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Effect of *Vernonia amygdalina* leaf meal on the reproductive indices of male rabbits

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ABSTRACT

The study evaluated the effect of *Vernonia amygdalina* leaf meal on semen indices, serum testosterone and sperm reserve of male rabbits. Forty rabbit bucks were randomly assigned into four groups and fed the experimental diets containing *Vernonia amygdalina* (VLM) at 0, 5, 10 and 15 % levels for 84days. Reproductive indices were evaluated using standard procedures. Data obtained were subjected to analysis of variance at $p<0.05$. All semen in rabbits fed 0, 5 and 10 % VLM had milky colour while 14.8% light green and 85.2% milky semen colour was observed in bucks fed 15%VLM. Libido score reduced in rabbits fed VLM diets. Bucks fed 15%VLM had significantly higher semen volume (0.47ml). VLM had no significant effect on spermatozoa mass motility, progressive motility and sperm concentration. Live sperm cells significantly increased in bucks fed 5 and 10% VLM diets. VLM had significant ($p<0.05$) effect on spermatozoa morphology. *Vernonia amygdalina* leaf meal had no significant ($p<0.05$) impact on testosterone, testicular and epididymal indices. In conclusion, up to 10%VLM can be adopted as feed ingredient for male rabbit breeder stock without deleterious effect on reproductive indices.

Keywords: Bitter leaf, Libido, Semen, Sperm reserve, Testes

INTRODUCTION

Leaf meals can be described as unconventional feedstuffs from plant sources that are relatively cheap with little or no competition between human and livestock for its use. Several plants' leaves with medicinal properties have been used in animal production either in its fresh (Attia et al. 2017, Olounlade et al. 2021), dried (Francis et al., 2013) or extracted form. (Abdulmalik and Oladapo, 2020) Some leaf meals have been reported to influence reproductive traits {Saalu et al., 2013; Adeyemi et al., 2014; Ogbuewu and Mbajiorgu, 2019} and promote growth (Ukawoko and Okehielem, 2016) of livestock when use as feed additive or feed components. *Vernonia amygdalina* popularly known as

bitter leaf is one of such leaves that have been used for its therapeutic properties. It is known in Nigeria for its dietary and diverse medicinal uses (Saalu et al., 2013). The phytochemical screening of *V. amygdalina* extract showed presences of tannins, saponin, cardiac glycosides, alkaloid and flavonoid as the most abundant phytochemical (Oboh, 2006; Imaga and Bamigbetan, 2013). Air dried *V. amygdalina* leaves contain 26.3% crude protein and 11.5% crude fibre (Akinwumi and Omotayo, 2016). Hence, it can be used as a feed component in rabbit diet since they have ability to digest fibrous feed stuff. The plant has been used in various forms in some animals with different effects. Oyeyemi et al., (2008) reported an adverse effect of aqueous extract of *V. amygdalina* on spermiogram and spermatozoa morphology in male wistar rats. Dietary *V. amygdalina* leaf meal in broiler finisher diet at a level up to 10% had no adverse effect on their performance (Durunna et al., 2011). *V. amygdalina* leaf extract (100 mg/kg) had no deleterious effects on rabbits' testes (Saalu et al., 2013). Dietary inclusion of bitter leaf at 100-150 g/kg of feed improves fertility in *H. bidorsalis* broodstocks which can enhance its seed production (Francis et al., 2013). Methanolic extract of *V. amygdalina* significantly decreased testosterone level, sperm motility and sperm count of male wistar rat (Oyedeji et al., 2013). Chronic administration of bitter leaf resulted in reversible antispermatogenic properties in male albino rats (Malviya et al., 2016). Rabbits administered *Vernonia amygdalina* extract had reduced superoxide dismutase (Abdulmalik and Oladapo, 2020). Several findings on *Vernonia amygdalina* leaf shows it contains substances that can influence livestock performance. With the rise in prices of conventional feed ingredients and the need to boost reproductive efficiency of the heterogenous rabbit stock available in the tropics, *Vernonia amygdalina* leaf (readily available, rich in nutrients and antioxidant properties) can serve as an unconventional feedstuff in rabbits' diet. This can contribute to improved spermatogenesis in male rabbits. This study therefore investigated the effect of dietary levels of *V. amygdalina* on semen production, morphology of sperm cells, serum testosterone and sperm reserve of rabbit bucks.

