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Growth performance, nutrient digestibility and haematological response of Captive grasscutters (*Thryonomys swinderianus*) to dietary bitter leaf (*Vernonia amygdalina*) meal

Sanni[a*], K.M., Okukpe[a], K.M., Biobaku[b], K.T., Ewetola[c], I.A., Jolayemi[a], O.G. and Adeyemi [a]. K.D.

[a] Department of Animal Production, Faculty of Agriculture, University of Ilorin, PMB 1515, Ilorin, Kwara State, Nigeria. [b] Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ilorin. [c] Department of Animal Nutrition and Forage Science, Michael Okpara University of Agriculture, Umudike.

***Corresponding Author:** modupetayo07@gmail.com; **Phone Number:** +2348034833438;*

Abstract

A feeding trial was conducted to determine the effects of dietary bitter leaf meal (BLM) supplementation on the growth performance, nutrient digestibility and haematological indices of weaner grasscutters. Forty grasscutters of mixed sexes weighing between 391.5g and 400.5g were assigned to four dietary treatments consisting of ten grasscutters per treatment in a completely randomized design. BLM was incorporated into the experimental diet at varying levels of 0, 20, 40, and 60g/kg representing T_1 (control), T_2, T_3 and T_4 respectively. Data collected were analyzed using one-way Analysis of Variance (ANOVA) Mean differences were significant at $p<0.05$. Growth performance of weaner grasscutters was significantly ($P<0.05$) improved by BLM at 40g/kg as indicated by highest final live weight, average daily weight gain and better feed conversion ratio (1037.9g, 9.11g/day and 4.09) respectively ($P<0.05$), while those on 20g/kg had the highest average daily diet feed intake (40.17g). Animals on 20g/kg BLM had significantly ($P<0.05$) improved nutrient digestibility. All haematological parameters were significantly ($P<0.05$) affected by dietary BLM except granulocytes and platelets. Packed cell volume, haemoglobin concentration, and red blood cells of the grasscutters increased across the dietary treatments as graded levels of BLM increased. Total leukocytes count significantly reduced with lowest value (7.53×10^9 /L) at 60g/kg while the lymphocytes were significantly ($P<0.05$) higher than the control. It was concluded that feeding grasscutters diets supplemented with BLM at 40g/kg improved their growth and health status as could be observed from their growth and haematological parameters, hence BLM can be used a natural growth promoting feed additives in grasscutter production.

***Key Words**: Grasscutter; Growth; Haematology; Bitter leaf meal; Supplement.*

Introduction

According to Swallah (2018), wildlife domestication is recommended to be a possible solution to address the increasing demand for meat in Nigeria in order to meet the recommended minimum daily animal protein requirements of 35-40g.

The grasscutter (*Thryonomys swinderianus*) is a member of the mammalian order and a monogastric herbivorous rodent (Swallah, 2018). The major factor limiting grasscutter production efficiency in captivity is nutrition (El-Hassan *et al.,* 2009). Poor feeding coupled with associated stress of captivity leads to slow growth rates, mortality especially in young ones, delayed sexual maturity and low productivity (Ansah *et al.*, 2012). To enjoy good quality protein supply from grasscutter production, efforts are required not only to multiply grasscutters but also to improve productivity through adequate nutrition (Opara, 2010).

The addition of artificial supplements and antibiotics to livestock feeds in a bid to increase productivity have been discouraged due to their residual effects in livestock products posing a potential threat to human health (Mohammed and Zakariya'u, 2012). Due to their abundance in the natural environment and lack of side effects, medicinal plants are recently being explored to be added to livestock feeds as supplements to replace synthetic antibiotics (Ndelekwute *et al.*, 2015). In the attempt to mitigate the problem of poor growth rate in captivity and synthetic antibiotics resistance, medicinal and nutritional plants like bitter leaf should be exploited as feed supplement in grasscutter diets to improve their productivity.

