

STRESS MODULATING EFFECT OF CRUDE LEAF EXTRACT OF VERNONIA AMYGDALINA IN CAPTIVE GRASSCUTTER (*Thryonomys swinderianus*)

Kehinde Matthias OKUKPE^{1*}, Alexander Dayo ADEYEMO¹, Mango ADUA², Oluwaseun Oludare OMOTAYO¹, Opeyemi Akinkunmi YUSUF¹, Temitayo Abosede ADERIBIGBE¹, Ibiyemi Omolola OPOWOYE¹, Fisayo Oludele OGUNSOLA¹ and Bamidele OSHO³

¹Department of Animal Production, University of Ilorin, Ilorin, Nigeria; ²Department of Animal Science, Nasarawa State University, Keffi, Nigeria; ³Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria

Corresponding author: - okukpekehinde@yahoo.com

The effect of administering graded concentration levels of *Vernonia amygdalina* (bitter leaf) crude leaf powder to captive grasscutters on growth performance, haematology, serum biochemistry and faecal worm egg-counts were evaluated. Animals were randomly assigned to four treatments with three replicates per treatment, each having three animals. Treatment A (control) was given distilled water while treatments B, C and D were given albendazole (according to manufacturers prescription), 25ml and 50ml *V. amygdalina* per litre of water respectively. The highest weight gain was observed in animals in treatment D compared to those in other treatments. The *Vernonia amygdalina* solution showed significant ($p<0.05$) improvement on haemoglobin, mean cell haemoglobin (MCH) and mean cell haemoglobin concentration compared to the control. The final live weight, final weight gain and daily weight gain were also significantly ($p<0.05$) affected by the treatments. The *Vernonia amygdalina* solution had significant effect only on Sodium, Potassium, Calcium, Phosphates, carbonates, Glucose and creatinine among the biochemical parameters studied. *Vernonia amygdalina* was more effective in controlling helminthes in weaner grasscutters at either of the levels used than the synthetic drug administered (albendazole) and has no deleterious effect on the gut microflora profile of the stomach, small intestine and caecum of the grasscutter.

It was concluded that *Vernonia amygdalina* leaf powder could improve the growth

performance of weaner grasscutters at any of the levels used without deleterious effect, though there is need to test the efficacy of higher doses on the animal.

Keywords: *Vernonia amygdalina*, Antibiotics, Dewormer, Growth-promoters

Grasscutter or cane rat (*Thryonomys swinderianus*) farming is a budding smallholder venture in West Africa (Baptist and Mensah 1986; Kyle 1987; Adu *et al.* 1999) and the meat is considered a delicacy (National Research Council, 1991). The current trend in grasscutter farming is towards increased stock levels and intensification of production practices. Accompanying the intensification of livestock management practices is increased incidence of diseases which often cause stress on the animal. As promising as micro-livestock integration sounds, helminthosis in grasscutters will threaten the practicability of production and availability of meat to the generality of the populace. It is with this in mind that this study was designed to find out the gastrointestinal parasites of domesticated grasscutter so as to aid prompt diagnosis with a view to reducing mortality and morbidity and thus improve food security in the country. A major parasitic condition of livestock under intensive management with significant economic consequences is helminthosis (Sykes, 1994). These evidences show that the health of captive grasscutters can thus no longer be taken for granted.

The possibility of using plant bioactive compounds to control helminth parasites in the gastrointestinal tract, either as

phytotherapeutic or nutraceutical options, has been a growing research area. There have been several review papers dealing with positive and negative effects of these compounds on animal physiology, performance and health (Hoste *et al.*, 2005; Mueller-Harvey, 2006; Rochfort *et al.*, 2008; Hoste *et al.*, 2012). Many researchers have used ethnoveterinary information to choose possible plant candidates which could be tested as AH against internal parasites of humans and animals (Waller *et al.*, 1998; Githiori *et al.*, 2005, 2006).

In recent years, interest has developed in different countries in the collection and extended use of medicinal plant extract for an alternative production purposes (Okukpe *et al.*, 2012). The use of herbal products for medicinal benefits has played an important role in nearly every culture on earth.

Vernonia amygdalina is a widely used local plant in Nigeria for both therapeutic and nutritional purposes. *V. amygdalina* grows in a range of ecological zones in Africa and produces large mass of forage and is drought tolerant (Bonsi *et al.*, 1995). The bitter taste is due to anti-nutritional factors such as alkaloids, saponins, tannins and glycosides (Butter and Bailey, 1973). The macerated leaves of the plants are consumed as vegetables and condiments while the water extract serves as tonic for the prevention of certain illnesses. *V. amygdalina* has been reported for its use by wild chimpanzee for the treatment of parasite related diseases in Tanzania (Huffman and Seifu, 1989). Philipson *et al.*, (1993) reported the antiplasmodial effects of sesquiterpene and steroidal constituents of *V. amygdalina* and some were also effective against *plasmodium falciparum in vitro*. Uhegbu and Ogbuehi (2004) reported the antidiabetic effects of aqueous extract of leaves of *Vernonia amygdalina*. Arising from their biodiversity and perhaps the rich complement of phytochemicals and secondary metabolites, plants have from antiquity been used as sources of medicament against various ailments. The plant has acquired special relevance recently, having been proved in human medicine to possess potent antimalarial and