MATERIALS AND METHODS

Preparation of Vernonia amgdalina Leaf Meal and Animal Management

Fresh leaves of *Vernonia amygdalina* were harvested from established plots, air dried, milled and incorporated with other feed ingredients at 0, 50, 100 and 150g/kg of diet to replace wheat bran. *Vernonia amgdalina* Leaf Meal (VLM) was added at these levels because no specific quantity has been established for rabbits; hence the need to start with moderate levels. The feeds were pelletized and the experimental diets (VLM0, VLM5, VLM10 and VLM15) are shown in Table 1.

Table 1. Composition of experimental diets

Ingredients	VLM0	VLM5	VLM10	VLM15
Maize	9.00	9.00	9.00	9.00
Corn bran	22.5	22.5	22.5	22.5
Rice bran	20.8	20.8	20.8	20.8
Soybean meal	9.90	9.90	9.90	9.90
Palm kernel cake	5.25	5.25	5.25	5.25
Wheat bran	30.8	25.8	20.8	15.8
Vernonia amygdalina Leaf Meal	0.00	5.00	10.0	15.0
Others	1.75	1.75	1.75	1.75
TOTAL	100	100	100	100
Calculated analysis:				
Crude protein (%)	16.1	16.4	16.6	16.9
Crude fiber (%)	9.04	9.19	9.35	9.30
Digestible energy (kcal/kg)	2468	2500	2532	2564

VLM- *Vernonia amygdalina* leaf meal; VLM_0- diet without VLM (control diet), VLM_5- diet with 5% VLM, VLM_{10}- diet with 10% VLM and VLM_{15}- diet with 15% VLM Others: Bone meal =1.10, Salt = 0.32 and Premix = 0.33

The rabbits were managed at the Rabbitry unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria. Forty rabbit bucks of heterogeneous population with an average weight of 1818.3g were used for this experiment. The experimental animals were subjected to same management system; and clean water was provided a*d libitum*. The rabbits were acclimatized for two weeks; thereafter allocated to four dietary groups replicated ten times in a completely randomized design and fed the experimental diets for twelve weeks (84 days).

Assessment of Reproductive Indices

The sexual drive (libido) of the rabbit bucks were determined weekly by introducing a teaser female into the males' cage and monitored using a stopwatch. The libido score of each buck was recorded as the number of mounts per minute. Semen was collected from the rabbits using an improvised artificial vagina once weekly and semen parameters (semen colour, volume, mass motility, progressive motility, sperm concentration and percentage live sperm cells) were assessed as described by Adeyemi et al. (2014). Spermatozoa morphology was determined using the method described by Oyedeji et al. (2013) and abnormalities observed in the head, midpieces and tail regions of the sperm cells were recorded. At the start and end of the experiment, five milliliters (5mls) of blood was sampled from each of twenty bucks (5 per groups) into plain collecting tubes and centrifuged at 3000rpm for 15mins to separate serum from the cell mass. This was assessed for serum testosterone level at 0 and 12th week of the experiment using Enzyme-Linked

Immunosorbent Assay (ELISA) technique and kit following manufacturer's instruction.

