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Effect of *Moringa Oleifera* Leaf Extract (Mole) on some Reproductive Parameters of Rabbits Reared in a Semi-Humid Environment

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Abstract- This research was conducted at the Kwara State Teaching and Research farm University, Malete, Ilorin, Kwara State. The purpose of the study was to investigate the effect of adding varying doses of *Moringa oleifera* leaf extract (MOLE) on some reproductive characteristics of male rabbits reared in a semi humid environment. A total number of 24 rabbits with an average age of 9 months and body weight of 600-800g were used in this study and randomly divided into four equal treatments (6 rabbits each) and gavaged with 0mls (Tr1, control), 30mls (Tr2), 60mls (Tr3) and 90 mls (Tr4) of MOLE. The sperm concentration, percentage normal and abnormal sperm cells, testicular morphometry and serum testosterone level were measured.

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Effect of *Moringa Oleifera* Leaf Extract (Mole) on some Reproductive Parameters of Rabbits Reared in a Semi-Humid Environment

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Abstract- This research was conducted at the Kwara State Teaching and Research farm University, Malete, Ilorin, Kwara State. The purpose of the study was to investigate the effect of adding varying doses of *Moringa oleifera* leaf extract (MOLE) on some reproductive characteristics of male rabbits reared in a semi humid environment. A total number of 24 rabbits with an average age of 9 months and body weight of 600-800g were used in this study and randomly divided into four equal treatments (6 rabbits each) and gavaged with 0mls (Tr1, control), 30mls (Tr2), 60mls (Tr3) and 90 mls (Tr4) of MOLE. The sperm concentration, percentage normal and abnormal sperm cells, testicular morphometry and serum testosterone level were measured. Results showed that the highest recorded values of the paired testis weight, volume and testis length were observed in rabbits given 90mls of MOLE. The testis width was significantly (P 0.05) higher in Tr4 (90mls MOLE) and lowest in Tr2 (30mls MOLE) compared to the control group. Observation showed that the sperm concentration of rabbits given 90mls of MOLE were significantly (P 0.05) improved, while the least value was recorded in Tr1 (0mls MOLE). 90mls of MOLE decreased significantly (P 0.05) the percentage abnormal sperm cells, while enhancing the percentage normal sperm cells. Testosterone concentration was insignificantly increased in Tr4 as compared to control group. At the end of the experiment, conclusion showed that *Moringa oleifera* leaf extract (MOLE) can be used at 90mls to enhance reproductive parameters in rabbit bucks.

Keywords: abnormal sperm cells, sperm concentration, testicular morphometry, moringa oleifera, testosterone.

I. Introduction

The availability of quality source of animal protein in suitable amount and at reduced cost in most low economic countries, especially Nigeria has remained a main problem to animal production (Ahemen et al., 2013). Rabbit production is a potential solution to the problem of meat shortage (Taylor, 1980; Lebas, 1983) because of its unique qualities as a highly prolific animal, highly nutritious with low cholesterol content. An obvious limiting factor to rabbit production in regions with hot climate is the susceptibility of this specie to heat stress (Ondrukas et al., 2011). These have been reported to negatively affect their feed intake, utilization, blood parameters, hormonal secretion and reproductive parameters (Fouad, 2005). The comfort zone for rabbit is 18 to 21°C (Habbeb et al., 1999) so, rabbits can withstand cold weather than warmer one. The metabolic rate increased by about 20% in rabbits when exposed to high air temperature ranging from 30 to 35°C (Gonzalez et al., 1971) with a marked decrease in feed intake (Rakes et al., 1988). It has been reported that reduction in feed intake occurs during heat stress causing a drop in nutrient intake, which adversely affects reproductive response (Habeeb et al., 1996; Marai et al., 2001). Although reactive oxygen and nitrogen species known as free radicals are essential in detoxification and immune function, an overproduction of this causes damage to valuable biomolecules such as DNA and lipid proteins (Aruoma, 1998). Oxidative stress has been known to influence reproductive function. Several strategies have been employed to reduce heat stress effect on animals. Some of which include environmental condition modification (evaporative cooling, use of sheds or fans),dietary, management and physiological modification of animals (Selim et al., 2003), but these practices are either complex to implement or expensive (Ahmad and Sarwar, 2006). There is therefore the need to exploit other practicable methods of countering the adverse effect of heat stress. Several plant extracts having different phytochemicals have been documented to have diverse antioxidant activity (Zheng and Wang, 2001) with low economic importance such as Moringa oleifera. Moringa oleifera has been documented to have multiple antioxidants with high levels such as phenolic acids (ellagic, chlorogenic, Gallic and ferulic acid), glucosinolate and flavonoids such as kaempferol, quercetin and rutin (Mbikay, 2012). It is also a valuable source of –carotene (vitamin A precursor) and vitamins B-complex, C, D and K (Dorgan and ǎ Tandon, 1975). These antioxidants are effective in preventing oxidative damage by enhancing antioxidant enzymes which reduces production of free radicals and lipid peroxidation (Sreelatha and Padma, 2009). According to Nimse and Pal (2015), antioxidants are substances which prevent free radical effect at the same time slow down or hinders cellular damage, therefore stand as a preventive measure against the deleterious effect of these free radicals to cellular component .This study is conducted to investigate the efficacy of *Moringa oleifera* leaf extract (MOLE) as a natural source of antioxidant on

