Bioscience Research Journal
Vol. 32, No. 1, February 29, 2020
Printed in Nigeria

0795-8072/2020 $10.00 + 0.00

http://www.niseb.org.ng/journals

BRJ 32105

Effects of *Vernonia amygdalina* Leaf Meal on Male Hormone and Reproductive Organ Weight of *Thryonomys swinderianus*

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(Received August 11, 2019; Accepted December 10, 2019)

ABSTRACT: Grasscutter (*Thryonomys swinderianus*) feeds mainly on green plants especially those with succulent stems. *Vernonia amygdalina* the most prominent species and one of the tropical tribes of the family Asteraceae, it is so unique that every part of it has an economic importance and has been shown to be valuable nutritionally. The aim of the experiment was to determine the effect of administering *Vernonia amygdalina* leaf diets on testis weight and reproductive hormones of grasscutter (*Thryonomys swinderianus*). A total of eighteen male weaner grasscutters weighing between 281.33g and 304.21g were allotted to six treatments in a completely randomized design (CRD). The animals in treatment A (control) were given feed without *Vernonia amygdalina* (VA) leaf meal, B had 1g VA/kg feed, C had 2g VA/ kg feed, D had 3g VA/kg feed, E had 4g VA/kg feed and F had 5g VA/kg feed. Feeding was done for a period of seven (7) weeks and at the end of the experiment, two animals were selected per treatment after starving for about 10hours, sedated with diethylether soaked in cotton –wool and placed close to nostril to reduce handling stress before slaughtering by severing the carotid artery and the jugular vein. Blood was collected into plain bottles, centrifuged at 500rpm for 5minutes to obtain serum for hormonal tests. The hormones tested for were luteinizing hormone, follicle stimulating hormone, oetrogen and testosterone. The penis and testis were weighed and recorded after excision and removal of adhering fats. Data collected were subjected to analysis using SAS 2008 model and mean differences separated with Tukey studentized range test. The reproductive hormones and testis weights were significantly ($p<0.05$) affected by the *Vernonia amygdalina* inclusions. It showed that Treatment F had the highest luteinizing hormone while treatment A had the lowest. Treatment D had the highest follicle stimulating hormone while treatment E had the lowest. Treatment A had the highest oestrogen level while treatment B had the lowest. Treatment A had highest testosterone while treatment B had the lowest. It was observed that the addition of *Vernonia amygdalina* leaf meal as feed supplement of grasscutter had no adverse effect on their reproductive parameters as could be observed from the reproductive organ weight and reproductive hormone values of the animal.

Keywords: Organ, hormone, *Vernonia amygdalina*, *Thryonomys swinderianus*, Grasscutter

Introduction

The Grasscutter (*Thryonomys swinderianus*) is an important source of meat and is acknowledged to be the preferred meat virtually throughout Nigeria and the West African Sub-Region (Martin, 1985; Baptist and Mensah, 1986; Ntiamoa-Baidu, 1998). The meat is appreciated because of its culinary

properties with demand consistently outstripping supply (National Research Council, 1991). Its savannah habitat has expanded as a result of encroachment on forest lands by crop farmers. Studies by Baptist and Mensah (1986) and Schrage and Yewadan (1999) showed that most of hitherto setbacks to its captive breeding can be overcomed. The male has a paired testicles situated abdominally and there is no scrotum (Addo *et al.*, 2002). Appertaining to the accessory sex glands are a pair of seminal vesicles, the prostate gland, composed of three lobes and a pair of compact pea-sized Cowper's glands.

There is another pair of glands (glands coagulate) between the base of the seminal vesicles and prostate glands (Addo *et al*., 2002). The Grasscutter feeds mainly on green plants especially those with succulent stems such as Elephant grass (*Pennisetum puerperium*), Guinea grass (*Panicum maximum*), and foliage of certain leguminous plants, maize husk, tubers of yam, cassava and other root crops. Often the animal suffers from health hazards as a result of stress and helminth infestations resulting in suboptimal production and sometimes death.

The use of antibiotics and antihelminths often result in residual deposits in carcass hence the restriction on its usage. This resulted in the search for a biological alternative, with the use of *Vernonia amygdalina,* a tropical plant of the family *Asteraceae*. It grows predominantly in tropical Africa especially in Nigeria, Zimbabwe and South Africa and it is domesticated in parts of West Africa. It is popularly called bitter leaves because of its bitter taste and is used as vegetables or as flavour decoction soups. The bitter taste of *Vernonia amygdalina* is as a result of its anti-nutritional components such as alkaloids, saponins, glycosides and tannins. The roots and leaves decoction of *Vernonia amygdalina* are commonly used in ethno medicine to treat fevers, hiccups, kidney problems, liver problems, stomach discomfort among other several uses. It is used in the treatment of diarrhoea, dysentery hepatitis and cough and as a laxative and fertility rejuvenator. The leaves are also used as a treatment against nematodes and other intestinal worms in humans and chimpanzees (Momoh *et al*., 2010).