antihelminthic properties (Abosi and Raseroka, 2003) as well as antitumorigenic properties (Izevbigie *et al.*, 2004) with an amazing antiparasitic efficacy in zoopharmacognosy. Pharmacological studies have also shown that the leaf extract has both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus (Akah *et al.*, 2004). The active components of the plant have been shown to be mainly sesquiterpene lactones like vernodaline and vernoamygdaline and steroid glycosides like vernonioside B1 and vernoniol B1 (Kupcham *et al.*, 1969). The World Health Organisation has however emphasized the fact that safety should be the over-riding criteria in the selection of herbal medicine for use in healthcare. Previous studies show the potentials of *Vernonia amygdalina* in improving the performance and health benefits of broilers when administered in drinking water over a period of time. Also antibacterial property of *V. amygdalina* was proved to be beneficial in a few applications. For example, Babalola and Okoh (1996) proposed that this plant may be suitable for use in beer industry, since it was able to inhibit the growth of *Bacillus circulans*, *Aerococcus viridans*, *Clostridium perfringens* and *Micrococcus sp* bacteria but promote alcohol production of brewer's yeast while Kola (2007) claimed that the antibacterial activity of its ethanolic leaf extract against *C. sporogenes* was able to revert the blood, protein and bilirubin urinary to basal level and increase neutrophil and white blood cell count as well as packed cell volume (PCV) in blood, thereby reducing haemolysis caused by infection. The effects of various concentrations of aqueous extract of *V. amygdalina* leaves on some biochemical indices of liver function in albino Wistar rats showed that *V. amygdalina* has nutritional, clinical and veterinary relevance considering the diverse applications of the plant in almost all African countries (Ojiako and Nwanjo, 2006). The crude extracts of *V. amygdalina* produce significant changes in packed cell volume (PCV), red blood cell and hemoglobin concentration. This extract also

significantly reduces the faecal egg count of helminths in the dung of chimpanzee (Uhegbu and Ogbuehi, 2004). It could be a potential source of a new lead on anthelmintic agent (Adedapo *et al.*, 2007). In the treatment of helminthiasis, anthelmintic drugs are used irrationally and recently, anthelmintics use has been found to produce toxicity even in human beings. High costs of conventional anthelmintics have led to limited effective control of the parasites. In some cases, wide spread use of low quality anthelmintics has enhanced development of resistance. This study therefore, was designed to evaluate the effects of *V. amygdalina* on growth performance, and its antelmintic effect in captive grasscutters (*Thryonomys swinderianus*).

MATERIALS AND METHODS

Preparation of *Vernonia amygdalina* powder

The experiment was conducted at the small animal unit of the Biotechnology Laboratory of the Faculty of Agriculture, University of Ilorin, Nigeria. Fresh leaves of *Vernonia amygdalina* was obtained from the University garden, washed, air-dried for four days and ground into powder using a food blender (Starlite, Model No: SL-999 CHINA). The powdered bitter leaf was diluted in 1litre of water (1:2w/v) and was left for 24hours. The filtrate was obtained by sieving extract with muslin cloth. The filtrate was then kept in the refrigerator before it was finally used at different inclusion level.

Sourcing of grasscutter (*Thryonomys swinderianus*)

A total of 36 weaned grasscutter of mixed sexes were purchased from The Republic of Benin. The animals were housed in a cage system and allowed 3weeks to acclimatize during which they were given a commercial feed (Pelleted Grower Topfeed^R) twice daily and their water changed daily.

Experimental design

The animals were randomly assigned to four treatments A, B, C and D with three replicates having three animals each.

Treatments A (negative control) and B (positive control) administered distilled water and 2.5g albendazole per litre of distilled water respectively. Treatment C and D were given 25 and 50mls of the extract per litre of distilled water respectively. Commercial pelleted grower feed were offered throughout the experimental period. The experiment was conducted for 10weeks. Live weight was recorded weekly while feed intake was recorded daily in grams. At the end of the experiment, three animals per treatment group were euthanized and blood sample was collected for haematological and serum biochemical examination. Faecal sample were collected at the start of the experiment and weekly to determine the faecal worm egg count and the level of worm infestation to determine the efficacy of the treatments applied. Twelve animals were randomly selected and slaughtered at the 10th week, with the small intestine, stomach and caecum separated for analysis of the gut bacteria/fungi population, identification and differentiation.

The serum total protein was determined by the Biuret method using a commercial kit (Randox Laboratories Ltd, U.K), while albumin value was obtained by bromocresol green method. The globulin and albumin-globulin ratio were determined according to the method of Coles (1986). The serum creatinine and urea nitrogen were estimated by deproteinisation and Urease-Berhelot colorimetric methods, using a commercial kit (Randox Laboratories Ltd, U.K). Also the free cholesterol was determined by nonane extraction and enzymatic colorimetric methods, respectively using commercial kit (QuimicaClinicaApplicada, S.A), while the serum enzymes; Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits. Faecal samples collected were subjected to the faecal egg counts using the McMaster method. The faecal culture, floatation and sedimentation techniques of faecal examination were carried out as described by Khin-Khin (2007). Faecal egg counts were recorded.

Statistical Analysis

The experiment followed a completely randomized design (CRD). The data obtained were analyzed by the Generalized Linear Model procedures of SAS Version 9.2 software (SAS, 2008). Means differences were considered significant at $p < 0.05$ and separated by Duncan multiple range test (Duncan, 1955).

RESULTS

Feed intake

The result for feed intake varies significantly with the increase in *Vernonia amygdalina* extract inclusion (Table 2). The total feed intake of grasscutters in treatment B increase numerically in comparison with those in treatments A, C and D, though not significantly ($p > 0.05$).

Table 1: Composition of the pelleted grower feed

Ingredients	Composition(Kg)
Maize	50.00
Soyabean meal	12.50
Fish meal (72%)	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	2810.67

Live weight gain

The effect of bitterleaf crude extract on the weight gain of the grasscutters shows that there was significant ($p < 0.05$) difference on the weight gain of the grasscutter administered orally with bitterleaf plant extract at different inclusion level of bitterleaf in animals in Treatment D (50ml/L) compared to those in treatment A (Control) for the period of 10 weeks of the experiment. However grasscutter given 50ml (treatment D) of bitterleaf extract had the highest weight gain (436g) followed by

those in treatment B (Albendazole) with 366.67g weight gain.

Feed to gain ratio

Feed conversion ratio was calculated as feed intake consumed per unit of gain. The feed conversion ratio of animals in treatment A had the highest gain (11.93) numerically compared to other treatments, though this was not significant ($p > 0.05$).

Haematological variables

Table 3 shows the haematological parameters of experimental animals. The packed cell volume (PCV) was highest in grasscutters administered 25ml/L of bitterleaf extract (treatment C) with 42.33% as compared to A, B and D but this was not significantly ($p > 0.05$) affected.

