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EFFECT OF GRADED GARLIC EXTRACT ON HAEMATOLOGY AND SERUM CHEMISTRY OF SHEEP IN SEMI-ARID BORNO STATE, NIGERIA

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ABSTRACT

The study was conducted to assess the effect of garlic extract on haematology and serum biochemistry of sheep fed concentrate corn cob diet in semi-arid region of Borno state. Sixteen (16) sheep of non-descript breed weighing on average 21.56 kg were used. The animals were divided into 4-groups and each group of 4-animals was randomly assigned to one of the treatments in a completely randomized design (CRD). The experiment lasted for 8 weeks. The treatments were 0.0ml, 2.5ml, 5ml, and 7.5ml of garlic extract for T_0 T_1, T_2, T_3, respectively. The results showed significant ($P<0.05$) differences among the treatments in Packed Cell Volume (PCV) Red Blood Cell (RBC), Haemoglobin (Hb),White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), , while Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell differential values were not influenced ($P>0.05$) by the levels of garlic extract. Total Protein, Globulin, Glucose, Urea, Creatinine, Cholesterol, Aspartate Aminotransferase and Alanine Aminotransferase were significantly different among the treatment groups ($P<0.05$) while albumin and alkaline phosphate ALP) were not different ($P>0.05$).Drenching with 5 ml garlic extract (treatment T3) has more effect in reducing serum cholesterol than drenching with 2.5 ml (treatment T2). It was concluded that garlic extract has significant effect on reducing serum cholesterol without negative effect on blood composition.

Keywords: Serum biochemistry; Haematology; Sheep; Garlic extract

INTRODUCTION

The importance of sheep production in Nigeria cannot be over emphasized since rearing small ruminants will provide employment and income as subsidiary occupation and are often regarded as producers of milk and meat. Production of sheep contributes immensely to livestock subsector of the Nigerian economy (Lakpini *et al*., 2002). Sheep play important role in the socioeconomic lives of the people around the world including Nigeria (Yakubu and Ibrahim, 2012).

Consumption of red meat is rampant among Nigerians as the major source of animal protein, most especially lambs and muttons. Lambs and mutton contain high fat content as

reported in the literature (Bernes and Stengarde, 2012; Bernes *et al.*, 2012). The level of lipid present in blood constituents of sheep needs to be regulated by the use of the natural antioxidants as feed additives in order to establish a normal level of lipid content of blood constituents. One of such is the use of garlic (*Allium sativum*) as feed additive.

Garlic (*Allium sativum*) has been used as a spice, it possesses bioactive components (Amagase *et al*., 2001) and these components have antibacterial, antifungal, antiviral, antioxidant, antithrombotic and vasodilator properties (Qureshi *et al*., 1983). Blood indices are important for the evaluation of the nutritive component of a given diet (Agbede and Aletor, 2003). The serum biochemistry and hematological components of the blood are influenced by the quantity and quality of feed, level of anti-nutritional elements or factors present and also in monitoring level of feed toxicity present in feed constituents that affect the formation of blood (Oyawoye and Ogunkuwe, 1998; Akinmutimi, 2004). The objective of the study was to assess the effect of Serum biochemistry and haematological indices of sheep administered with garlic extract in graded level in semi-arid Borno state

MATERIALS AND METHODS

Study Location

The study was conducted at the Department of Animal Science Teaching and Research Farm, University of Maiduguri. Maiduguri is located between latitude $11^{0}5^{1}$ and 12^{0} North and Longitude $13^{0}05^{1}$ and 14^{0} East and at altitude of 354m (1161ft) above sea level (DNMA, 2013). The area falls within the semi-arid zone of West Africa characterized by short duration of rainfall (3-4 months) which varies from minimum of 478mm to 500mm to maximum of 600mm-621mm (Afolayan *et al.,* 2013). The mean temperature is 34^{0}C, the maximum being 40-60^{0}C and the lowest 25^{0}C, which is in April and December, respectively.

Preparation of Garlic Extract

About 200 g of dried garlic were peeled and soaked overnight in 200ml of distilled water. The peeled garlic was soaked for easy pounding. The crushed garlic was left to stand for 2hours and later filtered. The garlic juice was used immediately because the active compound Allicin is highly volatile. The garlic juice was then diluted in water at the ratio of 8:2 making a concentration of 80%, according to the procedure adopted by Masamha *et al*. (2010).

