REPRODUCTIVE POTENTIALS AND SPERMATOZOA KINETICS OF RABBIT BUCKS FED *MORINGA OLEIFERA, PHYLLANTHUS AMARUS* AND *VISCUM ALBUM* HERBAL SUPPLEMENTS

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ABSTRACT
Three herbal supplements obtained from freshly air-dried *Moringa oleifera*, *Phyllanthus amarus* and *Viscum album* leaves were evaluated for their comparative effect on gonadal and extra-gonadal sperm reserves and spermatozoa kinetics of bucks. Forty bucks were allotted to four diets made up of standard grower rabbit ration without supplement and with 5% Moringa, Mistletoe and Phyllanthus supplementation for 84 days. Semen samples were collected from all bucks using artificial vagina, for semen quality and seminal oxidative stress markers. The organ weights, testicular and epididymal spermatozoa reserves were assessed to determine sperm production potentials using standard procedures. The result obtained revealed that spermatozoa concentration, motility, tranjectory and speed traits were significantly ($p<0.05$) higher in the group fed mistletoe supplemented diet compared to that recorded in the control groups. The gonadal and extra-gonadal sperm reserves of bucks fed the herbal supplements were depleted compared to bucks on control. Bucks on mistletoe supplementation rival the superior daily sperm production and testicular sperm reserve per testis in bucks without herbal supplements. In conclusion, the reproductive characteristics of bucks to the three herbal supplements differ. *Viscum album* supplements in bucks' diets encouraged daily sperm production, testicular sperm reserves, spermatozoa kinetics over other herbs supplements. *Phyllanthus amarus* supplementation in bucks' diet enhanced extra-gonadal sperm reserves compared to bucks on other herbal supplements. Thus strategic herbal supplementation would be required to meet different reproductive desires in bucks.

Keywords: Herbs; Spermatogenesis; Testicular; Epididymal; Spermatozoa kinetics

INTRODUCTION
Infertility has been a recurring problem among male and female individuals, with more than 90% of male infertility cases are due to low sperm counts, poor semen quality or both (Ekere *et al.*, 2013). The use of medicinal plants is becoming an object of increasing interest due to its food safety, as the use of antibiotics is becoming ever restricted because of its adverse effect on animals, residues in animal products and the development of antibiotic resistance (Omokore and Alagbe, 2019). Traditionally, herbs have been used to improve sexual performance or to treat infertility (Jimoh *et al*., 2020a). Some medicinal plants are extensively used as an aphrodisiac to relieve sexual dysfunction, or as fertility-enhancing agents. They provide a boost of nutritional value, thereby improving sexual performance (Sumalatha *et al*., 2010). In Nigeria, several plants have been identified to have medicinal and nutritional importance and been used to enhance the fertility of male and female animals (Ajuogu *et al*., 2018).

Feed additives rich in antioxidant could play a significant role in improving the health of rabbits (Jimoh *et al*., 2020b). Herbs such as *Moringa oleifera, Phyllanthus amarus* and *Viscum album* have proven to be of high therapeutic value and increase an animal's general performance (Daramola *et al*., 2018; Jimoh *et al*., 2018; Jimoh *et al*., 2020b). In comparison to commonly used conventional forages, they contain low protein, moderate fibre, and high minerals, flavonoids and alkaloids with the presence of several compounds in one plant producing a synergistic beneficial effect in the improvement of animal performance (Hossain *et al*., 2012). Given the consumption of *Moringa oleifera, Phyllanthus amarus,* and *Viscum album* leaves for the maintenance of health and well-being, the comparison of the effect of the herbs on spermatozoa reserves, sperm production efficiency and semen quality for the enhancement of male fertility is imperative, and necessitates the aim of this study

MATERIAL AND METHODS

Fresh leaves were harvested and shade-dried until crispy to touch and milled, after which they were referred to as Moringa leaf meal (MoLM), Phyllanthus leaf meal (PLM) and Mistletoe leaf meal (MiLM) respectively. Experimental diets were formulated and pelleted to meet the nutrient requirement of growing rabbits and were supplemented with the herbs at 5%. The whole experimental diets were fed to bucks at 4% body weight for respective treatments. T1 basal diet without leaf meal, T2 basal diet with 5% Moringa, T3 basal diet with 5% Mistletoe and T4 basal diet with 5% Phyllanthus. Forty mixed bred rabbits aged six weeks weighing 775±54.23g were used for the experiment. The bucks were assigned to four (4) dietary groups consisting of 10 replicates each, in a completely randomised design (CRD). The feeding trial lasted for 12 weeks, during which bucks were trained to serve artificial vagina from 9weeks. At the end of the trial semen samples were collected from each buck. Each sample was analysed for semen quality using tuberculin syringe to the nearest 0.1 ml. Sperm kinetics was determined using computer-assisted sperm analyser (SpermAnalyzeWin7 Xuzhou city, China, the setting of CASA as in 5th WHO manual, 51 sperm tracks, evaluated magnification x10, image acquisition rate: number frames/s 60). All bucks were euthanised, after which, the pairs of testes and epididymis were carefully separated and freed of all adhering connective tissues. They were separated into right and left testes, right and left Caput, corpus and caudal epididymis and weighed. The testes, caput, corpus and caudal epididymis were homogenised separately with normal saline solution (0.154M NaCl). The suspensions were mixed, filtered through a double layer cheesecloth and the filtrate transferred into test tubes. Sperm count was done haemocytometrically using the improved Neubauer haemocytometer. The gonadal and extra-gonadal sperm reserve, daily sperm production, sperm production efficiency was estimated using standard protocol. Data obtained from the study were subjected to Analysis of variance at $p = 0.05$. Duncan's Multiple Range Test was used to separate mean differences.