The hemoglobin was highest in animals in Treatment D (14.57g/dL) followed by those in Treatment C (12.87g/dL). This was not significantly ($P > 0.05$) affected with those in treatment B but significantly different from those found in the control. MCH (Mean cell haemoglobin) was significantly influenced by the bitterleaf extract. The total MCH of grasscutters in D (32.43pg) was significantly ($p < 0.05$) different from those in A (21.43pg) but identical with ($p > 0.05$) to those of B and C. The range goes between 21.43 and 32.43pg. Grasscutters in treatments A, B and D were identical ($p > 0.05$) but significantly ($p < 0.05$) higher than those in treatment C for MCHC values. Grasscutters in D (50ml/l of bitterleaf level) had the highest (35.67%) and this compared with those in T3 (30.37%) with 25ml/l of extract.

White blood cells (WBC) and Platelet (PLT) values were not significantly different across the treatments. Eosinophil values were generally lower across the treatments.

Biochemical variables

The result of the biochemical indices for the grasscutter is shown in Table 4. The value for serum sodium ranged from (137mmol/l) in B to (147.67mmol/l) in C. The values for serum potassium was highest in A (6.8mmol/l) which was not significantly ($p > 0.05$) different with those in B and D but significantly ($p < 0.05$) higher than those in C. Chloride shows higher numerical value (84.33mmol/l) in C (25ml/l of extract) than other treatments. Total protein was observed

Table 2: Effects of Vernonia amygdalina crude extract on growth of weaner grasscutter

PARAMETERS	A	B	C	D	±SEM
Initial wt(g)	1398.67	1411.33	1440.33	1433.33	9.73
Final wt(g)	1662.33 ^b	1778 ^{ab}	1734.33 ^{ab}	1869.33 ^a	3.16
TFI(g)	3105.28	3481.55	3404.04	3454.49	1.13
ADFI(g/animal)	44.36	49.74	48.63	49.35	1.62
TWG(g/animal)	263.67 ^b	366.67 ^{ab}	324 ^{ab}	436 ^a	2.61
ADWG(g)	3.77 ^b	5.24 ^{ab}	4.63 ^{ab}	6.23 ^a	0.37
FCR	11.93	10.39	10.54	7.92	0.71

¹a, b, means in the same row with different superscript differ significantly (P<0.05); FCR=feed conversion ratio, ADWG=Average daily weight gain, TWG= total weight gain, ADFI= Average daily intake, TFI=Total feed intake.

Table 3: Effects of Vernonia amygdalina crude extract on haematology of weaner grasscutter

PARAMETERS	A	B	C	D	±SEM
PCV (%)	22.67	37.33	42.33	40.67	3.46
Hb (g/dL)	6.77 ^b	12.30 ^a	12.87 ^a	14.57 ^a	1.14
RBC (g/dL)	3.09	4.36	4.79	4.57	0.37
MCH(pg)	21.43 ^b	28.33 ^{ab}	26.87 ^{ab}	32.43 ^a	1.50
MCHC (%)	33.17 ^{ab}	32.97 ^{ab}	30.37 ^b	35.67 ^a	0.77
MCV (fL)	67.33	82.33	88.33	73.67	3.55
PLT	351.33	327.33	331.67	317.67	2.60
WBC (10 ⁹ /L)	11.98	11.18	11.76	13.19	0.40
Lymphocytes (%)	43.00	48.00	51.00	56.67	2.47
Neutrophils (%)	54.00	47.00	46.67	41.33	2.28
Eosinophils (%)	0±0	0±0	0±0	0±0	0.00

^{a, b} - means in the same row with different superscript differ significantly (P<0.05); PCV=Packed cell volume; Hb = Heamoglobin; MCH= Mean Corpuscular Heamoglobin; MCHC= Mean Corpuscular Heamoglobin Concentration; RBC=Red Blood Cell; WBC= White Blood Cell; MCV=Mean Corpuscular Volume.

to have the highest numerical value (143.33g/l) in treatment D than other treatments but was not significantly affected. Highest numerical value was observed in D (51.33mmol/l) for Globulin than other treatments but was not significantly (p>0.05)

affected. Glucose value (19.9) was found to be highest for C and there was significant different from other treatments. Creatinine value (42mmol/l) for D was found to be lowest followed by values obtained for value C which are significantly different from A

and B. Values for ALT, AST and ALP was lower for D but no significant difference across the treatments.

Hydrogencarbonate (HCO^{-3}) ranged from 17.67mmol to 31.33mmol/l for all the treatments. HCO^{-3} was significantly higher in C and D followed by treatment B and least in treatment A.

Urea was numerically low in treatment C and D followed by B but highest in the control (A). Glucose was significantly high in treatment C, followed by B and A respectively with the lowest value in D. Globulin was numerically high in treatment D and B followed by C but lowest in the control (A). Albumin was numerically high

in the control (A) and B followed by D but lowest in the treatment C. Creatinine was significantly low in the treatments C and D and highest in the control. All the liver enzymes tested (ALT, ALP and AST) were not significantly affected across the treatments

The initial faecal analysis indicated that the experimental animals were naturally infested with worms at varying levels, ranging from the heavily infested to the moderately infested and also those with low infestation levels. The result (Table 5) showed that animals in treatments A (-ve control), B (+ve control), C (25ml of bitter leaf extract / litre of water), and D (50ml

Table 4: Effects of Vernonia amygdalina crude extract on some serum biochemical indices of weaner grasscutter