Bitter leaf (*Vernonia amygdalina*), is a small tree that reaches an average height of three meters and it is utilized as an edible vegetable in Nigeria and other sub regions of Africa (Igbakin, 2009). It belongs to the family *Asteraceae*, and can be used as food and also for medicinal purposes (Adaramoye *et al.*, 2008). Because of its numerous bitter components, it is usually referred to as bitter leaf (Ekpo *et al.*, 2007).

Some principal chemical constituents found in bitter leaf are steroid glycoside and vernonioside B1 (Tadesse *et al.*, 1993). Other phytochemicals present in bitter leaf include saponins, flavonoids, alkaloids and tannins which are believed to be responsible for the plethora of bioactivities possessed by the plant (Okeke *et al.*, 2015). Sanni, (2024) reported that proximate composition of air-dried bitter leaf contained 87.1% dry matter, 33.47% crude protein, 9.2% ash, 1.65% crude fibre, 4.92% ether extract and 37.86% carbohydrate. The activities of bitter leaf were studied in some animals such as chickens, rats and grasscutters by various authors, where it is used in the form of extract or as a leaf meal (Okukpe *et al.*, 2016; Ndelekwute *et al.*, 2017). Okukpe *et al.* (2019), observed some varied effects on the performance of weaner grasscutters fed air dried bitter leaf meal from 0 - 0.5% inclusion level and higher inclusion rates were recommended for further studies. Most research works done on the nutritional physiology of grasscutters have focused on the nutrient requirements of grasscutters, little or no work has been done on ameliorating stress and other factors that can affect adequate nutrient utilization (Sanni, 2024). Inadequate nutrient utilization can reduce the absorption of vital nutrients needed for growth in grasscutters (Sanni, 2024). The previous works done on the use of bitter leaf in grasscutters have shown that the leaf has great potentials in improving nutrient utilization and ameliorating stress but the effect is not significant at the low levels used, hence the need to examine the efficacy of the higher inclusion levels.

Therefore, the objective of this study was to determine the growth performance, nutrient digestibility and haematological response of weaner grasscutters to graded levels of bitter leaf meal (BLM) supplement.

Materials and methods

Experimental Site

The feeding trial was conducted at the biological garden of the Federal Polytechnic Offa, Kwara State**.** The polytechnic lies within the Savannah ecozone ecological zone on Latitude 8° 7'57.91"N and Longitude 4° 42' 32.32" E. Temperature ranges from 22.78 – 34.12^{o}C and relative humidity is 61.02%. The area has a tropical wet and dry or savannah climate and the average annual rainfall is 100.39 mm (Google Earth, 2022).

Source and Processing of the Test Ingredient

Fresh bitter leaves properly identified by Department of Plant Biology's Herbarium with Voucher Number UILH/001/972 were used for the study. Matured bitter leaves were harvested from Adewole Estate in Ilorin. The leaves were harvested for a duration of 12 months from January to December. They were cleaned and air dried under shade for 4 - 7days. The air-dried leaves was ground into powder using a food blender (Starlite, Model No: SL-999 CHINA) and stored in an airtight container at room temperature. The samples for proximate analysis and feeding were taken from the stored leaves. The powdered leaves were incorporated into a formulated diet as shown in (Table 1) at graded levels of 0, 20, 40, and

60g/kg making a total of 4 experimental diets (T_1, T_2, T_3, and T_4). The diets were formulated to meet the nutrient requirements for optimum production of weaner grasscutters as recommended by Adeniji, (2009) and Kusi *et al.*, (2012).

