



## Effects of *Moringa Oleifera* Linn Seed Administration on Sperm Production Rate and Gonadal Sperm Reserve in Rabbits

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### Authors' contributions

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

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### ABSTRACT

**Aim of Study:** To investigate the effects of oral administration of moringa seed on sperm production rate and gonadal sperm reserve in rabbits.

**Study Design:** 7 Month old mixed breed male rabbits were randomly assigned into two treatment groups. Group 1(n=30) was the control group whereas the rabbits in group 2 (n=30) were administered *Moringa oleifera* seed powder in drinking water (250mg/L). After 4 weeks, the gonadal sperm reserve, daily sperm production, sperm production per gram testes and testicular dimensions (weight, volume and density) was compared between the two groups.

**Place and Duration of Study:** Livestock farm, College of Agriculture, Lafia, Nasarawa state, Nigeria, from May to June 2013.

**Methodology:** Gonadal sperm reserve was estimated using a haemocytometer. Testicular weight and volume were determined using laboratory scale and water displacement method respectively. Parenchyma (1g) of each testis was sectioned and homogenized in 10ml of normal saline. The homogenate was filtered through 2 layers of loosely netted bandage. Spermatozoa number was determined using an improved Neubauer chamber. Daily sperm production (DSP) was estimated by dividing the gonadal sperm reserve by a time divisor of 3.66 corresponding to the time in days of the duration of the seminiferous epithelium cycle. Daily sperm production per gram testes (DSPG) was determined by dividing the DSP by the weight of testicular parenchyma.

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**Results:** The results indicate significantly higher gonadal sperm reserve and daily sperm production for the control group compared to the treatment, ( $4.37 \pm 0.18$  to  $3.27 \pm 0.32$  and  $1.20 \pm 0.06$  to  $0.77 \pm 0.09$ ) Mean values of daily sperm production per gram testes were however similar for all groups ( $0.53 \pm 0.03$  and  $0.47 \pm 0.20$ ). Testicular weight ( $2.20 \pm 0.06$  to  $1.43 \pm 0.12$ ) and volume ( $2.20 \pm 0.15$  to  $1.73 \pm 0.07$ ) were significantly higher for the control group compared to treatment ( $P=0.05$ ). Tissue density was similar for both groups ( $1.01 \pm 0.05$  to  $0.82 \pm 0.07$ ).

*Keywords: Rabbits; moringa seed; sperm reserve; testicular dimensions.*

## 1. INTRODUCTION

*Moringa oleifera* has the potential to improve nutrition, boost food security, foster rural development and support sustainable land use. It may be used as forage for livestock, a micronutrient liquid and a natural anthelmintic [1]. As an important tropical food crop, *moringa oleifera* is grown in sub-Saharan Africa for human consumption, herbal medicines, as a male aphrodisiac, water purification, livestock forage, a source of tannins and dyes [2]. The oil from dried seed showed antioxidant activity [3]. It has also been reported that *M. oleifera* seeds contain an estrogenic compound (Oleanolic acid) capable of disrupting the gonad function, differentiation and sexual maturation in Mozambique Tilapia, it is also associated with decreased sperm production and degeneration of testicular tissues [4]. *Moringa oleifera* roots are reported to have estrogenic effects, it inhibits maintenance and growth of the reproductive system [5]. All parts of Moringa tree are being consumed by humans with the secondary metabolites having the potential to affect health [6]. The secondary metabolites include alkaloids, glycoside, flavonoids, tannins, saponins, steroid and reducing sugar [7]. The objectives of this research is to investigate the effects of oral administration of moringa seed on sperm production rate and gonadal sperm reserve in rabbits.

## 2. MATERIALS AND METHODS

### 2.1 Location of Study

The research was conducted in the livestock farm of College of Agriculture, Lafia, Nasarawa state. It is located in the guinea savannah zone of northern Nigeria and found on latitude  $08^{\circ} 35'$  N and longitude  $08^{\circ} 33'$  E, respectively.

### 2.2 Experimental Animals and Design

A total of sixty mixed breed male rabbits (7 months old) were used for the study. The rabbits were individually housed in well ventilated wire cages, wooden stand was used to raise the cages above concrete floor. The animals were randomly assigned into two equal groups (30 each). Rabbits in group 1 ( $n=30$ ) serve as control and were given normal drinking water. Animals in group 2 ( $n=30$ ) were administered aqueous solution of *M. oleifera* seed powder at the rate of 50mg/kg/day (orally). *Moringa oleifera* seeds were collected from gardens around Lafia, Nasarawa State, Nigeria. The seeds were sun dried and milled to powder using a laboratory mill. The powder was dissolved in distilled water to make a solution of 25mg/ml. The experiment lasted for 4 weeks.

