Effects Of Tropical Herbs On Serum Biochemistry And Haematological Characteristics Of Weaner Grasscutters

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Abstract: This study was carried out to determine the effects of tropical herbs on the serum biochemistry and haematological characteristics of weaner grasscutters, using Ocimum gratissimum, Moringa oleifera, and Gongronema latifolia as supplementary sources of vitamins and minerals. A total of 20 weaner grasscutters comprising of four males and sixteen females at the age of eight weeks were used for the study. The experimental animals were allowed two weeks of stabilization period and feeding trials lasted for twelve weeks. Five grasscutters were randomly allocated to each treatment. The floor of the pens were cleaned daily, feed and water were provided ad libitum. Leaves were air dried under shade for ten days, all leaves were threshed carefully to separate leaves from twigs before blending. Twenty percent (20%) of M. oleifera, (20%) G. latifolia (20%) O. gratissimum leaf meals were added to each supplemented diet. Results on blood and biochemical analysis indicate that the mean values of haemoglobin concentration (HB), red blood cell count (RBC) and total white blood cell count (WBC) were affected by the treatments ($P<0.05$). The treatments did not affect ($P>0.05$) the mean packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean cell haemoglobin Concentration (MCHC) and Mean corpuscular volume (MCV) values in the blood of the grasscutters in various treatment groups. RBC and WBC values were higher (11.06 ± 0.59ul and 13.12 ± 1.39ul) for grasscutters in T_2 while grasscutters on T_1 recorded higher Hb concentration (17.65 ± 0.27g/dl). An increase in WBC and lymphocytes were recorded for grasscutters in the experimental groups compared with the control ($P<0.05$). Average NEU, MON, EOS, BAS values in the blood of grasscutters did not vary ($P>0.05$). The treatments significantly ($P<0.05$) increased the urea level in blood of grasscutters in the experimental group compared with control group. The study found that the leaf meals of herbs administered at the dosages used and for the duration of the experiment had significant treatment effects on the haemopoetic system of grasscutters with better effect on growth performance recorded for grasscutters on M. oleifera leaf and O. gratissimum leaf supplemented diets. The study recommends the use of tropical herbs as important supplements for improving the serum biochemistry and haematological parameters of grasscutters.