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some reproductive parameters of rabbits reared in a semi-humid environment.

II. Materials and Methods

The research was conducted at the rabbitry section, College of Agriculture, Kwara State University Teaching and Research Farm, from December to February, 2015. The study lasted for a period of 9 weeks.

a) Plant Collection and Preparation of MOLE

Fresh leaves of Moringa oleifera were collected early in the morning at Ita-alamu area of Ilorin, Kwara State. The fresh leaves were manually removed from the stem, cleaned and made free of sand and other impurities using distilled water. The fresh leaves were spread under a shade to dry for 10 days. The dried leaves were blended into powdered using an electric kitchen blender. Finely pulverized Moringa oleifera leaves weighing 300g was poured into a 2.5 litre macerating flask and 1.5 litre of distilled water added. The resulting mixture was thoroughly homogenized and sieved with a cheese cloth then filtered using whatman filter paper (24cm). The MOLE was then preserved in the freezer till needed.

b) Experimental Animals and Management

The experimental animals used were twenty four (24) male bucks with an averagely aged 9 months old, having average initial body weight ranging from 600-800g. The experimental animals were randomly allotted to four (4) groups comprising six experimental animals per treatment in a completely randomized design. Each group was divided into three (3) subgroups with 2 animals per subgroup. Experimental animals were acclimatized for ten (10) in the teaching and research farm before data collection commenced. Prior to the data collection, animal were given oxytetracycline (5%) intramuscularly twice, vitamin B complex intramuscularly and ivomec through subcutaneous route of administration. Treatment one (1) served as the control which received no extract of Moringa oleifera, while rabbits in treatment 2, 3 and 4 received the MOLE extract at 30, 60 and 90mls respectively via gavage method. The average temperature inside the research station at the peak of the experiment ranged from 33-360C monitored using wet and dry bulb thermometer. The relative humidity was 46±3% recorded daily using hygrometer. Feed and water was given ad libitum. The experimental layout design was completely randomised design (CRD).

c) Data collection

After slaughtering individual rabbits, the scrotum were cut-in to expose and bring out the testes.The testes were weighed and their the testes and volumes using Archimedes volume displacement, the lengths and circumferences were determined using thread and ruler. The epididymides were completely removed from other adjoining epithelial tissues. The cauda epididymides were then excised and several deep cuts were carried out on them to enhance the spermatozoa swim out easily. They were separately placed in glass beakers having 5ml physiological normal saline solution. The sperm cells were freed into the beakers containing physiological saline with many deep cuts made on them. The concentration of the sperm was obtained with the use of improved Neubaeur haemocytometer. Percentage normal and those sperm cells which were deviations from normal were determined by conventional methods (Zemjanis, 1977). Testosterone determination was done using ELIZA method.

d) Chemical Analysis

Proximate composition of diets fed to the animals were carried out in a reputable chemical analysis labouratory through the procedure stated by Association of Analytical Chemist (A.O.A.C, 1990).

e) Statistical Analysis

Data obtained were analyzed using one way analysis of variance (ANOVA) in a completely randomized design while significant means was separated using Duncan's New Multiple test of software (SAS, 2003).

