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Research Article

EFFECT OF GRADED LEVELS OF BITTER LEAF (*VERNONIA AMYGDALIN*) ON PERFORMANCE AND SEMEN CHARACTERISTICS OF YANKASA RAMS IN SUDANSAVANNAH ZONE NIGERIA

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Abstract

A total number of 15 Yankasa grower rams were allotted into five treatment groups with three animals per group and each animal were housed individually in a pen measuring 2m x 1m consist of treatment A with 0%, B with 2%, C with 4%, D with 6% and E with 8% graded levels of *Vernonia amygladina* in a completely randomized design (CRD). The feeding trial lasted for 49days were feed and water was provided to the animal *adlibitum*. The objectives of the study were to determine the performance and semen quality characteristics of yankasa rams fed graded level of *vernonia amygdalina.* Daily feed intake was measured and weight of the rams was also taken at two weeks interval. The results showed that treatment A(65.99g/day) had the best daily weight gain followed by treatment C and B while Treatment D and E were significantly ($p<0.05$) lowest average daily gain (ADG). Cost of feed kg/live weight was significantly lower ($p<0.05$) in treatment A and B and significantly high ($p>0.05$) in treatment C,D and E. The study concluded that diet A was better than other treatments, and then followed by diet B. The result revealed a significant ($P<0.05$) in sperm cell concentration, percentage motile sperm cell, percentage morphologically normal spermatozoa and scrotal circumference. However, no significant ($P>0.05$) differences was observed in semen volume and semen pH. The haematological result of the effect of *vernonia amygladina* in Yankasa rams shows a significant ($P<0.05$) differences in RBC, PCV, Hb and Lymphocyte. It is therefore concluded that feeding Yankasa ram with *vernonia amygladina* has a profound effect in performance, semen quality characteristics and haematological profile. It was recommended that longer period of feeding the animals with bitter leaf is needed to ascertain its ability to modify rumen environment and achieve improve feed utilization in yankasa rams.

Keywords: Bitter leaf, Performance, Semen and Yankasa Rams.

INTRODUCTION

Reproduction is central to the continued existence of animals on earth. In sexual animals, reproduction is important for both males and females for life to continue. Efficient reproduction is key to the success of any livestock enterprise (Garba*et al.,* 2022). Several research efforts have been made, targeted towards enhancing the reproductive performance of farm animals through scientific approaches involving some kind of genetic, nutritional and physiological manipulations or interventions (Herbert & Ukar, 2008; El-Azim & El-Kamash, 2011). One of the major issues on breeding in farm animals is fertility, and approximately 30% of the problems are related to the males (Lee *et al.,* 2012; Barkhordari *et al.,* 2013). To improve the productivity of Nigeria Livestock sub-sector animal scientists (Ubah *et al.,* 2016) are in the opinion that strategies will have to focus on combating infertility, since this is a major problem among others that attributed to the low productivity of Nigerian Livestock production system. Recently, a wide number of plant-derived pharmaceutical products are now being used in traditional medicine because of their beneficial properties in handling infertility (Yama *et al.*, 2011). Bitter leaf (*Vernonia amygdalina*) is an important plant in Nigeria that has a multiplicity of biological role (Saalu *et al.,* 2013) it has a unique nutritional and phytochemical property which has numerous physiological, biochemical and morphological benefits.

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It is known that consumption of vegetables is essential for a healthy life due to their antioxidative properties (Saalu *et al.,* 2011; Akunna *et al.,* 2011). Vernonia amygdalina has been observed to grow in Nigeria under harsh condition. However, in general it has been found have a strained taste, which affects its intake (Saalu *et al.,* 2013). The bitter taste is due to the anti-nutritional factor such alkaloid, saponins, tannins and glycosides (Saalu *et al.,* 2013). These compounds have been found to have a great affect the microbial (bacteria, protozoa, fungi) activities in the rumen thereby modifying the fermentation process. It has been reported that other species such as ginger, garlic have also been used for a similar work. There will be a better utilization of feed and consequent reduction in the cost of production, in addition, the emission of gases like methane and carbon dioxide will be reduced and subsequent preservation of the ozone layer. Bitter leaf botanically called *Vernonia amygdalina,* Bitter leaf is a medicinal plant, which rows in the humid tropical secondary forests of Africa. Bitter leaf is among several natural products used by traditional healers in Western Nigeria to treat a number of bacterial infections. The leaves are used as a leafy vegetable for preparing the popular bitter-leaf soup and the juice or extract serves as a tonic drink. It contains 18% protein, 8.5% fiber in a dry matter, and a good composition of macroelements1. Moreover, *Vernonia amygdalina* has been used in traditional medicine as an antihelminthes, an ant malarial, and a laxative herb. (Osho, *et al.,* 2014). This research is aimed at identifying the effect of graded levels of bitter leaf on performance and semen quality characteristics of Yankasa rams raised in sudan savannah ecological zone of Nigeria.

