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Effect of sampling time on Haematological Characteristics of Weaner rabbits fed diets containing differently processed *Crotalaria retusa* Leaves

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Target Audience: Animal scientists: livestock farmers;

Abstract

Three (3) processing methods; shade drying, oven drying and ensiling were used to investigate the effects of processing methods on the haematological profile in rabbits. Twenty four (24) weaner rabbits of mixed breeds with an average weight of 658.12g were randomly allocated to four (4) dietary treatments with six (6) rabbits per treatment in a Completely Randomized Design experiment. The leaves of Crotalaria retusa were harvested fresh and divided into three portions of 4kg each. The first portion was ensiled in a polyethene bag for 14 days, the second portion was oven dried at 60^{o} C for six (6) hours while the third portion was shade dried for three (3) days and then included in the experiment at 5% across all treatments except diet the control (which contained 0% C. retusa leaves). The experimental diets and clean drinking water were supplied to the rabbits ad libitum. Blood samples were taken at the commencement of the experiment (week 0), and subsequently at the fourth and eighth week respectively. Processing methods affected the proximate composition of the experimental diets. The ensiled Crotalaria retusa leaves recorded the highest Crude protein content (17.86%) and nitrogen free extract (57.52%). The Blood parameters measured were significantly (P<0.05) influenced by processing methods at different sampling time. Packed Cell Volume (PCV) of blood sampled at 4 and 8weeks were generally higher in rabbits fed ensiled C. retusa leaves diets. Total protein (TP) were generally higher in rabbits fed ensiled C. retusa leaves diets at all sampling time. Based on these results, growing rabbits could tolerate up to 5% processed Crotolaria retusa leaves without adverse effects on their haematological parameters.

Key words: *Crotalaria retusa,* Haematology, Processing methods, sampling time, Weaner Rabbits.

Description of Problem

The need to increase livestock production as a means of alleviating the overwhelming shortage of animal protein is very vital to humanity [1]. The demand for protein of animal origin in Nigeria is greater than the supply [2]. There is therefore acute shortage of animal protein in the diet of many Nigeria, demanding that effort should be

directed to livestock that are prolific and have short gestation interval such as rabbit. This is because livestock like cattle, sheep, goats and pigs take longer period to mature [3]. Many investigators have suggested ways of increasing the low animal protein intake of Nigerians. [4] reported that rabbits are being used in Nigeria as a valuable source of animal protein in rural communities and for scientific research in academic institutions. Rabbits have the ability to convert feedstuff such as forages, most agricultural by-products, kitchen wastes, etc that human beings cannot consume directly into highly nutritious meat.

The potentialities of rabbit rearing are that the cooked meat has a high nutritional value with high protein (56%), low fat (9%), low cholesterol, sodium and calories (8%) and contain 28% phosphorus, 13% iron, 16% zinc, 14% riboflavin, 6% thiamin, 35% B_{12} and 48% niacin – making it ideal meat for hypertensive patients. Also rabbitary requires comparatively low level of capital set up; requires a little space and is well-adapted to domestic rearing [5]. However, there is the need to urgently incorporate non-conventional feed stuff in rabbit diet for optimum performance and reduction in cost of production to make rabbit consumption viable and solve the problem of scarce and expensive conventional feed stuff [6]. One of such non-conventional feedstuff is the leaves of *Crotalaria retusa. Crotalaria retusa* Linn. is usually known as rattle box plant and is a leguminous plant. It belongs to the genus Crotalaria and it is widely found in tropical Africa in open places from mountains to semi-deserts with over 500 species among which is *Crotalaria retusa* L. [7]. In addition, [8] reported that the genus *Crotalaria* is distributed and grow/occur naturally throughout the ecological zones of Nigeria. They could be found in savanna and open forest areas usually waste places, cultivated and/or open areas. [9] reported that most of the species are annuals, semi-perennials with few perennials herb or shrubs, and they establish during the late rains (August – September). Similarly, [10] reported that some *Crotolaria* species(*C. retusa* L ., *C. lachnosema, C. Naragutensis)* in Zaria, Nigeria are used as forage for feeding of sheep, goats and cattle are allopatric in nature.

The examination of blood provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutritional and pathological status of an animal. Animals with good blood composition are likely to show good performance [11]. Laboratory tests on the blood are vital tools that help detect any deviation from normal in the animal's body [12]. According to [13] changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors. This study examined the effects of three different processing methods of *Crotalaria retusa* leaves on the haematological parameters of weaner rabbits.