Testicular parameters were assessed for right and left testis. The testes volume was determined using Archimedes principle of water displacement by immersing each testis in a measuring cylinder filled with normal saline solution up to 50ml, the displaced water volume was then recorded as the testis volume. Weight of the testis and epididymis were taken using a sensitive weighing scale and the values obtained were recorded in grams. Testicular and epididymal sperm reserves were estimated using the Neubauer haemocytometric technique as described in Adeyemi et al. (2014). The reproductive parameters were assessed on-farm and at the Reproductive Physiology Laboratory, Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

Statistical analysis

Data obtained on the effect of *Vernonia amgdalina* leaf meal on reproductive indices in male rabbits were analyzed using one-way ANOVA. The general linear model (GLM) procedures of SAS (2009) software were used in this statistical analysis. The linear model employed was:

$$Yij = \mu + Si + Eij$$

where Yij = observation of the dependent variables ij (reproductive indices), μ = fixed effect of population mean for the variable, Si = effect of diet (j = 4; VLM0, VLM5, VLM10 and VLM15), and Eij = random error associated with observation ij assumed to be normally and independently distributed. Means were separated using Tukey's Range Test with statistical significance established at $P<0.05$.

RESULTS AND DISCUSSION

Semen Indices

Milky semen colour was observed across the experimental groups, however bucks fed 15%VLM recorded 14.8% light green and 85.2% milky semen colour (Figure 1). This showed that 16 samples out of the 108 successful semen collections for the 12 weeks in this group had light green colour. The 14.8% light green semen colour recorded in animals fed 15%VLM diet implies that pigment in VLM at higher concentration can penetrate the seminal duct thereby altering seminal fluid colour. High percentage of semen with milky colour recorded in this study was similar to that of Ajuogu et al. (2018) and Oguike et al. (2019). A normal semen sample has a homogeneous white opalescent appearance (Boiti et al., 2005).

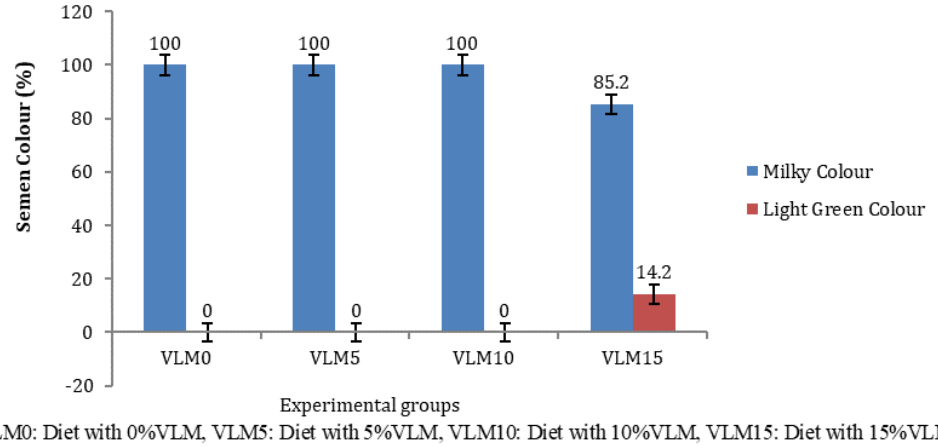


Figure 1. Semen colour of rabbit bucks fed *Vernonia amygdalina* leaf meal

As presented in Table 2, libido score was significantly ($p<0.05$) lower in male rabbits fed *Vernonia amygdalina* leaf meal (VLM) compared to those fed diets with no VLM.

Table 2. Libido score and semen indices of rabbit bucks fed dietary levels of *Vernonia amygdalina* leaf meal

Parameters	VLM0	VLM5	VLM10	VLM15	SEM	P value
Libido Score	7.73^{a}	6.64^{ab}	5.70^{b}	5.70^{b}	1.015	0.003
Semen Volume (ml)	0.43^{a}	0.34^{b}	0.36^{b}	0.47^{a}	0.01	<0.0001
Spermatozoa mass activity (%)	85.2	83.9	81.62	82.8	0.605	0.18
Progressive Motility (%)	79.4	79.8	77.8	77.9	0.664	0.62
Sperm Concentration (x10^{6}/ml)	303	310	337	355	48.10	0.63
Live Sperm Cells (%)	95.7^{ab}	97.9^{a}	97.9^{a}	95.2^{b}	0.444	0.04