MATERIALS AND METHODS

Experimental Location

The study was carried out in the ruminant unit, Prof. Lawal Abdu Saulawa, Livestock Teaching and Research Farm, Department of Animal Science, Federal University Dutsin-ma. The site lies between latitude 12°27'18' North and 7°29'29' East and 605 meters above sea level with an average rainfall of 700mm within the Sudan Savannah zone (Gaddafi *et al.,* 2019).

Experimental Animals and Their Management

Fifteen yankasa rams were purchased and use for the study. The experimental animals were quarantined for two weeks during which Bannath II dewormer (12.5g/kg body weight) were administered, sprayed against ecto-parasite and treated with oxyteracyline (a broad spectrum antibiotics) by injection.

Semen Collection

Semen samples used in this study were collected from the rams weekly for a period of four weeks consecutively using electro-ejaculator techniques as described by Oyeyemi *et al;* (2002) with little modification. Prior to the semen collection preliminary training were given to the rams. The animals were gently restrained, the rams were placed on its side and the penis was extended from the sheath by stretching the sigmoid flexure. The penis was than grasped with the sterile gauze and the gland penis was diverted into a 50ml disposable tube that was insulated by the hand of the collection technician. The animals were gently massaged the accessory glands by exerting a downward pressure on the bottom of the rectum for 10 to 15 second prior to the inserting electro-ejaculator. The lubricated electro-ejaculator probe was than inserted into the rectum of the animal and turned on were the voltage increased manually for three to eight seconds and then the animal is allowed to rest for 15-20 seconds and repeated till ejaculation. After ejaculation, the semen was covered to maintain its temperature and taken to the laboratory for processing.

Semen quality parameters determination

Semen Volume and Colour

Ejaculate volume was measured immediately after collection from the ram using graduated collecting tube while semen colour was determined by visual observation.

Semen pH

Initial semen pH will be obtained by means of comparative pH paper as described by Kamar *et al.,* (1979). The pH paper is calibrated from 1 to 14 with different colours. One inch of the paper will be inserted into each sample of the semen then it will be removed and checked for the colour match on the pH paper chart.

Sperm cell concentration

Spermatozoa concentration was determined with the aid of improved Neubaurhemocytometer. Where a cover slip was charged with the ram`s semen diluted with 0.05% formol-saline solution. The sperm cell heads in the 5 large cells of the hemocytometer were counted after viewed under microscope at x 40 objective.

Percentage sperm cell motility

A drop of semen diluted with a drop of sodium citrate was placed on a warm glass slide covered with clover slip. This was viewed using x 40 objective of light microscope and the percentage active, progressively motile cells were estimated as described by Chenoweth (2005).

Percentage morphological normal sperm cell

One drop of the collected semen was added to two drops of eosin-nigrosin stain, mixed thoroughly before a smear was made on a clean glass slide. The slides were examined at x 100 magnifications. Sperm cell with morphological aberrations were determined from a total count of 100 spermatozoa and the percentage number of normal cell were determined (Chenoweth, 2005)

Experimental Diets

Five diets were formulated containing graded levels of *vernonia amygladina* at 0, 2, 4, 6 and 8% for treatments A, B, C, D and E respectively with A as a control.

