Modulating Role of *Vernonia amygdalina* Leaf Meal on Spermatogenic and Steroidogenic Functions of West African Dwarf Bucks Testis

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Abstract

Vernonia amygdalina has a unique nutritional and phytochemical property with numerous physiological, biochemical and morphological benefits due to its anti-oxidative and anti-helminthic properties. This study evaluated the effects of *Vernonia amygdalina* leaf meal (VALM) on follicle stimulating (FSH), luteinizing hormones (LH) and testosterone regulation of seminal characteristics of West African dwarf (WAD) bucks. Twenty-four (24) WAD bucks were randomly assigned to four treatments (A, B, C and D) in a completely randomized design for a nutritional trial of eight weeks. Animals were given diets containing 0, 20, 30 and 40g VALM/kg feed. Animals were weighed and blood samples were collected at the beginning of the experiment and subsequently forth-nightly before the end of the experiment for hormonal assay. Semen volume, semen color, sperm concentration, sperm motility and sperm morphology were determined from the semen samples obtained forth-nightly by electro-ejaculation. All data collected were analyzed using the General Linear Model procedure of SAS, (2002). Means were separated using Duncan's Multiple Range Test of the same software. There were significant differences ($p<0.05$) in most of the hormones studied except testosterone which was not significantly affected by VALM. Sperm concentration, motility and morphology increased significantly ($P<0.05$) and was highest at 40 g of VALM/kg feed. The color of the semen from all the treatments was whitish grey. It was therefore concluded that VALM improved the FSH and LH production at 40 g of VALM/kg feed with improved sperm quality in WAD bucks, thereby enhancing the reproductive performance of the goats.

Keywords: *Vernonia amygdalina, semen quality, hormone, goats*

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1. Introduction

In recent times, much attention has been shifted from synthetic drugs to natural plants and their products (Atanasov*et al.*, 2021). Various plant parts that were once considered of little or no importance are now studied and subsequently developed into drugs with minimal side effects (Salmerón-Manzano *et al.*, 2020). Some wild herbs and species have been shown to be extremely effective, relatively non-toxic and have substantial scientific documentation to attest to their efficacy in infertility management (Jaradatand Zaid, 2019). The fertility improving capacity of plant extracts has been reported in numerous studies (Okukpe *et al.*, 2012). *Vernonia amygdalina* (Va), popularly called bitter leaf because of its bitter taste, are broadly devoured in Nigeria. The unpleasant taste of Va is due to its phytochemical constituents such as alkaloids, saponins, glycosides, and tannins (Ojo, 2021; Agidew, 2022). It develops prevalently in tropical Africa, particularly in Nigeria, Zimbabwe, and South Africa, and is tamed in parts of West Africa (Egharevba *et al*., 2014). The Va wood, especially from the root, has been shown to have fertility inducing properties (Kadir *et al*., 2020). *Vernonia amygdalina* has a very bitter taste which can be reduced by proper boiling, or in the case of young leaves by soaking in several changes of water (Grubben and Denton, 2004). It contains flavonoids and other plant compounds of nutritional value that promote overall health (Eziuche *et al*., 2021). The leaves have been found to contain bioactive chemicals such as Vernonioiside B and Myricetin which are mainly flavonolic compounds. A quantitative analysis also gave a total phenolic content of about 3.01 mg/g of dry matter (Gordian *et al.*, 2007). Other phytochemicals identified include vernodaline; vernomygdin; vernodalol; vernolide; hydroxyvernolide; tannins; vitamin C; luteolin; luteolin 7-O-b-glucoronide; leteolin 7-O-b-glucoside; vernonioside D and E; vernolepin; vernonioside A1 – A4; vernonioside B1 – B3; vernodalinol; alpha-muurolol; 1,5 dicaffeoyl-quinic acid, dicaffeoyl-quinic acid, chlorogenic acid and luteolin-7-O-glucosi (Djeujo *et al.,* 2023). Various parts of this plant have been used as therapeutic agents in different ailments and disease conditions including gastrointestinal problems (Czigle*et al.,* 2022). Proper nutrition is very important for the health and reproductive performance of animals and the foundation of successful production systems. It also impacts growth performance and development of reproductive organs. Testicular mass and semen characteristics can be influenced by feeding (Okukpe *et al*., 2012). Severe under-nutrition is one of the most common causes of impaired reproductive capacity in terms of semen production and quality (Ferramosca and Zara, 2022). A low plane of nutrition suppresses the production of gonadotropins by the pituitary gland and the secondary sex hormones, so that atrophy of the prostrate and the seminal vessels occur thereby affecting semen quality in terms of fluid volume and concentration (Anamthathmakula and Winuthayanon, 2020). Thus, the plane of nutrition is pivotal in determining the quality of buck semen. The problem of poor semen quality and low fertility caused by low plane of nutrition can be reduced with high plane of nutrition. *Vernonia amygdalina* is believed to have a stress modulating effect and contain essential nutrients necessary for reproductive enhancement. Analysis of seminal parameters such as semen volume, semen color, sperm concentration, sperm morphology and mass motility is particularly helpful in investigating male infertility, genital infections and pathologies (Akang *et al.*, 2023). The effect of *Vernonia amygdalina* leaf meal (VALM) in alleviating stress related reproductive disorders in West African dwarf goats exposed to environmental stressor has not been adequately researched. Therefore, the thrust of this experiment was to determine the effect of VALM on male sex hormones and semen quality in West African dwarf (WAD) bucks.