RESULTS AND DISCUSSION

Spermatozoa kinetics of rabbit bucks fed the herbal supplements reveal that bucks on mistletoe had better motility traits (Percentage motility, progressive motility), though was comparable to those of bucks on moringa and Phyllanthus supplements but were overall better than those of bucks with no herbal supplement. The motility, speed and trajectory traits of spermatozoa obtained from semen of bucks on Moringa and Phyllanthus supplements were similar with those fed no supplement.

Table 1: Semen Quality and spermatozoa kinetics of rabbit bucks fed three herbal supplemented diet

Parameters	**Control**	**Moringa**	**Mistletoe**	**Phyllanthus**	SEM
Volume (ml)	0.42^{a}	0.41^{a}	0.32^{b}	0.32^{b}	0.02
Sperm concentration ($x10^{7}$)	6.99^{b}	7.06^{b}	12.73^{a}	5.82^{b}	1.2
Percentage motility (%)	36.06^{b}	59.57^{ab}	71.50^{a}	62.80^{ab}	4.88
Non- Progressive motility (%)	3.03^{b}	1.51^{b}	9.00^{a}	2.82^{b}	1.09
Progressive motility (%)	32.03^{b}	58.05^{a}	61.50^{a}	62.80^{a}	5.06
Curvilinear velocity (VCL) (µm/s)	3.54^{b}	4.40^{b}	8.02^{a}	3.14^{b}	0.49
Average path velocity (µm/s)	3.24^{b}	3.89^{b}	7.19^{a}	3.07^{b}	0.61
Straight line velocity (µm/s)	2.33	2.66	3.47	2.68	0.19
Linearity (%)	71.33^{ab}	62.65^{ab}	51.41^{b}	89.59^{a}	5.21
Straightness (%)	71.78^{ab}	68.78^{ab}	53.05^{b}	90.50^{a}	4.46
Amplitude of lateral head (µm)	0.22^{b}	0.19^{b}	0.40^{a}	0.14^{b}	0.03
Beat cross frequency (Hz)	0.31	0.48	0.77	0.32	0.06
Wobble (%)	89.45	89.11	92.55	98.90	2.10
Liveability (%)	77.68	88.78	93.34	95.04	4.62
Acrosome integrity (%)	85.03	91.35	93.56	95.34	5.24

*means along the same row with different superscripts, differ significantly ($P<0.05$), SEM: standard error of mean

Herbal supplements did not influence the viability (liveability and acrosome integrity) of spermatozoa. Similar to the result on *Phyllanthus amarus* fed rabbits of high spermatozoa motility agrees with the report of Azubuike, *et al*., (2018) that male rats had higher sperm concentration,

motility following *Phyllanthus amarus* extracts gavage. Similarly, Khalifa *et al*. (2016) reported that the presence of flavonoids in herbs had been implicated to enhance male sexual behaviour. Spermatozoa concentration of semen obtained from bucks on mistletoe supplemented diet was superior to those obtained from Moringa and Phyllanthus supplemented groups.

This indicates that the mistletoe supplement possesses the ingredients to enhance spermatozoa concentration and motility traits in rabbit semen. The spermatozoa speed traits (curvilinear velocity and average path velocity) were superior in mistletoe supplement fed groups. However, among the spermatozoa trajectory traits, the only amplitude of lateral head was superior in mistletoe supplemented groups. In comparison, spermatozoa trajectory trait such as linearity and straightness of spermatozoa was superior in Phyllanthus supplemented groups. The trend of result shows that the extra-gonadal sperm reserves of bucks fed the herbal supplements were depleted compared to bucks not fed herbal supplements (Table 2). Among the herbal supplement, Phyllanthus supplemented groups tend to compare favourably with the non-supplemented groups. Sexual maturation in male rabbits is related to an increase in FSH secretion since FSH binds within the seminiferous tubules to facilitate spermatogenesis (Ojeda and Skinner, 2006). Increase in epididymal weight observed in bucks fed moringa supplement with low sperm reserves could be due to parenchyma tissues resulting from an increase in ROS healthy parenchyma tissues and detrimental to sperm maturation (Ewuola *et al*., 2014).