Keywords: Grasscutter, haematological characteristics, tropical herbs, serum

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1 INTRODUCTION

Haematology is the discipline of medical science that studies the blood and blood-forming tissues and is currently considered an integral part of clinical laboratory diagnostic support in avian medicine. The routine collection and processing of blood samples allows the evaluation of haematological responses to diseases (Howlett & Jaime, 2008). The use of blood examination as a way of assessing the health status of animals has been documented (Muhammed et al., 2000; Owoyele et al., 2003; Muhammed et al., 2004). This is because it plays a vital role in physiological, nutritional and pathological status of organisms (Muhammed et al., 2000). The effect of both raw and processed feed on the haematological parameters of animals have been reported (Muhammed et al., 2000; Owoyele et al., 2003; Muhammed et al., 2004). Haematological parameters are those parameters that are related to the blood and blood forming organs (Stenesh, 1975). Haematological parameters are blood characteristics, which affect both the health and nutritional state of an animal. The nutritional value of a feed stuff could therefore be reflected through parameters such as: white blood cell (WBC), red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), lymphocytes, and neutrophils. The full blood count (FBC), sometimes referred to as a full blood examination or complete blood count, is one of the most commonly performed blood tests, as it can tell us so much about the status of our health. It is important for diagnosing conditions in which the number of blood cells is abnormally high or abnormally low, or the cells themselves are abnormal (Stenesh, 1975). Studies have shown that certain factors influence haematological and biochemical parameters (Weldy et al. 1964). Haematological and biochemical analyses of an animal's blood represent a good diagnostic aid for the assessment of physiological, nutritional and pathological conditions of animals (Jain, 1986; Bush, 1991; Awah-Ndukum et al., 2001). Nutrition age, sex, genetics (breed and crossbreeding), reproduction, housing, starvation, environment factors, stress, transportation and diseases are known to affect haematological and biochemical values (Coles, 1986) and thought to play major roles in the differences in haematological and biochemical parameters observed between tropical and temperate animals (Ogunriade et al., 1981; Bush, 1991; Ogunsanmi et al., 1994; Opara & Fagbemi, 2008). Ogunsanmi et al. (2002), determined the haematological, plasma biochemical and whole blood electrolytes profile in the normal live-captive and rehabilitated adult African grasscutters. They reported no statistical evidence of sexual dimorphism in the values of these parameters of the cane rats, except plasma alanine transaminase (ALT), which was significantly higher ($p<0.001$) in the males (9.01 ± 0.73iu/l) than in the females (8.3 ± 1.03iu/l). Owolabi, (2002) and Opara et al. (2006), reported a significantly ($p<0.05$) higher lymphocyte (10.25 ± 1.0ul), eusinophil (0.1 ± 0.03) and basophil (0.05 ± 0.03) values for both the female and male wild grasscutters, compared with those of captive – reared lymphocyte (4.91 ±0.96ul), eosinophil (0.01 ± 0.02ul) and basophil (0.01 ± 0.02) for both female and male grasscutters. They equally reported a significantly higher white blood cell counts in female than male wilds grasscutters and attributed these differences to the free nature of the wild rodents which are more prone to all kinds of infections (Gotoh et al., 2001). The significantly high levels of basophils and eusinophils among the wild grasscutters (Opara et al., 2006) were due to the presence of inhabiting parasites in the animals. This study was carried out to determine the effects of tropical herbs on the haematological characteristics of weaner grasscutters (Thryonomys swinderianus). In particular, the study determined the effect of Ocimum gratissimum, Moringa oleifera, and Gongronema latifolia leaf meals as a supplementary source of vitamins and minerals, on the haematological and serum biochemical parameters of weanner grasscutters.

2 MATERIALS AND METHODS

The study was carried out at the grasscutter unit of the Department of Animal Science, University of Nigeria, Nsukka. Nsukka lies in the derived savannah region, and is located on Longitude 6°25'N and Latitude 7°24'E (Ofomata, 1975) at an altitude of 430m above sea level (Breinholt et al., 1981). The climate is a typical humid tropical setting with a RH range of 56.01- 103.83%. Average diurnal minimum temperature ranges from 22°C – 24.7°C while the average maximum temperature ranges between 33°C – 37°C (Okonkwo & Akubuo, 2007). Annual rainfall ranges from 1680mm – 1700mm (Breinholt et al., 1981). This study was carried out to determine the effects of tropical herbs on the serum biochemistry and haematological characteristics of weaner grasscutters. In particular, the study determined the effect of Ocimum gratissimum, Moringa oleifera, and Gongronema latifolia as supplementary sources of vitamins and minerals, on the haematological and serum biochemical parameters of weaner grasscutters. A total of 20 weaner grasscutters comprising of four males and sixteen females at the age of eight weeks were used for the study. The experimental animals were allowed two weeks of stabilization period and feeding trials lasted for twelve weeks. Five grasscutters were randomly allocated to each treatment. The floor of the pens were cleaned daily, feed and water were provided ad libitum. Leaves were air dried under shade for ten days, all leaves were threshed carefully to separate leaves from twigs before blending. Twenty percent (20%) of M. oleifera, (20%) G. latifolia (20%) O. gratissimum leaf meals were added to each supplemented diet. The experimental animals were allowed two weeks stabilization period and feeding trials lasted for twelve (12) weeks.