Management of Experimental Animals and Experimental Design

Twenty (20) sheep of non-descript breed weighing on average 21.56 kg were used for the study. All the animals were obtained from the flock of sheep kept at the Department of Animal Science Livestock Teaching and Research Farm, University of Maiduguri. They were weighed and identified using plastic ear tags. Feeding was done at 4% body weight once daily at 8:00 am with the left over being weighed before the next feeding. The animals were divided into 4-groups and each group of 4-animals was randomly assigned to one of the treatments in a completely randomized design (CRD). The animals were given prophylactic treatment against external and internal parasites. The animals were fed for 7

days as an adaptation before the commencement of the experiment. Oxytetracycline and multivitamin injection were administered at the rate of 1ml per 10 kg body weight. The animals were housed in pens, with wide windows for adequate ventilation. The experiment lasted for 8 weeks.

Experimental Diet Preparation and Dietary Treatments

The feed ingredients used for the formulation of the experimental diets were maize cob, wheat offal, cotton seed cake, poultry litter and exogenous enzyme. The diet formulated consisted of maize cob (40%), wheat offal (30%), cotton seed cake (15%), poultry litter (15%). The animals were given 0, 2.5, 5, and 7.5 ml of garlic extract for T_0 (Control), T_1, T_2 and T_3 respectively.

Table 1: Composition of experimental diet (%)

Ingredient	Treatments (graded level of garlic extract (ml)				
	T0(0)	T1 (2.5)	T2(5.0)	T3(7.5)	
Maize Cob	40	40	40	40	
Wheat Bran	30	30	30	30	
Cotton Seed Cake	15	15	15	15	
Poultry Litter	15	15	15	15	
Total	100	100	100	100	
Level of Garlic Extract	0 ml	2.5 ml	5 ml	7.5 ml	
Ingredient	Proximate composition (%) of feed ingredient				
	DM	CP	CF	EE	ASH
Poultry litter	95.20	10.11	25.00	3.00	4.00
Cotton seed cake	96.30	15.10	23.00	8.00	5.00
Wheat offal	94.30	8.31	19.00	3.00	5.00
Corncob	96.10	5.07	21.00	3.00	4.00

Blood Samples Collection and Analysis

At the end of the feeding trial, three animals were randomly selected from each treatment. The blood samples were collected from three (3) animals per treatment at the last day of the study before terminating the experiment. Blood samples were collected from each animal by jugular-veinpuncture using disposable syringes and sterile needles (18 gauges). Prior to feeding in the morning, bleeding was done and an average of 10 ml of blood was collected from each animal. The blood samples were placed in two vacutainers. One contained ethylene diamine tetra-acetic acid (EDTA) for haematological studies as described by (Al-Eissa and Alkahtani, 2011), the second bottles contained no anticoagulant and it received the remaining blood which was allowed to stand for about 2 hours at room temperature. The universal bottles were thereafter centrifuged at 700xg for 15 minutes, the serum separated were decanted and stored in a freezer at -10ºC for blood biochemical analysis as reported by Gambo *et al*. (2011).MCV, MCH and MCHC were deduced according to Jain (1986) as follows: *MCV (fl) = PCVX10/RBCX10; MCH (pg) = HbX10/RBC (10^6); MCHC (%) = HbX100/PCV.*

From the centrifuged blood sample in plain bottles, serum was collected for biochemical assay. Total protein and albumin were determined by *Biuret method* and

Bromocresol Green method respectively. Blood Urea Nitrogen (BUN), Creatinin, Bilirubin as well as activities of the liver enzymes (AST and ALT) were determined by Standard Enzymatic method as outlined by Bush (1991). Serum Cholesterol was determined by *Burchad reaction.*

Chemical and Statistical Analyses

Samples of the experimental diets were analyzed for proximate analysis using the procedures of AOAC (2002). Data generated from the haematological indices and serum biochemical profiles were subjected to one way analysis of variance (ANOVA). Significant difference between treatment means were separated using Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Haematological Indices

The heamatological parameters of sheep administered varying levels of garlic extract is shown in Table 2. There were significant (P<0.05) differences among the treatments in Packed Cell Volume (PCV) Red Blood Cell (RBC) values, Haemoglobin (Hb),White Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), while Mean Corpuscular Haemoglobin Concentration (MCHC) and White Blood Cell differential values were not influenced (P>0.05) by the treatments.