The reported activity of *Vernonia amygdalina* is attributable to the complex active secondary plant compounds that are pharmacologically active such as flavonoids, tannins, steroids and cardiac glygosides (Orabi and Shawky, 2014). It contains significant quantities of lipids (Ejoh *et al*., 2007), proteins with essential amino acids (Igile *et al*., 1994) as well as carbohydrates (Eleyinmi *et al*., 2008) and carotenoids, though not in large quantities (Udensi *et al*., 2002). It contains essential elements such as calcium, iron, protein, potassium, phosphorus, manganese, copper and cobalt (Bonsi *et al*., 1995). Despite the nutritional potential of *Vernonia amygdalina,* there is little information on its utilization in the diets of Grasscutter for enhancement of reproductive potential. The present study evaluates the effects of *Vernonia amygdalina* on reproductive hormone and organ weight of male grasscutters fed diet with graded levels of *Vernonia amygdalina* leaf meal.

Materials and Methods

Experimental site: The experiment was carried out at the grasscutter unit of the Biotechnology laboratory, Department of Animal Production, University of Ilorin, Nigeria.

Experimental diets: Fresh matured *Vernonia amygdalina* leaves were harvested from the university garden, rinsed, air dried for four days retaining the greenish coloration. It was ground into powder using a food blender and later incorporated into a formulated commercial grower mash at graded levels. It was then mixed evenly after which a liquid binder (local hot water starch) was added, mixed and forced through an extruder to form pellets, it was then sun dried for two days.

Vernonia amygdalina (VA) leaf powder was added at different levels per kilogram commercial feed (Grower Topfeed^{R}) i.e. treatment A – Control (VA not added), B – 1g of VA per kg of feed, C – 2g of VA per kg of feed, D – 3g of VA per kg of feed, E – 4g of VA per kg of feed and F – 5g of VA per kg of feed. Feed was made to last for four weeks.

Table 1: Gross Composition of the Experimental grower feed (Top feed^{R})

Ingredients	Composition (kg)
Maize	50.00
Soyabean meal	12.50
Fish meal	1.00
Wheat offal	11.45
Palm kernel cake	7.00
Corn bran	6.00
Groundnut cake	8.00
Bone meal	2.50
Oyster shell	1.00
Salt	0.30
Vitamin premix	0.25
Total	100
Calculated CP	18.44%
Calculated ME (Kcal/kg)	2810.67

Experimental animals and management: A total of eighteen (18) male weaner grasscutters were procured from the Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria at 12weeks of age. They were randomly allotted to six treatments with three grasscutters per treatment. The grasscutters were weighed to determine their initial body weight. The grasscutters were housed in wire-net cages with a floor space of 8.3m× 5.5m, L×B per replicate. They were offered experimental diets and water *ad libitum* for a period of seven weeks. They were given *Pennisetum purpureum*, *Panicum maximum* and *Citrullus lanatum* in the acclimatization period during which the test ingredient was gradually administered to the animals before the start of the experiment. At the end of the experiment, two animals were selected per treatment after starving for about 10hours, sedated with diethylether soaked in cotton –wool and placed close to nostril to reduce handling stress before slaughtering by severing the carotid artery and the jugular vein. Blood was collected into plain bottles, centrifuged at 500rpm for 5minutes for serum collection for hormonal tests. The hormones tested for were luteinizing hormone, follicle stimulating hormone, oestrogen and testosterone. The penis and testis were excised with adhering fats removed, weighed and recorded.

Statistical Analysis: All data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) 2008 Model. Means of the treatment were separated using Tukey studentized range test.

Results

The proximate composition of the treated diets showed non-significant differences ($p>0.05$) in the dry matter, ash, crude fibre and fat while the crude protein and carbohydrate was significantly affected by the inclusion of the *Vernonia amygdalina* leaf meal. There was an increase in crude protein with the addition of the leaf meal while there was a reduction in carbohydrate level. There were significant differences in the values obtained for luteinizing hormone (LH), follicle stimulating hormone (FSH), oestrogen and testosterone with the feeding of *Vernonia amygdalina* (Va) leaf meal diets. Most of the hormone increased significantly ($p<0.05$) with increase in Va leaf inclusion except oestrogen which reduced with additional increase in Va administration. The penis weight was not significantly ($p>0.05$)

affected by Va but testis weight was significantly (*p<0.05*) affected with a reduction in weight in treatments B and D while the others were not different from the control.

Table 2: Proximate Composition (%) of graded levels of *Vernonia amygdalina* Leaves treated diet

Parameters (%)	A	B	C	D	E	F	±SEM
Dry matter	90.80	90.60	90.90	91.00	90.80	90.60	0.08
Ash	4.20	4.13	4.33	4.00	4.13	4.20	0.07
Crude fibre	1.60	1.40	1.60	1.50	1.40	1.50	0.09
Crude protein	26.25^{c}	27.20^{c}	28.69^{bc}	29.94^{b}	30.62^{ab}	32.81^{a}	0.53
Fat	14.00	15.00	16.00	17.00	15.00	16.00	0.95
Carbohydrate	44.65^{a}	42.90^{ab}	40.30^{bc}	38.56^{ec}	39.68^{bcd}	36.09^{ed}	0.75

a,b,c,d,e - means within the same row having different superscripts differ significantly($p<0.05$) while those with the same superscripts are not significantly different($p>0.05$). SEM – Standard Error of Mean.