2.1 Haematological Investigations on Experimental Grasscutters

At the end of the study, a veterinarian was invited from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka for haematological investigation. A total of three (3) grasscutters/ treatment were randomly selected. For the four (4) treatments under investigation, twelve (12) blood samples were collected. Each animal had a separate glass test tube to avoid contamination of blood samples between treatments, while a separate microcapillary tube was used each time blood was drawn from any of the grasscutters as a precautionary measure against transmission of infectious agents from grasscutter to grasscutter. Blood sample for haematological determination was collected from the retro-bulbar plexus of the medial canthus of eye of the grasscutter. A microcapillary tube was carefully inserted into the medial canthus of the eyeat 30˚-40˚ angle towards the back of the eye to puncture the retro-bulbar plexus and thus enable outflow of blood into a sample bottle containing ethylene-diamine-tetra-acetic acid (EDTA). The sample bottle was shaken gently to mix up the blood with EDTA and prevent clotting. Name of the method used in haematological determination: Orbital technique Leucocytes count (total white blood cells) and Erythrocytes count (red blood cells) were determined using haemocytometer method (Schalm et al., 1975) while Haemoglobin concentration was determined using cyanomethaemoglobin method (Kachmar, 1970). Packed cell volume (haematocrits) were determined using microhaematocrit method (Coles, 1986) while Differential leucocyte count was determined using Leishman Technigue (Schalm et al., 1975). The mean cell haemoglobin Concentration (MCHC), Mean Corpuscular haemoglobin (MCH) and the Mean corpuscular volume (MCV) were calculated according to Mitruka & Rawnsley (1977). The equations (1, 2 & 3) are shown below:

$$\text{Mean Corpuscular Volume (MCV)} = \frac{\text{pcv}(\%)\text{X } 10}{\text{RBC count (millions)}} \quad (1)$$

$$\text{Mean Corpusucular Haemoglobin (MCH)} = \frac{\text{Hb}\left(\frac{\text{g}}{\text{dl}}\right)\text{X } 10}{\text{RBC count (millions)}} \quad (2)$$

$$\text{Mean Corpusucular Haemoglobin Concentration (MCHC)} = \frac{\text{Hb}\left(\frac{\text{g}}{\text{dl}}\right)\text{X } 100}{\text{PCV}} \quad (3)$$

2.2 Methods used in determining clinical serum chemistry: Orbital technique

Blood urea were determined using modified method of Berthelot-Searcy for the in vitro using a Quimica Clinica Applicada (QCA) Enzymatic urea test kit (QCA, spain) while Total protein was determined using direct Biuret method (Lubran, 1978) and Creatinine were determined using modified Jaffe method using the Quimica Clinica Applicada (QCA) creatin test kit (QCA), Spain) (Blass et al., 1974). Serum albumin and serum globulin were determined using Bromocresol green method using Quimica Clinica Applicada (QCA) Albumin test kit (QCA) (Doumas et al., 1971).

3 RESULTS AND DISCUSSION

3.1 Haematological indices of Weaner Grasscutters

Results of the haematological implications of the inclusion of GLLM, OGLM and MOLM in the diet of weaner grasscutters are shown in Table 1.

Table 1: *The effect of administration of OGLM, GLLM, and MOLM on Haematological indices of Weaner Grasscutters*

	TREATMENT			
PARAMETERS	T1	T2	T3	T4
PCV(%)	54.00 ± 1.73	58.17 ± 1.42	53.33 ± 2.94	55.5 ± 3.55
RBC(ul)	9.45 ± 0.80^{ab}	11.06 ± 0.59^{a}	7.70 ± 1.18^{b}	9.60 ± 0.92^{ab}
Hb(g/dl)	17.65 ± 0.27^{ab}	15.19 ± 3.29^{b}	16.22 ± 0.69^{ab}	18.09 ± 1.07^{a}
WBC(ul)	11.77 ± 2.11^{ab}	13.12 ± 1.39^{a}	8.53 ± 0.74^{b}	6.75 ± 0.85^{b}
MCHC	32.69	26.11	30.41	32.59
MCH	18.68	13.73	21.06	18.84
MCV	57.14	52.58	69.26	57.81
Differential white blood cell counts (%)				
NEU	3.35 ± 0.31	3.46 ± 0.49	2.38 ± 0.28	2.22 ± 0.40
LYM	8.08 ± 1.89^{a}	9.22 ± 0.72^{a}	5.99 ± 0.45^{b}	4.39 ± 0.44^{b}
MON	0.03 ± 0.03	0.11 ± 0.11	0.05 ± 0.04	0.00 ± 0.00
EOS	0.31 ± 0.80	0.16 ± 0.10	0.12 ± 0.76	0.12 ± 0.34
BAS	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.03 ± 0.27

a,b,c = Means ± SEM on the same row with different *superscript are significantly (p<0.05) different. T_1= MOLM, Moringa oleifera leaf meal, T_2= GLLM, Gongronema latifolia leaf meal, T_3= OGLM, Ocimum gratissimum leaf meal and T_4= CONTROL, NEU=Neutrophil, LYM=Lymphocytes, MON=Monocytes, EOS=Eosinophils, BAS=Basophils.*