Table 2: Haematological parameters of sheep fed varying levels of Garlic extract

Parameters	Treatments (graded level of garlic extract) (ml)				
	T0(0)	T1 (2.5)	T2(5.0)	T3(7.5)	SEM
PCV %	28.3^{a}	24.69^{b}	27.67^{ab}	27.0^{ab}	1.29^{*}
Hb g/dl	9.40^{a}	8.20^{b}	9.17^{ab}	8.97^{ab}	0.7^{*}
RBC g/dl	9.27^{c}	9.70^{c}	12.57^{b}	13.43^{a}	0.26^{*}
MCV fl	20.1^{a}	30.94^{b}	26.0^{ab}	22.08^{ab}	1.44*
MCH pg	10.0^{b}	6.9^{a}	8.4^{ab}	7.3^{ab}	3.4^{*}
MCHC	33.2	33.17	33.2	33.1	0.2^{NS}
WBC	6.00^{b}	10.60^{a}	8.80^{a}	10.43^{a}	0.77^{*}
Neutrophil	40.33	34.67	44.0	31.0	13.13^{NS}
Eosinophil	2.33	1.00	1.00	1.00	1.62^{NS}

a,b= Means in the same row with different superscript differ significantly (P<0.05)
NS= Not significant; *=Significant (P<0.05); SEM=Standard error of means; PCV=Packed Cell Volume; Hb=Haemoglobin; RBC=Red Blood Cell; WBC= White Blood cells;
MCV=Mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MCHC=Mean corpuscular haemoglobin Concentration

The PCV value of sheep Administered varying levels of garlic extract ranged from 24.69 to 28.3%. The PCV values obtained in this study were in contrast with the value 43.8 ±0.6%, reported by Egbe *et al*, (2000) but within the range value of 27 – 45% as reported by Jain (1993). Packed Cell Volume is important in the diagnosis of anaemia (Chineke *et al.*, 2006). The higher PCV values obtained in this study might likely be a sign of healthier sheep.

The haemoglobin values ranged from 8.20 to 9.40 g/dl. The haemoglobin values were within the normal range (8 – 16g/d*l*) of haemoglobin for healthy sheep (Greenwood, 1977).The values obtained for all the treatment groups indicate nutritional adequacy of all diets since values did not indicate mal-or-under nutrition (Churche*t al*., 1984).The Red blood cell count obtained in this study ranged from 9.27 to 13.43 g/dl for treatments T_0 and T_3 respectively. These values were slightly above the RBC values for lamb (4.44 – 8.69 g/dl) reported by Njidda *et al*. (2014). Red blood cell indices provide information about the haemoglobin content and size of red blood cells. Abnormal values indicate the presence of anaemia and the type of anaemia (Gernsten, 2002). Mean corpuscular Volume MCV values obtained for T_0 and T_1were 20.1 - 30.94 f*l*, respectively which were lower than the normal physiological MCV range for sheep (35.3 – 43.7f*l*) reported by Borjesson *et al.* (2000).The values of mean corpuscular haemoglobin obtained in the study ranged from 6.9 to 10 pgT_1 and T_0 respectively which were also lower than the values (12-20 pg) for lamb as reported by Njidda *et al.* (2014). The higher MCH and MCV values might be due to age of the animals (Egbe, 2000). The values of MCV and MCH are very important in the diagnosis of anaemia and also serve as useful index of the capacity of the bone marrow to produce red blood cells (Awodi *et al*., 2005).Mean corpuscular Haemoglobin Concentration (MCHC) showed no significant ($p > 0.05$) differences among the treatments. The values were within the normal range value of 30.89 ± 0.33 reported by Anosa and Todd (1952).

The white blood cell values ranged from 6.00 and 10.60 for T_0 and T_1 respectively. The white blood cell (WBC) were within the normal range values of sheep $7.36 \pm 0.23\%$, reported by Egbe *et al.* (2000). The higher WBCs count recorded in the animals in treatment T_0 might be due to the response of the animals as protection against invading pathogens. This showed that the animals were healthy because decrease in number of WBC below the normal range is an indication of allergic conditions, while elevated values (leucocytosis) indicate the existence of a recent infection, usually with bacteria (Ahamefule*et al.*, 2008).The WBCs or leucocytes are the mobile unit of the body in protecting system (Aiello, 2000).

