower the values obtained in control treatment and 30 g GL/kg diet. Percentage hatched eggs values obtained in 15 g MO/kg and 30g MO/kg were the same (P>0.05), but significantly higher than values recorded in 30 g GL and control treatment respectively.

Results of the combined effect of *M. oleifera* and *G. latifolium* on fertility and egg hatchability of turkey tom's semen are shown in Table 9 and Figure 3. Percentage of dead-shell-embryo were higher in B and D treatment compared to other treatments. Percentage hatchability was higher in treatment C and E compared to other treatments.

The results of the main effect of *Moringa oleifera* on semen quality traits of turkey toms are presented in Table 5. From the results, semen quality traits improved significantly in favor of the toms on Moringa leaf powder diets. Semen color from *M. oleifera* treated toms and those on control diet had the same (P>0.05) white creamy color and viscous semen, demonstrating good quality semen that contains a lot of spermatozoa. According to Bearden *et al.* (2004), gross appearance of ejaculated semen is used to evaluate semen for quality. According to the same authors, good quality turkey semen should be creamy white and viscous. Semen volume increased significantly in treated toms compared to those on 0 g MO/kg diet. The value of semen volume observe in the current study was greater than value of 0.18ml observed by Ngu *et al.* (2014) in local turkey toms. The findings of Fatoba *et al.* (2013) who observed a substantial rise in semen volume of male treated toms with Moringa root extract, was in agreement with the increase in semen volume documented in the current study. However, efficacy of the Moringa to stimulate sperm production may be related to the rise in the semen volume in toms on Moringa leaf powder diets. This suggests that dietary *M. oleifera* may have caused an in improvement in hormone functions, growth, activity of the seminiferous tubules and the interstitial cells of toms. According to nutritional assessment by González-Burgos *et al.* (2021), Moringa is composed of 1.108 mg β-carotene, 25.02% proteins, 10.42% fat, 15.2 mg/100 g vitamin C, 11.83% dietary fiber, 326.4 µg/100 g vitamin B1 and 28.50% carbohydrates. These compounds contained in Moringa helps in promoting semen production and quality in farm animals. Progressive motility improved higher in toms on Moringa leaf diet compared to those on control diet.

Figure 1 Sperm collection method

Table 1 Main effect of *Moringa oleifera* on semen quality traits of turkey toms

Treatments	CS	SV (mL)	PM (%)	SC (x10^9/mL)	LS (%)	DS (%)	NS (%)	ABS (%)
0 g *Moringa*/kg	1.00	0.39^c	81.31^c	3.78^b	87.73^c	12.27^a	82.33^b	17.67^a
15 g *Moringa*/kg	1.00	0.49^b	87.93^b	4.11^{ab}	89.58^b	10.42^b	84.90^a	15.10^a
30 g *Moringa*/kg	1.00	0.58^a	92.59^a	4.82^a	94.13^a	5.87^c	91.38^a	8.62^b
SEM	0.04	0.02	1.57	0.09	0.68	0.63	0.12	1.87
P-values	0.34	0.03	0.01	0.05	0.04	0.00	0.00	0.04

CS: semen color; SV: semen volume; PM: progressive motility; SC: sperm cell concentration; LS: live sperm cells; DS: dead sperm cells; NS: normal sperm cells and ABS: abnormal sperm cells.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Main effect of *Gongronema latifolium* (GL) on semen quality traits of turkey toms

Treatments	CS	SV (mL)	PM (%)	SC (x10^9/mL)	LS (%)	DS (%)	NS (%)	ABS (%)
0 g GL/kg	1.00^b	0.43^a	88.42^a	3.65^a	90.69^a	9.31^b	80.89^a	19.11^b
15 g GL/kg	1.47^a	0.38^a	71.80^b	2.41^b	88.91^b	11.09^a	76.44^b	23.56^a
30 g GL/kg	1.47^a	0.28^b	71.22^b	2.21^b	87.S57^b	12.43^a	75.78^b	24.22^a
SEM	0.04	0.03	1.57	0.09	0.68	0.63	0.12	0.12
P-values	0.01	0.03	0.00	0.04	0.00	0.03	0.01	0.01

CS: semen color; SV: semen volume; PM: progressive motility; SC: sperm cell concentration; LS: live sperm cells; DS: dead sperm cells; NS: normal sperm cells and ABS: abnormal sperm cells.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Combined effect of *M. oleifera* and *G. latifolium* on semen quality traits of turkey toms