Experimental Animals and Management

Forty (40) mixed sex 8 weeks old grasscutters purchased from Fagnon farms, Republic of Benin were used for this study. The animals were housed in a wooden one-step cage with cells partitioned with wood, and floors made of wire mesh. The cages were placed inside a closed pen made of concrete. The diameter of each cell was 40cm×80cm×50cm. Thirty -two (32) female grasscutters and eight (8) males in ratio 4:1 were assigned randomly into four (4) treatment groups. The groups consist of eight females and two males per treatment with five replicates per group and two grasscutters per replicate in a completely randomized design (CRD), after two weeks of acclimatization. Experimental diets (concentrates) and water were provided *ad libitum* throughout the experimental period. Elephant grass (*Pennisetum purpureum*) was offered to the animals once in the evening to meet their forages requirement as monogastric herbivore. The elephant grass contains 29.3% dry matter, 9.25% crude protein, 9.28% ash, 1.17% ether extract, 26% crude fibre, and 34.30% nitrogen free extract. Strict hygiene was maintained in the animal house by cleaning the animal cages, feeding troughs and drinkers daily, while the pens were cleaned and disinfected weekly with soap, disinfectant and water. Ten weeks were allotted to the trial and it was conducted with the approval of Ethical Review Committee of University of Ilorin, with the **Protocol Identification Code** and **UERC Approval Number** UERC/AGR/157 and UERC/ASN/ 2019/1636 respectively.

Analytical methods

Proximate analysis of the experimental diets (Table 1) was carried out at Central Research and Diagnostic Laboratory Tanke, Ilorin. The samples were replicated in three places; proximate analysis which includes the determination of the dry matter, crude protein, crude fibre, ash, and ether extract was determined according to the analytical methods of AOAC, (2005).

Table1: Gross composition of basal experimental diets

Ingredients /Nutrients (%)	T_1(0g)	T_2(20g)	T_3(40g)	T_4 (60g)
Maize	50.00	50.00	50.00	50.00
Corn bran	6.00	6.00	6.00	6.00
Wheat offal	11.45	11.45	11.45	11.45
Fish meal	1.00	1.00	1.00	1.00
Soybean meal	12.50	12.50	12.50	12.50
Palm kernel cake	7.00	7.00	7.00	7.00
Groundnut cake	8.00	8.00	8.00	8.00
Bone meal	2.50	2.50	2.50	2.50
Oyster shell	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30
Vitamin premix	0.25	0.25	0.25	0.25
BLM(g)	0.00	20.00	40.00	60.00
Proximate composition				
Dry matter	94.23	94.20	93.86	94.14
Ash	5.04	5.07	4.56	5.12
Ether extract	5.51	5.42	5.48	5.45
Crude fibre	5.43	4.96	4.94	4.67
Crude protein	18.12	18.78	19.31	21.50
NFE	60.13	59.97	59.58	57.40

NFE= Nitrogen Free Extract, Bitter Leaf Meal =BLM. T_1; 0g BLM/kg = control; T_2; 20g BLM/kg; T_3; 40g BLM/kg and T_4; 60g BLM/kg.

Data Collection

Growth Performance

The initial body weight of each grasscutter was recorded in grams before the commencement of the feeding trial while the final body weights were taken at the end of 10 weeks. Daily feed intakes of each grasscutter were derived by taking note of the amount of feed served and the quantity of leftovers with the aid of a Mettler weighing scale (Model No: SNR K67861 Switzerland).

The feed conversion ratio (FCR) was calculated at the end of the feeding trial using the formula:

$$FCR = \frac{feed\ intake\ (g)}{Body\ weight\ gain\ (g)}$$

Nutrient Digestibility

Nutrient digestibility trial was done at the 9th week of the experiment. Four grasscutters (2males and 2 females) were randomly selected from each treatment and allocated in to separate experimental cages for easy collection of faeces. The feacal samples were collected daily and bulked for a period of 7days. Feed intake and the quantity of faeces voided were determined daily in grams. The faecal samples per treatment were mixed together, oven dried and ground, from which a representative sample of 50g each was taken to the laboratory for proximate analysis. Proximate composition of the feeds, elephant grass and faecal samples were evaluated using the method of AOAC, (2005). The nutrient digestibility was determined using the formula below.

$$Digestibility\ (\%) = \frac{Nutrient\ intake\ (g) - Nutrient\ in\ faeces\ (g)}{Nutrient\ intake\ (g)} \times 100$$

Blood Collection for Haematology

At the 10th week of the experiment, four animals consisting of 2 males and 2 females were randomly selected per treatment. The animals were euthanized and 2mL blood samples was collected using a 5mL sterilized disposable syringes with 21 gauges and 1.5 inches-long needles for each animal into ethylene diamine tetra acetic acid (EDTA) coated plastic tubes for haematology. Haematological variables determined are; White blood cell (WBC), Red blood cell (RBC), Haemoglobin (Hb), Packed cell volume (PCV), Platelets (PLT), Neutrophils and Lymphocytes were determined using an Axiom® Automated Haematology Analyzer 7886A3 (Axiom Med U.K.). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) were estimated from the values obtained.