VLM – *Vernonia amygdalina* Leaf Meal, VLM0: Diet with 0%VLM, VLM5: Diet with 5%VLM, VLM10: Diet with 10%VLM, VLM15: Diet with 15%VLM: $^{a, b}$:Means within each row with different superscript are significantly different by Tukey's multiple comparison method ($p< 0.05$)

Semen volume and live sperm cells were significantly ($p<0.05$) influenced by the levels of VLM fed to the bucks. Values obtained for spermatozoa mass motility, progressive motility, sperm concentration and percentage live sperm cells were not significantly ($p>0.05$) different among the bucks in all the groups. Fertility indices of males such as efficiency of semen production, libido and quality of sperm tend to remain uniform throughout the reproductive life of an animal but may be significantly altered

by age, nutrition, environment, health status, drugs and chemicals (Togun and Egbunike 2006). In this study, dietary levels of *Vernonia amygdalina* leaf meal (VLM) influenced average libido score (5.7 to 7.73 mounts/minutes) of male rabbits. A decline in the average libido score of the rabbit bucks with increase in dietary *Vernonia amygdalina* leaf meal (0 to 15%) was recorded. This is in contrast with the findings of Oguike et al. (2019) who observed increased libido score of rabbit bucks fed *Aspilia africana* leaf meal up to 20%. Evaluation of semen provide index for prediction of quality and fertility status of animals (Ajayi et al., 2011). A decrease in the semen volume of rabbit buck fed 5 and 10 % VLM implies that *Vernonia amygdalina* contain substances that may inhibit secretion of seminal fluid from the accessory sex glands since concentration of sperm cells in the semen did not reduce in the VLM group. This result is in agreement with that of Ogbuewu et al. (2009) who reported decrease in semen volume of rabbit bucks fed *Azadirachta indica* at 5 to 15%. However, the semen volumes recorded in this study were within normal range (0.3 to 0.6ml) for rabbit breeds (Lebas et al., 1997). Mass activity (77.7 to 79.43%) recorded was higher than 65.00 to 68.30% (Ahemen et al., 2013); 62.50 to 75.00% (Ogunlade et al., 2019) when 5 to 15% water spinach leaf meal and *Moringa olerifera* leaf meal were fed to rabbit bucks in the studies. The progressive motility of sperm cells in this study is similar to the findings of Ogbuewu et al. (2009) also reported decreased sperm motility in rabbit bucks fed diets with Neem leaf meal.

High concentration of spermatozoa is an indication of high fertility rate because of the number of spermatozoa available at the time of copulation or insemination (Oyeyemi and Okendran, 2007; Attia et al. 2017). Sperm concentration (303.53- 355.42 $\times 10^6$/ml) reported in this study was higher than 88.00 - 116.83$\times 10^6$/ml reported by Oguike et al. (2019) from the effect of *Aspilia Africana* leaf meal and 140.50 - 250.45$\times 10^6$/ml reported by Ogunlade et al., (2019) with *Moringa olerifera* leaf meal supplementation in male rabbits. The significant effect of VLM on live sperm cell in this study is in agreement with that of Oyedeji et al., (2013) who recorded a non-significant decrease in live sperm cell of rat administered *Vernonia amygdalina* extract for 30days. Good semen samples have a minimum of 75% live sperm (Ajala et al., 2001). Therefore, the percentage live sperm cells obtained in this study can be termed suitable for efficient reproduction. This shows that *Vernonia amygdalina* leaf meal had no adverse effect on semen production, however this leaf may not serves as an efficient spermatogenesis booster.