Parameters	Treatment combinations					SEM	P-values
	A	B	C	D	E		
CS	1.00	1.00	1.00	1.00	1.00	0.06	0.43
SV (mL)	0.40^b	0.30^c	0.52^a	0.30^c	0.34^{bc}	0.02	0.00
PM (%)	81.20^b	83.13^b	87.99^a	72.47^c	88.87^a	1.98	0.00
SC (x10^9/mL)	3.16^b	3.14^b	4.14^a	3.17^b	3.07^b	0.14	0.01
LS (%)	89.28	87.58	87.87	86.93	94.29	1.05	0.96
DS (%)	10.36^c	12.21^b	10.06^c	13.03^a	10.10^c	0.41	0.04
NS (%)	84.05^b	82.48^b	89.00^a	76.05^c	84.33^b	1.40	0.00
ABS (%)	15.45^b	17.01^b	11.00^c	23.45^a	15.16^b	1.35	0.02

A: control; B: 15 g MO + 15 g GL/kg; C: 30 g MO + 15g GL/kg; D: 15 g MO/kg + 30 g GL/kg; E: 30 g MO + 30 g GL/kg; CS: semen color; SV: semen volume; PM: progressive motility; SC: sperm cell concentration; LS: live sperm cells; DS: dead sperm; NS: normal sperm and ABS: abnormal sperm cells.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

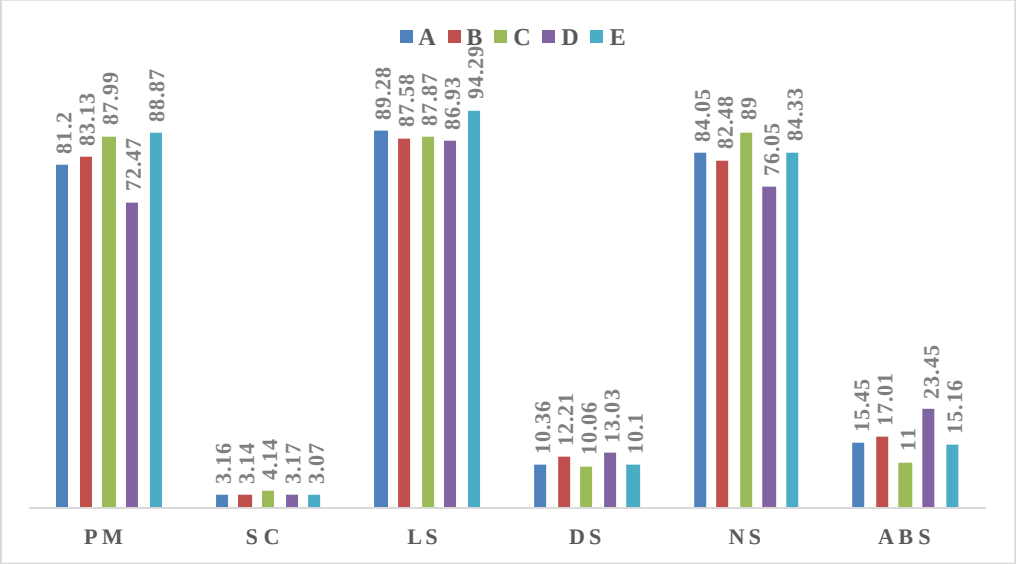

Figure 2 Combined effect of *M. oleifera* and *G. latifolium* on semen quality traits of turkey toms
PM: progressive motility; SC: sperm cell concentration; LS: live sperm cells; DS: dead sperm; NS: normal sperm and ABS: abnormal sperm cells

Table 8 Main effect of *M. oleifera* and *G. latifolium* inclusion on fertility and hatchability of turkey eggs

Fertility indices	Treatments					SEM	P-values
	0 g	15 g MO	30 g MO	15 g GL	30 g GL		
Total eggs	25.00	25.00	25.00	25.00	25.00	-	-
Percentage infertile eggs	19.90^{b}	12.91^{c}	10.33^{c}	21.98^{ab}	25.18^{a}	1.88	0.01
Percentage fertile eggs	80.10^{b}	87.69^{a}	89.17^{a}	78.02^{ab}	74.82^{c}	1.89	0.02
Dead-in-shell embryos (%)	19.20^{b}	12.67^{c}	14.33^{c}	10.72^{c}	23.32^{a}	1.56	0.01
Percentage hatched eggs	60.32^{b}	74.52^{a}	75.35^{a}	64.75^{b}	51.23^{c}	3.10	0.00