Haematological determination

Blood will be collected at the end of experiment I (12 weeks post trail) by using sterile syringe and needle using jugular venepuncture of four bucks per treatment and will be put into well labelled blood collection bottles, which contained ethylene diamine tetraacetic acid (EDTA). The blood samples will be put in an ice pack and transported to the haematology unit of Medical Laboratory of Federal Medical Centre, Katsina State for determination of haematological parameters. Which includes: the haemoglobin content, white blood cells, red blood cells, neutrophils, lymphocytes, eosinophil, monocytes, basophils and packed cell volume will be determined by the procedure outlined by Decie and Lewis (2001)

Dietary Treatment

The experimental animals were allotted into the five treatment groups of three rams per group and one ram per replicate in a completely randomized block design (CRD) (17). Data were analysed using analysis of variance (ANOVA) and DMRT were used to separate the treatment mean using statistical analysis system (SAS, 2002) each animal were housed in a pen measuring 2m x 1m , which was disinfected prior to the commencement of the research

Table 1. Gross Composition of Experimental Diet

Parameters	Treatment				
Ingredients %	**A**	**B**	**C**	**D**	**E**
Poultry litter waste	10	10	10	10	10
Sorghum husk	15	13	11	24	9
Cowpea Husk	24	24	24	30	24
Wheat offal	15	20	20	19	18
Groundnut Hay	26	30	30	10	30
Batter leaf	0	2	4	6	8
Salt	1	1	1	1	1
Calculated Energy (ME/kca)	2569	2297	2343	2732	2524

The energy was calculated using pausanguard formula (ME=36x CP% X CF% + 81.5 X EE% + NFE%

RESULTS AND DISCUSSION

Characteristics of the experimental diet

The crude protein level used in the present study was 11-12%. This was in accordance with the recommended level of the crude protein for fattening of sheep and goat of 11% reported by ARC (AOAC, 1990). The either extract ranged from 4.59-5.02 in the study, which is not comparable of 3.90% reported by Maigandi (2001),while the crude fibre varied from 5.5-25.21 which is less than 34.80% when fed 20% FSD as replacement for cowpea husk in the diet of Uda sheep.

utilization of nutrients in the diet. Treatment D and E had lower feed intake and lower ADG which is due to the high percentage of bitter leaf of 6% and 8%,which leads to poor palatability and ultimately low feed intake . Economics analysis of the performance experimental animals in this study showed that cost per kg live weight gain lower in treatment A(N761.02)with zero percent bitter leaf, followed by treatment B(1241.58)with 2% bitter leaf and 4%(N1246.43) in treatment C and then treatment D(N1654.09) with 6% bitter leaf. Treatment E (N2095.56) recorded the highest cost of feed/kg live weight gain. The results of the study indicate that as the level of bitter level inclusion increase, the cost feed per kg live weight gain increases.

Table 2. Proximate composition of experimental diet

Parameter	Treatment					
	A	B	C	D	E	SEM
Dry matter	94.14	93.78	94.9	94.1	25.13	93.69
Crude protein	27.86	26.50	27.73	27.33	26.73	10.88
|Either extract	3.23	2.06	2.26	2.00	1.60	4.63
Crude fibre	65.99^{a}	42.18^{ab}	46.82^{ab}	40.82^{ab}	32.65^{b}	20.68
Ash	15.40^{ae}	23.69	23.52	22.15	22.93	8.11
Nitrogen free extract	67.52	44.3	42.97	53.17		49.39

Table 3. Performance and nutrient intake of Experimental diet

Parameters	A	B	C	D	E	SEM
Initial Body weight (kg)	24.63	24.43	25.46	25.33	25.13	2.61
Final Body weight (kg)	27.86	26.50	27.73	27.33	26.73	2.59
Body weight gain (kg)	3.23	2.06	2.26	2.00	1.60	0.39
Average daily gain (g)	65.99^{a}	42.18^{ab}	46.82^{ab}	40.82^{ab}	32.65^{b}	7.90
Feed Grain Ration	15.40^{ae}	23.69	23.52	22.15	22.93	0.07

Means with the same letter are not significantly different (p>0.05) along the same row.
key: a=control b=2% bitter leaf, c= 4% bitter leaf, d=6% bitter leaf and e=8% bitter leaf.

