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Potential of *Moringa oleifera* leaf meal in improving reproductive efficiency of rabbit bucks in hot climate

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***Target Audience:** Rabbit Farmers, Livestock researchers, Nutritionist, Rabbit breeders*

Abstract

Several parts of the plant Moringa oleifera are used for therapeutic purposes in farm animals. The leaves have been traditionally used as an ingredient in some herbal formulations as blood purifier, cholesterol reducing agent, immune and possibly reproductive enhancers in farm animals. This experiment was conducted to determine the effect of varied levels of supplemental Moringa oleifera leaf meal (MoLM) on sperm storage and production potential of rabbit bucks in hot climate (26.8-30.7 ℃). In a 168-day feeding trial, a total of 20 rabbit bucks were allotted to four dietary treatments containing 0, 2.5, 5.0 and 7.5% MoLM with five replicates per treatment in a completely randomised design. At the end of the feeding trial, the rabbits were sacrificed; testes and epididymides excised, weighed and homogenized separately for sperm cell count in the homogenate to determine sperm reserves. Daily sperm production was estimated from testicular sperm reserves. Testicular and epididymal sperm reserve of bucks were significantly ($p<0.05$) influenced by the dietary treatments. The testicular weight was not significantly different among the dietary treatments. However, testicular sperm reserve ($x10^6$sperm cells/ml) in the left, right and paired testes of the bucks fed treatments 1(52.96±11.12) and 2 (55.22±16.78) were not significantly different from each other but they were significantly ($p<0.05$) higher than those fed treatments 3 (15.52±3.53) and 4 (14.4±1.53). Epididymal sperm reserves ($x10^6$sperm cells/ml) of bucks fed T1 (118.95±4.01) and T2 (120.16±3.24) were significantly ($p<0.05$) higher than those on T3 (76.56±6.18) and T4 (68.65±6.58). The daily sperm production ($x10^6$ sperm cells) in paired testes of bucks fed Treatments 1 (15.40±0.46) and 2 (16.10±0.05) were not significantly different from each other but both were significantly ($p<0.05$) higher than those fed Treatment 4 (4.20±0.07). This suggest that feeding rabbits with Moringa oleifera leaf meal up to 2.5% as a supplement improved sperm production and sperm reserves, while above this level it reduced sperm storage potential and daily sperm production.

Keywords: Moringa leaf meal, Sperm reserves, Sperm production, Rabbits

Description of Problem

Rabbit meat production has been on the increase in Nigeria in recent years. The rabbit (*Oryctolagus cuniculus*) is one of the most productive meats producing specie among all domesticated animals. In addition to this, rabbits have several other characteristics that might be advantageous to subsistence farming system, such as their small body size, short generation interval with a relatively short gestation period average of 30-31 days. The daily weight gain is high in proportion to the body weight, which gives them a rapid growth rate, and makes them to attain sexual maturity

early. These factors result in the rabbit reaching the weight of a sexually mature animal 30% faster than other animals [1] and make rabbits suitable as meat producing small livestock in developing countries [2].

Recently, there has been interest in the utilization of Moringa (*Moringa oleifera*) as a protein source for livestock [3, 4]. *Moringa oleifera* leaves have quality attributes that make it a potential replacement for soybeans meal or fish meal in non-ruminant diets. The effect of Moringa leaf meal on growth and blood profile of some animal species has been documented [5, 6]. However, the available reports in these areas outweighed information on the influence of the leaf meal on the reproductive potential of animals. Hence, the need to assess the sperm storage capability of rabbit bucks fed varied supplemental levels of *Moringa oleifera* leaf meal.

Materials and Methods

Processing of moringa leaves

The harvested Moringa leaves were air dried under a shed until they were crispy to touch, while retaining their greenish coloration. The leaves were then milled using a sieve to obtain a product herein referred to as *Moringa oleifera* Leaf Meal (MoLM) for use.