MO: *M. oleifera* and GL: *G. latifolium*.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The results agree with the previous discoveries of Upendra *et al.* (2000) who conducted study on the effect of herbal preparation called SPEMAN forte VET in male breeder broiler and detected a substantial rise in sperm motility when compared with the control. The significant differences reported for progressive motility in favor of the treatment group highlighted the positive effect of Moringa in enhancing sperm motility by providing the substrate (ATP) needed for motility. The increased progressive motility from toms fed Moringa leaf powder diet may also be attributed to vitamin E (Sebola *et al.* 2017) and selenium found in Moringa. Hansen and Deguchi (1996) stated that dietary selenium causes an increase in sperm concentration, sperm motility and sperm capacity in farm animals (including poultry species). Plant material like Moringa contains substantial amounts of beneficial antioxidants, phytochemicals, minerals and vitamins known to increase growth and stimulate reproduction in animals, including poultry species (Mahfuz and Piao, 2019).

Sperm concentration was highest in toms on Moringa leaf powder diets. The results compare with those reported by Yusuf (2014) who reported a significant effect in favor of the treatment group on sperm concentration when incorporated dietary *M. oleifera* leaf meal in the diet of turkey toms. Bearden *et al.* (2004) claimed that low sperm concentration has been associated with low fertility.

Therefore, the higher sperm concentrations recorded for toms on Moringa leaf powder diet suggest that testicular development and proper hormone balance in toms were triggered by the inclusion of Moringa leaf powder diet.

Increased % of live and normal sperm cells in treated toms could be linked to the protective effects of Moringa leaf powder against damage of semen by oxidative stress. Results of the nutritional assessment of Moringa shows that it contains vitamin C and E, an antioxidant and anti-stress agent that works to combat heat and oxidative stress, which have the potential to have degenerative effect on semen (Sebola *et al.* 2015).

Table 9 Combined effect of *M. oleifera* and *G. latifolium* inclusion on fertility and egg hatchability of turkey tom's semen

Fertility indices (%)	Treatment combinations					SEM	P-values
	A	B	C	D	E		
Overall eggs	25.00	25.00	25.00	25.00	25.00	-	-
Percentage infertility	22.83	24.70	13.60	29.75	19.94	0.26	0.66
Percentage fertility	76.17	75.17	86.39	69.25	80.06	5.68	0.23
Dead-shell-embryos (%)	19.98^{b}	28.06^{a}	16.99^{b}	30.13^{a}	19.12^{b}	5.36	0.02
Percent hatchability	76.67^{c}	75.17^{c}	88.39^{a}	66.87^{d}	83.33^{a}	3.27	0.00

A: control; B: 15 g MO + 15 g GL/kg; C: 30 g MO + 15g GL/kg; D: 15 g MO/kg + 30 g GL/kg and E: 30 g MO + 30 g GL/kg.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

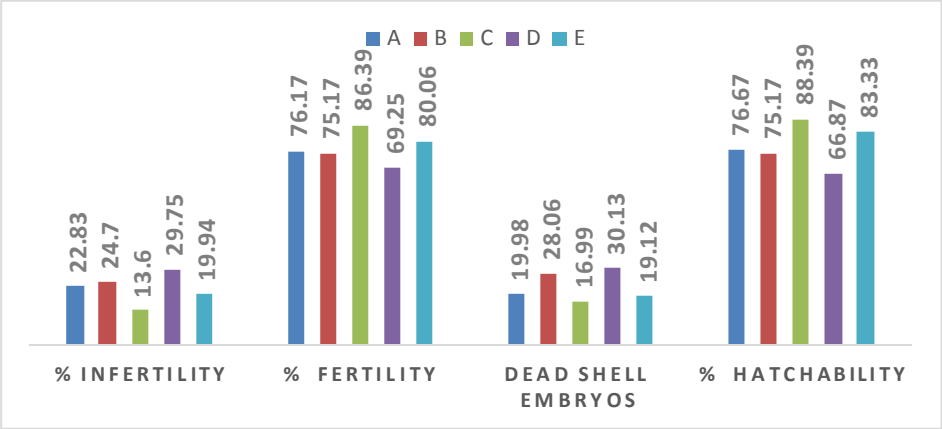

Figure 3 Combined effect of *M. oleifera* and *G. latifolium* inclusion on fertility and egg hatchability of turkey tom's semen