2. Materials and Methods

2.1 Location of the Study

The feeding trial was conducted from the month of February – May, 2021 at the University Teaching and Research farm located (14^{o} N 11^{o} E) in sub-humid tropical environment with an annual rainfall range of 1500-1700 mm and average maximum and minimum temperature readings of 18 °C and 25 °C, respectively. Blood and semen samples were collected at the beginning of the experiment

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and every forth night for sexual hormone assessment in serum and sperm quality parameters, respectively.

2.2 Processing of *Vernonia amygdalina* leaf meal for inclusion in concentrate diet

Vernonia amygdalina leaf was collected from the University of Ilorin Teaching and Research farm, the leaves were air-dried at room temperature, picked without the stalks and ground using an electric blender LG^{R} model.

2.3 Animals and treatments

Twenty-four (24) adult male West African Dwarf goats with initial weights of between 10.63 and 12.50 kg were divided into four groups, balanced for body weights. The goats were assigned to four dietary groups in a randomized complete block design A, B, C and D. Goats in each group received a concentrate diet (Table 1) containing 0, 20, 30 or 40 g *Vernonia amygdalina* leaf meal (VALM)/kg feed. The concentrate was fed at 500 g/10 kg body weight in two equal rations (0800 and 1600 hours) daily as supplement to basal *Panicum maximum* hay (9.18% CP). Goats were housed individually in concrete-floored pens (1 x 1.5 m^2) during the first 49 days and thereafter transferred into metabolic pens equipped with facilities for individual animal separate feeding, provision of water and collection of faeces and urine over the last seven days. The goats were treated against external and internal parasites before the commencement of the feeding trial.

Table 1: Composition of the basal experimental feed

Ingredients, %	A	B	C	D
Maize	20.00	20.00	20.00	20.00
Wheat offal	15.00	15.00	15.00	15.00
Groundnut cake	8.20	8.20	8.20	8.20
Palm kernel cake	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00
Methionine	0.05	0.05	0.05	0.05
Salt	0.08	0.08	0.08	0.08
Lysine	0.05	0.05	0.05	0.05
Vitamin premix	0.05	0.05	0.05	0.05
VALM	0.00	4.00	6.00	8.00
Proximate composition of feed (%)				
Nutrients, %	**A**	**B**	**C**	**D**
Dry Matter	94.94	96.34	95.90	95.88
Crude protein	18.48	23.48	22.29	20.42
Crude fibre	10.42	9.03	11.30	9.48
Ether extract	3.08	4.26	4.46	3.37
Ash	7.12	7.10	7.14	7.18
Neutral Detergent fibre	246.00	228.00	236.00	216.00
Acid Detergent fibre	149.00	158.00	156.00	172.00