Experimental diets

The control diet was formulated to contain 15.90% crude protein, 10.84% crude fibre and 2519.28 kcal/kg digestible energy and the four experimental diets were as follows: T1 = 100% control diet + 0% MoLM; T2 = 97.5% control diet + 2.5% MoLM; T3 = 95.0% control diet + 5.0% MoLM; and T4 = 92.5% control diet + 7.5% MoLM [12]. The calculated nutrients for each treatment are presented in Table 1.

Table 1: Calculated nutrients in the experimental diets with the varied levels of *Moringa oleifera* leaf meal.

NUTRIENT	T1 CD (0% MoLM)	T2 CD+2.5% MoLM	T3 CD + 5.0% MoLM	T4 CD + 7.5% MoLM
Crude Protein (%)	15.90	16.20	16.50	16.80
Crude Fibre (%)	10.84	10.85	10.86	10.87
Digestible Energy (Kcal/kg)	2519.28	2488.70	2458.12	2427.54

CD – Control Diet, MoLM – *Moringa oleifera* Leaf Meal

Experimental animals and management

The study was carried out at the rabbitry unit of Teaching and Research Farm of the University of Ibadan, Ibadan in hot climate (26.8-30.7℃). A total of 20 rabbit bucks were used for this experiment**.** The bucks were allocated randomly into four (4) treatments with 5 rabbit bucks per treatment. They were housed in individually in separate hutches raised from the floor. The animals were fed their respective diet twice a day at 8.00am and 2.00pm. The experiment lasted for twenty-four weeks.

Estimation of testicular sperm reserves

The right and left testis of each rabbit were carefully removed after slaughtering, trimmed and weighed. The left and right testes were homogenized separately, in physiological saline (0.154 M NaCl) at 200mg/ml. The suspension was mixed and sieved through a double layer of sterile gauze into calibrated test tube and analyzed the same day. Testicular sperm reserve was estimated as the total number of late spermatids and spermatozoa in the testicular homogenate. All sperm reserves were expressed in millions [7].

Estimation of epididymal sperm reserves

The left and right epididymides were carefully separated from the testis. It was thereafter separated into the 3 regions: caput, corpus, and cauda. Each section was homogenized in the normal saline. The homogenate was filtered using a cheese cloth into a test tube and analysed immediately. Epididymal sperm reserve was determined by haemocytometric counting of sperm cells in the homogenate. Daily sperm production (DSP) was estimated from the testicular sperm reserves using this formula [7];

$$DSP = \frac{[Testicular\ Sperm\ Reserve]}{[Time\ Divisor\ (3.43)]}$$

Data analysis

Data obtained were subjected to one-way analysis of variance at P=0.05 and differences were separated using Duncan multiple range test.

Results

Testicular weight and sperm reserves of bucks fed supplemental *Moringa oleifera* leaf meal

The results of testes weight and testicular sperm reserves of rabbit bucks fed varied supplemental levels of *Moringa oleifera* leaf meal are presented in Table 2. The weight of left, right and paired testes was not significantly different among the treatments. The sperm reserves in the left, right and paired testes were significantly ($p<0.05$) higher in rabbits on the control and T2 than those on T3 and T4. However, the testicular sperm reserves of rabbits fed T3 was not significantly different from that of bucks fed T4. The testicular sperm reserves of rabbits fed T2 was not significantly different from that of bucks fed the control diet.

Table 2: Testis weight and sperm reserves of bucks fed varied levels of *Moringa oleifera* leaf meal

Parameters	T1 CD+0% MoLM	T2 CD+2.5% MoLM	T3 CD + 5.0% MoLM	T4 CD + 7.5% MoLM
***Relative Testis weight (%):**				
Left testis	0.14 ± 0.03	0.18 ± 0.04	0.12 ± 0.05	0.25 ± 0.02
Right testis	0.14 ± 0.04	0.16 ± 0.04	0.11 ± 0.03	0.12 ± 0.01
Paired testes	0.27 ± 0.06	0.35 ± 0.08	0.23 ± 0.07	0.37 ± 0.02
Testicular sperm reserve (×10⁶/g testis):				
Left testis	30.88 ± 5.47^{a}	29.82 ± 9.10^{a}	6.40 ± 1.21^{b}	7.68 ± 1.02^{b}
Right testis	22.08 ± 6.34^{a}	25.60 ± 8.71^{a}	9.12 ± 2.63^{b}	6.72 ± 0.78^{b}
Paired testes	52.96 ± 11.12^{a}	55.22 ± 16.78^{a}	15.52 ± 3.53^{b}	14.40 ± 1.53^{b}

a,b – means in the same row with different super script are significantly different (P<0.05)
CD – Control Diet, MoLM – *Moringa oleifera* Leaf Meal; *Relative to liveweight