The percentage of morphologically intact sperm found in treated toms was consistent with the acceptable range for turkey sperm reported by Bearden *et al.* (2004). However, the decrease in proportion of normal spermatozoa in toms on 0 g MO/kg diet may be as a result of a large production of aberrant spermatozoa caused by partial or total degeneration of the sperm tubules, according to Anderson (2001). However, aging of spermatozoa leading to loss of membrane integrity as a result of peroxidation in the vas deferens can also be linked an increase in defective sperm (Noirault and Brillard, 1999) observed in toms on 0 g MO/kg diet (Figure 4).

The result of the main effect of *Gongronema latifolium* on semen quality traits of turkey toms are shown in Table 6. There is paucity of information on the use of *Gongronema latifolium* in local turkey. The semen color of the control group (1.00) was white creamy and viscous, whereas, toms on 15g GL and 30GL/kg produced watery, less viscous, clear semen (1.47). This was an indication that *Gongronema latifolium* contains substances that affect semen quality even though it also contains several compounds that are of nutritional benefits. The % semen volume of toms on control diet had a mean value of 0.43 mL which was slightly higher than 0.18 mL (Ngu *et al.* 2014) for indigenous breeds of toms.

Findings of Ikpeme *et al.* (2012) similarly noted a significant decline in semen quality in rats treated with *G. latifolium*, particularly at higher inclusion and longer duration. When plants with antimalarial qualities are employed, some semen quality metrics are reduced (Ezeonwu and Dahiru, 2013), which is typical of plants with antifertility properties that negatively affect semen quality. Therefore, *G. latifolium* may contain some substances that affect semen quality negatively. Percent progressive motility dropped in toms on *G. latifolium* compared to those on control diet. The drop in sperm motility in treated toms may have caused by a lengthy course of treatment which may have led to decrease in sperm motility due the loss of spermatogenesis resulting to reduction in testosterone discharge (Oyeyipo *et al.* 2011).

Although it appears that there has not yet been any research on *G. latifolium* impact on the semen characteristics of male turkeys, research on male albino rats has shown that it reduces sperm motility (Oyeyipo *et al.* 2014). According to Oyeyipo *et al.* (2014) decreased sperm concentration, motility and normal sperm morphology of treated rats demonstrates that nicotine (95 % alkaloid) which *G. latifolium* has impaired some semen qualities.

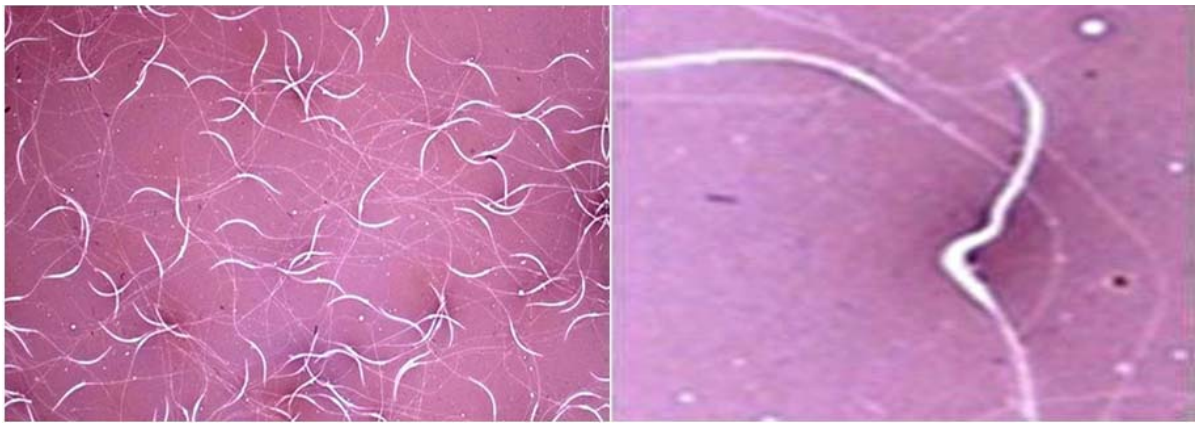

Figure 4 Viability and morphological examination of stained spermatozoa (stain used: eosin/nigrosine)