Table 3: Effect of garlic extracts drench on Biochemical Indices of Sheep

Parameters	Treatments (graded level of garlic extract) (ml)				
	T0(0)	T1 (2.5)	T2(5.0)	T3(7.5)	SEM
Total Protein (g/L)	61.67^{b}	59.33^{b}	67.00^{a}	61.67^{b}	2.59*
Albumin (g/L)	33.67	31.00	33.67	30.33	5.30^{NS}
Globulin (g/L)	28.00^{b}	28.33^{b}	33.33^{a}	31.33^{ab}	1.77*
Glucose (mmol/L)	3.33^{ab}	2.50^{c}	2.57^{bc}	3.87^{a}	0.08*
Urea (mg/dl)	4.80^{a}	3.27^{c}	4.20^{b}	3.33^{c}	0.30*
Creatinine (µ/L)	84.00^{a}	75.67^{b}	85.67^{a}	75.33^{b}	3.30*
Cholesterol (mmol/L)	78.33^{ab}	85.00^{a}	62.67^{b}	72.67^{ab}	49.26*
ALP (µ/L)	36.33	36.33	41.00	36.67	9.43^{NS}
ALT (µ/L)	38.67^{a}	17.67^{b}	23.67^{b}	26.00^{b}	19.32*
AST (µ/L)	71.67^{b}	105.00^{a}	77.33^{ab}	59.33^{b}	134.47*

a, b, means in the same row with different superscript differ significantly (P<0.05); NS=Not significant; *=Significant (P<0.05); SEM=Standard error of means; ALP= alkaline phosphate; AST=Aspartate Aminotransferase; ALT= Alanine Aminotransferase.

Biochemical Indices

The serum biochemistry of sheep fed all-concentrate corn cob diets is shown in Table 3. The Total Protein, Globulin, Glucose, Urea, Creatinine, Cholesterol, Aspartate Aminotransferase and Alanine Aminotransferase were significant ($P<0.05$) among the treatment groups, while albumin and alkaline phosphate ALP) were not ($P>0.05$).

The total protein of the animals ranged from 59.33 to 67 g/L. The values for total protein, albumin and Globulinobtained in this study were within the normal range reported for normal healthy balami rams (Njidda *et al.*, 2014). The total protein values were similar to the range value (60 – 93g/*l*) for sheep (Borjesson *et al.*, 2000). Milne and Scott (2006) reported a normal physiological range of 30 – 38g/*l* albumin comparable to the values obtained in the present study. Oboh *et al*. (2011) reported that serum total protein of animals are indirect indices for measuring nutritional protein adequacy in farm animals. Dairo (2005) reported that albumin is an important blood clotting factor due to its ability to prevent haemorrhage, therefore the higher the value the better for the animals. This could be the reason why all the animals have comparable total protein content among the different treatment groups. This observation agreed with Allison (1955) and Anon (1980) who reported changes in protein reserve in animal and indicated that serum total protein is associated with alterations in albumin fraction.

The values of the blood glucose as shown in table 2 ranged from 2.5 -3.87mmol/L. These values were within the normal range (2.0-3.0 mmol/L) reported by Njidda *et al*. (2014).

The urea values obtained in this study were close to the average (4.40 mg/dl) reported by Njidda *et al*. (2014) for balami rams. The decrease in the blood urea concentration of the animals across the treatments might be due to good ammonia utilization. The higher value obtained in serum urea for animals on control diet was an indication poor of efficiency of utilization of nitrogen and urea recycling and could affect the amino acid balance. The blood urea levels in the study were within the recommended limits and this suggests that the kidneys and liver of the animals were normal. Creatinine values obtained ranged 75.33- 85.67 µ/L for T3 and T2, respectively, which fell within the normal range (Davis and Burndt, 1994).The urea and creatinine concentration in the blood were used for kidney function (Davis and Burndt, 1994).

Serum cholesterol values obtained ranged from 62.67- 85.00 mmol/L for T2 and T1 respectively. The values of cholesterol obtained in the study were within the normal range reported for healthy ewes (Mitruka amd Rawnsley, 1977).

The serum aspartate amino transferase (AST) and serum alanine amino transferase (ALT) activity values observed in this study were within the normal range (40.0-123 IU/l) and (25.0– 70 IU/l) respectively reported for healthy ewes (Mitruka amd Rawnsley, 1977). The serum alkaline phosphatase (ALP) activity did not show significant differences among treatments, and values were not within the normal range (IU/l) reported for clinically healthy ewes (Mitruka amd Rawnsley, 1977).

CONCLUSION

It was concluded that garlic extract has significant effect on reducing serum cholesterol without negative effect on blood composition.

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