Sperm morphology

Table 3 shows the sperm morphology (%) of rabbit bucks fed dietary levels of *Vernonia amygdalina.* The result shows that VLM had significant ($p<0.05$) effect on the head, midpiece and tail of the sperm cells based on the percentage abnormalities recorded among the groups. Head abnormality was

significantly (p<0.05) higher (1.55%) in buck fed 15% VLM than that of bucks fed 10%VLM (0.73%). Bucks fed 0% VLM diet had significantly (p<0.05) higher midpiece abnormality. Percentage abnormality of the tail region followed the same trend with that of the midpiece. Rabbit bucks fed 0% VLM had the highest total abnormalities (3.52%) with the lowest (1.34%) recorded in bucks fed 10%VLM. Sperm defect is attributable to the activities of free radicals (Ligha et al., 2013). These radicals cause conditions like lipid peroxidation, protein oxidation, DNA damage and cellular degeneration. The activities of these free radicals can be checked by anti-oxidants in *Vernonia amygdalina* (Hamzah et al., 2013; Abdulmalik and Oladapo, 2020). Presence of anti-oxidant in VLM might have contributed to the lower sperm abnormalities observed in this study.

Table 3. Sperm morphology of rabbit bucks fed dietary levels of *Vernonia amygdalina* leaf meal

Parameters (%)	VLM0	VLM5	VLM10	VLM15	SEM	P value
Head abnormality	1.19^{ab}	1.09^{ab}	0.73^{b}	1.55^{a}	0.085	0.0105
Mid piece abnormality	0.94^{a}	0.24^{b}	0.11^{b}	0.40^{b}	0.057	<.0001
Tail abnormality	1.39^{a}	0.37^{b}	0.49^{b}	0.64^{b}	0.112	0.0035
Total abnormalities	3.52^{a}	1.69^{b}	1.34^{c}	2.57^{b}	0.164	<.0001

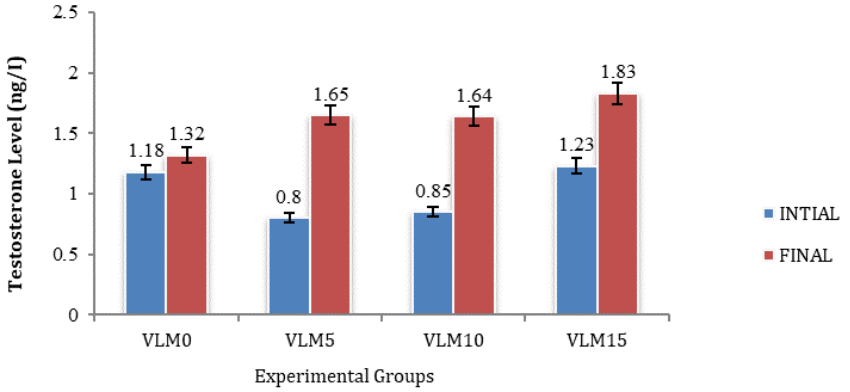
VLM – *Vernonia amygdalina* Leaf Meal, VLM0: Diet with 0%VLM, VLM5: Diet with 5%VLM, VLM10: Diet with 10%VLM, VLM15: Diet with 15%VLM: $^{a, b}$: Means within each row with different superscript are significantly different by Tukey's multiple comparison method (p< 0.05)

The percentage significant total abnormal sperm cells recorded ranged from 1.34 to 3.52%, which is lower than the upper limit of 20% suggested as the least threashold recommendable for good reproductive potential and fertility in either normal mating or artificial insemination (Oyeyemi and Okediran, 2007).

Serum testosterone and sperm reserve

Figure 2 shows the serum testosterone levels (baseline and final) in rabbit bucks fed dietary of *Vernonia amygdalina* leaf meal. The mean values obtained for testosterone concentration at the end of the study were not significantly (P<0.05) influence by VLM levels among the experimental groups. Male rabbits fed 15%VLM had the highest testosterone concentration with the least recorded in bucks fed 0%VLM. Testosterone is the major hormone of the testes and it stimulates sperm production (Abdel-Khalek et al., 2005). Obianime and Uche, (2009) reported that flavonoid possesses pharmacological properties such as anti-oxidant properties, anti-microbial

and anti- inflammatory activities which enable it to enhance fertility. Findings from this study corroborate the non-significant increase in serum levels of Luteinizing Hormone and testosterone in wistar rat administered bitter leaf extract (Saalu et al., 2013).



