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Haematogical Indices and Serum Biochemistry of Sheep Fed a Concentrate Diet Supplemented with Graded Levels of Exogenous Enzyme in the Semi-Arid

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Abstract- The study was conducted to determine the effect hematological indices and serum biochemistry of Sheep fed a rich diet supplemented with graded level of exogenous enzyme in the semi-arid region of Nigeria. Twenty Sheep of the non-descript breed used for the study. The animals were weighed, and divided into four groups. Each group of 5-animals randomly allotted to one of the 4-treatments in a Completely Randomized Design. The exogenous enzyme included in the diets at the level of 0, 200, 400, and 600g in TI, (Control), T2, T3 and T4, respectively. The result showed that PCV, Hb, WBC, and Neutrophil differed considerably ($P<0.05$) among the treatments, while RBC, MCV, MCH, MCHC, Eosinophils and lymphocyte were not significantly, ($P>0.05$) different between the treatments. There were significant ($P<0.05$) differences among the treatments in blood urea, Alanine Amino Transferase and Alkaline phosphate, while creatinine, Total Protein, Albumin, Globulin and Aspartate amino Transferase were not significant ($P>0.05$) among the treatment groups. However, the level of inclusion of an exogenous enzyme at 400g/100kg of corn cob based diets showed the best result. The utilization of the corn cob based diet treated with exogenous enzyme, had no deleterious effect on the health condition as demonstrated by the serum biochemical and hematological parameters of the Sheep.

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Abstract- The study was conducted to determine the effect hematological indices and serum biochemistry of Sheep fed a rich diet supplemented with graded level of exogenous enzyme in the semi-arid region of Nigeria. Twenty Sheep of the non-descript breed used for the study. The animals were weighed, and divided into four groups. Each group of 5-animals randomly allotted to one of the 4-treatments in a Completely Randomized Design. The exogenous enzyme included in the diets at the level of 0, 200, 400, and 600g in TI, (Control), T2, T3 and T4, respectively. The result showed that PCV, Hb, WBC, and Neutrophil differed considerably (P<0.05) among the treatments, while RBC, MCV, MCH, MCHC, Eosinophils and lymphocyte were not significantly, (P>0.05) different between the treatments. There were significant (P<0.05) differences among the treatments in blood urea, Alanine Amino Transferase and Alkaline phosphate, while creatinine, Total Protein, Albumin, Globulin and Aspartate amino Transferase were not significant (P>0.05) among the treatment groups. However, the level of inclusion of an exogenous enzyme at 400g/100kg of corn cob based diets showed the best result. The utilization of the corn cob based diet treated with exogenous enzyme, had no deleterious effect on the health condition as demonstrated by the serum biochemical and hematological parameters of the Sheep.

I. Introduction

Ruminant animals play a significant role in the agricultural economy of Nigeria. With their inherent qualities thriving under harsh environments and low capital investment by the agricultural farmer under the free-range system. Ruminants act as an insurance against crop failures and provide alternative sources of livelihood to the farmers all year round (Selvamand Safiullah, 2002).

The feed resources that provide the bulk of ruminant feed in the semi-arid zone of Nigeria include natural grasses and crop residues. These characterized by low intake and digestibility. The general awareness on the use of exogenous enzyme is due to their ability to increase the efficiency of digestion by improve the digestibility and feed energy (Anon, 2015). The exogenous proteins, like other feed enzymes, are of natural origin and non-toxic. They are mostly commercial products of microbial fermentation which are safe, inexpensive and straightforward, substantial, reliable and agro-industrial resources (Bhat and Hazlewood, 2001)

The use of blood examination is a way of assessing the health status of animals has been documented (Muhammad *et al*., 2000). It plays a vital role in the physiological, nutritional, and pathological state of organisms. Enzyme addition tends to increase the differential lymphocyte count (Colombatto *et al*., 2003). The objective of this study is to determine the performance and blood indices of Sheep fed concentrate diet supplemented graded level exogenous enzymes.