Figure 5 Hatched live poult and dead in-shell embryos

The sperm concentration values of toms on control diet fall within the acceptable range when compared to the 3.91 × 10^9/mL reported for chicken (Modupe *et al.* 2013), however it is greater than the 2.81 × 10^9/mL observed for local toms. Furthermore, the considerable decrease in testosterone secretion brought on by *G. latifolium*, as noted earlier by Oyeyipo *et al.* (2011) may be related to the disruption of spermatogenesis and thus leading to reduced semen quality in *G. latifolium* treated toms. This agrees with the findings that *G. latifolium*, specifically at higher doses, causes a decrease in sperm level in rats (Ugonna, 2013). Such negative impacts of artificially impairing spermatogenesis on sperm are linked to plants having anti-malaria characteristics and significant levels of alkaloids (Ezeonwu and Dahiru, 2013). The results reveal that toms fed *G. latifolium* had a considerable decline in the percentage of live sperm cells compared to those on control diet. The results was in consistent with the findings of Ugonna (2013), who reported a reduction in the percentage of viable sperm following administration *G. latifolium* in male poultry.

The detrimental impacts of *G. latifolium* on the health of semen according to the findings of Ikpeme *et al.* (2012) who found that administration of *G. latifolium* extract to albino rats significantly reduced sperm viability when compared to the control validates the fact that *G. latifolium* contains some substances that are detrimental to semen quality. The reduction in the proportion of live sperm cells implies in the current study suggest that the *G. latifolium* is effective in altering spermatogenic processes and pathways as also noted by (Raji *et al.* 2003). According to Ekaluo *et al.* (2009), the drop in live sperm cells in the treated toms was a sign that rate of induced mutation on sperm cells increases during spermatogenesis. Percentage of normal sperm cells was higher in toms on control diet compared to *G. latifolium* treated toms. Ikpeme *et al.* (2012) similarly reported that the concentration of both *P. biglobosa* and *G. latifolium* extracts in the treated male rats considerably increased the aberrant sperm morphology. Premature sperm cells and the appearance of multinucleated giant cells, and sign of degeneration, were found in the tes-

tes of chicken fed Neem seed kernel meal but not in the control group (Mohan *et al.* 1997). Additionally, as reported by (Ekaluo *et al.* 2009), the altered epididymal environment may have contributed to the observed rise in the percentage of sperm heads with abnormalities and subsequent decline in semen traits.

The combined effect of *G. latifolium* and *M. oleifera* on semen quality traits of toms are shown in Table 7. The phytochemicals that are present in both plants may have been responsible for the enhancement in semen quality observed in some treatment combinations in the current study. As a nerve tonic, *M. oleifera* controls neuro hormonal operations, regulates the gametogenic and androgenic functions of the testes, and increases the activity of the seminiferous tubules. Upendra *et al.* (2000) showed that male broiler breeders had significantly larger semen volume and other semen characteristics as observed in toms on treatment C. In the main effect of *G. latifolium* on semen quality traits of turkey toms (Table 6), it was discovered that toms on *G. latifolium* had lower semen quality as a result of substances contained in *G. latifolium* which are detrimental to semen quality when compared to those on control diet. But, in its combination with Moringa up to 30 g/kg, % semen motility improved in toms on treatment C and E. This was as a result of the ability and enough of Moringa leaf powder being available to counteract the negative effect *G. latifolium* which would have had detrimental effect on some of these semen quality traits such as sperm motility. In treatment combination of 15 g MO g and 30 g GL/kg, the lower inclusion (15 g MO/kg) of *M. oleifera* was unable to counteract the detrimental impact of *G. latifolium* as a result, toms on treatment D had the lowest mean value (72.47%) for progressive motility. So, it was scientific to conclude that the enhanced % sperm motility recorded in toms on treatment C and E suggested that *M. oleifera* could have provided protection against the detrimental effects of *G. latifolium* on semen quality. The findings of Ekaluo *et al.* (2011) who noted that plants with greater alkaloid and glycoside contents have substantial lethality and may have antifertility effects on the treated animal, are consistent with this conclusion. The findings of Amaglo *et al.* (2010), who reported a positive connection between flavonoids, availability of vitamins, minerals, quality amino acids and sperm production, were consistent with the observed boost recorded in the current study with regards to increased semen volume, % sperm concentration, progressive motility, live sperm, normal sperm cell and reduced dead and abnormal sperm cell in toms on treatment B, C and E. Research has shown that Moringa leaves contain high levels of amino acids, methionine, and cysteine, which are important for the development of germ cells (Ebisch *et al.* 2006) as well as the amino acid arginine for spermatogenesis. Moringa leaves contain poly unsaturated fatty acids which functions to maintain sperm cell viability, sperm maturation, and fertility (Conquer *et al.* 2000). Toms on treatment E showed a reasonable improvement in sperm concertation compared to control group, indicating that *M. oleifera* may have helped to lessen the negative effects of *G. latifolium*. Ikpeme *et al.* (2012) indicated a reduction in sperm count in rats given a high dose of *G. latifolium* extract combined with *P. biglobosa*, which was due to interruption in spermatogenic processes and alteration in the epididymal environment as reported by Ekaluo *et al.* (2009). It can be concluded that toms receiving a combination of 30 g MO + 15 g GL/kg had higher percentage value of normal sperm cells than other those on the other treatment combinations and the control diets. A considerable rise (23.45%) in dead sperm and reduction in the percentage of normal sperm cells (76.05%) were observed in the combination at 15 g MO + 30 g GL/kg. compared to other treatments. High inclusion of *G. latifolium* may be responsible for the high increase in degenerative sperm cells as a result of its contents of substances that are detrimental semen and quantity of 15 g MO/kg was not enough to counteract the detrimental effect *G. latifolium* has on semen. However, the paired treatment's results showed that *M. oleifera,* at an inclusion of 30 g/kg, may have provided defense against *G. latifolium*-induced deformities on the spermatozoa and thus reducing the number of % dead sperm cells, abnormal sperm cells and increased % of live sperm cell as observed in toms on treatment E. According to research by Saalu *et al.* (2011), rats co-treated using an extract of *M. oleifera* leaves and hydroxyurea had their testicles protected against the morphologic, spermatogenic, and oxidative alterations.