### 2.3 Evaluation of Gonadal Sperm Reserve

The rabbits were euthanized by cervical dislocation. Gonadal sperm reserve was estimated using a haemocytometer [8]. The connective tissues adhering to the testes were separated. Testicular weight and volume were determined using laboratory scale and water displacement method respectively. Parenchyma (1g) of each testis was sectioned and homogenized in 10ML of normal saline. The testicular homogenate was filtered through 2 layers of loosely netted bandage. The spermatozoa numbers were determined using an improved Neubauer chamber. Daily sperm production (DSP) was estimated by dividing the gonadal sperm reserve by a time divisor of 3.66 corresponding to the time in days of the duration of the seminiferous epithelium cycle (4). Daily sperm production per gram testes (DSPG) was determined by dividing the DSP by the weight of testicular parenchyma.

### 2.4 Statistical Analysis

The significance of differences ( $P=0.05$  or  $P<0.01$ ) was examined using t-test. Statistical analysis was run on SPSS 2010 [9].

## 3. RESULTS AND DISCUSSION

The effects of *Moringa oleifera* seed on sperm reserve is shown in Table 1. The results showed significantly higher mean values of gonadal sperm reserve and daily sperm production for the control group compared to the treatment ( $P=0.05$ ). Mean values of daily sperm production per gram testes are however similar for all groups.

**Table 1. Effects of *Moringa oleifera* seed on sperm production rate and gonadal sperm reserve**

Parameters	Control	Treatment
	Mean	Mean
Gonadal sperm reserve ( $10^9$ )	4.37 <sup>a</sup> ±0.18	3.27 b±0.32
Daily sperm production( $10^9$ )	1.20 <sup>a</sup> ±0.06	0.77 b±0.09
Daily sperm production per gram testes( $10^9$ )	0.53 <sup>a</sup> ±0.03	0.47 <sup>a</sup> ±0.20

Means within the same raw bearing different superscripts are significantly different ( $P=0.05$ )

Mean values of testicular weight and volume are significantly higher for the control group compared to treatment ( $P=0.05$ ). The values of tissue density are similar for both groups (Table 2).

**Table 2. Effects of *Moringa oleifera* on testicular dimensions**

Parameters	Control	Treatment
	Mean	Mean
Weight of paired testes (g)	2.20 <sup>a</sup> ±0.06	1.43b±0.12
Volume of paired testes (cm <sup>3</sup> )	2.20 <sup>a</sup> ±0.15	1.73 b±0.07
Tissue density	1.01 <sup>a</sup> ±0.05	0.82 <sup>a</sup> ±0.07

Means within the same raw bearing different superscripts are significantly different ( $P=0.05$ )

Earlier reports indicate that *M. oleifera* seeds contain bioactive chemicals capable of disrupting the gonad function, differentiation and sexual maturation of Mozambique tilapia. It

has also been associated with decreased sperm production and degeneration of testicular tissues [4]. Similarly; it has also been reported to cause disintegration of testicular tissues thereby acting as an effective sterility agent [6]. *Moringa oleifera* seeds contain an estrogenic compound called oleanolic acid as reported by [4]. Testicular atrophy has also been reported in rats administered exogenous estrogens [10,11]. A positive correlation between testicular weight, gonadal sperm reserve and daily sperm production have also been reported in different species of animals [12,13]. The reduced testicular mass observed in this study is also an indication of testicular degeneration. It is well known that gonadotropins are the major regulators of spermatogenesis. Luteinizing hormone targets the leydig cells to stimulate production of androgens namely testosterone which in turn act on androgenic receptors in the seminiferous epithelium to control spermatogenesis. Follicle stimulating hormone target receptors within the sertoli cell to regulate spermatogenesis. The initiation and maintenance of normal spermatogenesis and thus fertility rely on a delicate balance of the hypothalamo-pituitary-testis axis [9]. The negative feedback effect of testosterone on both hypothalamus and pituitary to regulate gonadotropin is well established. It has been reported that a major component of the negative feedback action of androgens on gonadotropin secretion is mediated via aromatization to estrogen [9]. Administration of estrogen is reported to cause reduction in circulating gonadotropin releasing hormone and profound suppression of follicle stimulating hormone and luteinizing hormone [14]. Similar findings indicate that estrogen administration resulted in atrophy of the seminiferous tubules and a marked decrease in Leydig cells, correlated with low plasma gonadotropin levels. In all cases, the rete testis appeared hyperplastic and the other components (efferent ductules, epididymis) well preserved [15].

#### 4. CONCLUSION

The study showed that *M. oleifera* seed administration result in reduction of daily sperm production and gonadal sperm reserve associated with diminished testicular weight. Sperm production per gram testes was not affected by treatment. There is need for caution in the consumption of *M. oleifera* seed for medicinal purposes.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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