2.4 Collection of samples and analyses

Average daily feed intake for the individual goat was measured as the difference between the amount of feed offered and the amounts refused over 24 hours during the 7-day collection period. Body weight measurements were recorded before early morning feeding on the first day of the 56-day feeding trial and subsequently at 14-day intervals. The difference between two consecutive measurements was used to estimate body weight gain over the interval period. Total faeces voided by each goat were collected during the 7-day digestibility period and about 10% preserved for chemical

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analyses. Blood samples were collected through the jugular vein from individual goats. The blood collected was put into plain bottles, taken to the laboratory and centrifuged at 1200 x g for 20 minutes for serum using Minifuge RF, Heraeus, and Hannover, Germany. Separated serum was stored frozen at -4 °C until assayed. Hormone concentrations were measured by using an auto analyzer (Hitachi 747, Boehringer Mannheim, Madrid, Spain) which followed the principles of enzyme linked immunosorbent assay, ELISA kit (Lifespan Biosciences, Inc).

Urine was collected into a plastic bucket acidified with 100 ml of N Hcl and about 10% of the daily urine production was composited for each goat. Samples of feeds and orts were composited every two weeks, oven-dried (60 °C) to constant weights and allowed to air-equilibrate before being ground to pass through 1-mm sieve. About 10% of the ground feed and orts samples were preserved for later dry matter and proximate analyses. Preserved feed and faeces subsamples were analyzed for dry Kjeldahl nitrogen, crude fibre, ether extracts and total ash (AOAC, 2005). Percentage nitrogen in the urine subsamples was measured (AOAC, 2005). Wet faecal samples were used for the determination of dry matter digestibility (Van Soest *et al*., 1991). Empirical data were subjected to analysis of variance of a randomized complete block designed experiment using SAS (statistical Analysis software) package, 2002 model. Differences between treatment mean was separated using Duncan`s Multiple Range Test of the same model.

3. Results

As observed in Table 2, the nutrient digestibility coefficients were significantly different ($p < 0.05$) in crude fibre (CF), crude protein (CP), ether extract and NDF, but not in ash and ADF ($p > 0.05$). The ash digestibility coefficient in this study was numerically high, ranging from 76.27 to 83.1. The treatments B (20g/kg) and C (40g/kg) both had the highest ash, crude fibre and ether extract digestibility values. CP digestibility value of 40.3% was the highest in treatment B, with treatment C and D having similar values, and lowest in treatment A with a value of 17.37%. Crude protein digestibility was increased more significantly ($p<0.05$) by the inclusion of 20%VALM/kg feed in the diet. Treatment A was the lowest in ash, CP, ether extract and ADF digestibility values. NDF digestibility value of 53.69 and 51.26% in both treatment A and B respectively were the highest, with 40.54% in treatment D as the lowest. Treatments C and D were the highest in ADF digestibility value of 68.59% and 68.31% respectively. The effects of *Vernonia amygdalina* leaf meal (VALM) on male sex hormones of West African Dwarf goats are shown in Table 3. There were significant differences ($p<0.05$) in oestrogen, follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone values in animals administered *Vernonia amygdalina* leaf meal. There was significant reduction in oestrogen in animals administered 20 and 30 g VALM/kg feed compared with the control and treatment D with 40 g VALM/kg feed. Goats administered higher levels of VALM had significantly higher levels of testosterone compared with the control. Conversely, there were significant reductions in FSH and LH with an increase in VALM inclusion in the feed with the least value of both found in treatment D.

Table 2. Nutrient digestibility coefficients (%) of WAD bucks fed *Vernonia amygdalina* leaf meal

Digestibility coefficients (%)	A (0 g/kg)	B (20 g/kg)	C (40 g/kg)	D (60 g/kg)	± SEM
Ash	76.27	83.1	82.20	79.68	1.98
Crude fibre	94.94^{b}	95.89^{a}	96.07^{a}	94.92^{b}	0.29
Crude protein	17.37^{b}	40.30^{a}	31.76^{ab}	33.37^{a}	5.04
Ether extract	75.59^{c}	92.17^{a}	90.61^{a}	83.64^{b}	2.16
NDF	53.69^{a}	51.25^{a}	48.29^{a}	40.54^{b}	1.88
ADF	57.37	66.14	68.59	68.31	1.54

$^{a, b, c}$ –means values having different superscript along the same row are significantly different ($p<0.05$). NDF - Neutral Detergent Fibre; ADF - Acid Detergent Fibre. A – 0 g Vernonia amygdalina leaf meal (VALM) to 1 kg concentrate feed, 20 g VALM/kg concentrate feed,40 gVALM/kg concentrate feed, 60 g VALM/kg concentrate feed.