Table 4. Cost of Feed

Parameters	A	B	C	D	E	SEM
Cost of feed (N/kg)	50.22^{a}	53.39^{a}	57.66^{ab}	67.52^{c}	68.42^{c}	0.54
Feed consumed(kg)	1.00^{a}	0.88^{a}	0.96^{c}	0.83^{a}	0.74^{b}	1.53
Cost of feed consumed (N/day)	41.85^{c}	38.77^{d}	46.20^{c}	46.58^{a}	41.73^{b}	2.50
Cost of feed per kg	761.02^{d}	1241.58^{c}	1241.58^{e}	1654.09^{h}	2095.56^{a}	0.50

Means with the same letter are not significantly different (p>0.05) along the same row. key: a=control b=2% bitter leaf, c= 4% bitter leaf, d=6% bitter leaf and e=8% bitter leaf

Performance characteristics of the experimental animals

The result indicates that high feed intake in the in treatment A and B. The reason behind high feed intake in the treatment A is as a result of high proportion of Groundnut Hay and absence of bitter leaf *(Vernonia amygdalina)* in the diet which have a high palatability. While treatment B has only 2% of bitter leaf *(Vernonia amygdalina)* which gives it a better palatability than treatment C,D and E, which had 4%,6% and 8% levels of bitter leaf respectively . Another reason in difference in feed intake may be due to individual differences in adjusting to a particular test ingredient or diet. Payne (1990) and lynch *et al.,*1992 (12)had earlier reported that individual variation in sheep and other animals affect the rate of feed intake. The experiment shows that average daily gain(ADG)was higher in the treatments A which has zero levels of bitter leaf *(vernonia amygdalina)* followed by treatment B which had 2% of bitter leaf *(VERNONIA amygdalina).* Abil *et al.* (1992) who reported an ADG of 53.40g/day when they replaced cotton seed cake and maize with wheat brain in the diets of sheep.ADG of animals recorded in this experiment compares with other reports like Maigandi (2001) who obtained 68g/day when fed FSD as replacement for cowpea husk in diet of Uda sheep (Maigandi, 2001). One of the basic explanations for good ADG recorded in the treatment A was as a result of high

This is in contrast with the report that unconventional feeds can reduce cost of livestock production as observed by Maigandi and Tukur (2002) when they used FSD in the diet of growing sheep (Maigandi and Tukur, 2002).

Effect of bitter leaf on Yankasa ram semen quality characteristics

Table 5 shows the effect of bitter leaf on yankasa ram semen quality characteristics. Semen colour indicate that bitter leaf has a profound effect on milky appearances of semen while control group (0 % bitter leaf) appeared creamy in colour, Semen volume and pH shows non-significant (P>0.05) difference in this study, although numerical difference exist between group where treatment D and E has the highest numerical values of semen volume and pH. The pH measurement was used to measure the hydrogen ion concentrations produced by sperm cells metabolic activities after collection and/or during storage. Therefore, the impact of pH on survival of sperm cells cannot be overlooked because there are some sources of biochemical changes on sperm cells which include: Increased concentration of solute, dehydration and changes temperature. Despite non-significant difference in the semen pH values in this study are still fall within the normal ranges of ovine semen pH of 5.9-7.3 reported by

Table 5. Effect of bitter leaf on Yankasa rams semen quality characteristics

Parameters	A	B	C	D	E	SEM	LOS
Semen colour	Creamy	Milky	Milky	Milky	Milky	-	-
Semen volume (ml)	0.70^{a}	0.50^{a}	0.75^{a}	0.85^{a}	0.95^{a}	0.12	NS
Semen Ph	6.00^{a}	6.15^{a}	6.35^{a}	6.55^{a}	6.60^{a}	0.17	NS
Sperm cell concentration (10^{6}/ml)	210.00^{d}	233.50^{c}	246.50^{bc}	268.00^{b}	289.00^{a}	6.77	*
Motile Sperm cell (%)	67.00^{b}	66.00^{b}	72.00^{ab}	75.00^{a}	77.00^{a}	1.61	*
Morphologically normal spermatozoa (%)	81.50^{b}	81.50^{b}	84.50^{ab}	85.50^{a}	87.50^{a}	0.81	*
Scrotal circumference	24.05^{b}	25.25^{ab}	25.55^{a}	25.90^{a}	26.65^{a}	0.41	*