III. Results and Discussion

Table1: Proximate composition of *Moringa oleifera* leaves

Parameters	Composition
Dry matter %	91.78
Moisture content (%)	28.43
Crude protein (%)	28.43
Crude fat (%)	6.40
Crude fibre (%)	9.15
Total ash (%)	9.09
NFE	46.93

NFE- Nitrogen free extract

The results of proximate analysis in Table 1 showed that Moringa leaves had an appreciable crude protein content (28.43%), crude fibre (9.15%), ash (9.09%), dry matter (91.78%), NFE (46.93%), but low content of ether extract (6.40%). Dry matter content of MOLE in this study was lower than the values reported by Mutayoba et al. (2011) who reported dry matter values of 93.7% . The crude protein (CP) value of MOLE obtained in the present research is higher compared with the value observed by Olugbemi et al. (2010) which was 27.44%, although Mutayoba et al. (2011) recorded a higher (30.65%) crude protein value. The crude fat and ash values (6.40% and 9.09%) recorded in this research were higher than the values 2.11% and 7.93% reported by Ogbe et al. Crude fibre value of 9.15% reported here was higher than 5.43% which was reported in the study

conducted by Sodamade et al. (2013). These differences in values of MOLE have been observed in the previous studies stated to be due to differences in the soil type, climatic conditions, stage of maturity and their genetic make-up.

Table 2: Testicular morphometry of rabbits fed *Moringa oleifera* leaf extract (MOLE)

Parameter	T_1	T_2	T_3	T_4	SEM	P-value
Paired Testis Weight (g)	10.83	11.50	12.67	16.00	0.84^{NS}	0.169
Paired Testis Volume (cm^3)	6.67	2.50	5.00	7.50	0.76^{NS}	0.128
Testis Length (cm)	8.67	7.75	8.67	8.75	0.22^{NS}	0.486
Testis Width (cm)	3.43^{a}	3.25^{a}	5.33^{ab}	6.50^{b}	0.44^{S}	0.001

$^{a-b}$ mean bearing different superscript in the same row differ significantly($P < 0.05$). T_1 = Control, T_2 =30mls of Moringa oleifera leaf extract (MOLE), T_3 =60mls of M. oleifera leaf extract (MOLE), T_4 = 90mls of M. oleifera leaf extract (MOLE), SEM = Standard error of means, NS = No significant different, S = Significant different

The influence of the Moringa oleifera leaf extract on testicular morphometry of rabbits are shown in Table 2. This research was used to determine the testicular morphometry and epididymal sperm indices of rabbit bucks given varying concentration of *Moringa oleifera* leaf extract (MOLE). Weight of the pair of testes in observed this study were not significantly ($p<0.05$) affected by experimental material, having values ranging between 10.83 and 16.00 g. The observed figures are in consonance with the submissions of Bitto and Gemade (2001) who recorded a non significant influence of pawpaw peel meal (PPM) up to 30% on testicular morphometry of rabbit bucks. This is as well in agreement with the observation of Ogunlade et al. (2006) who showed a non significant difference in the weight of testis among rabbits fed fumonisin contaminated diets. Observation also showed that MOLE did not influence the testis length and testis volume of the rabbits, although those given 90mls of MOLE had the highest recorded values of both the testis length and volume respectively. There is a striking similitude between the result of this study and the report of Ahemen et al. (2013) who observed no significant (P 0.05) differences among the testis length, width, weight and volume of rabbit bucks when given different doses of water spinach. The findings however, is noted to be different from the reports of Ajayi et al. (2009) who showed significant influence of experimental diets (Blood-wild sunflower leaf meal mixture diet) on testicular length of rabbits. The mean testicular width of rabbits given 90mls of MOLE was significantly (P 0.05) higher compared to those of the control (0mls of MOLE) and those given 30mls of MOLE (T2). The information relating to morphometric parameters of the reproductive tract have been observed to give an invaluable information on adjudging the breeding and fertililizing ability of animals (Ogbuewu et al., 2009). According to Gage and Freckleton (2003), the testes size, length and width of mammals are described as favourable pointer to the present and future spermatozoa production. Knowledge of the important morphometric qualities of the reproductive organ is important to enhance the opinion and forecast not only of sperm production ability, but likewise the storage potential and fertilizing capability of the breeder male. Moreira et al. (2001) verified in a study of Santa Ines sheep, that changes in testicular length and scrotal circumference is considered viable indicators of the effect of thermal stress on gonads. In accordance with Ezekwe (1998) and Perry and Petterson (2001), testes size, length and width are high quality indicators of present and future sperm production. This enhances increased fertilizing potential in rabbits