The administration of GLLM, OGLM and MOLM to weaner grasscutters influenced significantly (P<0.05) the Hb, RBC, and WBC, indices of the grasscutter's blood. Control had higher Hb values of (18.09g/dl), T_1 (17.65g/dl), T_2 (15.19g/dl) and T_3 (16.22g/dl) respectively. WBC had T_1 (11.77ul), T2 (13.12ul), T_3 (8.53ul) and control had lowest values of (6.75ul). No significant differences (P>0.05) among treatments were recorded in the PCV, MCHC, MCH and MCV concentration in the blood of the grasscutters. The mean RBC content of the blood of grasscutter on MOLM (9.45ul) and control (9.60ul) were similar (P > 0.05). However, their values differed (P < 0.05) from those of grasscutters fed on GLLM (11.06ul), and OGLM (7.70ul). There were significant differences (P<0.05) in the mean RBC between grasscutters on GLLM and those on OGLM. Grasscutters on GLLM diet recorded the highest RBC value while those on OGLM recorded the minimum. The mean WBC content of the blood of grasscutter fed on MOLM (11.77ul), and GLLM (13.12ul) differ significantly (P < 0.05). However, their values were significantly higher (P < 0.05) than that of the control grasscutter (concentrate). The result shows that MOLM (32.69) and control (32.59) had similar and higher (P<0.05) MCHC values than OGLM (30.41) and GLLM (26.11) in the blood of grasscutters. However, MOLM (18.68) and control (18.84) had similar MCH values which differed (P<0.05) from GLLM (13.73) and OGLM (21.06) that had the highest MCH in the blood of the grasscutters fed on different plant diets. OGLM had higher MCV value (69.26) and this differed significantly from (P<0.05) from that of grasscutters fed on MOLM (57.14), GLLM (52.58) and control (57.81). The results on differential white blood count shows that only lymphocytes count was significantly (P<0.05) affected by treatments with GLLM (9.22 ± 0.72) having higher lymphocyte count than MOLM (8.08 ± 1.89), OGLM (5.99 ± 0.45) and control 4.39 ± 0.44). However, neutrophils, eosinophils, monocytes and basophil were not significantly (P>0.05) affected by treatments. The packed cell volume in the blood of grasscutters at the end of the experiment for the control group was 55.5% on the average and 53.33% (OGLM), 58.17% (GLLM), 54.00% MOLM on the average for the experimental groups. This shows that the packed cell volume (PCV) was not significantly (P<0.05) affected by treatment. These packed cell volume values were significantly higher than those values (20.3% to 30.4%) reported by Ogunsami et al. (2002) in captive grasscutters. Probably the grasscutters were able to maintain constancy in the amount of PCV in the blood despite the presence of antinutritional and other chemicals in the experimental diets containing the leaf meals. The red blood cell counts in this study were 9.6/ul for the control group and mean values of 7.70ul, 11.06ul, 9.45ul for the experimental groups. The values indicate an increase RBC counts on treatment 2 (GLLM) compared to the control group. Treatment 1 (MOLM) had similar values with the control group. The increased RBC values obtained in treatment 2 were comparable with the findings of Ogunsanmi et al. (2002), these authors reported RBC values of (12.36ul ± 0.52) in captive grasscutters. The increased condition is known as polycythemia or erythrocytosis. Red blood cell count is an indication of feed quality. High red cells can increase the delivery of oxygen to the tissues, and the animal experience full calories of energy. However, the increased number of cells in blood causes the blood to become thickened and sticky. This may put a huge strain on the heart, and can cause heart attacks and heart failure. The haemoglobin values in the blood of the grasscutters at the