SEM= Standard error mean, LOS= Level of significance, NS= Not significant

Table 6. Effect of bitter leaf on Yankasa rams haematological profile

Parameters	A	B	C	D	E	SEM	LOS
RBC (10^{6}/µl)	3.86^{b}	3.90^{b}	3.96^{b}	4.10^{a}	4.15^{a}	0.06	*
PCV (%)	29.27^{c}	30.12^{b}	30.84^{b}	33.75^{a}	33.97^{a}	0.21	*
Hb (g/dl)	9.32^{c}	9.56^{bc}	10.02^{ab}	10.23^{a}	10.63^{a}	0.17	*
WBC (10^{3}/ul)	9.81^{a}	9.84^{a}	9.72^{a}	9.15^{b}	9.35^{ab}	0.15	*
Neutrophil (%)	56.00^{a}	49.00^{a}	45.00^{a}	45.00^{a}	32.85^{a}	0.25	NS
Lymphocyte (%)	47.75^{a}	48.35^{ab}	45.65^{ab}	44.25^{ab}	40.70^{b}	1.45	*
Monocyte (%)	5.10^{a}	5.00^{a}	4.15^{a}	4.21^{a}	4.25^{a}	0.02	NS

RBC= Red blood cell, PCV= Packed cell volume, Hb= Hemogolobin, WBC= White blood cell, SEM= Standard error mean, LOS= Level of significance, NS= Not significant

ptasynska, (2009). Therefore, the semen pH values obtained in this study could be said to be of very good quality based on the report of Frunza et al. (2008) who reported that pH that is higher than 8 denotes poor quality semen. The interesting finding here is linear increase of sperm cell concentration with increases graded level of bitter leaf which was statistically ($P<0.05$) higher in experimental rams in 8% bitter leaf group. Treatment E (8% graded level of bitter leaf) had the highest ($P<0.05$) percentage of motile sperm cell (77.00%) followed by 75.00%, 72.00%, 67.00% and 66.00% for treatment D, C, A and B respectively. There are linear increases ($P<0.05$) with increases graded level of bitter leaf on percentage morphological normal sperm cell of Yankasa rams in this study, where treatment E had the highest (87.50%) morphologically normal spermatozoa while treatment A and B had the same lowest value of 81.50% respectively. Significantly ($P<0.05$) higher scrotal circumference was observed in this study with increases graded level of bitter leaf in Yankasa rams. The scrotal circumference determination in this study was to use as one of the indices for predict sperm production, Camela et al. (2019) states that scrotal parameters like the scrotal length and scrotal circumference have been used as indices for sperm production where a decrease in both parameters could indicate testicular degeneration and its attendant poor semen quality (Barth, 2017). Therefore, the linear increases in scrotal circumference observed in this study clearly indicate absence of testicular degeneration or other form of pathological injuries.

Effect of bitter leaf on Yankasa rams haematological Profile

The mean red blood cell (RBC), packed cell volume (PCV), haemaglobin (Hb), white blood cell (WBC) and lymphocyte values obtained for rams in this study were significantly ($P<0.05$) different. However, these haematological values were within the normal ranges for healthy Yankasa sheep as reported by Babashani *et al.* (2015) who reported 26.20-35.25% PCV, 5.60-11.75(g/dl) Hb, 9.60-10.62 (10^{3}/ul) WBC, 42.00-50.05% Neutrophils, 47.90-60.08% lymphocytes and haematological values obtained in this study are slightly higher than haematological values reported by Ajayi *et al.* (2021) who reported ram has 4.10(10^{6}/ul) RBC, 27.58% PCV, 9.18 (g/dl) Hb and 7.88 (10^{3}/ul) WBC. A linear increases was observed in RBC, PCV and Hb with increases inclusion level of bitter leaf this clear indicated that bitter leaf may not have adverse effects on the bone marrow, kidney and haemoglobin metabolism rather it improves its functions since it has been reported that only substances which significantly affect the values of RBC and associated parameters would have effects on the bone marrow, kidney and haemoglobin metabolism (Longe and Momoh, 2015)

Conclusion

Thereare significant differences in the performance of the treatments. Treatment A and B with 0% and 2% performed better than other Treatments. This could be due to higher feed intake of the feed dueto the better palatability. Treatment A was recorded as the best feed gain ration, lowest feed/live weight gain. This was followed by treatment B. This short period could have been responsible for the poor performance with bitter leaf. It can be concluded that feeding the animals with bitter leaf over a longer period of time will improve bitter leaf use in modifying the rumen environment. It is therefore concluded that feeding Yankasa ram with vernonia amygladina has a profound effect in performance, semen quality characteristics and haematological profile.

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