The results of the main effect of *M. oleifera* and *G. latifolium* inclusion on fertility and hatchability of turkey eggs are presented in Table 8. From the result, % of infertile eggs were lower in 15 and 30 compared values obtained in 15 and 30 GL/kg and control. The antioxidant and nutritional advantages of *M. oleifera* leaves meal, which are able to sustain the spermatozoa's fertilizing potential and also protect it from oxidative damage, may be responsible for the decrease in the percentage of infertile eggs observed in the study in toms fed *M. oleifera.* However, infertility in poultry chickens has frequently been linked to oxidative stress (Aitken, 2018). At the cellular or individual level, an imbalance between pro-oxidants and anti-oxidants leads to oxidative stress (Bisht *et al.* 2017). The anti-oxidant capabilities of various plant pigments, such as carotinoids, lutein, alpha- and beta-carotene, xanthins, and chlorophyl, have been reported by Fuglie (1999). Poor quality semen may be responsible for rise in the percentage of sterile eggs observed in some treatment combinations. Based on the results of the study, it can be concluded that some chemical

substances found in plants have a negative impact on the quantity and quality of the animal's semen, lowering its fertility rate. The anti-anti semen properties of *G. latifolium* may be responsible for the increase in % of infertile egg observed in 15 and 30 g GL. Nevertheless, increased in % of infertile egg obtained in 15 and 30 g GL/kg could therefore attributed to the poor quality semen used for insemination as well as the physiology of the hen at the time of the insemination (Keith, 2008). Reduced % of dead-in-shell embryos fertilized by semen from toms on 15 g GL could be that negative impact of *G. latifolium* was not enough to disrupt the fertilizing power of semen of toms as a result of its little inclusion compared to 30 g GL/kg. The higher values of % of dead-in-shell for the control group and 30 g GL supplemented toms, respectively, may be caused by the poor semen quality with greater percentage of dead sperm and thus unable to support the sustainability of embryos (Figure 5). Toms treated with 30 g G. *latifolium* had higher % value of dead-in-shell embryo than control toms and this can be attributed to earlier data showing a greater percentage of aberrant sperm in *G. latifolium* treated toms. The results of the study were consistent with those of Keith (2008) who found that while only one sperm is needed to fertilize an egg, sufficient numbers of morphologically healthy sperm are needed to ensure hatchability. Devegowda (2009) cited a decrease in the number of sperm available to fertilize an egg and a drop in sperm quality as causes of embryonic death. The highest % of egg hatchability rate, however, was for eggs laid by hens inseminated with Moringa-treated toms at 15 and 30 g MO/kg. A quality sperm's capacity to carry out fertilization is typically directly tied to an embryo's chance of surviving and eventual hatching. The quality of the semen is therefore crucial for ensuring a high percentage of hatchability. The percentage of hatched eggs recorded in this study was a greater than the range of 22–51% reported by Machebe *et al.* (2013) and lower than the range of 95–100 (%) reported for exotic turkeys (Keith, 2008). The percentage hatchability for the control group was 60.32%, which was higher than the value of 56.25% for local toms reported by Ngu *et al.