PARAMETERS	A	B	C	D	±SEM
SODIUM (mmol/ L)	140 ^b	137 ^b	147.67 ^a	143.67 ^{ab}	1.53
POTASSIUM (mmol/ L)	6.80 ^a	6.30 ^a	4.80 ^b	6.40 ^a	0.27
CALCIUM (mmol/ L)	2.27 ^a	2.10 ^{ab}	1.97 ^b	2.27 ^a	0.05
PHOSPHATE (mmol/ L)	1.23 ^b	2.1 ^a	1.97 ^a	2.00 ^a	0.13
CHLORIDE (mmol/l)	67.33	72.67	84.33	82.00	2.94
HCO^{-3} (mmol/ L)	17.67 ^c	26.00 ^b	30.00 ^a	31.33 ^a	1.65
UREA (mmol/ L)	5.13	4.63	4.07	4.10	0.22
GLUCOSE (mmol/ L)	12.87 ^{bc}	14.53 ^b	19.90 ^a	12.00 ^c	0.98
TOTAL PROTEIN (g/L)	118.0	123.33	94.33	143.33	8.17
GLOBULIN	30.33	41.33	32.00	51.33	4.27
ALBUMIN (g/L)	78.67	74.00	58.00	70.00	4.06
AST (IU/L)	46.67	40.33	47.00	46.00	3.46
ALT (IU/L)	26.67±2.52	25.67±7.23	17±3.61	24.67	2.14
ALP (IU/L)	404.67	256.33	181.33	111.00	5.18
CREATININE (mmol/L)	78.00 ^a	56.67 ^b	43.00 ^c	42.00 ^c	4.62

^{a, b, c} - means in the same row with different superscript differ significantly (P<0.05); AST= aspartate aminotransferase, ALP= alkaline phosphatase, ALT= alanine aminotransferase.

Table 5. Effects of *Vernonia amygdalina* crude extract on *Trichuris* and *Strongyles* faecal egg counts in weaner grasscutter.

Helminths	Treatments	Before	After	±SEM	Significance	Remark
<i>Trichuris</i>	A	580.00	750.00	15.03	0.36	NS
	B	313.33	153.33	11.65	0.22	NS
	C	146.67	16.67	5.08	0.14	NS
	D	53.33	6.67	4.67	0.02	S
<i>Strongyles</i>	A	53.33	103.33	6.06	0.30	NS
	B	93.33	13.33	1.10	0.32	NS
	C	1280.00	66.67	19.01	0.23	NS
	D	0.00	0.00	0.00	0.00	NS

All values represent mean; Comparison for significance between the treatments was done using paired sample t-test.
 Keys: SEM = Standard Error of Mean; NS = Not significant; S = Significant

Table 6. Effects of *Vernonia amygdalina* crude extract on the bacteria and fungi count of grasscutter gut microflora.

Treatments	Bacteria	Fungi
A	<i>Klebsiella spp.</i> <i>Escherichia coli.</i> <i>Proteus spp.</i>	<i>Aspergillus spp.</i> <i>Yeast spp.</i>
B	<i>Klebsiella spp.</i> <i>Escherichia coli</i> <i>Proteus spp.</i> <i>Pseudomonas spp</i>	<i>Yeasts spp.</i> <i>Aspergillus spp.</i>
C	<i>Klebsiella spp.</i> <i>Escherichia coli</i> <i>Proteus spp</i>	<i>Yeast spp.</i> <i>Aspergillus spp.</i>
D	<i>Klebsiella spp.</i> <i>Escherichia coli</i> <i>Proteus spp</i>	<i>Aspergillus spp.</i> <i>Yeast spp.</i>

Table 7. Effects of *Vernonia amygdalina* crude extract on the microbial counts of grasscutters stomach, small intestine and caecum.

TREATMENTS	STOMACH	SMALL INTESTINE	CAECUM
A	142 (38.67%)	107 (29.17%)	118 (32.16%)
B	52 (45.61%)	47 (41.23%)	15 (13.16%)
C	95 (37.40%)	62 (24.41%)	97 (38.19%)
D	63 (49.61%)	51 (40.16%)	13 (10.24%)

of bitter leaf extract / litre of water) had 1900, 1220, 4280, and 260 egg per gram (epg) respectively.

Eggs of two helminth species *Trichuris trichiura* (*Trichuris*) and *Strongyloides stercoralis* (*Strongyles*) were the predominant eggs discovered in the animals. It could therefore be deduced that treatment A had a moderately high level of worm infestation, treatment B, a moderate

level of worm infestation, treatment C, a high level of worm infestation, and treatment D, a low level of worm infestation. Administration of synthetic drug i.e. albendazole for B and aqueous extract for C and D respectively produced a significant reduction in the worm burden of the grasscutters at the 9th week of the experiment, while the worm count burden increased for treatment A.

Treatment A had a 23.9% increase in the total worm count of *Trichuris spp.*, while it increased the worm count of *Strongyle spp* by 75%; B had a reduced the total worm count of *Trichuris spp.* (51.06%) and *Strongyle spp.* (85.71%); C had a total worm count of *Trichuris spp* of 88.63% and *Strongyle spp* was 94.79%; while D reduced the total worm count of *trichuris spp* by 82.5%, with that of *Strongyle spp* undetermined. This trend showed that aqueous extract obtained from *Vernonia amygdalina* was more efficacious than synthetic drug in deworming grasscutter, which establishes the fact that *Vernonia amygdalina* leaves has certain chemicals that are of high antihelmintic attributes. Three mortalities were recorded, with A, C and D recording one mortality each, this was however not directly related to the level of worm infestation in the three treatments. Table 6 and 7 shows effects of the treatments on bacteria and fungi count and the organ microflora of grasscutters respectively. The result showed that *Klebsiella spp*, *Escherichia coli*, *Proteus spp* were the bacteria present in the stomach, small intestine and caecum of the grasscutters except that of animals in treatment B where *pseudomonas spp.* was discovered. The fungi profile of the animals in the four treatments was similar, with *Aspergillus spp.* and *Yeast spp.* being the fungi present. The microbial count (Table 7) varied among the four treatments, grasscutters administered aqueous extract of *Vernonia amygdalina* had higher microbial counts when compared to grasscutters administered treatments A and B respectively, with those found in treatment D being too numerous to count, and those in treatment B having the least microbial count.

DISCUSSION

The increase in weight gain with low FCR in this study corresponds with that of Durunna *et al.* (2011) who observed an increased weight gain with low feed conversion ratio in broilers given 75mls/litre of bitter leaf extract. It also supports the findings of Tangka (2003) that reported improved

growth performance of animals fed bitter leaf. The increase in the weight correlates with the lower FCR observed in the treated group. The lower the FCR the higher it is for the animals to convert feed consumed to meat. Olobatoke and Oloniruha (2009) reported that inclusion of bitter leaf powder in cockerels feed significantly improved FCR. Although this finding is in contrast with that of Mohammed and Zakariyau (2012) who observed that inclusion of bitter leaf as a feed additive did not significantly improve weight gain and FCR in broilers.