end of the experiment were (18.09 g/dl) on the average for the control group and (16.22, 15.19, and 17.65 g/dl) on the average for the experimental groups. This shows a reduction in the haemoglobin level, and this is in consonance with the finding of (Ephraim et al.,2000) in which the hemoglobin value decreases significantly after administration of aqueous extract of Ocimum gratissimum to rabbits. The haemoglobin values were higher than the reports Opera et al., (2006) in wild grasscutters (14.17 ± 0.52g/dl) and the reports of Ogunsami et al., (2002) in captive grasscutters (12.36 ± 1.65g/dl). Thus haemoglobin (Hb) concentration in this study fell within the normal range of (12 – 18g/dl) normally contained in the blood of grasscutters. Haemoglobin has the unique property of combining reversibly with oxygen and is the medium by which oxygen is transported within the body. It takes up oxygen as blood passes through the lungs and releases it as blood passes through the tissues. The observed difference in control group and T_2 (GLLM) group suggests that the oxygen carrying capacity of the blood was high in grasscutters on control diet, suggesting that GLLM leaf meal caused a decline in the oxygen carrying capacity. The white blood cell count gave a mean value of 6.75uL for grasscutters in the control group at the end of the experiment and mean values of 8.53ul, 13.12ul and 11.77ul respectively for grasscutters on MOLM, GLLM and OGLM diets. A significant increase in the WBC count was observed in the experiment group as compared to the control. Ephraim et al. (2008) reported a decrease in WBC count by their work on captive grasscutters. The presence of high levels of vitamin A, vitamin C, vitamin E and phytates that are known to have antioxidant properties and useful in maintaining good health, may have been responsible for the increase in WBC values reported in the experimental groups. According to Iweala & Obidoa (2009), phytosterols and flavonoids in G. latifolia leaves possibly interferes with the process of WBC synthesis resulting to increased presence of WBC in the blood. In addition, Duthie et al. (1996) reported that antioxidant phytochemicals play a protective role on the lymphocytes and also decrease their destruction in the blood. The presence of high levels of vitamin A (40.82 mg/100 g), vitamin C (15 mg/100 g), vitamin E (tocopherol) (3.71 mg/100 g), β-carotene (6.80 mg/100 g) and phytate (6.5 mg/100 g) that have been indicated to have antioxidative properties (Traber & Atkinson, 2007) and useful in maintaining good health, have been suggested as possible causes of increased WBC in the blood. Leukocyte counts have been reported to increase due to any form of stress, exercise, feeding, age, breed and wide variety of other conditions (Dellmann & Brown, 1987). The mean corpuscular haemoglobin concentration MCHC, MCH, MCV had no significant differences between the control group and the experimental groups suggesting that the amount and quantity of leaf meals (20%) added to the diets did not have any detrimental effect on the internal physiological milieu of the grasscutters. Thus the grasscutters on some of the experimental diets were able to perform better in some parameters compared with the control. In this study, the lymphocyte counts of the reared young grasscutters were higher than neutrophil counts. The mean values of neutrophil (NEU), monocyte (MON), Eosinophil (EOS) and Basophils (BAS) in the blood of the grasscutters were not significantly different (P>0.05) between the control group and the experimental groups suggesting that the amount and quantity of the leaf meal (20%) added to the diets did not have any effect on the physiology of the grasscutters.