Statistical Analysis

Data obtained for proximate analysis were analyzed using descriptive statistics. All the data obtained during the feeding trial were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) using SAS (2008). Mean differences were considered significant at p<0.05. Significant means among variables were separated using Duncan Multiple Range Test of the same software

The model was:

$y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

where: y_{ij} = observation, μ= Overall mean, α_i ; = Effects due to population and ε_{ij}= random error

Results and Discussion

Results

Growth Performance of grasscutters fed dietary bitter leaf meal

The growth performance of weaner grasscutters fed dietary bitter leaf meal is presented in Table 2. Dietary BLM had significant (P<0.05) influence on virtually all the growth parameters investigated in this study. The concentrate and total feed intakes values obtained in grasscutters fed 20g BLM/kg recorded the significantly (P<0.05) highest value when compared with other dietary treatments. However, higher dietary BLM inclusion levels significantly (P<0.05) decreased the concentrate intake, while the forage dry matter intake increases significantly as the BLM inclusion increases. The final live weight and weight gain of the grasscutters varied significantly (P<0.05) across the dietary treatment in which those fed 40g/kg BLM

(1037.90g and 637.40g) recorded the highest value when compared with other dietary treatments. However, the weight gain of animals on 60g BLM/kg was lowest though comparable with the control. The concentrate feed conversion ratio (CFCR) and total feed conversion ratio (FCR) were significantly lower in dietary BLM groups compared with those on control, with those on 40g/kg BLM having the lowest value of 4.09 and 6.33 respectively.

Table 2: Growth performance of grasscutters fed dietary bitter leaf meal

PARAMETERS	T_1	T_2	T_3	T_4	SEM	P values
Initial wt(g)	395.50	391.50	400.50	393.00	9.98	0.93
Final wt(g)	781.90^{c}	871.90^{b}	1037.90^{a}	767.10^{c}	21.9	0.00
TWG(g)	387.40^{bc}	480.40^{b}	637.40^{a}	376.10^{c}	24.5	0.00
ADWG(g)	5.53^{bc}	6.86^{b}	9.11^{a}	5.37^{c}	0.35	0.00
ADFDMI(g)	19.38^{c}	19.60^{c}	20.15^{b}	20.85^{a}	0.08	0.00
ADCI(g)	38.96^{a}	40.17^{a}	36.74^{b}	34.53^{c}	0.47	0.00
TFI(g/)	58.34^{b}	59.76^{a}	56.89^{c}	55.38^{d}	0.49	0.00
CFCR	7.65^{a}	5.94^{b}	4.09^{c}	6.51^{ab}	0.42	0.00
FCR	11.45^{a}	8.83^{b}	6.33^{c}	10.45^{ab}	0.63	0.00

a, b, c, means with different superscripts are significantly different (p<0.05) along the row; TWG= Total weight gain, ADWG= Average daily weight gain, ADFDMI= Average daily forage dry matter intake, ADCI = Average daily concentrate intake, TFI= Total feed intake (Diet and forage), CFCR= Concentrate feed conversion ratio, FCR= feed conversion ratio. SEM = Standard Error of Mean, Bitter Leaf Meal =BLM. T_1; 0g BLM/kg = control; T_2; 20g BLM/kg; T_3; 40g BLM/kg and T_4; 60g BLM/kg

Nutrient digestibility of grasscutters fed dietary bitter leaf meal

Table 3 shows the nutrient digestibility of grasscutters fed dietary BLM. Dietary BLM had significant (P<0.05) influence on the nutrient digestibility. Grasscutters on 20g BLM/kg had the highest digestibility of all the nutrients except crude protein. The dry matter, crude protein, ash, crude fibre and nitrogen free extract digestibility of animals on dietary BLM groups (20g, 40g and 60g/kg) were significantly (P< 0.05) higher compared to those on control. Animals on 20g/kg BLM had the highest ether extract digestibility, while those on 60g/kg had the lowest values.