VLM0: Diet with 0%VLM, VLM5: Diet with 5%VLM, VLM10: Diet with 10%VLM, VLM15: Diet with 15%VLM

Figure 2. Serum testosterone levels of rabbit bucks fed dietary levels of *Vernonia amygdalina* leaf meal

Table 4. Testicular and epididymal indices of male rabbits fed dietary levels of *Vernonia amygdalina* leaf meal

Parameters	VLM0	VLM5	VLM10	VLM15	SEM	P value
Volume of Testis (cm^3)						
Right	0.19	0.22	0.26	0.16	0.016	0.1587
Left	0.22	0.21	0.18	0.23	0.014	0.6455
Weight of Testis (g)						
Right	2.06	2.19	2.28	2.02	0.075	0.6216
Left	2.14	2.26	2.29	1.86	0.104	0.4721
Testicular Sperm Reserve ($x10^6$)						
Right	14.00	15.50	14.75	13.75	1.27	0.9687
Left	15.00	13.25	16.25	23.00	2.50	0.5727
Weight of Epididymis (g)						
Right	0.71	0.54	0.51	0.55	0.036	0.2131
Left	0.72	0.57	0.53	0.91	0.092	0.4887
Epididymal Sperm reserve ($x10^6$)						
Right	107.6	167.75	124.75	108.75	14.42	0.4431
Left	165.5	175.50	151.75	160.00	14.57	0.9587

VLM – *Vernonia amygdalina* Leaf Meal, VLM0: Diet with 0%VLM, VLM5: Diet with 5%VLM, VLM10: Diet with 10%VLM, VLM15: Diet with 15%VLM

However, methanolic extract of *Vernonia amygdalina* significantly decreased testosterone level in male wistar rat (Oyedeji et al., 2013). This implies that the androgenic properties of *Vernonia amygdalina* can influence testes and its functions especially in concentrated form as reported from the use of its extracts.

Table 4 shows the testicular volume, testes and epidydimis weight; and sperm reserve of rabbit bucks fed levels of *Vernonia amydaglina* leaf meal (VLM).

Levels of VLM had no significant ($p>0.05$) effect on the testicular and epididymal indices assessed. Levels of VLM had no adverse effect on testicular indices. This shows that sperm production in the testes was not significantly improved which also resulted in a non-significant increase in spermatozoa storage in the epididymis. There was no significant boost in testes size that may result in higher testicular efficiency. The non-significant effect observed in testicular and epididymal indices implies that mature rabbit diets can be fed dietary VLM up to 15% without any deleterious effects on reproductive organs and their functions.

CONCLUSION

This study showed that *Vernonia amygdalina* leaf meal (VLM) can be fed to male rabbits up to 10% with no deleterious effect on reproductive indices. Good semen production and quality can be maintained with VLM as a feed ingredient and up to 10% may be adopted for optimum performance based on the parameters assessed in this study.

REFERENCES

Abdel-Khalek, E.A., Abu El-Hamd, M.A., Omara, M.E., 2005. Reproductive performance of New Zealand white bucks fed diets containing different types of silage*. Journal of Agriculture* Sciences *Mansoura University.* 30 (4), 1979-1991.

Abdulmalik, O., Oladapo, O. O. 2020. The effect of aqueous extract of *Vernonia amygdalina* (bitter leaf) on antioxidants and the liver of rabbits on high cholesterol diet. *International Journal of Biochemistry Research and Review*, 29(9), 91-100. https://doi.org/10.9734/ijbcrr/2020/v29i930228

Adeyemi, A.A., Ewuola, E.O., Tewe, O.O., 2014. Testosterone, libido assessment and semen characteristics of rabbits fed supplementation *Moringa oleifera* leaf meal. Nigerian Journal of Animal Science. 16 (1), 13 - 19.

Ahemen, T., Abu, A.H., Orakaaya, T.T., 2013. Sperm quality and testicular morphometry of rabbits fed dietary levels of Water Spinach (*Ipomoea aquatica*) leaf meal. Agriculture and Biology Journal of North America. 4(3), 352-357

Ajala, O.O., Oyeyemi, M.O., Akusu, M.O., Eimunjeze, H.E., 2001. The effects of scrotal insulation on the testicles and spermatozoa characteristics of West African Dwarf goats. Sokoto Journal of Veterinary Sciences. 3(1), 44-50.

