Hypo and hyper thermic stress effects on *Moringa* extract extender from male turkeys administered *Moringa* leaves and seeds extracts.

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Abstract
The thermic effects on semen samples of male turkeys administered *Moringa* leaves and seeds extracts were examined after being diluted with *Moringa* extract as an extender at different levels (0%, 50% and 100%). A total of 36 male local turkeys were used for the experiment. The male turkeys were administered aqueous extract from *Moringa olerifera* seeds and leaves. The turkeys were assigned into four treatment groups consisting of T1 (0% extract), T2 (100%seed extract) T3 (50%seed and 50%leaf extract) and T4 (100% leaf extract). The treatments was replicated three times. Each treatment had nine turkeys with three turkeys per replicate. Semen collection was done using locally fabricated tools. The collected semen were subjected to thermal stress at 37^0C (hypo) and 46^0C (hyper) for a period of 90minutes. Progressive motility was evaluated very 30minutes with an aliquot of each treatment sample under a warm microscope. The semen was combined with the extender at the rate of 1:5 (semen: extender). The result showed that there were no significant ($p > 0.05$) on the extenders as well as the temperature variation effect from the water bath. However there were significant difference ($P<0.05$) on sub group effect of on hyper thermic conditions, which is an indication that the administered extract had a significant positive effect on the motility rate of sperm cells of local turkeys in the hyper thermic (46^0C) condition. The leaves extracts alone (T4) were significantly highest, while the seeds extracts (T2) was better than the combination effect (T3) when compared with the control (T1). Therefore since there were no significant difference among the extenders (B, C) and the control (A). It then becomes imperative to conclude that; to reduce cost and use locally available materials, using *Moringa* leaves and seeds extracts will go a long way to boost local turkey production by an average Nigerian farmers at the rate of 50% *Moringa* extract to real extender at 46^0C.

Introduction
Low fertility and poor hatchability due to poor semen quality resulting from semen oxidative stress affects turkey production in Nigeria (Bucak-Louis *et al*. 2010). Sexual dimorphism in the size of turkeys contributes largely to their low fertility rate. Therefore artificial insemination (AI) is an option yet to be explored at large. Storage which is an integral part of AI is affected by temperature, as fertility levels of turkey semen drops after 6 hours of storage at refrigeration temperature and those of chicken after 24 hours (Donoghue and Whishart, 2000). Besides, turkey semen are active only in aerobic conditions whereas chicken spermatozoa are active in anaerobic conditions. This makes turkey semen more efficient because it has higher oxidation rate and lower lactic acid accumulation in the presence of oxygen which is the major difference in terms of their metabolism (Douard *et al*., 2000). The use of various plant extracts in animal feeding trails has over the years witnessed increased exploitation due to many health benefits which are well documented (Chia *et al*., 2018). These phytobiotics or phytogenic including herbs and spices and plant extracts are safe and available substitutes to synthetic antibiotics, they are well known for their pharmacological effects and used as feed supplements or medicines in poultry industry (Morna *et al.,* 2017). *Moringa oleifera* is one of such plants that has been used in this regard over the years due to its rich nutritional composition (Moyo *et al.,* 2011). Hence using the leaves and seeds extracts from *Moringa olerifera* as an extender at various temperatures was geared towards improving local productivity of turkeys among rural famers in the least possible way.

MATERIALS AND METHOD
Experimental materials
The *Moringa olerifera* leaves and seeds were procured from the metropolis of Owerri. The leaves and seeds were handpicked, shade dried in an open air room for about one week at room temperature. The leaves seeds were

grounded separately into powder. The ground leaves and seeds were stored in their various designated air-right containers. 1 gram of *Moringa* was diluted in 1000mls of clean water over night. Cheese cloth was used to sieve of the plant particles in the morning and future diluted with 4000ml of clean water. This served as drinking water for each replicate.

Experimental birds and management

A total of thirty six (36) six (6) weeks old male local turkey were purchased from a reputable farm and were quarantined for 2 weeks to enable them adapt and stabilize. The local male turkeys were assigned to 4 treatment groups replicated three times with nine local turkeys per treatment and three turkeys per replicate. The turkeys were separated in each replicate in a deep litter pen spread with wood shavings. The feed and water were given ad-libitum throughout the period. The experiment lasted for seven months (196 days).

Experimental treatments

Treatment one (TI) had no *Moringa* extracts. Treatment two (T2) had only 1 gram of *Moringa* seed powder. Treatment three (T3) had a combination of leaf and seed powder (500 mg of each powder) while treatment four (T4) had only 1 gram of leaf powder.