Table 3: Nutrient digestibility of grasscutters fed dietary bitter leaf meal

Parameters (%)	T_1	T_2	T_3	T_4	SEM	P values
Dry Matter	42.12^{c}	60.81^{a}	56.79^{a}	49.92^{b}	1.40	0.00
Crude protein	41.92^{d}	61.54^{b}	65.93^{a}	56.85^{c}	1.09	0.00
Ash	12.70^{c}	37.82^{a}	27.35^{b}	15.62^{c}	2.38	0.00
Ether extract	73.66^{b}	82.03^{a}	74.61^{b}	69.34^{c}	0.84	0.00
Crude fibre	55.65^{d}	71.23^{a}	67.29^{b}	63.71^{c}	1.10	0.00
NFE	40.87^{d}	59.78^{a}	53.34^{b}	47.85^{c}	1.47	0.00

a, b, c, d, means in the same row with different superscript differ significantly (p<0.05). NFE=Nitrogen free extract, SEM = Standard Error of Mean, Bitter Leaf Meal =BLM. T_1; 0g BLM/kg = control; T_2; 20g BLM/kg; T_3; 40g BLM/kg and T_4; 60g BLM/kg.

Haematological parameters of grasscutters fed dietary bitter leaf meal

The haematological indices of the grasscutters are shown in Table 4. Dietary BLM had significant (p<0.05) influence on all haematological parameters except granulocytes and platelet counts. PCV, Hb and RBC values increased as the graded levels of BLM supplement increases. Animals on 40g/kg BLM recorded the highest MCV values which was significantly ($p<0.05$) higher when compared to other dietary treatments. The MCH and MCHC values of animals on control and 20g/kg BLM are similar and were significantly ($p< 0.05$) higher than those on 40g and 60g/kg BLM. The WBC values of the control group were significantly ($p<0.05$) higher than those on dietary BLM groups. Lymphocytes counts of grasscutters on BLM supplement were identical and were significantly ($p< 0.05$) higher compared to the control group.

Table 4: Haematological indices of grasscutter fed dietary bitter leaf meal

PARAMETERS	T_1	T_2	T_3	T_4	SEM	P values
PCV (%)	38.70^b	42.05^b	54.48^a	56.55^a	1.06	0.00
Hb (g/dL)	14.38^{ab}	14.90^a	15.00^a	13.03^b	0.38	0.01
RBC (10^{12}/L)	6.03^b	6.65^b	8.37^a	8.83^a	0.18	0.00
MCV (fL)	64.15^{ab}	63.23^b	65.13^a	64.05^{ab}	0.38	0.03
MCH (pg)	23.93^a	22.40^a	16.98^b	15.58^b	0.36	0.00
MCHC (%)	37.28^a	35.43^a	26.53^b	23.93^b	0.67	0.00
WBC (10^9/L)	12.98^a	10.78^b	8.70^c	7.53^c	0.51	0.00
Lymphocytes (%)	55.18^b	82.63^a	91.95^a	91.33^a	2.30	0.00
Granulocytes (%)	3.05	1.40	1.03	1.00	0.70	0.18
PLT (10^9/L)	577.50	841.75	639.00	698.50	70.28	0.10

a,b,c - means in the same row with different superscripts are significantly different ($P< 0.05$); PCV= Packed Cell Volume; Hb = Haemoglobin; RBC= Red Blood Cell; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Haemoglobin; MCHC= Mean Corpuscular Haemoglobin Concentration; WBC= White Blood Cell; PLT= platelets. SEM = Standard Error of Mean, Bitter Leaf Meal =BLM. T_1; 0g BLM/kg = control; T_2; 20g BLM/kg; T_3; 40g BLM/kg and T_4; 60g BLM/kg.

