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Organ weight, serum biochemistry and gut microbial counts of *Thryonomys swinderianus* fed diet supplemented with *Vernonia amygdalina* leaf meal

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Abstract

The study investigated the effect of Vernonia amygdalina (VA) leaf supplementation on organ weight and gut microbial count of Thryonomys swinderianus. Thirty-six weaner grasscutters of mixed sexes were allotted randomly to six treatments (A, B, C, D, E, and F) in a completely randomized design for a nutritional trial of 8 weeks. At the end of the experiment, caecum, kidney, liver, lungs, intestine and the heart were excised to determine their weights and the intestinal/caecal contents and scrapings were obtained to determine the fungi and bacteria population. Data collected were analyzed using one-way Analysis of Variance (ANOVA). The VA supplementation had significant effect ($p<0.05$) on relative organs with a range of 0.47 – 0.60%, 0.40 – 0.62%, 1.20 – 2.83%, 0.52 - 0.58%, 5.53 – 12.91%, 714.00 – 960.00g, 494.00 – 781.00g and 479.00 – 747.00g for lungs, heart, liver, kidney, intestine, live weight, eviscerated and carcass weight of the grasscutter respectively, with a smaller relative weight of the kidney from 3g/kg VA supplementation upwards. The serum biochemical parameters were also significantly affected by VA supplementation. There was a decrease in alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase but were within the normal range required for rodents. Total protein and Albumin were significantly improved by the supplement while total bilirubin was variable with a significant decrease prior to 4gVA supplementation. Microbial populations in the intestine and ceacum were reduced to varying extent by the supplement. It could be concluded that VA supplementation reduced intestinal and caecal microbes and increased the live and carcass weights but decreased relative weights of selected organs in the grasscutter.

Keywords: Grasscutter, *Vernonia amygdalina*, Organ weight, Microbial counts

Introduction

The requirement for adequate animal protein has brought attention to the rearing of wild animals (Akinloye, 2005) as wildlife has great potentials for meat production especially in Africa (Asbey and Addo, 2000). Grasscutter is the choice among the wildlife and it accounts for the greater proportion of bush meat sold in most parts of West Africa (NRC, 1991). Nevertheless, its collection from the wild is attended by destruction of the environment bushfires set by hunters (Ntiamo-Baidu, 1998). To conserve wild population of the grasscutter and preserve its habitat, attempts are being made in the sub-region to domesticate the animal (Addo, 2002) for its economic exploitation. Studies showed that the animals could be kept in captivity with increased litter size (Ntiamo-Baidu, 1998). However, regular and adequate feeding of the animal has been the major factor that determines its productivity and health under domestication (Asibey and Addo, 2000; Okukpe *et al*., 2016). 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 high mortality. *Vernonia amygdalina popularly called bitter leave* grows in the humid tropics of Africa and is among several herbs used by traditional healers in Western Nigeria to treat a number of bacterial infections. Moreover, *Vernonia amygdalina* has been used in traditional medicine as an antihelminth, antimalaria, and a laxative herb (Jisaka *et al*., 1992; Akinpelu, 1999; Abosi and Raseroka, 2003). A wide array of phytochemicals which include tannin, oxalate, phytate, saponin, flavonoid, luteolin and glucoside has been shown to be present in VA (Harborne, 1973; Ohigashi, 1994; Igile *et al.,* 1995; Tona *et al*., 2004; Ejoh *et al.,* 2007; Eleyinmi *et al*., 2008; Njan *et al*., 2008). These phytochemicals are believed to be responsible for the plethora of bio-activities possessed by the plant. In addition,VA contains 18% protein, 8.5% fiber (dry matter), and a good composition of macroelements (Partanen and Mroz, 1999; Ezekwe and Obidoa, 2001). Due to its nutritional qualities, it could serve as source of invaluable nutrients especially crude protein and fiber in grasscutter nutrition. It can also improve the overall health of the animal through supply of phytochemicals. Thus this study investigated the weight gain, organ weight, serum biochemistry and gut microbial population of grasscutter fed VA supplemented diets.