Preparation and use of the extender

This involved collection of semen from each treatment. The collected semen was pool and divided into six (6). The pooled semen samples were gradually subjected to higher (46^0C) and lower (37^0C) temperature for a period of 1hour. The semen trait was assessed after every 30 minutes. Semen samples from turkey toms was diluted with egg yolk extender in the ratio of 1:5 (semen : extender) The egg-yolk extender consisted of penicillin (0.028g), 1g of glucose, egg yolk (20ml) and distilled water made up to 100ml as control. After dilution, the semen samples were drawn into Eppendorf tubes sealed and maintained at 46^0C for one (1) hour. 50mls of *Moringa* leaves and seeds aqueous extracts were collected in separate test tubes and centrifuged at 3000 revolution per minutes for ten (10) minutes. The clear supernatant fluid of these extract was decanted into a clean beaker. The extender was supplemented at 0% (A), 50% (B) and 100% (C) respectively, and each of the test tube was subjected to hyper (46^0C) and hypo (37^0C) thermic conditions in a water bath. Examined after, zero (0), thirty (30) and sixty (60) minutes at 46^0C and 37^0C, the samples were assessed for progressive motility.

Data collection

At about of twenty six (26) weeks of age, all the male turkey from each treatment group were trained for semen collection using the abdominal massage techniques as was described by Burrows and Quinne (1937) and Baskst and Long (2010). This involves massaging the cloacae region to achieve phallic tumescence. After which the region surrounding the cloacae is gently squeezed (Cloacal stroke) to express the semen (Kalamah *et al.*, 2002). The collected semen was examined for both physical and microscopic parameters. Prior to collection of semen, turkey toms were denied of feed and water for some hours to avoid faecal and urea contamination.

Data evaluation

An aliquot of each sample from extender A (0%), B (50%) and C (100%) was evaluated for progressive motility after every 30minutes.

Sperm motility

The motility of the collected semen was evaluated immediately after collection by taking a normal saline solution with the aid of micropipette on a clean warm (38^OC) glass slide. A clean glass rod was then used to take a very small dab of the whole semen and dropping it on the buffer. A clean cover slip was placed on the drop, then it was allowed to spread under the cover slip but not beyond it. The slide was then viewed under electronic microscope with low magnification (× 400). The observed view was scored in percentage.

Statistical analysis

The 2x3x4 factorial in completely randomized design (CRD) experiment was analyzed using simple T-test and netted design described by Ogbeibu (2014).

Result and Discussion

Table 1; shows the effect of standard extender (A) and *Moringa* leaves and seeds augmented extenders (B and C) on the motility rate of turkey semen over time, under hyper thermic (46^0C) and hypo thermic (37^0C) conditions. The progressive motile sperm of the *Moringa* administered treatment groups was in line with the observations of Zaharadden *et al.,* (2005) 80.17±2.63 – 84.25±2.23 in local turkey breeds. It was observed that there were no significant (P>0.05) effect of extender on the hypothermic and hyper thermic conditions on motility rate of the sperm cells. Although numerically the extender B (50% *Moringa* extract) and C (100% *Moringa* extracts) subgroup effects were far better than the control under both hypo and hyper thermic stress conditions. Best motility rate was observed in extender B under effect of seeds extracts in hypo thermic condition. While extender B with male turkeys administered leaves extracts thrived better in hyper thermic conditions. This may imply that *Moringa* extract really improved the motility rate at both higher and lower body temperatures. There were no significant difference (P>0.05) on both thermic conditions on which the samples were subjected. However there were significant difference (P<0.05) on sub group effect of on hyper thermic conditions. This indicates that the administered extract had a significant positive effect on the motility rate of sperm cells of local turkeys in the hyper thermic (46^0C) condition. The leaves extracts alone (T4) were significantly highest, while the seeds extracts (T2) was better than the combination effect (T3) when compared with the control (T1). These findings are in line with those of Alemade *et al.,* (2014) on use of *Moringa* leaves on rabbit reproductive response. Therefore since there were no significant difference among the extenders (B, C) and the control (A). It then becomes imperative to conclude that, to reduce cost and use locally available materials and enhance productivity, using *Moringa* leaves and seeds extracts will go a long way to boost local turkey production by an average Nigerian farmers at the rate of 50% *Moringa* extract to real extender at 46^0C.

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