Discussion

Growth Performance of Grasscutters

The concentrate intake values were higher than the forage dry matter intake values. This shows that the concentrate diet contributes more to the dry matter and total feed intake of the grasscutters. This may be due to higher concentration of essential nutrients such as energy and proteins in the concentrate. This finding is consistent with reports of other studies that feed intake in rabbits (a pseudo ruminant cousin of the grasscutter) is determined by the concentration of energy and other nutrients in their feed and by the need to satisfy their nutritional demands (Wogar, 2015). These finding is similar to the observation of Wogar, (2015) and Gbose, (2021) on forage and concentrates intakes of grasscutters. Previous studies have shown that secondary metabolites present in bitter leaf, such as tannins and alkaloids, can influence feed palatability and intake when present at high concentrations (Yacout, 2016). The daily feed intake (34.53- 40.17g) observed in this study were lower than the range of (44.36-49.35g) reported by Okukpe *et al.* (2016) in weaner grasscutters. This finding contrasts with the report of Okukpe *et al.* (2016), who observed different intake responses in grasscutters administered aqueous bitter leaf extract. Similarly, Ndelekwute *et al.* (2017) reported no significant differences in daily feed intake of finisher broilers fed diets containing 25–75 g/kg bitter leaf meal.

The differences in the results could be connected to the extraction method and processing of the bitter leaf. The extraction of active principles in aqueous solution might result to a very high concentration of the secondary metabolites which could influence the gastric hormones and metabolism of the experimental animals. This and other environmental factors might interact to result in the outcomes previously observed. Unlike this present study, the BLM supplemented was obtained from air dried leaves, it was not concentrated or extracted. The cellulose and other constituents in the leaf would result to bulking of the intestine, influencing the peristalsis and sensitizing the autonomic nervous system of the intestine directly due to the roughages. This directly or indirectly would have influence on the quantity of feed consumed daily (Adaramoye *et al*., 2008).

There was a progressive rise in weight gained and lower feed conversion ratio as the supplementation of BLM increases up to 40g/kg diets. The lower the feed conversion ratio the better the rate at which the animals convert feed consumed to meat. This might be linked to high quality of protein present in the BLM supplement that supplies additional amino acids in adequate quantities and right proportion for tissue synthesis leading to high weight gain. There was a decline in the weight gained for grasscutters on diets supplemented with 60g/kg BLM. The drop in the weight gain might be attributed to the supplemental level which was high and thus correspond to lower feed intake of the group. The increased weight gained with lower FCR obtained in this study agrees with the findings of Ndekwelete *et al.* (2017) who reported significant increase in weight gain with the lowest FCR in broilers offered 75g/kg diet of bitter leaf meal. Similarly, Osho *et al.* (2014) reported improved growth performance of broilers administer 5g-10g/ litre of bitter leaf extract. In another study conducted previously by Okukpe *et al.*, (2016) they observed improved growth performance of weaner grasscutters administered 50ml/litre of bitter leaf extract with non-significant FCR of 7.92-11.93 which was higher than 4.09-7.65 FCR observed in this study. This can also be attributed to the beneficial effects of phytochemicals present in bitter leaf which have been reported to enhance the activities of gastro intestinal enzymes to boost the breakdown and absorption of nutrients, which will improve growth performance (Adaramoye *et al*., 2008).

Nutrients Digestibility of Grasscutters fed dietary bitter leaf meal.