Materials and methods

The experiment was carried out at the grasscutter unit of the biotechnology laboratory, Department of Animal Production, University of Ilorin, Nigeria. Fresh *Vernonia amygdalina* leaves were harvested from the university garden, rinsed, air dried and ground into powder using a food blender. The powdered leaves were incorporated into a formulated commercial grower mash (Grower Topfeed[R]) at graded levels of 0 - 5g /kg making a total of 6 experimental diets.

Table 1: Composition of the commercial grower feed (Top feed [R])

Ingredients	Composition (kg)
Maize	50.00
Soybean 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	100.00
Calculated Crude protein (%)	18.44
Calculated Metabolizable energy (Kcal/kg)	2810.67

Thirty-six weaner grasscutters of mixed sexes were procured from the Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria at 12weeks of age. Six animals, housed in wire-net cages with a floor space of 8.3m× 5.5m were randomly allotted to each of the six treatments with two replicate of three grasscutters per replicate. The grasscutters were weighed at the beginning of the experiment to determine their initial body weight. They were fed experimental diets alongside *Pennisetum purpureum*, *Panicum maximum* and *Citrullus lanatum while* water was provided *ad-libitum* for a period of 8 weeks. Records of daily feed intake and weekly weight gain were taken. At the end of the experiment, four animals were selected per treatment. The animals were starved for 10hours and slaughtered by severing the carotid artery and the jugular vein. Blood was collected into plain and Ethylene diamine tetracetic acid (EDTA) coated bottles for haematology and biochemical tests, respectively. The animals were then skinned and eviscerated to harvest internal and external organs which were weighed using electric weight balance. In addition, different parts of the intestine and the caecum were collected for microbial evaluation. The relative organ weight was calculated using the equation below:

$$\text{Relative organ weight (\%)} = \frac{\text{Weight of the organ}}{\text{Live Weight of the animal}} \times 100$$

Intestinal and caecal bacterial and fungal count was determined by counting colony forming units incubated in Nutrient Agar and Potato Dextrose agar and Malt Extract agar respectively.

Statistical analysis

Data collected were analyzed using one-way Analysis of Variance (ANOVA). Means were separated using Tukey's Studentized Range (HSD) Test at 95% significant level (SAS, 2008).

Results

The effects of *Vernonia amygdalina* (VA) supplementation on organ weights of grasscutter is presented in Table 1. VA supplementation at higher dosage (D - F) decreased weight of kidney, heart, lungs and stomach. Live, eviscerated and carcass weights were similar to control at high dosage though they were reduced at low dosage (B and C). Intestinal weight increased as level of supplementation increased with the exception of the animals fed treatments E and F. VA supplementation had significant (P<0.05) but indefinite effect on serum parameters of treated grasscutters (Table 2). Alkaline phosphate (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), albumin (Alb) and bilirubin (Bil), especially at high dosage, were significantly (P<0.05) reduced in treated animals compared to the control. However, total protein (TP) was significantly higher (P<0.05) than the control at higher supplementation (D - F). Similar to serum biochemistry, VA supplementation significantly (P<0.05) reduced bacteria and fungi population in the intestine and caecum of experimental grasscutters (Table 3). However, the pattern among treatments was indefinite as some levels of supplementation had similar effect on microbial population as that of the control.

Table 1: Effects of *Vernonia amygdalina* leaf powder on rdative organ, live and carcass weight of *Thryonomys swinderianus*

Parameters	A (0gVA/kg feed)	B (1gVA/kg feed)	C (2gVA/kg feed)	D (3gVA/kg feed	E (4gVA/kg feed)	F (5gVA/kg feed)	± SEM
Kidney (%)	0.66^{a}	0.60^{abc}	0.64^{ab}	0.58^{bcd}	0.54^{cd}	0.52^{d}	0.02
Heart (%)	0.44^{bc}	0.42^{c}	0.60^{ab}	0.44^{c}	0.40^{c}	0.62^{a}	0.03
Liver (%)	1.20^{b}	1.20^{b}	2.20^{a}	2.09^{b}	2.83^{a}	1.96^{b}	0.06
Lungs (%)	0.59^{ab}	0.60^{a}	0.60^{a}	0.51^{bc}	0.58^{ab}	0.47^{c}	0.02
Stomach (%)	4.05^{a}	2.86^{bc}	2.30^{cd}	2.10^{d}	1.89^{d}	3.35^{b}	0.13
Intestine (%)	5.53^{e}	7.93^{c}	11.21^{b}	12.91^{a}	6.59^{d}	7.37^{c}	0.16
Live Weight (g)	911.00^{a}	714.00^{c}	786.60^{b}	914.00^{a}	960.00^{a}	930.00^{a}	12.77
Eviscerated Weight (g)	708.00^{c}	494.00^{f}	643.00^{d}	621.00^{e}	781.00^{a}	736.00^{b}	3.84
Carcass Weight (g)	676.00^{b}	479.00^{d}	592.00^{c}	595.00^{c}	747.00^{a}	665.00^{b}	3.07