Blood represent a means of assessing clinical and nutritional health status of animals and the haematological variables most commonly used include packed cell volume (PCV), red blood cell (RBC), Hb, MCH and MCHC. The significant increase in haemoglobin, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration reflect the ability of the extract as well as the positive control to improve oxygenation of body cells and growth of animal compared to the negative control. Other haematology parameters studied where not significantly affected though within standard reported by other researchers (Osho *et al.*, 2014) and fall within normal range reported by Baynet *et al.* (2008) for captive grasscutters. The ability of the extract to numerically alter the distribution and occurrence of lymphocyte, neutrophil and basophil reflect capability of the extract acting as an immuno-stimulant. Leucocyte counts have been reported to increase from any form of stress, exercise, feeding, age, and wide variety of other conditions (Okukpe *et al.*, 2012b). Basophils and eosinophils play a role in regulating allergic and inflammatory processes and host defense responses against parasitic infections, like helminthiasis and ectoparasitic infestation (Butterworth, 1999). The basophilic cells have been reported to be rare in the blood (Burke *et al.*, 2003). Aregheore *et al.* (2009) suggested that the presence of some phytochemicals in bitter leaf extract induces the animal to respond as if it has infection.

Serum biochemical indices are used to determine the level of heart attack, liver damage and to evaluate protein quality and amino acid requirements in animals as reported by Harper and Dugaiczky (1983). In the serum biochemical indices (Table 7), Sodium (Na) level obtained was significantly high (147.67mmol/L) for treatment C compared to other treatments and are within the normal range of values for reared grasscutter reported by Baynet *et al.*, (2008). The serum K level was higher in all the treatment studied except for treatment C which showed lower K (4.8mmol/l) value but still within the normal range. The electrolytes are known to regulate osmotic pressure, maintain membrane potentials and acid base balance and transmit nerves impulses. Sodium and Potassium deficiency affect the tubes of kidney resulting in inability to concentrate urine (Burke *et al.*, 2003). The values for chloride show significant variation but are all within close range. This was found to be highest in treatment C while others fall below the normal range. The urea level in the study shows that treatment A had (5.13mmol/l) highest value. The high level of serum urea might be attributed to excessive tissues protein catabolism associated with protein deficiency. Urea concentration which characterizes renal function was higher in grasscutters in the control group but are not significantly ($p>0.05$) affected. Serum glucose was highest and differs significantly in treatment C compared to other treatments. When glucose is lower than the normal range it is an indication of hypoglycemia while higher levels are indication of hyperglycemia (Uhegbu and Ogbuehi, 2004). The glucose level for all treatment groups fall within normal ranged but the numerical reduction observed in treatment D was in agreement with the finding of Osinubi (2006) and Owen and Amakiri (2011) who reported that *Vernonia amygdalina* has hypoglycemic effect. Akah and Okafor (1992) reported that bitter leaf possess both hypoglycemic properties. The hypoglycaemic properties observed in the *Vernonia amygdalina* extracts suggest synergistic interaction of phytochemicals and

antioxidants. According to Abraham *et al.* (2011), the phytochemical composition of *Vernonia* extracts show presence of polyphenols which suppress post-prandial hyperglycaemia and glucose transport across the small intestine. Saponin is known to delay glucose transfer from stomach to small intestine there by controlling the rate of absorption (Pandey and Rizvi, 2009). Epicatechin has a restorative effect on the pancreatic beta cells against alloxan damage by producing more insulin to quicken the uptake of glucose into the cells. Also flavonoid as an antioxidant reduces/reverse the effects of diabetes on the rats' pancreatic cells. These observations agrees with the report of Akah *et al.* (2004) that some phytochemicals in *Vernonia amygdalina* contribute to the blood glucose lowering by reversing pancreatic damage caused by induction of diabetes and increase insulin production.

No significant difference was observed from values for ALP, AST and ALT across the treatment groups. Their uses for clinical purpose are primarily for the diagnosis of liver diseases (Burke *et al.*, 2003). The effect of the extract on some biochemical indices of liver function for this research is in line with the findings of (Ojiako and Nwanjo, 2006) who reported that the effect of various concentrations of aqueous extract of *V. amygdalina* leaves on some biochemical indices of liver function in albino Wister rats showed that *V. amygdalina* has nutritional, clinical and veterinary relevance considering diverse applications of the plant in almost all African countries. In this investigation the level of these marker enzymes (ALT, AST and ALP) were reduced though there was no significant difference, thus improving renal and hepatic functions. This observation is consistent with earlier report on hepatoprotective potentials of leaf extracts of *V. amygdalina* in mice (Iwalokun *et al.*, 2006).

Anthelmintic drugs such as albendazole, are used to treat parasitic infection in livestock. The regular use of these drugs and sometimes improper use make the issue of resistance inevitable. According to (Shalaby, 2013) it has been observed that

frequent use of the same group of anthelmintic, use of anthelmintics in suboptimal doses, prophylactic mass treatment of domestic animals, and frequent and continuous use of a single drug have contributed to the widespread of anthelmintic resistance in helminthes.

The different doses of aqueous extract of *Vernonia amygdalina* showed a non significant relationship ($P < 0.05$) between the faecal worm egg count of *Trichuris spp.* in treatment C before and after the experiment, however, a numerical decrease was observed in the faecal worm egg count. A significant relationship ($P < 0.05$) existed between the faecal worm egg count of *Trichuris spp.* in treatment D before and after the experiment, a numerical decrease was observed in the faecal worm egg count of grasscutters administered this treatment. Treatment A however showed a non-significant relationship ($P < 0.05$) between the faecal egg count of *Trichuris spp.* before and after the experiment with numerical increase in the faecal worm egg count. Treatment B (albendazole) also showed a non significant relationship ($P < 0.05$) between the faecal worm egg count of *Trichuris spp.* before and after the experiment, however, a numerical decrease was observed in the faecal worm egg count.