Table 3: Semen characteristics of rabbit bucks fed *Moringa oleifera* leaf extract (MOLE)

Parameter	T_1	T_2	T_3	T_4	Std. Mean Error	P-value
Concentration ($x10^6$/ml)	138.50^{a}	161.75^{ab}	178.33^{ab}	190.00^{b}	6.63^{S}	0.000
Abnormal cell (%)	15.33^{b}	14.50^{b}	12.00^{ab}	9.50^{a}	0.76^{S}	0.001
Normal cell (%)	84.67^{a}	85.50^{a}	88.00^{ab}	90.50^{b}	0.76^{S}	0.001
Testosterone (ng/ml)	5.24	5.78	6.95	8.42	0.48^{NS}	0.073

$^{a-b}$ means bearing different superscript in the same row differ significantly($P < 0.05$). Keys: T_1 = Control, T_2 =30mls of Moringa oleifera leaf extract (MOLE), T_3 =60mls of M. oleifera leaf extract (MOLE), T_4 = 90mls of M. oleifera leaf extract (MOLE). SEM = Standard error of means. S = Significant different.

The results of some sperm characteristics and testosterone of rabbits given Moringa oleifera leaf extract (MOLE) are presented in Table 3. The sperm concentration of rabbit bucks given MOLE is significantly (P 0.05) influenced by the experimental diet. The values of sperm concentration observed in this research ranged from 138.50 to 190.00 x106/ml and were similar to the recorded values of 136.00 to 184.00 x106/ml stated by Ajayi (2009). Oyeyemi and Okediran (2007) reported that 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. The percentage normal sperm cells in this research ranged from 84.67 to 90.50% and were significantly affected by experimental inclusions. 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%). Arthur et al. (1989) discovered that high quality semen samples show an average of 25% dead sperms. The average value of percentage normal sperm cells reported in this research was within the range of high quality samples. The percentage live sperm cells are those present for use during fertilization (Ajala et al., 2001). The percentage abnormal sperm cells values in this research ranged from 9.50 to 15.33%. Values obtained were within the range of 6.00 to 16.00% as reported by Ajayi (2009). The percentage of abnormal sperm cells in this research were lower than the upper limit of 20% suggested as the least quantity recommendable for good reproductive potential and fertility in either normal mating or in artificial insemination (Oyeyemi and Okediran, 2007). Ajayi et al. (2009) established the influence of quality feeding on sperm characteristics of rabbits. Oyeyemi et al. (1998) 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.The testosterone values recorded was not significant across the treatment, although the highest recorded values were observed in rabbit group given 90mls MOLE. Testosterone hormone is produced by the interstitial cells of the testis and necessary for the completion of spermatogenesis. The result is in consonance with the report of Sajjad et al.(2007) who reported that the levels of blood serum testosterone were correlated with scrotal circumference and semen volume in buffalo bulls of 14 years of age. El-Hanoun et al. (2014), also reported that a good relationship exist between increased testostosone concentration and increased libido of male rabbits.

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