a,b,c,d,e - means on the same row with different superscript are significantly different (p<0.05); VA – *Vernonia amygdalina* ; SEM – Standard Error of Mean

Table 2: Effect of *Vernonia amygdalina* leaf meal on the serum parameters of *Thryonomys swinderianus*

Treatments/ Parameters	A (0gVA/kg feed)	B (1gVA/kg feed)	C (2gVA/kg feed)	D (3gVA/kg feed)	E (4gVA/kg feed)	F (5gVA/kg feed)	±SEM
ALP, U/L	34.96^{a}	7.60^{bc}	8.36^{bc}	3.04^{c}	4.18^{bc}	9.50^{b}	0.69
ALT, U/L	128.71^{a}	108.13^{b}	105.25^{b}	84.10^{c}	84.29^{c}	90.44^{c}	1.69
AST, U/L	377.89^{a}	342.11^{b}	279.47^{c}	239.47^{d}	265.79^{c}	278.42^{c}	2.81
Bil direct,µmol/L	2.46^{ab}	2.21^{b}	2.22^{b}	2.13^{b}	2.84^{a}	2.33^{b}	0.05
Bil total, µmol/L	5.40^{ab}	4.83^{b}	4.86^{b}	4.66^{b}	6.33^{a}	5.11^{ab}	0.17
TP, $mgdl^{-1}$	8.96^{b}	8.47^{bc}	8.03^{c}	10.16^{a}	10.51^{a}	9.18^{b}	0.09
Alb, U/L	4.02^{bc}	4.67^{a}	4.35^{ab}	3.56^{cd}	3.40^{d}	2.44^{e}	0.06

a,b,c,d,e - means on the same row with different superscript are significantly different ($p < 0.05$); ALP – alkaline phosphate, AST - aspartate amino transferase , ALT - alanine amino transferase, TP - total protein, Alb - albumin, Bil - bilirubin , VA – *Vernonia amygdalina* , SEM – standard error of mean.

Table 3: The effect of *Vernonia amygdalina* leaf meal on intestinal and caecal microbial count of *Thryonomys swinderianus*

Parameters	A (0gVA/ kg feed)	B (1gVA/ kg feed)	C (2gVA/ kg feed)	D (3gVA/ kg feed)	E (4gVA/ kg feed)	F (5gVA/ kg feed)	± SEM
TBC LI (cfu)	$7.0\times10^{3\,d}$	$1.2\times10^{4\,cd}$	$4.5\times10^{4\,bc}$	$5.5\times10^{5\,a}$	$1.7\times10^{4\,cd}$	$7.0\times10^{4\,b}$	0.42
TFC LI (cfu)	$4.7\times10^{3\,a}$	$1.2\times10^{2\,c}$	$3.3\times10^{2\,c}$	$1.3\times10^{3\,d}$	$1.1\times10^{2\,c}$	$0.0\times10^{0\,c}$	38.64
TBC SI (cfu)	$4.2\times10^{5\,a}$	$1.5\times10^{4\,c}$	3.3×10^{b}	$3.6\times10^{5\,b}$	$9.9\times10^{3\,c}$	$4.3\times10^{4\,c}$	0.62
TFC SI (cfu)	1.1×10^{3c}	$8.0\times10^{2\,c}$	$5,7\times10^{a}$	$2.3\times10^{3\,b}$	$0.0\times10^{0\,c}$	$0.0\times10^{0\,c}$	76.25
TBC S (cfu)	1.1×10^{4b}	$1.0\times10^{3\,e}$	$6.9\times10^{3\,c}$	$1.6\times10^{3\,a}$	$3.0\times10^{3\,d}$	$1.3\times10^{2\,c}$	114.53
TFC S (cfu)	$3.6\times10^{3\,b}$	$4.0\times10^{2\,d}$	$5.7\times10^{3\,a}$	$2.5\times10^{3\,c}$	$0.0\times10^{0\,e}$	$4.9\times10^{2\,d}$	62.68
TBC C ($cfu\times10^{5}$)	3.30^{a}	3.10^{a}	1.60^{b}	1.60^{b}	1.00^{c}	1.50^{c}	11626. 92
TFC C ($cfu\times10^{2}$)	57.03^{a}	14.00^{bc}	7.40^{c}	20.00^{b}	3.00^{c}	3.00^{c}	241.66