Ajayi, F.O., Agaviezor, O., Ajuogu, P.K., Harcourt P., 2011. Semen characteristics of three strains of local cocks in the humid tropical environment of Nigeria. International Journal of Animal and Veterinary Advances. 3(3), 125 -127.

Ajuogu, P.K., Herbert, U., Ibeh P.C., Nodu, M.B., Ukpabio, U.H., Onyegbule, C.G., Akintola, A.O., 2018. Semen quality characteristics and testosterone levels of rabbit bucks fed costusafer leaf. African Journal of Biotechnology. 17(2), 24-28. http://doi.org/10.5897/AJB2016.15833

Akinwumi, O.A., Omotayo, F.O. 2016. Proximate analysis and nutritive values of ten common vegetables in South-West (Yoruba Land) Nigeria. Communication in Applied Sciences. 4(2), 79 – 91.

Arhoghro, E.M., Ekpo, K., Anosike, E.O. Ibeh, G.O., 2009. Effect of aqueous extract of bitter leaf (*Vernonia Amygdalina* Del) on Carbon Tetrachloride (CCl4) induced liver damage in albino wistar rats. European Journal of Scientific Research 26(1):122-130.

Attia, Y.A., Hamed, R.S., F. Bovera, A. E. Abd El-Hamid, M. A. Al-Harthi, H.A. Shahba, 2017. Semen quality, antioxidant status and reproductive performance of rabbits bucks fed milk thistle seeds and rosemary leaves. Animal Reprod Sci. 178-186. doi:10.1016/j.anireprosci

Boiti, C., Castellini, C., Theau-Clement. M., Besenfelder, U., Liguori, L., Renieri. T., Pizzi, F., 2005. Guidelines for the handling of rabbit bucks and semen. World Rabbit Science. 13(2), 71-91

Durunna, C.S., Chiaka, I.I., Ebenebe, O.E., Udedibie, A.B.I., Uchegbu, M.C., Durunna, M.O., 2011. Value of bitter leaf (*Vernonia amygdalina*) meal as feed ingredient in the diet of finisher. International Journal of Agriculture and Rural Development. 14(2), 722-726

Francis, O.M., Akinlolu, A.A., Kehinde, O.A., 2013 Assessment of Bitter Leaf (*Vernonia amygdalina*) as fertility enhancer in the Giant African catfish (Heterobranchus bidorsalis) broodstock. Academia Journal of Biotechnology. 1 (2) 36 - 40.

Hamzah, R.U., Jigam, A.A., Makun, H.A., Egwim, E.C., 2013. Antioxidant properties of selected African vegetables, fruits and mushrooms: A Review. Mycotoxin and Food Safety in Developing Countries. 203-209.

Imaga, A.O., Bamigbatan, D.O., 2013. In vivo biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter Leaf). International Journal of Nutrition and Metabolism. 5(2), 22-27. http://doi: 10.5897/ijnam12.0001.

Lebas, F., Coudert, P., Rouvier, R., De Rochambeau, H., 1997. The Rabbit: husbandry, health, and production Rome: *Food and Agriculture organization of the United Nations*. 45-60.

Ligha, A.E, Kola-Ojo, B.O., Jegede, A.E., 2013. Amelioration of histopathological effect of lead induced testicular damage by *Vernonia amygdalina* leaves. Research Journal of Pharmaceutical, Biological and Chemical Sciences 4:800–808.

Malviya, N., Malviya, S., Jain, S., Vyas, S., 2016. A review of the potential of medicinal plants in the management and treatment of male sexual dysfunction. Andrologia. 48(8), 880-893. http://doi: 10.1111/and.12677

Obianime, A.W., Uche, F.I., 2009. The Phytochemical constituents and the effects of methanol extracts of Phyllanthus amarus Leaves (Kidney Stone Plant) on the hormonal parameters of male guinea pigs. Journal of Applied Sciences and Environmental Management. 13(1), 5-9.

