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Impact of Supplementing Fresh *Moringa oleifera* Leaves in Diet to Improve Semen Quality in Rabbit Bucks

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ABSTRACT

Background: This research focused on assessing the effects of *Moringa oleifera* leaves (MOL) as a dietary supplement on the quality and characteristics of semen in rabbits.

Methods: Eighteen (n=18) breeding bucks of New Zealand white, of similar age group, were used for the study. Three feeding regimes, (i) 100% commercial rabbit pellets (CRP)-Group I (ii) 90% CRP + 10% fresh MOL on a dry matter (DM) basis - Group II and (iii) 80% CRP + 20% fresh MOL on a DM basis – Group III, were adopted and the trial continued for 21 days. After adaptation to the diet, semen was collected from each buck and subjected to evaluation using computer- assisted semen analyzer.

Result: The sperm count, normal sperm morphology and sperm motility increased in comparison with the control. The inclusion of 20% *Moringa oliefera* in the diet (Group III) caused a significant increase ($P<0.05$) in sperm concentration (Control =136.2 M/ml; Group III=297.2 M/ml). There was no significant difference ($P>0.05$) in sperm motility and semen volume among the groups. The findings indicate that adding 20% fresh *Moringa oliefera* leaves on a dry matter basis to commercial rabbit pellets can enhance the quality and traits of semen in breeding bucks.

Key words: *Moringa oliefera*, Morphology, Motility, Rabbit bucks, Semen.

INTRODUCTION

Enhancing reproductive efficiency in rabbit bucks relies on various factors including diet, genetic lineage, health condition, management practices, seasonality and age. These factors lead to differences in semen quality (EL Deeb Mariam *et al.,* 2015). *Moringa oleifera*, commonly known as the horse radish tree, is utilized as an inexpensive source of protein for animal feed (Sarwatt *et al.,* 2002). It is rich in carotene, ascorbic acid, vitamin B, calcium, potassium, selenium, zinc and iron. Rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins, leaves are the most utilized portion of plants. The insular tropical climate is ideal for the plant's survival. In addition to thriving in hot, dry regions and the wet tropics, it can also endure on less rich soils and is not greatly impacted by drought (Anwar *et al.,* 2007). Due to this fact, *Moringa oleifera* can be grown for inclusion into an animal's diet. This plant has reportedly been extensively used in ethnoveterinary and traditional medicine (Tabassam *et al.,* 2014). The leaves are well known for its antimicrobial effects. The *Moringa olifera* leaves extract (MOLE) may be a good substitute for the antibiotic component of traditional bovine semen extenders when refrigerated at refrigerated temperature. It may improve semen quality, reduce microbial load and help maintaining fertilization potential (Chatley *et al.,* 2025).

Moringa leaf extract (MLE) is one of the natural ingredients that can improve the quality of preserved semen by preventing sperm lipid oxidation and increase the sperm storage time (Laskar *et al.,* 2025). One of the most crucial aspects of reproduction is sperm viability and inadequate nourishment might produce semen of dubious quality. Poor environmental conditions cause farm animals' fertility and semen quality to decline (Rasooli *et al.,* 2010). Significant reductions in sperm motility and plasma membrane integrity, as well as notable increases in sperm abnormalities and DNA damage that results in infertility, have been linked to the accumulation of free radicals (Potts *et al.,* 2000).

Various natural feed resources have been employed for practical, safe and cost-effective purposes to mitigate adverse effects on male rabbits' semen quality (Elnagar *et al.,* 2010; Hashem *et al.,* 2013). Prior research demonstrated that in stressed male rats, Moringa Oleifera ethanolic extract (MOEE) might increase libido and sperm quality as well as sexual activity and testosterone levels (Prabsattroo *et al.,* 2015). As it relates to semen characteristics and its quality, it is very important that semen and its compositions remain of the best quality at all time, for good reproductive

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performance. Determining the concentration in a semen sample is crucial because, if the ejaculate quality is sufficient for insemination, this parameter is utilized to calculate how many spermatozoa will be good enough for insemination (Di-Iorio *et al.,* 2014).