Nutrients digestibility value is used to determine feed efficiency. The high digestibility values in BLM supplemented groups might be linked to high crude protein content of meals supplemented with BLM, since high protein tends to increase digestibility of nutrients and enhance growth performance. Protein helps in apparent dry matter digestibility, through the process of digestion, ingested protein is broken down in to free amino acids and are used for metabolism by the animals (Annor *et al.*, 2013). The decreased digestibility of nutrients at higher inclusion levels of 40g and 60g/kg BLM might be due to increased amount of some secondary metabolites found in bitter leaf that have been observed to reduce nutrient digestibility. For instance, tannins and saponins have depressive effect on protein digestion and utilization while phytates affect utilization of minerals (Yacout, 2016). However, animals on 40g/kg BLM had the best performance. This means that the digestible nutrients at this level were better retained, absorbed and utilized. The values observed for crude protein, crude fibre and ash digestibilities were lower than the values reported by Okukpe *et al.*, (2017) who fed grasscutters varying levels of energy. However, some values were higher when compared with the findings of Eniolorunda *et al.* (2018) who fed grasscutters cassava meal fortified with fermented protein sources. The differences between this result and that of other authors might be due to differences in the physiological age of the animals and type of feed used. Animals have been observed to retain nutrient base on their nutritional needs and also on the sufficiency of each nutrient in the diet supplied (Okukpe *et al.*, 2017). The high to moderate digestibility rates of most nutrients observed in this study especially in the BLM fed groups suggested that the feed offered to the animals are well balanced in all the nutrients required by the animals for proper growth. These could also be attributed to the BLM used in the study which contains easily digestible nutrients, especially crude protein that has a very high biological value.

Haematological Indices of Grasscutters fed dietary bitter leaf meal

Blood parameters are reliable indicators of the physiological, nutritional, and health status of animals in feeding trials. In this study, the significant increases in packed cell volume (PCV), haemoglobin (Hb), and red blood cell (RBC) counts in grasscutters fed bitter leaf meal (BLM) agree with the findings of Owen and Amakiri (2011), who reported similar improvements in haematological indices with increasing BLM inclusion. The PCV values for control (38.7%) and 20g/kg BLM (42.05%) fell within normal range values (29-44%) reported for young grasscutters (Byanet *et al.*, 2008), while values obtained at 40 and 60 g/kg BLM, though higher, were comparable with those reported for grasscutters fed tropical herbs (Lucky *et al.*, 2018). The Hb values were within the standard range of (12 -18g/dl) usually found in the blood of healthy grasscutters (Lucky *et al.*, 2018). However, the observed reduction at 60g/kg BLM may suggest a slight decline in oxygen-carrying capacity at higher inclusion levels. Red blood cell counts increased progressively with increasing levels of BLM supplementation relative to the control. These values were comparable with the earlier findings of Lucky *et al.* 2018); that indicated improved oxygen delivery and nutrient utilization, reflecting good feed quality. On the other hand, blood thickens and becomes sticky when cell count increases. This could severely stress the heart, increasing the risk of heart attacks and heart failure. This is not the case in this study because red blood cell counts were within healthy range for grasscutters (Lucky *et al.*, 2018). The PCV, Hb and RBC values were higher when compared with the report of Okukpe *et al.*, (2016) who administer crude bitter leaf extract to weaner grasscutters. The observed significant increases in PCV, Hb, and RBC of grasscutters on BLM-supplemented groups indicated that BLM enhances blood volume, improves oxygen transport, and promotes efficient nutrient assimilation, thereby supporting animal growth. This effect may be attributed to the presence of haemopoietic nutrients and phytochemicals in bitter leaf, including iron, vitamin C, folate, flavonoids, and saponins (Hauwa *et al.*, 2023).

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are critical indicators of red blood cell production and anaemia status (Osho *et al.*, 2014). The MCV, MCH and MCHC values observed were lower than the range of values (67.33-88.33fL, 21.43-32.43pg, and 30.37-35.67%) reported by Okukpe *et al.* (2016) for weaner grasscutters administered crude bitter leaf extract. However, they were comparable with the range of values (52.58- 69.28, 13.73-21.08, 26.11-32.69 respectively) observed by Lucky *et al.* (2018) in weaner grasscutters fed tropical herbs. Several researches documented variations in haematological studies of grasscutters because of their highly sensitive response to the environment (Noah *et al.*, 2020). According to Olabanji *et al.* (2007), normal values of Hb, PCV, RBC, MCH, MCV, and MCHC may indicate that the test materials are safe and nutrient supplied are adequate. Reduction or elevated levels of Hb, PCV, RBC, MCH, MCV and MCHC may indicate anaemia, low protein intake, liver damage, or parasitic infection (Noah *et al.*, 2020). This observation suggested that the diets were well-balanced and fortified to provide the animals with enough nutrients, and that the amount of bitter leaf meal added to the diets had no adverse effects on the grasscutters' internal physiological status and environment.