a,b,c,d,e - means on the same row with different superscript are significantly different ($p<0.05$); TBC-Total bacteria count, TFC -Total fung al count, LI-Large Intestine, SI -Small Intestine, S - stomach, C-Caecum, VA – *Vernonia amygdalina* , SEM- Standard Error of Mean, cfu- colony form unit.

Discussion

The inclusion of *Vernonia amygdalina* leaf powder in the diet of grasscutter had a significant effect ($p<0.05$) on the kidney, heart, lungs, stomach and intestine weight. This supports the work of Agboola (2000) that improved feeding in terms of nutrition and health enhances weight gain of the grasscutter. Conversely, the increase in organ weight in this study was similar to the findings of Jisaka *et al.* (1992) and Addo (2002) on improved growth performance in animals fed bitter leaf. Serum parameters are indicative of the incidence and severity of heart and liver disease and protein quality in animals. Hence the lower the levels of these markers in the serum allude to the better quality of these organs and the overall good health of the treated animals in comparison to the control. The findings from this study corroborate the observations of Babalola *et al.* (2001) and Iwalokun *et al. (*2006) that various concentrations of aqueous extract of *VA* leaves improved the liver function of albino Wistar rats. This might be due to the stress modulating effect of *VA as alluded to by* Okukpe *et al*. (2016). Findings of this study showed that the microbial profile of the gastrointestinal tract of grasscutters comprises bacteria and fungi and confirms the conclusion of Tona *et al*. (2004) and Alo *et al*. (2012). VA supplementation significantly reduced intestinal and caecal bacteria and fungi population in the treated animals in agreement with Jisaka *et al*. (1992) and Abosi and Raseroka (2003) who reported that VA due to its antimicrobial effects reduced fungi population. Currently, different additives including plant extracts such as VA leaf powder or acidifiers have been used as alternative to AGPs. The potency of plants extract is due to their wide antimicrobial effect which has been demonstrated in different *in- vitro* and *in - vivo* studies (Smith-Palmer *et al*., 1998; Tona *et al*., 2004). Generally, the effect of VA on the intestinal and caecal microbes might be similar to dietary acidifier which can selectively terminate pathogenic microbes (Van Immerseel *et al*., 2006) while enhancing the growth of beneficial ones in the caecum (Partanen, 2001) thereby enhancing protein digestion and performance (Partanen and Mroz, 1999; Njan *et al*., 2008). Fungi grow and develop in the presence of starch (Alabi *et al*., 2005); the development of a good mold fungus produces the enzyme cellulose spurred in large quantities that can be used to remodel and lower crude fibre (Adams, 1993). Thrope and Beal (2001), supported by Li *et al.* (2008) reported that cellulase is an enzyme that can decide β-glycosides bond (1,4) on cellulose and Binns (2013) concluded that high population of fungus can increase the crude protein content of the substrate as the mold is a source of single cell protein. This may be part of the reasons for the increased carcass weights with increase in VA inclusion.

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 microbial profile of the grasscutters was similar to the reports of Akinpelu (1999), Tona *et al*. (2004) and Foo *et al.* (2014) on the presence of normal gastrointestinal microbial population. *VA supplementation* did not have any negative effect on the grasscutters but assisted in stabilizing the level of microbes in the gastrointestinal tract.

Conclusion

The inclusion of the VA leaf powder caused a general decrease in organ weight and in bacterial and fungal load. Furthermore, the best inclusion level with respect to cumulative effect on all parameters investigated was observed at 2g/kg feed. Therefore, supplementation of VA leaf at 2g/kg can be recommended in grasscutter diet for improved productivity and health status of grasscutters.

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