One of the most crucial characteristics linked to semen fertilization capacity is unquestionably sperm motility. The most popular and original measure of sperm function is still sperm motility (Partyka *et al.,* 2012). Sperm viability is also a key determinant of sperm quality and a prerequisite for successful fertilization. In addition to visual assessment, computer-assisted semen analysis (CASA) or photographic analysis can be utilized to assess sperm concentration, motility, morphology and volume (Verstegen *et al.,* 2002; Rijsselaere *et al.,* 2003). In order to control feed and improve animal reproductive performance, new feed sources had to be explored due to the scarcity of grain and the high price of imported feed additives.

Thus, the purpose of this study was to find out how much fresh *Moringa oleifera* leaves were added to commercial rabbit pellets and how that affected the semen qualities of rabbit bucks in Trinidad's tropical climate.

MATERIALS AND METHODS

Animals

The experiment was carried out using eighteen (n=18) sexually matured New Zealand white rabbit's bucks weighing 3200 g-3900 g. The animals were acquired from the rabbitry unit of the field station of the University of the West Indies, Trinidad and Tobago during the academic year 2020-21 and were placed in hutches with a size of 30″ × 25″. Throughout the period of the experiment, bucks were exposed to subtropical conditions, where the average minimum temperature ranged from 21.0 to 23.0°C and the average maximum temperature was 32.0 to 35.0°C. Good ventilation and fresh air were provided to minimize ammonia levels in the house, as well as hygienic elimination of wastes.

Plant collection and preparation of *Moringa oleifera* leaves

Fresh leaves of *Moringa oleifera* were collected in the morning from the University field station farm, Mt. Hope, UWI. The plants were identified and fresh leaves were manually removed from the stem, cleaned to remove sand and other impurities. The fresh leaves were harvested a day before to feeding and air-dried under shade to reduce the moisture content.

Supplement of *M. oleifera* leaves in rabbit's diet

All the rabbits were fed on commercial food pellets (Master Mix® Ltd.) and provided *ad libitum* drinking water during whole experimental periods. The diet composition on a dry matter basis contains 17.0% crude protein, 15% crude fiber, 2.5% crude fat along with trace elements such as zinc, copper sulphate, niacin, trace of cobalt and many other elements.

The rabbits were divided into three groups of 6 rabbits each. The first group received 100% commercial food pellets (concentrate) and served as control (Group 1). The second group have received a dosage of 90% concentrate and 10% (40 g) fresh *Moringa oleifera* leaves (Group 2). The third group were received 80% concentrate and 20% (80 g) fresh *Moringa oleifera* leaves (Group 3). The bucks were fed with the respective diets for three weeks, following which semen was collected artificially after eight weeks of feeding.

Semen collection

Semen was collected from six sexually mature male rabbits in each group by using a specially constructed artificial vagina, which was filled with warm water. A matured doe was placed in the mating pen with the buck to stimulate libido and to increase sperm concentration. After a couple of strokes done by the buck on the does, the penis was then guided into the artificial vagina for collection of semen. Immediately after collection, the ejaculates were transferred to the laboratory and care was taken to avoid exposure of the semen to any unfavorable conditions during or after collection. A different artificial vagina was used for each collection and was collected under hygienic conditions to prevent bacterial and environmental contamination. The animals were ejaculated twice a week in the morning hours. Semen volume was read off the collection tube and recorded in milliliters.

The semen collection was carried out at Artificial Breeding Centre (ABC), Aripo, which is a government farm specially designed for the collection of animal semen for distribution via artificial insemination to the farming public.

Semen evaluation

Semen was evaluated for determination of semen volume, sperm motility, sperm morphology, sperm concentration and live sperm count using Computer Assisted Semen Analyser (CASA), a computerized program called HT CASA II, Hamilton Thorone, USA.