White blood cells (leukocytes) are essential components of the body’s defense system, produced in the bone marrow and lymphoid tissues and transported via the bloodstream to sites of inflammation to combat infectious agents (Hall, 2006). A reduction in total leukocyte count may result from bone marrow disorders, viral or bacterial infections, or exposure to certain chemical agents such as antibiotics (Esievo, 2017). In this study, the significantly lower WBC counts observed in the BLM-supplemented groups compared with the control may indicate an antibiotic-like response to bitter leaf meal. This observation aligns with the antimicrobial effects of BLM on the gut microflora of the grasscutters. Importantly, the WBC values remained within the normal physiological range (8.6–12.7 ×

10^3/L) reported for young grasscutters (Byanet *et al.,* 2008), indicating the absence of leukopenia. Leukocytes counts within the standard ranges suggested that the feeding patterns do not negatively affect the immune system. The reduction in WBC may be attributed to the anti-inflammatory properties of phytochemicals such as flavonoids, tannins, and saponins found in bitter leaf as leukocytes counts can decrease when there is no inflammation. Total and differential leukocyte counts in both health and disease conditions are influenced by various factors such as stress, infection, exercise, feeding, age, breed and species variations (Esievo, 2017). Lymphocyte counts observed in all the groups were higher than the normal range of 27- 46% reported by Byanet *et al.* (2008) in young grasscutters. The reason for the high lymphocyte counts in this study compared with other authors may be due to age differences because lymphocyte count is noted to be generally high in young animals than adults being the antibody formation stage. Lymphocytes works majorly in association with the immune system by attaching to specific invading organisms and destroying them (Hall, 2006). Immunity is the ability to protect the body tissues from damage that can be caused by all types of organisms or toxins. Higher lymphocyte counts observed in BLM groups might be linked to the antimicrobial and anti-inflammatory properties of bitter leaf. The suppressive effect it had on pathogens will lower the body temperature, leading to reduced inflammation in the body. Granulocytes play a great role in regulating allergic and inflammatory processes, host defense response against bacteria and parasitic infections and general diseased conditions (Noah *et al.*, 2020). Neutrophils accounts for a larger percentage of circulating WBC. Lower secretion of neutrophils in the absence of infection and inflammation will elevate the lymphocyte counts (Esievo, 2017). High values of leukocytes and its differentials has been described as a sign of high resistance to disease (Alagbe, 2018). This could mean that bitter leaf meal stimulates the immune system of the grasscutters to fight diseases by significantly increasing the lymphocytes counts of BLM fed groups. High concentrations of saponins, flavonoids, vitamin C and E, which are known to have antioxidant qualities and are beneficial for preserving health together with zinc which is essential for immunity in bitter leaf may be responsible for the increase in lymphocyte values reported in the BLM supplemented groups (Lucky *et al.*, 2018). Antioxidant phytochemicals protect lymphocytes and reduces their destruction in the blood, according to the report of Duthie *et al.* (1996). Increased lymphocyte counts without corresponding increase in WBC cannot be linked to infections or toxicity. This indicates that BLM supplement was not toxic to the animals. There were no significant differences in the granulocyte and platelet counts. However, the platelet counts of the BLM groups were numerically higher than the control, which means that the leaf meal enhances the blood clotting ability of the animals.

Conclusion and Recommendations

It was concluded that BLM supplement can be considered as a growth promoter as it improved the performance of grasscutters by increasing their weight gain and better FCR up to 40g/kg BLM of feed. It also significantly increased the digestibilities of nutrients at optimal inclusion level of 20g/kg diets. The supplement significantly improved the PCV, Hb, and RBC of grasscutters up to 60g/kg but slightly reduces the WBC without having adverse effect on their immunity. It can therefore be recommended from the result above that bitter leaf meal should be supplemented in the diet of weaner grasscutters to enhance their productivity and health at 40g/kg feed.

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