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Table 3. Effect of *Vernonia amygdalina* leaf meal on male sex hormones of West African Dwarf Goats.

Parameter/Treatments	A	B	C	D	±SEM
Oestrogen, pg mL^{-1}	1236.00^{b}	794.67^{d}	1136.0^{c}	3615.33^{a}	17.84
Testosterone, ng mL^{-1}	0.66^{c}	0.61^{d}	0.77^{b}	0.88^{a}	0.02
Follicle Stimulating Hormone, mg mL^{-1}	55.56^{a}	21.49^{b}	18.66^{c}	18.16^{d}	0.42
Luteinizing Hormone, mg mL^{-1}	48.81^{a}	40.76^{b}	40.69^{b}	25.61^{c}	0.42

^{a,b,c,d} - mean with different subscript within row differs significantly (p<0.05)

The effects of graded levels of *Vernonia amygdalina* (Va) leaf meal on semen quality of West African Dwarf bucks was presented in Table 4. There were significant differences (p<0.05) in sperm motility, concentration and morphology, but not in semen volume. There were significant (p<0.05) increases in sperm motility, concentration and morphology with concomitant increase in VALM in the animal diets.

Table 4. Effects of Graded Levels of *Vernonia amygdalina* Leaf Meal on Semen quality of West African Dwarf Bucks

Parameters/Treatments	**A**	**B**	**C**	**D**	**±SEM**
Semen volume (ml)	0.16	0.14	0.16	0.15	0.055
Semen color	Whitish-grey	Whitish-grey	Whitish-grey	Whitish-grey	
Sperm motility (%)	78.18^{b}	80.91^{b}	81.00^{b}	86.12^{a}	0.774
Sperm concentration (million/ml)	51.40^{b}	54.10^{ab}	54.90^{ab}	55.60^{a}	0.908
Sperm morphology (%)	86.93^{ab}	83.60^{c}	85.78^{bc}	89.45^{a}	0.716

a, b, c – means in the same row with different superscripts are significantly different(p< 0.05)

4. Discussion

As observed in Table 2, the nutrient digestibility coefficients were significantly different (p < 0.05) in crude fibre (CF), crude protein (CP), ether extract and neutral detergent fibre (NDF). There was a significant reduction in NDF digestibility with increased VALM inclusion in the diet contrary to the report of Yousuf *et al*. (2013) that apparent CP digestibility was influenced significantly with a high inclusion rate of *V. amygdalina* leaf meal with improved digestibility of NDF with increasing VALM. Kedir (2011) and Adugna *et al.* (2023) confirmed that the digestibility of CP increased with increasing level of the supplemented *V. amygdalina.* Animals in treatments B and C were more efficient in nutrient digestibility, corroborating the report of El-Nomeary *et al.* (2021) and Jaapar *et al.* (2023) that crude fibre and crude protein of composite diet determine best the digestibility of feed in sheep and goats.

Although the reasons for male infertility are multifaceted, the major cause is hormonal abnormality (Okonofua *et al*., 2005; Chaudhuri *et al.*, 2022). The hormonal abnormality may be caused by the disturbance or suppression of the hypothalamic-pituitary-adrenal axis, which may arise in part from the uncontrolled or excessive use of hormone production inhibitory substances in feed or environmental factors (Chen *et al.*, 2013; Sheng*et al.*, 2021). As could be observed in Table 2, there was a significant reduction in oestrogen in animals administered 20 and 30 g VALM/kg feed compared with the control and treatment D with 40 g VALM/kg feed. Goats administered higher levels of VALM had significantly higher levels of testosterone compared with the control. Conversely, there were a significant reduction in FSH and LH with an increase in VALM inclusion in the feed with the least value of both found in treatment D. VALM has a unique nutritional and phytochemical property which has numerous physiological, biochemical and morphological benefits. It is known that the consumption of vegetables is essential for a healthy life due to their anti-oxidative properties (Saalu *et al*., 2013; Gariballa *et al*., 2021). The result of this research shows the modulating effect and role of

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bitter leaf meal extract in a dose-dependent manner. Studies have also shown that treatment with antioxidants improves steroidogenesis by enhancing the primary effect of the Leydig cell endocrine function along with increased circulatory testosterone production and stimulation of spermatogenesis (Martin and Touaibia. 2020). This assertion supports the result obtained in the present study where significant increase in testosterone was obtained with an increase in the levels of VALM in the animals feed.