This equipment gives a detailed evaluation of a semen sample immediately after collection. The first measure of evaluation done is the volume of the semen ejaculated. Semen was then diluted with 1:5 in an extender (Cortalap® IMV Technologies) using a commercial buffer solution. Before the buffer was used, it was placed in a water bath at a temperature of 30°C as to prevent shock. Semen was then aspirated from collecting tube using a micro pipette and then gently placed in a four- chambered slide for evaluation.

Statistical analysis

All data produced from the experiment were analyzed using the linear models Statistical Package for Social Sciences (SPSS) software. Semen characteristics were analyzed using one- way analysis of variance (ANOVA) tests for comparisons among means between the three treatments. Duncan is multiple range tests were used to locate points of significant differences between groups.

RESULTS AND DISCUSSION

Results of semen quality assessment, total volume (ml), sperm concentration (10^6/ml), motility (%), live sperm (%), abnormal sperm (%) and total sperm output (10^6/ ejaculate) are presented in Table 1. Significant differences (P<0.05) were observed on the effects due to inclusion levels of *Moringa oleifera* leaves (*MOL*) in rabbit diet on the sperm concentration and total sperm output. There were no significant differences (P>0.05) observed on the effects due to the inclusion levels of *Moringa oleifera* leaves on the other parameters like reaction time, semen volume, forward motility, live sperm, sperm abnormalities, semen appearance and semen viscosity.

This research demonstrates that feeding of *Moringa oleifera* leaves at a level of 10% and 20% in commercial rabbit pellets in male rabbit (bucks) caused a significant increase in sperm concentration and total sperm output in the rabbit.

Sperm concentration and total sperm output (TSO) was significantly (P<0.05) influenced by treatment. The highest sperm concentration and TSO (297.2×10^6/ml) were observed in Group 3, followed by Group 2 (221.0×10^6/ml and 183.4×10^6/ml) and control Group 1 (136.2×10^6/ml).

The effect of *Moringa oleifera* leaves on semen morphology is shown in Fig 1 and Table 1. The effect of *MOL* on semen morphology manifested in treatment groups 2 and 3 compared to group 1 (control). The result illustrated that *MOL* inclusion in the rabbit diet increased the normal morphology of rabbit sperm (Fig 1). It also shows that *MOL* reduced sperm abnormality like bent tail, coiled tail, DMR (distal mid-piece reflex), distal droplet and proximal droplet, as observed in the difference between

Table 1: Effect of different levels of *Moringa oleifera* leaves on physical parameters of semen of rabbit bucks.

Parameters	Group 1 (Control)	Group 2 (10% inclusion of MOL)	Group 3 (20% inclusion of MOL)
Reaction time (s)	2.6	2.4	2.1
Volume (ml)	1	0.8	1
Sperm concentration (10^6/ml)	136.2[a]	221.0[b]	297.2[b]
Forward motility (%)	81.6	89.4	89.9
Live sperm (%)	76	88.3	91.2
Abnormal sperm (%)	6.4	5.2	5
Tail abnormality (%)	5.3	4.8	4.4
Head abnormality (%)	1.1	0.4	0.7
*TSO (10^6/ejaculate)	136.2[a]	183.4	297.2[b]
Semen appearance	Milky	Milky	Milky
Viscosity	Normal	Normal	Normal

*Total sperm output (TSO) = Semen ejaculate volume (ml) × semen concentration (10^6/ml)

[a,b]Means within a row with different superscript letters are significantly different (P<0.05).

MOL= *Moringa oleifera* leaves.