Materials and Methods

Experimental site

The experiment was carried out at the Rabbitary Unit of the Teaching and Research Farm of the Department of Animal Science, Ah madu Bello University, Samaru, Zaria, Kaduna State, Nigeria. The area is located in the Northern Guinea Savannah zone of Nigeria and is situated on latitude 11°12'N and longitude 7 °37'E at an elevation of 610m above sea level [14]

Experimental materials

The leaves of *Crotalaria retusa* were collected from the Institute for Agricultural Research (IAR) research farm Zaria, Nigeria. The leaves were separated from the stalks and divided into three (3) portions of 4kg and then subjected to three (3) different processing methods which include: shade drying, oven drying and ensiling.

Shade drying

The harvested *C. retusa* leaves were air-dried under the shade for three (3) days until when the leaves becomes crisp to preserve it nutritive value as much as possible. The samples were then ground using hammer mill and stored in an air tight plastic bags before taken to the laboratory for proximate analysis.

Oven drying

The leaves were oven dried at 60° C for six (6) hour as over exposure to heat can make them lose their nutrient content. Samples were taken from the over dried leaves and then ground using hammer mill and stored in an air tight plastic bags in readiness for proximate analysis.

Ensiling

Fresh leaves of *Crotalaria retusa* were ensiled by putting them in a polyethene bag which was properly tied to exclude air. The sample in the bag was then transferred into an air-tight container with cover and then buried in the ground for fourteen days. Samples were also taken for proximate analysis after the ensiling process.

Experimental animals and management

A total of twenty four weaned rabbits of mixed breeds aged between 6 to 7 weeks old with an average weight of 658.12g were obtained from the Livestock farm, Department of Animal Science, Ahmadu Bello University Zaria, Nigeria were used for this study. The rabbits were certified apparently healthy and were identified with tags and then weighed individually. Six rabbits per treatment were housed individually in rabbit hutches made of wooden frames and wire mesh for easy management. The experimental diets and clean drinking water was provided *ad libitum*. The experiment lasted for 63 days.

Experimental design and data collection

The experiment was a Completely Randomized Design (CRD) with four treatments. The treatments consisted of the control which contained 0% *C. retusa* leaves, 5% ensiled *C. retusa* leaves, 5% oven dried *C. retusa* leaves and 5% shade dried *C. retusa* leaves representing T_1, T_2, T_3 and T_4, respectively, with six rabbits randomly assigned to each treatment. Blood sample was collected from each of the rabbit in the treatment groups before the commencement of the experiment(0 Weeks), 4 weeks and 8 weeks of the

experiment using a 2ml plastic syringe through the marginal ear vein into well labeled sample bottles that contained ethylene diamine tetra-acetic acid (EDTA) as anticoagulant. The packed cell volume (PCV), white blood cell (WBC) and the haemoglobin (Hb) concentrations were measured using the Wintrobes Microhaematocrit, improved Neubauer haemocytometer and Cyanomethaemoglobin methods respectively [15]. Similarly, blood samples collected without anti coagulant were used for the determination of total protein using commercially available analytical kits. The proximate analyses of the diets were determined according to methods decribed by [16].

Table 1: Ingredient composition and calculated analysis of experimental diets fed to weaner rabbits.

	Dietary Treatments			
	1	2	3	4
Ingredients (%)	**Control**	**Ensiled *C. retusa***	**Oven-dried *C. retusa***	**Air-dried *C. retusa***
Maize	38.00	36.00	36.00	36.00
Maize Offal	10.00	10.00	10.00	10.00
Rice Offal	21.00	20.00	20.00	20.00
Groundnut cake	15.20	11.20	11.20	11.20
Blood Meal	5.00	5.00	5.00	5.00
BDG	7.00	9.00	9.00	9.00
Crotalaria retusa leaves	0.00	5.00	5.00	5.00
Bone Meal	5.00	5.00	5.00	5.00
Salt (Na Cl)	0.30	0.30	0.30	0.30
Lysine	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**
Cost/kg (₦)	**59.10**	**51.65**	**51.65**	**51.65**
***Calculated Analysis* (%)**				
Crude Protein	17.09	17.57	17.39	17.35
Crude Fibre	12.08	13.09	12.73	12.36
Ether Extract	4.06	4.32	4.19	4.12
ME (kcal/kg)	2,527	2,516	2,522	2,517
Ca:P	1:7	1:7	1:7	1:7
Lysine	0.88	0.87	0.83	0.83
Methionine	0.70	0.67	0.60	0.60