II. Materials and Methods

a) Experiment Site

The study was carried out at the Department of Animal Science Teaching and Research Livestock Farm, University of Maiduguri. Maiduguri, the capital of Borno state, is situated between latitude 11°51' North, Longitude 13° 5' East and an altitude, of 354m above sea level (DNMA, 2013). The area falls within the Sahelian region (semi-arid zone) of West Africa, which is characterized by the short duration of rainfall (3-4 months). The rainfall varies from a minimum of 478mm-500mm to maximum of 600mm-621mm (Afolayan *et al*., 2012). The relative humidity (RH) is 32% in the morning. The minimum relative humidity (RH) is 11% in March, and the maximum is 64% in August (Afolayan *et al*., 2012; DNMA, 2013). The mean temperature is 34℃, the maximum being 40-60℃, and the lowest 25℃, which is in April and December, respectively. The average dew point is 52%, the minimum being 32% in February, the maximum is 72% in August (Afolayan *et al*., 2012; DNMA, 2013).

i. Animals and Experimental design

Twenty (20) Sheep of the non-descript breed weighing on average 22.65kg used. The animals obtained from the flock of Sheep kept at the Department of Animal Science Livestock Teaching and Research

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Farm, University of Maiduguri. They were weighted and identified using plastic air tags. Feeding was done at 4% body weight once daily at 8:00 am with the leftover being weighed before the next feeding. The animals divided into 4-groups, and each group of 5-animals randomly assigned to one of the treatment in a completely randomized design (CRD). The study lasted for 11 weeks.

ii. *Treatments (Experimental diets)*

The feeding ingredients used for the formulation of the experimental diets were maize cob, wheat offal, cottonseed cake, poultry litter, and exogenous enzymes. The diet formulated consisted of maize cob (40%), wheat offal (30%), cotton seed cake (15%), poultry litter (15%). The enzyme included in the diet a level of 0, 200, 400 and 600g in T1 (control), T2, T3 and T4 respectively.

Table 1: The composition of the Experimental Diets (%)

Ingredients	Treatments and level of Enzyme supplementation (g)			
	T1(0)	T2 (200)	T3 (400)	T4 (600)
Corncob	40	40	40	40
Wheat offal	30	30	30	30
Cotton seed cake	15	15	15	15
Poultry litter	15	15	15	15
total	100	100	100	100

iii. *Blood Sample Collection*

a. *Blood Sample collection and analysis*

At the end of the feeding trial, three Sheep randomly selected from each treatment. The blood sample was collected from three (3) animals per treatment on the last day of the study before terminating the experiment. Blood samples were collected from each animal by jugular-venipuncture using a disposable syringe and sterile needles (18 gauge). Before feeding in the morning, bleeding done and an average 0f 10ml of blood was collected from each animal by jugular-venipuncture using disposable syringes and sterile needles (18 gauge). Before feeding in the morning bleeding done, and 10 ml of blood collected from each animal. The blood sample placed in two vacutainers. One contained ethylene diamine tetra-acetic acid (EDTA) for hematological 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 there that centrifuged at 700xg for 15 minutes, the serum separated were decanted and stored in a freezer at -10C for blood the biochemical analysis as reported by Gambo *et* al. (2011). MCV, MCH and MCHC were deduced according to Jain (1986) as follows: MCV (*Fl*) = PCVX10/RBC; MCH (*pg)* = Hb10/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* and *Bromocresol Green Methods,* respectively. Blood Urea Nitrogen (BUN), Creatinin, Bilirubin as well as activities of the liver enzymes (AST and ALT) were determined by Enzymatic method as outlined by Bush (1991). Burchadrection determined serum Cholesterol

b. *Statistical Analysis*

The data generated were subjected to analysis of variance (ANOVA) using the complete randomized design (CRD), and the Duncan multiple range tests, was used for the mean separation.

III. Results and Discussion

Table 2: Hematological Parameters and differential count of Sheep supplemented with graded levels of the enzyme

Parameters	Treatments and level of Enzyme supplementation (g)				
	T1(0)	T2 (200)	T3 (400)	T4 (600)	SEM
PCV (%)	25^{b}	32^{a}	28^{b}	28^{b}	1.0*
Hb(g/dl)	8.30^{b}	10.53^{a}	9.30^{b}	9.20^{b}	0.33*
RBC ($\times 10^{6}$)	11.67	13.53	12.22	10.60	1.01^{NS}
MCV (fl)	22.12	23.65	22.91	26.42	2.00^{NS}
MCH (pg)	7.11	7.78	7.62	8.67	0.80^{NS}
MCHC (%)	33.20	33.91	33.21	32.85	7.89^{NS}
WBC ($\times 10^{3}$)	14.13^{a}	10.67^{ab}	7.60^{b}	9.67^{ab}	1.87*
Neutrophil (%)	66.67^{a}	48.67^{b}	51.00^{ab}	65.33^{a}	4.81*
Lymphocyte (%)	33.33	49.33	47.00	33.67	5.2^{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;

a) *Hematological Parameters and differential count of Sheep supplemented with graded levels of the enzyme*

The result of the hematological parameter of Sheep is present in table 1. The PCV, Hb, WBC, and Neutrophil differed significantly (P<0.05) among the treatments, while RBC, MCV, MCH, MCHC, Eosinophils and lymphocyte were not significantly(P<0.05) different between the treatments.