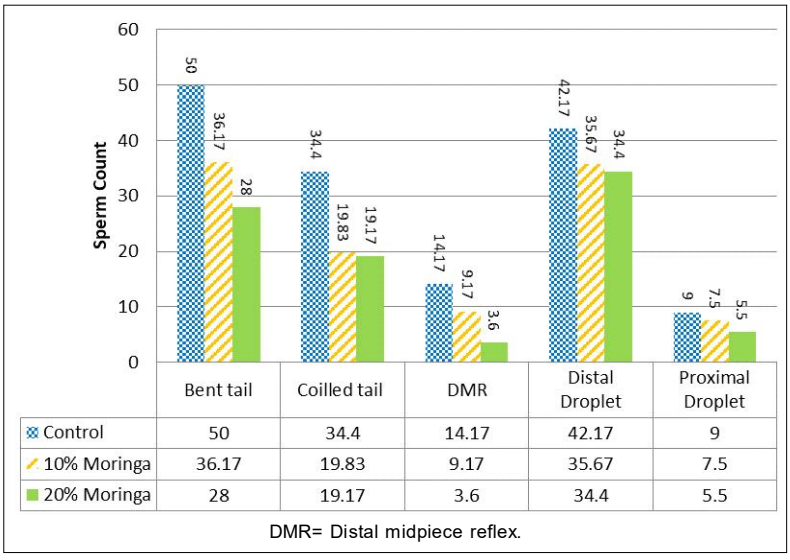

Fig 1: Effect of *Moringa oleifera* supplement on abnormality of spermatozoa in rabbit's semen.

group 1 (control) and rabbit fed with *MOL* (group 2 and 3). Overall, the effect of *MOL* on sperm abnormalities (tail abnormalities, head abnormalities) manifested in treatment Groups 2 and 3, were less when compared to Group 1 (control).

The results of sperm motility were also influence by dietary treatment. The forward motility was higher (89.9%) in rabbit fed with the highest level (20%; Group 3) of *MOL* followed by 89.4% (10%; Group 2) and 81.6% in control (Group 1). Also, the results show that there was an increase in live spermatozoa percent in rabbits fed varying levels of *M. oleifera* in treatment Group 2 (88.3%) and Group 3 (91.2%) when compared to those in the control group (76.0%).

The present finding suggests that the supplement of *Moringa oliefera* leaves could be integrated at a 10 to 20% level without any a negative effect on reproductive efficiency and performance of rabbit bucks. EL Deeb Mariam *et al.* (2015) has suggested that *Moringa Oleifera* leaves (*MOL*) could be integrated at 4% without negative effect on reproductive efficiency and performance of male rabbits intended for breeding purposes. Also, Ewuola *et al.,* (2014) stated that inclusion of *MOL* in rabbit's diet up to 7.5% improved semen quality but also recommended that the inclusion level of 2.5% MOL can enhance reproductive efficiency in male rabbits (Ewuola *et al.,* 2014). Meanwhile, Abu *et al.,* (2013) also reported that using *MOL* at a 15 % level doesn't cause any adverse effect on semen quality; these supplement ranges are in agreement with the current findings (Castellini and Lattaioli, 1999).

In the present study, all semen quality parameters as concentration, motility, live sperm count and total sperm output (TSO) tend to follow an upward trend in bucks received 20% *MOL* compared to control animals. It was stated that the number of sperms and their motility are the most important parameters of fertility (Brun *et al.,* 2002; Ajayi *et al.,* 2009). The high nutritive value of *MOL* incorporated into buck's diets possess the potential to improve the reproductive efficiency of rabbit bucks and subsequently enhance fertility performance. In the present study, not many differences were recorded in the volume, appearance and viscosity due to either treatments.

In current finding the sperm concentration of rabbit bucks significantly ($P<0.05$) influenced by the experimental diet containing *MOL* at 20% level. The values of sperm concentration observed in this research ranged from 136.2 to 297.2 $\times$ 10^6 ml and were more or less similar to the recorded values of 136.00 to 184.00$\times 10^6$ ml in rabbits fed with sunflower leaf meal (Oyeyemi *et al*., 2007). An increased concentration of spermatozoa is a signal to a possible high fertility rate by the reason of the number of spermatozoa available during service or insemination (Ojo *et al*., 2017). Oyeyemi *et al.* (2007) also declared that quality nutrition with high percentage of protein will improve motility and concentration of spermatozoa and *Moringa* leaves is known to have high crude protein content (Arthur *et al.,* 1975).