FSH in the male serum was high in the controlled bucks than other treatments; there was a decrease in the level of FSH serum with an increase in the dosage of *Vernonia amydalina* administered. According to Oduwole (2021), LH helps to stimulate the Leydig cells of the testis to produce testosterone and increase androgen output. It is therefore emphasized that LH is the only hormone capable of stimulating testicular steroidogenesis in the absence of the other hormones. According to the report of Santi *et al.* (2020), FSH acts on the seminiferous tubules to control spermatogenesis and the growth of androgen dependent accessory reproductive glands as well as support the action of LH on testosterone production.

The effects of graded levels of *Vernonia amygdalina* leaf meal (VALM) on semen quality of West African Dwarf bucks was presented in Table 3. There were significant differences ($p<0.05$) in sperm motility, concentration and morphology, but not in semen volume. One of the factors that have been reported to affect semen quality is the quality of feed consumed by the animal at a particular time (Ribas-Maynou*et al*., 2023). This report was corroborated by the significant ($p<0.05$) increases in sperm motility, concentration and morphology with concomitant increase in VALM in the animal diets. The result obtained was comparable to the report of Saalu *et al.* (2013) that VALM caused a significant ($p> 0.05$) increase in sperm concentration, percentage motility, morphology and percentage live sperm produced. He inferred that higher dosage of VALM could be deleterious to the testes when administered for a long period. According to Ajala and Owoyemi, (2016) VALM increase sperm concentration and motility at a low level (10%) in *Clariasgariepinus* (catfish) for 45days with low morphological abnormalities Silvestre *et al.* (2004) and Dcunha *et al.* (2022) reported that semen intended to be used for artificial insemination should not have sperm motility less than 60% if high semen quality is required. Sperm motility was lower in the control than any other treatment in this study. The direction of movement of spermatozoa is important in semen evaluation as the percentages of progressive motility have direct effect on fertilizing capacity (Bearden and Fuquay, 1997; Bjorndahl, 2010). It could therefore be inferred that *Vernonia amygdalina* elicits increased sperm motility and this could be due to the lipid, carbohydrate and vitamin C contents of the leaves. Vitamin C deficient diet is known to reduce sperm motility (Kadir, 2020) and the Carbohydrate content of *Vernonia amygdalina* is a rich source of energy which is needed to produce high sperm motility. According to Tanga *et al.*(2021) semen with less than 80% sperm morphology should not be used for breeding purposes. Animals in treatment D (40 g VALM) had the highest sperm morphology and it can be used for artificial insemination. Semen obtained from other treatments was also of good quality.

According to Brazilian College of Animal Reproduction guidelines (CBRA, 1998), goats' semen can display different colours ranging from white to citrine-yellow. All treatments had whitish grey semen colour. It shows that the level of VALM inclusion in the feed does not have any appreciable effect on semen colour.

According to Anderson (1992) and Villani *et al.*(2021) semen produced with less than one million sperm concentration should not be used for artificial insemination because of its low fertility. The semen collected from treatments A, B, C and D was of good quality and can be used for artificial insemination.

Treatment D could be used for artificial insemination because it gives the best sperm motility, sperm concentration and sperm morphology and this might be due to its 40 g inclusion level of *Vernonia amygdalina* leaf meal.

For reproduction to be successful, the production of viable sperm cells that enable successful fertilization is necessary. This experiment improved viable sperm production in WAD bucks using

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graded levels of *Vernonia amygdalina* leaf meal. The result for this study showed that *Vernonia amygdalina* improved semen quality in WAD bucks. Therefore, *Vernonia amygdalina* at 40g inclusion level can be used to improve viable sperm production in WAD bucks.

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