For the *Strongyle spp.*, the faecal worm egg count for treatments A, B, and C showed a non significant relationship ($P < 0.05$), although numerical increase in the faecal worm egg count was observed in treatment A, numerical decrease in the faecal worm egg count was however observed in B and C. The effect of treatment D in controlling *Strongyloides* class of worm was however undetermined as it was not detected in the faecal samples analysed before and after the experiment.

The results obtained from the study showed that the plant *V. amygdalina* has antihelminthic effects in grasscutters, with satisfactory efficacy at 25ml – 50ml/ litre of water. Studies on the antihelminthic effects of *Vernonia amygdalina* carried out by other researchers with different outcomes include (Alawa *et al.*, 2003,

2010) and (Danquah *et al.*, 2012). Alawa *et al.*, (2010) reported about 59.5% activity of *V. amygdalina* against helminths in cattle while (Danquah *et al.*, 2012) showed *V. amygdalina* antihelminthic activity against earthworm. Farombi and Owoeye (2011) also cited that the shrub has been used in the treatment of various helminth diseases. Also studies by (Nalule *et al.*, 2013; Oyeduntan and Emmanuel, 2015), have shown the efficacy of *Vernonia amygdalina* in controlling antihelminths.

It was however observed that literature on the effect of this plant on gastrointestinal helminthes in grasscutter is limited, hence this report on the effect of *Vernonia amygdalina* as an antihelminthic agent in grasscutters. The implication of this is that the plant could be a good alternative to the commonly used synthetic anthelmintics (Waller *et al.*, 2004). The plant is also readily available and it is found as a shrub in most rural backyard gardens in developing countries to which Nigeria belongs (Lawal-Adebawale, 2012).

The microflora content of specified organs i.e the stomach, small intestine and caecum showed that the microbial profile of the gastrointestinal tract of the grasscutters were similar, comprising of bacteria (*Klebsiella spp.*, *Escherichia coli*, *Proteus spp.*, and *Pseudomonas spp.*) and fungi (*Aspergillus spp.* and *Yeast spp.*). This implies that administration of aqueous *Vernonia amygdalina* extract to weaner grasscutters in treatments C and D respectively did not alter the microbial profile of the grasscutter as it had similar profile with the grasscutters in treatment A (control) supporting the work of Namso and Okaka (1998) and Opara and Fagbe (2008) that helminthes are location specific in grasscutter. Also, the microbial profile of grasscutters in treatment B was similar to the others; save for the presence of *Pseudomonas spp.*, which suggest the ability of synthetic anthelmintics to eliminate/substitute microbes and the intolerable nature of the synthetic drug. The microbial profile also shows that there were no pathogenic or parasitic microbes in the gastrointestinal tract that could be of

concern to the animal or human's health. The fungi profile however did not show any variation amongst the four treatments. The microbial profile of the grasscutters was similar to works carried out by Mpoame (1994), Wedge *et al.* (2000), and Yaro *et al.* (2011) on the normal gastrointestinal microflora. The administration of aqueous extracts of *Vernonia amygdalina* did not have any negative effect on the grasscutters but assist in stabilizing the level of microbes in the gastrointestinal tract. It was also observed that microbes were higher in the stomach, this suggests that more microbes were concentrated in the stomach because of the intense digestive activities that goes on in the stomach, and the microbial count of the small intestine and the caecum were similar.

CONCLUSION

In conclusion, the inclusion of crude bitter leaf extract in the water of grasscutter had no adverse effect on the performance traits as could be observed from the haematology and serum biochemistry of the animals. Furthermore, inclusion of the extract cause an increase in body weight with a reduction in serum glucose and worm burden as the extract level increases. The best administration level with respect to cumulative effect on all parameters investigated was 50 mL L⁻¹ of drinking water. This implies that the administration of this natural herb at this level of inclusion could be important in improving the productivity and health status of grasscutters. *Vernonia amygdalina* could therefore be a better replacement for synthetic drugs such as albendazole. The inclusion level of treatment D (50mL L⁻¹ of drinking water) gave the best performance in terms of weight gain, reduction in blood sugar and urea. This could be a good indicator in its use in the control of helminthes and heart diseases. Further studies should be carried out with doses above 50ml/L of drinking water.

REFERENCES

1. Abosi, A.O., Raseroka, B.H. (2003). *In vivo* antimalarial activity of *Vernonia amygdalina*. *Br. Journal of Biomedical Sciences* 60: 89-91.
2. Abraham, G., Odunze, U. O., Mahammed, A. (2011). Phytochemical and antimicrobial studies on *Vernonia blumeoides* hookfil (asteraceae) ethanol extract. *Nigerian Journal of Pharmaceutical Sciences* 10(1): 50-56.
3. Adedapo, A.A., Otesile, T., Soetan, K.O. (2007). Assessment of anthelmintic efficacy of an aqueous crude extract of *Vernonia amygdalina*. *Pharm. Biol.* 45: 564-568.
4. Adu, E.K., Alhassan, W.S., Nelson, F.S. (1999). Smallholder farming of the greater cane rat, *Thryonomys swinderianus*, Temminck, in southern Ghana: A baseline survey of management practices. *Tropical Animal Health and Production* 31: 223-232.
5. Akah, P., Njoku, O., Nwanguma, A., Akunyili, D (2004). Effects of aqueous leaf extracts of *Vernonia amygdalina* on blood glucose and triglyceride level of alloxan-induced diabetic rats (*Rattus rattus*). *Ani. Res. Intl.* 1(2): 90-94.
6. Akah, P.A. and Okafor, C.L. (1992). Blood sugar lowering effect of *Vernonia amygdalina* Del. in an experimental rabbit model. *Phytother. Res.* 6: 171-173.
7. Alawa C.B.I., Adamu A.M., Gefu J.O., Ajanusi O.J., Abdu P.A. and Chiezey N.P. (2010). *In vivo* efficacy of *Vernonia amygdalina* (compositae) against natural helminth infection in Bunaji (*Bos indicus*) calves. *Pak. Vet. J.* 30(4): 215-218.
8. Arhoghro, E.M., Ekpo, K.E., Anosike, E.O., Ibeh, G.O. (2009). Effect of aqueous extract of bitter leaf (*Vernonia amygdalina* Del.) on