The percentage normal sperm cells in this research ranged from 93.6 to 95.0% which showed increased pattern at 20% inclusion level but were not significantly affected by experimental inclusions of *MOL*. The percentage normal sperm cells value was significantly ($P<0.05$) higher in T4 (90.50%) compared with T1 (84.67%), T2 (85.50%) and T3 (88.00%) of *Moringa Oleifera* leaves (MOLE) treatment, which supports our findings (Ajala *et al.,* 2001). The percentage of live sperm cells in this study range from 76.0 to 91.2% and were not significantly influenced by dietary supplement of *Moringa oleifera* leaves. Arthur *et al.,* (1975) discovered that high quality semen samples show an average of less than 25% dead sperm and the average value of percentage normal sperm cells reported in present study was within the range of high quality samples (Odeyinka *et al.,* 2008). The percentage live sperm cells are those available for use during fertilization (Dougnon *et al.,* 2012). The percentage of abnormal sperm in present study ranged from 5.0 to 6.4 %. The value obtained was more or less within the range of 9.50 to 15.33% and 6.00 to 16.00% (Ojo *et al*., 2017). Whereas the percentage of abnormal sperm cells in the present finding were lower than the upper limit of 20% abnormality (Arthur *et al.,* 1975), which suggests that the semen could be recommended for good reproductive potential and fertility in either normal mating or in artificial insemination. On the other side, this improvement in buck reproductive performance may be due to the fact that *Moringa oleifera* leaves have valuable minerals such as Fe and vitamins A, B complex, C, K, E which contribute in enhancing semen quality and reaction time (George *et al.,* 2017; Herbert *et al.,* 1992).

Motility is the movement of spermatozoa in the semen medium during reproduction and it is a good indicator of viability. Motility basically estimates the proportion of active spermatozoa in the semen, at least fifty percent (50%) or more of the sperm should be motile. In the current study the sperm motility were influenced by the dietary treatment with MOL. However, motility was found to be higher in rabbit fed with the highest level (20%) of *Moringa oleifera*. George *et al.* (2017) also observed similar finding and reported that significant improvement in sperm motility (90.7%) was noticed in rabbit fed with the highest level (30 g) of *Moringa oleifera*. This is in agreement with our results. However, Abu *et al*. (2013) has observed that *Moringa oleifera* leaf meal did not significantly influence sperm active motility, which is not in agreement with our finding.

Our results show that appearance of the rabbit semen and viscosity was not influenced by the treatments, as semen usually exhibit milky and homogeneous stickiness. Herbert also reported that the appearance of semen is a part of important characteristic of quality. Good quality semen should have a uniformly milky appearance which gives the indication of high sperm concentration. Semen viscosity is the liquid flow of semen. After ejaculation, it should take 30 minutes to an hour for semen to liquefy from its gel-like state. High semen viscosity causes low semen mobility, if the sperm does not liquefy or remains very thick, fertilization maybe difficult due to the sperm not being able to reach an

egg. Our results also shows viscosity of the rabbit semen was not influenced by either of the treatments.

CONCLUSION

The current findings support the traditional use of *Moringa oleifera* as a medication to enhance sexual function and corroborate the good effects of *Moringa oleifera* leaf meal on rabbit semen parameters. This may also indicate that *Moringa oleifera* leaf meal is a reasonably safe traditional treatment that may be used to improve the nutritional management of rabbit bucks' semen production, which in turn improves the reproductive performance of inseminated rabbit does. Therefore, *Moringa oleifera* might be a safe and efficient substitute for locally accessible feed in enhancing rabbits' semen quality and reproductive efficiency. Consequently, up to 20% of rabbits' diets can contain *Moringa oleifera* leaf meal without negatively affecting their performance.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Compliance with ethical standards

Campus ethical committee, University of the West Indies, St. Augustine, approved the protocol before the study commenced.

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Author contributions

The authors have contributed in research work, data interpretation and drafting of manuscript.

Conflict of interest

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