Oboh, G. 2006. Nutritive Value and Haemolytic Properties (in vitro) of the leaves of *Vernonia amygdalina* on human erythrocyte. Nutrition and Health. 18(2), 151-160. https://doi:10.1177/026010600601800207

Ogbuewu, I.P., Okoli, I.C., Iloeje, M.U. 2009. Semen quality characteristics, reaction time, testis weight and seminiferous tubule diameter of buck rabbits fed Neem (*Azadirachta indica A. Juss*) Leaf Meal Based Diets. International of Journal Reproductive BioMedicine. 7(1), 23- 28.

Ogbuewu, I.P., Mbajiorgu, C.A. 2019. Potential of leaf and seeds of tropical plants in chicken diets: effect on spermatozoa and egg production. Tropical Animal Health and Production 51: 267–277 https://doi.org/10.1007/s11250-018-1715-3

Oguike, M.A., Onuta, S.C., Amaduruonye, W., Akpan, I.U. 2019. Impact of Aspilia africana on semen and testicular characteristics of rabbit bucks. Journal of Advanced Agricultural Technologies. 6(2), 144-149. https://doi: 10.18178/joaat.6.2.

Ogunlade, J.T., Adeusi, A.S., Ogunleye, O.E., Olatunji, M.A., Busari, K.F., Arobadi, J.O., Ojo, J.O., Alamuoye, O.F., Akinsola, K.L., 2019. Effects of graded levels of *Moringa oleifera* leaf meal on growth performance, semen quality indices, blood profile and carcass characteristics of rabbit bucks. International Journal of Life Science. 8(3), 63 -71.

Olounlade, P.A., Saka Konmy, B.B., Bertrand Azando E.V., Doko Allou, S., Baba-Moussa, L. 2021. *Moringa oleifera*, *Ocimum gratissimum* and *Vernonia amygdalina* as a natural antiparasitic alternative in growing rabbits. *Livestock Research for Rural Development. 33, (114).* http://www.lrrd.org/lrrd33/9/33114abido.html

Oyedeji, K.O., Bolarinwa, A.F., Azeez, A.A., 2013. Effect of methanolic extract of *Vernonia amygdalina* on reproductive parameters in male albino rats.

Research Journal of Pharmacology 7 (1), 7-11 http://doi:10.36478/rjpharm.2013.7.11.

Oyeyemi, M.O., Oluwatoyin, O., Ajala, O.O., Leigh, O.O., Adesiji, T., Fisayo, T., 2007 The spermiogram of male wistar rats treated with aqueous leaf extract of *Vernonia amygdalina*. Folia Veterinarian 51(3-4): 126-129

Oyeyemi, M.O., Okediran, B.S., 2007 Testicular parameters and sperm morphology of chinchilla rabbits fed with different planes of soy meal. International Journal of Morphology. 25 (1), 139-144.

Saalu, L.C., Akunna, G.G., Oyewopo, A.O., 2013. The histo-morphometric evidences of *Vernonia amygdalin*a leaf extract-induced testicular toxicity. International Journal of Morphology. 31(2), 662 - 667. http://dx.doi.org/10.4067/S0717-95022013000200052

SAS, 2009. Statistical analytical system institute, SAS/STAT User's guide statistical, SAS Institute, Cary. North Carolina, USA.

Togun, V.A., Egbunike, G.N., 2006. Seasonal variations in the sperm production characteristics of zebu (White Fulani) cattle genitalia in the humid tropical environment. *Middle-East. Journal* of *Scientific Research.* 1, 87-95.

Ukawoko, A.I., Okehielem, O.V. 2016. Effect of Gmelina (*Gmelina arborea*) leaf meal based diets on growth performance of West African Dwarf bucks. Asian Journal of Animal Sciences. 10 (2), 154-158. http://doi: 10.3923/ajas.2016.154.158.