Table 2: Extra-gonadal sperm reserves of rabbit bucks fed herbal supplemented diet

	Control	**Moringa**	**Mistletoe**	**Phyllanthus**	**SEM**
Left caput sperm reserve ($x10^7$ Spz)	2.50^{b}	2.13^{b}	3.45^{a}	2.04^{b}	0.34
Left corpus sperm reserve ($x10^7$ Spz)	20.11^{a}	11.72^{b}	12.92^{b}	16.61^{a}	7.38
Left caudal sperm reserve ($x10^7$ Spz)	39.44^{a}	27.14^{b}	22.52^{b}	13.02^{c}	3.16
Right Caput sperm reserve ($x10^7$ Spz)	1.60^{b}	2.01^{b}	1.64^{b}	3.96^{a}	0.50
Right Corpus sperm reserve ($x10^7$ Spz)	9.73^{ab}	13.31^{a}	6.74^{b}	11.84^{a}	1.65
Right Caudal sperm reserve ($x10^7$ Spz)	30.93^{a}	17.14^{b}	25.93^{a}	19.63^{ab}	5.64
Total caput sperm reserve ($x10^7$ Spz)	4.10	4.14	5.10	6.00	0.56
Total corpus sperm reserve ($x10^7$ Spz)	29.81^{a}	24.72^{ab}	19.60^{b}	28.54^{a}	3.84
Total caudal sperm reserve ($x10^7$ Spz)	70.22^{a}	44.22^{b}	48.53^{b}	32.63^{b}	10.71
Right Epidydermal sperm reserve ($x10^7$ Spz)	42.20	32.24	34.33	35.43	5.80
Left Epidydermal sperm reserve ($x10^7$ Spz)	62.00^{a}	40.93^{b}	38.82^{b}	31.72^{b}	8.86

*means along the same row with different superscripts, differ significantly ($P<0.05$), SEM: standard error of mean, Spz: Spermatozoa.

The gonadal sperm reserves and sperm production potentials of bucks on herbals supplements decreased drastically in comparison with bucks on control (Table 3). The decrease in testicular and epididymal sperm reserve observed in herbal supplemented bucks might reflect the spermatogenic cell death (Alya and Azhar, 2013). Bucks on mistletoe supplemented groups rival the superior daily sperm production and testicular sperm reserve in bucks without herbal supplements. This shows mistletoe supplemented bucks had comparable daily sperm production and testicular sperm reserve as bucks without supplement. This could be likened to the report of Ekere *et al*., (2013) which stated that sperm production in albino rats increased due to methanolic leaf extract from *D. arborea.* The result obtained in Moringa supplemented bucks could be due to Sertoli cells malnourishment for the growth and survival of sperm cells and inhibiting testosterone production by the Leydig cells leading to lower daily sperm production and gonadal sperm reserves (Ewuola *et al*., 2014). Phytochemical screening of medicinal plants reveals they possess alkaloids, saponins, tannins, terpenes, alkaloids, flavonoids, carbohydrates and cardiac glycosides, which have been implicated in male reproductive toxicology (Obembe and Raji, 2018).

CONCLUSION

The gonadal and extra-gonadal sperm reserves of bucks fed the herbal supplements were depleted compared to bucks on control. The difference in the elicited response by the bucks to the three herbal supplements implies that strategic herbal supplementation would be required to meet different reproductive desires in rabbit bucks. *Viscum album* supplements in bucks' diets encouraged daily

sperm production, testicular sperm reserves, spermatozoa kinetics over other herbs supplements. *Phyllanthus amarus* supplementation in bucks' diet enhanced extra-gonadal sperm reserves compared to bucks on other herbal supplements.

Table 3: Sperm production and gonadal sperm reserves of rabbit bucks fed herbal supplemented diets

	Control	Moringa	Mistletoe	Phyllanthus	SEM
Left Testis sperm reserve ($x10^7$ Spz/testis)	21.32^{a}	6.54^{c}	11.73^{bc}	16.71^{ab}	1.89
Left Testis sperm reserve ($x10^7$ Spz/g testis)	61.20^{a}	5.58^{c}	10.61^{c}	33.81^{b}	9.80
Right testis sperm reserve ($x10^7$ Spz /testis)	27.43^{a}	7.04^{b}	24.40^{a}	9.28^{b}	4.66
Right testis sperm reserve ($x10^7$ Spz /g testis)	57.84^{a}	6.88^{c}	26.03^{b}	18.22^{b}	8.11
Testisicular sperm reserve ($x10^7$ Spz/ testis	41.93^{a}	13.26^{b}	36.31^{a}	25.94^{b}	4.79
Testicular sperm reserve ($x10^7$ Spz/g testis)	105.43^{a}	12.54^{c}	36.61^{bc}	52.03^{b}	13.92
Epididermal sperm reserve ($x10^7$ Spz)	104.00^{a}	73.14^{b}	73.22^{b}	67.12^{b}	11.63
Daily Sperm Production (Spz $x10^7$)	12.24^{a}	3.96^{c}	10.54^{a}	7.56^{b}	1.40
Sperm Production Efficiency (Spz $x10^7$)	30.53^{a}	3.63^{c}	10.74^{b}	15.24^{b}	4.04

*means along the same row with different superscripts, differ significantly ($P<0.05$), SEM: standard error of mean, Spz: Spermatozoa

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