Key: BDG = Brewer's Dried Grain; ME = Metabolizable Energy; Ca:P = Calcium -Phosphorus ratio

Results and Discussion

Proximate composition

The proximate composition of the experimental diets and the differently processed leaves of *Crotalaria retusa* is shown in Table 2. The dry matter (DM) content of the experimental diets ranged from 91.28% in treatment containing the shade dried *C. retusa* leaves to 94.38% in treatment containing the oven dried leaves while the DM content of the processed *C. retusa* leaves ranged from 93.60% in the ensiled leaves to 97.90% in the oven dried leaves. The dry matter content of the differently processed *C. retusa* leaves obtained in the present study compared favourably with the findings of [9] who reported a DM

values ranging from 96.67 to 99.42% of rattle box plant. The differences observed in the DM content of the processed leaves could be due to the processing methods adopted.

Table 2: Proximate composition of the experimental diets and processed *C. retusa* leaves

	Dietary Treatments				Processing methods		
Parameters	T_1	T_2	T_3	T_4	ECRL	OCRL	SCRL
Dry matter	93.66	94.18	94.38	91.28	93.60	97.90	95.06
Crude protein	16.32	16.58	17.16	17.18	17.86	16.14	16.48
Crude Fibre	13.48	13.21	14.07	13.59	12.60	14.67	14.87
Ether extract	3.49	3.66	3.36	3.92	0.96	0.89	0.62
Ash	11.20	10.61	12.48	12.06	3.26	3.68	3.40
NFE	55.51	55.94	52.93	53.45	57.52	52.12	52.67

Key: ECRL = Ensiled *C. retusa* leaves, OCRL = Oven dried *C. retusa* leaves, SCRL = Shade dried *C. retusa* leaves. NFE= Nitrogen free extract

On dry matter basis, crude protein (CP) content of the experimental diets ranged from 16. 32% in the control diet to 17.18% in the diet containing the shade dried *C. retusa* leaves. The CP values reported in the present study for oven dried and shade dried *C. retusa* leaves (16.14 and 16.48%) respectively, were slightly higher than 15.44 and 14.00% reported by [9] for oven and shade dried *C. retusa* leaves respectively, the differences could be due to season of harvest. In the present study, the CP is within the range of 9.0 – 29.7% reported by [18] for growing rabbits in tropical environments.

The crude fibre (CF) values of the experimental diets ranged from 13.21% in the ensiled to 14.07% in the diet containing oven dried leaves. The CF of the differently processed *C. retusa* leaves alone ranges from 12.60% in ensiled to 14.87% in the shade dried leaves. The values of the CF content reported in this study were lower than the (24.42 and 29.91%) reported by [9] for oven and shade dried *C. retusa* leaves. Variations in the crude fibre contents of the processed leaves might be due to leafiness, time of harvest and processing methods adopted. Levels of 13 – 45% CF content is known to be satisfactory for growing rabbits (Lebas *et al*., 1986). Generally, the CF content of the experimental diets are within the recommended range of 10 – 20% for optimum growth and performance of rabbits [19].

The nitrogen free extract (NFE) content of the expermental diets varied from 52.93% in the diet containing the oven dried *C. retusa* leaves to 55.94% in the diet containing the ensiled leaves. The control and ensiled diets recorded the highest NFE content of (55.51 and 55.94%) respectively. [9] reported similar values of (52.26%) NFE content in oven dried leaves of the same plant. The high content of NFE in the experimental diets is an indication that the plant material have higher energy content because NFE consists of mainly

sugars and starch among other components. The variability in the nutrient content of forage legumes like *Crotalaria retusa* have been attributed to the state of hydration (fresh, wilted, dry) and drying procedure [20] age of cutting, season and geographical location [21].