- carbon tetrachloride induced liver damage in albino wistar rats. *Eur. J. Sci. Res.* 26: 122-130.
9. Babalola, G.O. and Okoh, A.I. (1996). Assessment of *Vernonia amygdalina* as a substitute for hop brewing. *Tech Q. Master Brew Assoc. Am.* 33: 44-46.
 10. Baptist, R. and Mensah G.A. (1986). The cane rat - farm animal of the future? *World Animal Review* 60: 2-6.
 11. Bonsi, M.L.K., Osuji, P.O., Tuah, A.K., Umunna, M.N. (1995). *Vernonia amygdalina* as supplement of teff straw (*Eragrostis tef*) fed to Ethiopian Menz sheep. *Agroforestry Syst.* 31: 229-244.
 12. Burke, D.G., Chilibeck P.D., Parise, G., Candow, D.G., Mahoney, D., and Tarnopolsky, M. (2003). "Effect of creatine and weight training on muscle creatine and performance in Vegetarians" *Medicine and Science in sports and exercise* 35(11): 1946-55
 13. Butter, G.W. and Bailey, R.W. (1973). *Chemistry and Biochemistry of herbaye*, Vol. 1 Accident Press London and New York.
 14. Butterworth, A.E. (1999). Cell mediated damage to helminthes. *Advanced Parasitology* 23: 143
 15. Byanet, O., Adamu, S., Salami, S. O., Obadiah, H. I. (2008). Haematological and plasma biochemical parameters of the young grasscutter (*Thyonomys swinderianus*) reared in northern Nigeria. *Journal of Cell and Animal Biology* 2 (10): 177-181
 16. Coles, E.H. (1986). *Veterinary Clinical Pathology*. 4th Edn., W.B. Saunders Co., Philadelphia, London, ISBN: 978-0721618289, Pages: 486.
 17. Danquah C.A., Koffuor G.A., Annan K. and Ketor E.C. (2012). The Anthelmintic Activity of *Vernonia amygdalina* (Asteraceae) and *Alstonia boonei* De Wild (Apocynaceae). *J. Med. Biom. Sci.* 1(1): 21-27.
 18. Dellmann H, Brown EM (1987). Blood and bone marrow. In: *Textbook of Veterinary Histology*, 3rd Ed Lea Febiger, Philadelphia. pp. 71-86.
 19. Duncan, D.B. (1995). Multiple range and multiple tests *Biometrics* 11: 1-42.
 20. Durunna, C.S., Chiaka, I.I., Ebenebe, O.E., Udedibie, A.B., Uchegbu, M.C., Durunna, O.N. (2011). Value of bitter leaf (*Vernonia amygdalina*) meal as feed ingredient in the diet of finisher. *Int. J. Agric. Rural Dev.* 14: 722-726.
 21. Farombi E.O. and Owoeye O. (2011). Antioxidative and Chemopreventive Properties of *Vernonia amygdalina* and *Garcinia biflavonoid*. *International Journal of Environ. Res. Publ. Health.* 8: 2533-2555.
 22. Githiori, J.B., Athanasiadou, S., Thamsborg, S.M. (2006). Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet. Parasitol.* 139: 308–320.
 23. Githiori, J.B., Höglund, J., Waller, P. (2005). Ethnoveterinary plant preparations as livestock dewormers: practices, popular beliefs, pitfalls and prospects for the future. *Anim. Health Res. Rev.* 6: 91–103.
 24. Harper M.E. and Dugaiczky A. (1983). "Linkage of the evolutionarily-related serum albumin and alpha-fetoprotein genes within q11-22 of human chromosome 4". *Am. J. Hum. Genet.* 35 (4): 565–72.
 25. Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C.A.

- (2012). Direct and indirect effects of bioactive tannin rich tropical and temperate legumes against nematode infections. *Vet. Parasitol.* 186: 18–27.
26. Hoste, H., Torres-Acosta, J.F., Paolini, V., Aguilar-Caballero, A., Etter, E., Lefrileux, Y., Chartier, C., Broqua, C. (2005). Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Res.* 60: 141–151.
 27. Huffman M.A and Seifu M. (1989). Observations on the illness and composition of a possibly medicinal plant *Vernonia amygdalina* (Del.) by a wild chimpanzee in the Mohale Mountains National Park, Tanzania. *Primates* 30: 51–63.
 28. Iwalokun, B.I., Alibi-Sofunde J.A., Odunala T., Magbagbeola O.A. and Akinwande, A.I.(2006). Hepatoprotective and anti-oxidant activities of *Vernonia amygdalina* on acetaminophen-induced hepatic damage in mice. *J. Med. Food* 9(4): 526–530.
 29. Khin-Khin, L. (2007). Prevalence of cryptosporidia, giardia, and other gastrointestinal parasites of dairy calves in Mandalay, Myanmar [MPh Thesis], Chiang Mai University, Freie Universität Berlin,.
 30. Kola, O.M. (2007). Anti-inflammatory activity of ethanolic leaf extract from *Vernonia amygdalina* on the immune system of swiss albino rats dosed with *Clostridium sporogenes* (NCIB 532). *Res. J. Med. Sci.* 1: 127–131
 31. Kupchan, S.M., Hemingway, R.J., Karim, A., Wermer, D. (1969). Tumour inhibitors XLVII vernodalin and vernomygdin, two new cytotoxic sesquiterpene lactones from *Vernonia amygdalina* Del. *J. Organic Chem.* 34: 3908–3911.
 32. Kyle, R. (1987). Rodents under the carving knife. *New Scientist* 114: 58–62. 25.
 33. Lawal-Adebawale, O. A. (2012). “Dynamics of ruminant livestock management in the context of the Nigerian agricultural system,” In: *Livestock Production*. K. Javed, Ed., InTech, Rijeka, Croatia,
 34. Mohammed, A.A. and Zakariyau A.S. (2012). Bitter leaf (*Vernonia amygdalina*) as a feed additive in broiler diets. *Res. J. Anim. Sci.* 6: 38–41.
 35. Mpoame, M. (1994). Gastro intestinal helminthes of the cane rat *Thryonomys swinderianus*, in Cameroon. *Trop. Anim. Health Prod.* 26: 239–240.
 36. Mueller-Harvey, I. (2006). Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric.* 86, 2010–2037.
 37. Nalule, S. A. Mbaria, J. M. and Kimenju, J. N. (2013). “In vitro anthelmintic potential of *Vernonia amygdalina* and *Secamone Africana* on gastrointestinal nematodes” *Agriculture and Biology Journal of North America* 4(1): 54–66.
 38. Namso, M.E. and Okaka, C.E. (1998). A survey of naturally occurring parasites of cane rats. *Niger. J. Parasitol.* 28: 28–29.
 39. National Research Council (NRC) (1991). *Microlivestock: Little-Known small animals with a promising economic future.* (Vietmeyer Noel, ed). National Academy Press, Washington D. C. pp: 147–155.
 40. Ojiako O.A and Nwanjo H.U. (2006). Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *Afr. J. Biotech.* 5: 1648–1651.
 41. Okukpe, K.M., Adeloye, A.A., Belewu, M.A., Alli, O.I., Adeyina, O.A. and Annongu, A.A. (2012):