* (2013). Thus, it can be concluded that adding healthy plant herbs such as Moringa with strong antioxidants to the diet of turkey toms can considerably increase the percentage of egg hatchability of turkey, which is the ultimate goal of any breeder. According to research, natural antioxidants including vitamin E and C, selenium, and carotenoids found in *M. oleifera* play important roles in avian reproduction by maintaining the antioxidant defenses of the spermatozoa and embryonic tissues and this could be responsible to the % increase in fertile egg laid by hens inseminated with semen from toms on 15 and 30 g GL/kg compared to control group and 30 g GL/kg. Moyo *et al.* (2011) showed that Moringa contains high levels of zinc and vitamin E and Amen and Al-Daraji (2011) reported that zinc and vitamin E could play a beneficial role in the hatchability of eggs. Zinc helps with the protection of genetic material structure or deoxyribonucleic acid (DNA) chromatin in the sperm nucleus, which is an important structure for successful fertility.

Results of the combined effect of *M. oleifera* and *G. latifolium* on fertility and egg hatchability of turkey tom's semen are shown in Table 9.

Mahmoud and Hazim (2011) found that supplementing breeder male diets with zinc boosted fertility, hatchability of total eggs, hatchability of viable eggs, sperm egg penetration, and a decrease in embryonic mortality in a trial with broiler breeders. The findings of the current study imply that the decrease in the percentage of dead-in-shell embryos in treatment C may be related to the potent antioxidants found in Moringa. Larger quantities of *G. latifolium* in the mixture resulted in a larger percentage of dead-in-shell embryos, which may be a result of the herb's lowering semen quality. By maintaining the antioxidant defenses of the spermatozoa and embryonic tissues, natural antioxidants including vitamin E and C, selenium, and carotenoids play key roles in avian reproduction (Surai *et al*. 2006). According to Agarwal *et al*. (2004), seminal plasma is the primary source of antioxidants defending the seminal components against oxidative damage. Additionally, according to Sreelatha and Padma (2009), antioxidants found in mature and green leaves both provide superior defense against free radicals and function similarly to pharmaceutical-grade antioxidant preparations. On the other hand, % egg hatchability in treatment C (30 g MO+15 g GL/kg) and E. (30 g MO+30 g GL/kg) were the highest. This could be linked to the various phytochemical, vitamins and mineral contained in Moringa. The combination C and E treatment may have caused increase in embryonic survival. The finding was consistent with that of Durape (2007), who found that broiler breeder hens inseminated with the sperm of males who had received polyherbal supplementation had an improvement in hatchability from 57.2% to 59.1%. Large amounts of vitamin C in *Moringa* may help with fertility because of its capacity to shield cells from free radicals as a potent antioxidant. The idea that oxidative stress harms eggs, sperm, reduces fertility and hatchability of eggs is a recurring one in the reproductive literature.

CONCLUSION

A study was conducted to determine the semen quality, fertility and egg hatchability in local Nigeria turkey toms fed Moringa and Gongronema leaf powder diets. Toms on 30 g MO/kg had highest ($P<0.05$) improvement in semen quality traits. In the main effect of Gongronema on semen

quality, toms on control diet had highest values in semen quality. In combined effect of Moringa and Gongronema semen volume, sperm cell concentration and normal sperm cell values of toms on treatment C were higher ($P<0.05$) compared to other treatments, % fertility values and egg hatchability were highest at 15 and 30 g MO/kg. % fertility and egg hatchability of turkey tom's semen was higher ($P<0.05$) in toms on treatment C and E. It was concluded that M. *oleifera* at 30g/kg diet and combination of *M. oleifera* + *G. latifolium* at 30 g MO kg + 15 g GL/kg. Diet be used to improve semen quality of turkey toms.

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