The PCV value of Sheep supplemented with varying levels of exogenous enzyme ranged from 25.0 to 32.0 %. The PCV values obtained in the study were in contrast with the value 43.8+ 0.6% reported by Egbe-Nwiyi*et al*, (2000), while in line within the range value (27- 45%) as published by Jain. (1993) Packed cell volume is essential in the diagnosis of anemia (Chineke *et al*., 2006). The higher PCV values obtained in this study might likely be a sign of healthier Sheep.

The hemoglobin of the Sheep ranged from 3.30 to 10.53 g/dl. The hemoglobin values were in the normal range (8-16g/dl) of hemoglobin for healthy Sheep (Greenwood, 1977).

The red blood cell count obtained in this study ranged from 10.60 to 13.53 g/dl. The values were higher than the range value (4.44 – 8.69 g/dl) reported by Njidda *et al*. (2014). Red blood cell provide information about the hemoglobin content and size of red blood cells. Mean corpuscular volume, Mean corpuscular hemoglobin, Mean corpuscular Hemoglobin Concentration (MCHC) had showed no significant (p> 0.05) differences among the treatments. The values obtained for all the treatment groups indicate nutritional adequacy of all diets. Since the benefit did not indicate mal-or-under nutrition (Church *et al*. 1984). Mean corpuscular volume (MCV) values obtained ranged from 22.12 – 26.42 fl. According to Research Animal Resources (2009), the values fell within normal physiological range 23-48f*l* for Sheep. But reports of Borjesson *et al*. (2000) which is lower than the normal physiological MCV range for Sheep (35.3 – 43.7 f*l*). The value of mean corpuscular hemoglobin obtained in the study ranged from 7.11 to 8.67 pg T0 and T3 respectively, which were also lower than the values for lamb (12-20 pg) as reported by Njidda *et al*. (2014), but the study is inconformity with result 8-12 pg according to Research Animal Resources (2009). The value of MCV and MCH are significant in the diagnosis of anemia and also serve a useful index of the capacity of the bone marrow to produce red blood cells (Awodi *et al*., 2005). Mean Corpuscular Hemoglobin Concentration obtained in this study fell within the range of 31-38% as reported by Research Animal Resources (2009). Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration low level in blood is an indication of anemia (Aster, 2004).

The white blood cell values for the Sheep ranged from 7.60 to 14.13. The white blood cell (WBC) values were within the average range values of Sheep (4-12%) reported by Research Animal Resources (2009). The higher WBCs count recorded in the Sheep not

supplemented with the enzyme may be due to the response of the animals to protect themselves against invading pathogens. This study shows that the animals were healthy because, the decrease in number of WBC below the normal range is an indicator 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 9: Effect of Graded Level of the Exogenous Enzymes inSheep fed a concentrate diet

Parameters	Treatments and level of Enzyme supplementation (g)				
	T1(0)	T2 (200)	T3 (400)	T4 (600)	SEM
Total protein g/l	61.66	57.66	57.66	63.33	1.21 ^{NS}
Albumin g/l	38.00	31.33	35.00	35.33	1.44 ^{NS}
Globulin g/l	23.66	26.33	24.66	28.00	1.17^{NS}
Urea	4.20^{c}	5.53^{b}	3.83^{c}	6.23^{a}	0.30*
Creatinine	85.66	101.00	94.66	101.33	2.83 ^{NS}
ASAT	74.66	62.00	66.66	69.33	3.95 ^{NS}
ALAT	21.00 ^{b}	44.66^{a}	38.66^{a}	41.66^{a}	3.29*
ALK-phos	124.00^{a}	60.33 ^{b}	116.66^{a}	80.66 ^{b}	8.93*

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, AST – Aspartate Aminotransferase, ALT – Alanine Aminotransferase.

b) Serum Biochemistry Indices

The result of the serum biochemical indices of Sheep fed graded levels of the exogenous enzyme is presented in table 9. There was significant (P>0.05) difference among the treatment groups in blood urea.