- Investigation of phytochemical potential of some selected tropical plants. *Research Journal of Medicinal Plant* 6(6): 425 – 432.
42. Okukpe, K.M., Adeloye, A.A., Abayomi, F.D., Alli, O.I., Belewu, M.A. and Adeyina, O.A. (2012b): Physiological effects of *Myristica fragrans* extract on West African Dwarf Bucks. *Medicinal Plants* 4(1): 1-5.
 43. Olobatoke, R.Y. and Oloniruha J.A. (2009). Haematological assessment of bitter leaf (*Vernonia amygdalina*) efficiency in reducing infections in cockerels. Proceedings of the World Congress on Medicinal and Aromatic Plants, November 9-14, 2008, Cape Town, South Africa, pp: 472-473.
 44. Opara, M.N. and Fagbemi, B.O. (2008). Observations on the Gastrointestinal Helminth parasites of the wild Grasscutter (*Thryonomys swinderianus*, Temminck) in Imo State, Nigeria. *Int. J. Trop. Agric. Food Syst.* 2: 105-110
 45. Osho, I.B., Akindahunsi, A., Igbasan, F.A., Adekunle, D.J. (2014). Effect of orally administered bitter leaf (*Vernonia amygdalina*) extract on the growth performance and haematological parameters of broiler chicken. *J. Vet. Med. Anim. Health*, 6: 251-256
 46. Osinubi A.A. (2006). Effects of *Vernonia amygdalina* and chlorpropamide on blood glucose. *Med. J. Islam World Acad. Sci.*, 16: 115-119.
 47. Owen, A.J. and Amakiri A.O. (2011). Serological and haematological profile of broiler finishers fed graded levels of bitter leaf (*Vernonia amygdalina*) meal. *Adv. Agric. Biotechnol.* 1: 77-81.
 48. Oyeduntan A. A. and Emmanuel C. U. (2015). Effectiveness Evaluation of Levamisole, Albendazole, Ivermectin, and *Vernonia amygdalina* in West African Dwarf Goats. *Journal of Parasitology Research* 15: 1-5.
 49. Pandey, K.B. and Rizvi, S.I. (2009). Plant phytochemicals as dietary antioxidants in human health and disease. *Oxidative Medicine and cellular Longevity* 2(5): 270-278.
 50. Phillipson, J.D., Wright, C.W., Kirby, G.C., Warhurst, D.C. (1993). Phytochemistry of some plants used in traditional medicine for the treatment of protozoal diseases. Abstracts, Int Symposium of the Phytochem. Soc. of Europe; University of Lausanne, Switzerland, p. L3.
 51. Rochfort, S., Parker, A.J., Dunshea, F.R. (2008). Plant bioactives for ruminant health and productivity. *Phytochemistry* 69: 299–322.
 52. Rwangabo P.C, Laekman G, Claeys M, Tote J, Pieters L, Vanden- Berghe D, Herman A, Vlietinck A.J. (1986). Phytochemical and pharmacological investigation of the active fraction from the flowers of *Vernonia amygdalina* Del. *Planta Medica*. 5: 431.
 53. SAS (2008). SAS Institute Inc. 2008. ASA/STAT users Guide version 9.2 for windows. Carry, North Carolina, USA. SAS Institute Inc.
 54. Shalaby, H. A. (2013). “Anthelmintics resistance; how to overcome it?” *Iranian Journal of Parasitology* 8(1): 18–32.
 55. Sykes A.R. (1994). Parasitism and production in farm animals. *Animal Production*, 59: 155-172.
 56. Tangka, J.K., (2003). Analysis of the thermal energy requirements for the extraction of leaf protein concentrate from some green plants. *Biosyst. Eng.* 86: 473-479.
 57. Uhuegbu F.O. and Ogbuehi K.J. (2004). Effect of aqueous extract (crude) of leaves of *Vernonia*

- amygdalina (Del.) on blood glucose, serum albumin and cholesterol levels in diabetic albino rats. *Global J. Pure Appl. Sci.*, 10: 189-194.
58. Wall M.E., Wani M.C., Manikumar G., Abraham P., Taylor H., Hughes T.J., Warner J., MacGivney R. (1998). Plant antimutagenic agents, flavonoids. *J. Nat. Prod.* 51: 1084-1089.
59. Waller P.J., Coles G.C., Bauer C., Borgsteede F.H.M., Geerts S., Klei T.R., and Taylor M.A. (2004). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) methods for the detection of anthelmintic resistance in nematodes of Veterinary importance. *Veterinary Parasitology* 44: 35-44.
60. Wedge, D.E., Galindo, J.C.G., Macias, F.A. (2000). Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. *Phytochem.* 53: 747-757.
61. Yaro M., Addo P., and Barnes A.R. (2011). Effect of Sex and Age on Normal Microflora population dynamics of Gastrointestinal Tract in Grasscutters (*Thryonomys swinderianus*). *J. Phys. Pharm. Adv.* 2(4): 184-194.