3.2 Serum Biochemical Content of Grasscutters fed MOLM, GLLM and OGLM Supplemented Diets

The results of the serum biochemical contents of grasscutters fed on MOLM, GLLM and OGLM supplemented diets are presented in Table 2 below.

Table 2: *The Effect of the administration of MOLM, GLLM and OGLM on Serum Biochemistry of Weaner Grasscutters*

	TREATMENT			
PARAMETERS	MOLM	GLLM	OGLM	CONTROL
Urea (mg/dl)	11.95 ± 1.84[a]	12.87 ± 0.92[a]	11.77 ± 0.40[a]	5.24 ± 0.89[b]
Total protein (g/dl)	4.85 ± 0.32	5.12 ± 0.55	4.49 ± 0.44	5.06 ± 0.19
Creatinine (mg/dl)	1.00 ± 0.25	1.73 ± 0.18	1.23 ± 0.15	1.50 ± 0.25
Albumin (g/dl)	3.04 ± 0.57	2.95 ± 0.32	2.85 ± 0.06	3.32 ± 0.38
Serum Globulin (g/dl)	1.80 ± 0.25	2.17 ± 0.23	1.63 ± 0.49	1.74 ± 0.42

a,b,c = Means ± SEM on the same row with different superscript are significantly (<P 0.05) different. MOLM= Moringa oleifera leaf meal, GLLM= Gongronema latifolia leaf meal, OGLM= Ocimum gratissimum leaf meal.

The Urea nitrogen in the blood of grasscutters fed on MOLM (11.95mg/dl), GLLM (12.87mg/dl) and OGLM (11.77mg/dl) diets were similar (P >0.05). However, they differed (P<0.05) from those of the control treatment (5.24mg/dl). The total protein, creatinine, albumin and serum globulin in the blood of the grasscutters on MOLM, GLLM, OGLM and control treatment do not differ significantly (P > 0.05) among treatments. The mean value of urea was 5.24mg/dl for the control group and mean value of (11.77, 12.87, 11.95mg/dl) in experimental groups 1, 2 and 3 respectively. These values are lower than the findings of Ogunsanmi et al. (2002), and Opara et al. (2006) for wild grasscutters. Opara reported mean urea values of 21.87mg/dl on wild grasscutters, and Ogunsanmi had mean urea values of 27.00mg/dl in their work on captive grasscutters. The cause of the higher blood urea in the captive grasscutters fed on the experimental diet may be due to increased production of urea in the liver, or to increased protein breakdown or decreased blood flow through the kidney. Urea is a function of protein quality and high urea level depicts low protein quality fed. In the present study, lower concentration of urea in the grasscutters served the experimental diets indicated that the quantity of the leaf meals given to the gasscutters were not harmful to the grasscutters, concentrations of tannin, phytate and hydrocyanic acid were appreciable in the experimental diets fed to the grasscutters. Liver is the predominant source of urea production in the body. Urea is a waste product produced during protein synthesis in the liver, the kidney plays a major role in the filtrating process to help remove the waste found in the body, which primarily leaves through the urine. Therefore too much protein can put extra strain on these organs (Rao et al., 2007). If blood urea nitrogen is high, it often means eating too much protein. Factors that could be responsible for BUN increment are, increased catabolism, increased production of urea in the liver due to high protein diet, increased protein breakdown, decreased elimination of urea due to decreased blood flow through the kidney (Atkinson & Bourke, 1987). These authors also suggested that hepatic urea synthesis, which consumes HCO_3, plays an important role in acid-base homeostasis. The results obtained in this work for creatinine, albumin, serum globulin were not significantly different among treatments. The values for these parameters were similar to the findings of Oyewale (1997), for reared grasscutters and Opara et al. (2006) for wild grasscutters. Variations in the haematological indices of animals may occur due to genotype differences, age, physiological condition and nutrition (Machebe et al., 2009).

4 Conclusion

This study was carried out to determine the effects of tropical herbs on the serum biochemistry and haematological characteristics of weaner grasscutters. In particular, the study determined the effect of Ocimum gratissimum, Moringa oleifera, and Gongronema latifolia as supplementary sources of vitamins and minerals, on the haematological and serum biochemical parameters of weaner grasscutters. Findings of this study suggest the importance of tropical herbs as supplements in animal diets. The use of tropical herbs is comparatively viable as herbs are readily available. In particular, this study recommends that Moinga oleifera and Ocimum gratissimum leaf meals be incorporated into the feeds of grasscutters as supplements without any detrimental hematological effect. The study recommends increased research on tropical herbs as feed supplements with a view to improving the haemopoetic system and growth performance of grasscutters and other farm animals. The study recommends the use of tropical herbs as important supplements for improving haematological parameters of grasscutters.

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