Epididymal sperm reserves of bucks fed supplemental *Moringa oleifera* leaf meal

The results of epididymal sperm reserves of bucks fed varied levels of Moringa oleifera leaf meal are presented in Table 3. Sperm reserve ($x10^6$) in the three regions of the epididymis was significantly ($p<0.05$) altered among the treatments. At caput, the sperm reserves in left caput were significantly ($p<0.05$) higher in rabbits fed T2 and control than those fed T3 and T4. However, the sperm reserves in the right caput epididymis of

rabbits fed T2, T3 and T4 were not significantly different from one another. Sperm reserve in the left corpus epididymis of rabbits fed T2 and T3 were not significantly different from that of bucks fed the control diet. Sperm reserves in caudal epididymis of rabbits fed T2 were not significantly different from that of the control rabbits but were significantly higher than those fed T3 and T4. The epididymal sperm reserves of rabbits fed T2 was not significantly different from that of rabbits fed the control, but both was significantly ($p<0.05$) higher than that of rabbits fed T3 and T4.

Table 3: Epididymal sperm reserves of rabbit bucks fed varied levels of *Moringa oleifera* leaf meal

Parameters ($x10^6$)		T1 CD + 0% MoLM	T2 CD+ 2.5% MoLM	T3 CD + 5.0% MoLM	T4 CD + 7.5% MoLM
Caput:	Left	10.99 ± 0.14^{a}	10.56 ± 0.37^{a}	5.76 ± 0.25^{b}	4.00 ± 0.12^{b}
	Right	8.11 ± 0.65^{a}	6.08 ± 1.90^{ab}	6.51 ± 3.47^{ab}	5.30 ± 2.06^{b}
Corpus:	Left	5.01 ± 1.61^{a}	3.71 ± 0.49^{ab}	3.31 ± 1.48^{ab}	2.23 ± 0.76^{b}
	Right	3.63 ± 0.35	3.81 ± 0.45	3.20 ± 1.35	2.27 ± 1.29
Caudal:	Left	35.41 ± 0.78^{ab}	51.79 ± 0.37^{a}	20.05 ± 0.68^{b}	19.45 ± 1.12^{b}
	Right	52.80 ± 1.15^{a}	44.79 ± 1.3^{ab}	37.73 ± 1.26^{b}	35.40 ± 2.25^{b}
Epididymal sperm reserves		118.95 ± 4.01^{a}	120.16 ± 3.24^{a}	76.56 ± 6.18^{b}	68.65 ± 6.58^{b}

a,b – means in the same row with different superscripts are significantly different ($P<0.05$)
CD – Control Diet, MoLM – *Moringa oleifera* Leaf Meal

Daily sperm production of rabbit bucks fed supplemental *Moringa oleifera* leaf meal

The result of daily sperm production (DSP) of rabbit bucks fed varied levels of *Moringa oleifera* leaf meal are presented in Table 4. The daily sperm production ($x10^6$ spermatozoa) from right, left and paired testes were significantly ($p<0.05$) different among the treatments. The highest value for DSP of paired testes was observed in bucks fed T2 (16.10±0.50) which was not significantly different from those bucks fed control diet (T1), while the least (4.20±0.07) value was recorded in bucks fed T4.