Table 3: Effects of graded levels of *Vernonia amygdalina* leaf meal diet on some reproductive hormones of *Thyronomys swinderianus*

Treatments/Parameter	A	B	C	D	E	F	±SEM (10^{-2})
LH (pg/ml)	2.40^{e}	8.30^{d}	2.60^{e}	11.65^{c}	12.06^{b}	12.45^{a}	7.19
FSH (pg/ml)	10.01^{c}	10.25^{b}	9.93^{d}	10.96^{a}	9.91^{e}	10.26^{b}	0.19
Oestrogen (ng/ml)	1.02^{a}	0.94^{d}	0.99^{b}	0.96^{c}	0.97^{c}	0.97^{c}	0.24
Testosterone (ng/ml)	14.42^{a}	11.96^{f}	13.76^{b}	12.49^{e}	13.33^{c}	12.88^{d}	136.09

a, b, c, d, e, f - means having different superscript along the same row are significantly different ($p<0.05$); SEM-standard error of mean; LH- luteinizing hormone, FSH- follicle stimulating hormone.

Table 4: Effects of graded levels of *Vernonia amygdalina* leaf meal diet on some reproductive organ weight of *Thyronomys swinderianus*

Treatments/ Parameter	A	B	C	D	E	F	±SEM(10^{-2})
Testis (g)	1.00^{a}	0.70^{b}	1.00^{a}	0.90^{b}	1.00^{a}	1.00^{a}	5.27
Penis (g)	0.80	3.70	0.77	0.70	0.70	0.90	124.66

a, b, c, d, e, f - means having different superscript along the same row are significantly different ($p<0.05$); SEM-standard error of mean.

Discussion

Luteinizing hormone helps in sexualization by stimulating leydig cell production of the testosterone and acts synergistically with Follicle Stimulating Hormone which is not needed in excess in the body (Addo *et al.*, 2002). The inclusion of *Vernonia amygdalina* in the diet of grasscutter does not really have effect on the luteinizing hormone which normalizes its production in the body of grasscutter, because it is normally high in woman at menopause stage of life. It also causes infertility. The treatment diet seems to stabilize testosterone secretion which was significantly lower than the control. This was in line with the report of Saalu *et al.* (2013) that treatment of animals with antioxidants improves steroidogenesis by enhancing the primary effect of leydig cells endocrine function along with increased circulatory testosterone production and stimulation of spermatogenesis (Prasad and Rajalakshmi, 1989).

In male grasscutter, Follicle Stimulating Hormone (FSH) acts on the sertoli cells of the testis to stimulate sperm production. The inclusion of *Vernonia amygdalina* does not increase the level of the FSH but seems to help in stabilizing its production. Reduction in FSH can also cause delayed puberty and limited sperm production (Addo *et al.*, 2002). It has been reported by many researchers that FSH and oestrogen stimulate fat deposition in animals which does not translate to concomitant increase in weight gain (Okukpe *et al*., 2017). The male hormones influences muscle deposition and have a direct effects on body weight (Okukpe *et al*., 2011)

High Oestrogen level in males contributes to fat lay-down, prostate dysfunction and heart disease as well as gynecomastia (enlarged breasts). As the testosterone is transformed into Oestrogen, the low levels of testosterone can cause many unpleasant symptoms including loss of muscle mass, fatigue, low libido and erectile dysfunction. So therefore the inclusion of *Vernonia amygdalina* does not have a negative effect on the grasscutter for these reasons because it normalizes the secretion of all these hormones and does not have significant effect on penis weight but slightly affect testis weight in some of the treatments, though negatively. This result corroborates the report of Saalu *et al.* (2013) that there was a significant effect on sperm qualities of rats with the administration of extracts from *Vernonia amygdalina*.

Although, it was a reported increase in sperm qualities which invariably must have had a positive effect on the testis weight. It has been reported that an increase in testis weight was directly proportional to positive effect on sperm output and qualities (Okukpe *et al*., 2016. This also supports the submissions of Longe *et al*. (1983) and Oyeyemi *et al*. (2008) that there was a drastic increase noticed in all the sperm parameters of the animals treated with bitter leaf. The increase could be due to the presence of antioxidants, flavonoids and vitamins in the bitter leaf which were found to maintain the morphology, survival and functions of the cells located in the reproductive organs and hormones (Igile *et al*., 1994; Adu and Wallace, 2001). The addition of *Vernonia amygdalina* leaf had no negative effect on the reproductive organ weight of the animals and maintained the reproductive hormone level of the animal. Also, the best administration level with respect to cumulative effect on all parameters investigated was observed at treatment F (5g Va/kg of feed).

Conclusion

The study showed that the addition of *Vernonia amygdalina* leaf meal supplement to grasscutter feed could help to stabilize reproductive function at this level actually normalize their growth, productivity and the well-being or health of the animals. It is recommended that *Vernonia amygdalina* leaf supplement can be used in improving reproductive hormone and organ weight that might enhance reproductive function of the grasscutter.

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