Total protein values were not significant (P>0.05) among the treatments groups. The Total protein values ranged from 57.66 to 63.33 (g/L). The result of total protein in this study fell within the normal range (59-78g/l) as reported by Latrimer *et al*., (2003). Albumin values were not significantly (P>0.05) different among the treatment groups. The albumin values ranged from 31.33 to 38.00g/l in T2 and T1, respectively. The value recorded in this study fell within the range of 27-37g/l as reported by Latrimer *et al*., (2003). Globulin value was not significantly different (P>0.05) among the treatment groups. The globulin values ranged 23.66 to 28.00 g/dl in T1 and T4, respectively; T4 had the highest amount of globulin. This result could probably be due to the enzyme level in the various treatment groups. Globulin in the blood can be diagnostic of malfunction in the body or specific disease, such as liver disease (Anon, 1980). The values in this study fell within the average range 32-50 g/dl as reported by Latrimer *et al*., (2003). The normal values for albumin, total protein, and globulin obtained in this study indicate nutritional adequacy of the dietary protein for utilization (Apata 1990).

The values of urea were significantly different (P<0.05) among the treatment groups. In this study, the urea values ranged from 3.83 to 6.23 mmol/l for T3 and T4, respectively. The blood urea values fell within the normal range (3.5 – 10.7mmol/l) for a healthy animal, according to Sirois, (1995). The highest value (6.23) of blood urea obtained in this study is an indication of the inferiority of efficient utilization of nitrogen and urea recycling which might have affected the amino acid balance (Cetin *et al*., 2009). It probably enzyme helps in the breakdown cell wall and increase amino acid content. The creatinine values were not significantly (P>0.05) different among the treatment groups. Creatinine values ranged from 85-66 to 101.33 mmol/l of T1 and T4, respectively. The result of thisstudy is in contrast with the value 62.56 reported by Njidda (2014). The result is in agreement with Latrime *et al*., (2003) who said the blood creatinine level of 76-174 mmol/l in Sheep. High creatinine value is indicator of inadequate protein and amino acid metabolism that can lead to impaired and cardiac infarction (Gray and Howarra, 1980). The high level of corn cob in the diet which contains lignin and probably might have had an effect on the Sheep.

Aspartate Amino Acid Transferase (ASAT) values were not significantly (P>0.05) different among treatment groups. T1 (control) had the highest value of 74.66 lu/l, while T2 T3 and T4, had lower values of Aspartate Amino Transferase level in the blood. The values of Aspertate Amino Transferase fell within the average range value (40.0-123) lu/l for healthy animals (Mitruka and Rawnsley, 1977) ASAT indices is used for diagnosing hepatic damage in animals (Mohgoub *et al*., 2008).

Alanine Amino Transferase (ALAT) in the blood were significantly different (P<0.05) among the treatment groups. The ALAT values ranged from 21.00 to 44.66lu/l in T1 and T2, respectively. The values fell within the normal range of 15-44 lu/l as reported by Latrimer*et al*., (2003). T2 had the highest value of ALAT, while the lowest value recorded in T1 (control). ALAT is a liver-specific hepatocellular enzyme used to assess liver

damage (Mahgoub *et al*., 2008). The inclusion of the enzyme might probably affect the ALAT value of the animal.

Alkaline phosphate values were significantly different ($P<0.05$) among the treatment groups. The alkaline phosphate values ranged from 80.66 to 124.00 lu/l in T4 and T1. T1 (control) had the highest value of alkaline phosphate, which probably might be due to the diet without enzyme. Kaneko *et al*., (1997) reported higher values of Alkaline phosphate which were found in the 40 days of life, due to more intense bone remodeling and leakage of the enzyme from the growing bone and intestine into the blood.

IV. Conclusion

The utilization of the corn cob based diet treated with an exogenous enzyme. The study showed that no deleterious effect on the health conditions as determined by the serum biochemical and hematological parameters of the Sheep. However, the result showed that the level of exogenous at 400g/100kg of diet had the best result. The nutritive value of poor quality crop residue can be proved through the use of exogenous enzymes. Further research should be conducted to ascertain the level of inclusion of exogenous enzyme in ruminant diets.

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