Table 4: Daily sperm production of rabbit bucks fed varied levels of *Moringa oleifera* leaf meal

Parameters	T1 CD + 0% MoLM	T2 CD+ 2.5% MoLM	T3 CD + 5.0% MoLM	T4 CD + 7.5% MoLM
Right testis ($x10^6$sperm cells/mL)	6.44 ± 2.63^{b}	8.19 ± 5.44^{a}	7.46 ± 3.35^{ab}	1.96 ± 0.35^{c}
Left testis ($x10^6$sperm cells/mL)	9.00 ± 2.29^{a}	7.87 ± 0.49^{ab}	5.78 ± 3.88^{b}	2.24 ± 0.37^{c}
Paired testes($x10^6$sperm cells/mL)	15.40 ± 0.46^{ab}	16.10 ± 0.50^{a}	13.2 ± 0.07^{b}	4.20 ± 0.07^{c}

a,b,c – means in the same row with different super script are significantly different ($P<0.05$)
CD – Control Diet, MoLM – *Moringa oleifera* Leaf Meal

Discussion

Efficiency of spermatogenesis has been reported to be the estimated number of spermatozoa produced per day per gram of testicular parenchyma [8]. In this study, it was observed that testicular sperm reserve decreased with the inclusion of 5.0 and 7.5% MoLM in the diet of rabbit bucks. Rabbits fed 7.5% MoLM with the highest paired testes weight (0.37%) had the least testicular sperm reserve (14.4 x 10^6 sperm cells/g of testes). This result was at variance with the findings of Ekeocha [9] who reported that within specie, sperm production is a function of testicular size. It has been reported that prolonged consumption of MoLM or its extract at high concentration predisposes organs to damage [10], thus resulting in impaired function. Reduced testicular sperm reserve observed in rabbit bucks fed 5.0 and 7.5 % MoLM levels in this study may be due to the toxic effect of some anti-nutritional components of the Moringa leaf meal such as tannin [3]. Ewuola and Egbunike [7] reported tendency of reduced sperm reserve and daily sperm production when an animal is exposed to feed that contain potentially toxic substance. However, the significant increase in the testicular sperm reserves observed in rabbit bucks fed 2.5% MoLM could be attributed to beneficial effect of the moringa leaf in stimulating spermatogenesis and subsequent sperm cell formation in the testes. The leaf has been reported to contain phytochemicals like β-carotene and tocopherol, which enhances sperm formation [11, 12]. The significant increase in epididymal sperm reserves of rabbits fed 2.5% MoLM than those on higher inclusion levels could be attributed to treatment effect. This indicates that the leaf is best use as supplement at lower inclusion level to prevent accumulation of its inherent anti-nutrient in the animal. Also, it will encourage proper utilization of its antioxidants and other beneficial phytochemicals [13]. This in turn helps to mitigate reactive oxygen species and free radicals induced by hot climate, which affect reproductive efficiency of male rabbits in the tropics [14, 15]. The result from this work is at variance with the findings of Abu *et al*. [16] who reported that Moringa leaf meal did not have adverse effect on epidydimal sperm reserves in rabbit when they were fed up to 15% level for 10 weeks probably because of differences in the exposure period. However, Ogbuewu *et al*. [17] and Amao *et al*. [18] reported reduced sperm reserve and daily production in rabbit bucks when fed similar levels of neem leaf meal as compared to expected daily sperm production which was estimated to be 40.5 x10^6 per gram of testis [19]. Reduction in daily sperm production was observed with increase in the inclusion level of MoLM above 2.5%. The daily sperm production of rabbits fed 2.5% MoLM compared favourable with that of bucks not fed MoLM, thus indicating that the use of the leaf meal at this level did not adversely altered the nutritional quality of the diet at the expense of the sperm production in the rabbit bucks. This also agrees with the earlier report of no adverse effect of the crude moringa leaf extract on the blood profile and health status of gestating and lactating does [20].

Conclusion and Applications

1. This study showed that prolonged consumption of *Moringa oleifera* leaf meal at 2.5% in rabbit diets improved testicular sperm reserves, sperm storage capability and increased daily sperm production.
2. Inclusion of MoLM above 2.5% depressed reproductive potential of rabbits when fed for a prolonged period probably due to accumulation of inherent antinutritional factors over the period of administration.
3. It is therefore recommended that feeding Moringa leaf meal as a supplement should not exceed 2.5% for male rabbits intended for breeding purpose.

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