Haematological parameters

The results of the haematological indices of rabbits at the commencement of the study is presented in Table 3. There were significant differences ($p<0.05$) among the treatment groups for all the haematological parameters measured, except for packed cell volume (PCV) and haemoglobin (Hb). The PCV values (41.33 to 49.33%) were within the range of 30 – 50% reported by [22] and 33 to 50% reported by [23] for growing rabbits. The white blood cell (WBC) count ranged from 4.71 to 9.23 $x10^3/mm^3$, the values were within the range of 5.0 to 13 $x10^3/mm^3$, reported by [23] for healthy young rabbits. These results indicate that the animals were healthy at the beginning of the experiment because decrease in number of WBC below the normal range is an indication of allergic conditions, anaphylactic shock and certain parasitism, while elevated values (leucocytosis) indicate the existence of a recent infection, usually with bacteria [24].

The haemoglobin (Hb) concentration compared favourably with the values of 10 to 15 g/dl reported by [25] and 8.0 to 17 g/dl reported by [26]. Total protein (TP) values (5.03 to 5.09 g/dl) were within the range reported by [27] but lower than 5.81 – 6.75 g/dl reported by [28]. The neutrophil and lymphocyte values show significant difference ($p<0.05$) among treatment groups. The neutrophil values for all the treatment groups were lower than the values reported by [29] while the lymphocyte values agrees with the findings of [25].

Table 3: Haematological characteristics of rabbits fed the experimental diets at 0 week.

Parameters	Dietary Treatments processed *C. retusa* leaves				SEM
	Control	Ensiled	Oven dried	shade dried	
PCV (%)	41.83	41.33	42.66	49.33	0.75
WBC ($x10^3$/ mm^3)	6.58^{ab}	4.71^{b}	8.30^{a}	9.23^{a}	0.42
Hb (g/dl)	13.91	13.73	12.86	13.70	0.30
Total Protein (g/dl)	5.52^{ab}	5.28^{ab}	5.03^{b}	5.90^{a}	0.12
Neutrophils (%)	18.16^{b}	20.00^{ab}	21.66^{a}	21.00^{ab}	0.46
Lymphocytes (%)	80.00^{a}	79.50^{ab}	76.00^{b}	77.83^{ab}	0.61

^{abc} Means in the same row having different superscripts are significantly different ($P<0.05$)

Key: PCV = Packed Cell Volume, WBC = White Blood Cell, Hb = Haemoglobin, SEM = Standard Error of Mean;

The results of the haematological indices for the growing rabbits fed the experimental diets at 4 and 8 weeks are presented in Table 4 and 5, respectively. All the blood parameters measured (PCV, WBC, Hb, TP, Neutrophil and Lymphocyte) for rabbits on all the treatment groups fell within normal ranges (Table 4 and 5). Among treatments diets, PCV value obtained for rabbits fed ensiled leaves *Crotalaria retusa* ranged from 49.00% at the fourth week of age to 45.60% at eighth week of age. However, this figure did not differ

significantly ($p>0.05$) from the normal range of 30 – 50% reported by [25]. This suggest that ensiling method was good enough for detoxification of *C. retusa* leaves as may have been demonstrated in the normal PCV range values observed for the growing rabbits.

The WBC count ranged from 7.1 to 12.51 $x10^3/mm^3$ reported by [23] for healthy young rabbits. This showed that the experimental animals were healthy as decrease in number of WBC below the normal range is an indication of allergic conditions. The haemoglobin (Hb) concentration of rabbits at weeks 4 (13.86-17.11 g/dl) and 8 (11.70-16.60 g/dl) compared favourably with the values of 8.0 – 17.0 g/dl reported by [26] but higher than the range of 10 – 15 g/dl reported by [25]. Animals given shade dried *C. retusa* leaves had the highest Hb concentration, though it was within the normal range. The normal ranges of values for Hb observed in this experiment showed that the vital physiological relationaship of haemoglobin with oxygen in the transport of gases (oxygen and carbondoxide) to and from the tissues of the body has been maintained and was normal [30].

Table 4: Haematological characteristics of rabbits fed the experimental diets at 4 week.

	Dietary Treatments processed *C. retusa* leaves				
Parameters	Control	Ensiled	Oven dried	shade dried	SEM
PCV (%)	45.33b	49.00[a]	41.67[b]	39.00[c]	0.74
WBC ($x10^3$/ mm^3)	12.51	10.69	9.33	8.26	0.60
Hb (g/dl)	15.92[a]	16.96[a]	13.86[b]	17.11[a]	0.30
Total Protein (g/dl)	5.40[b]	5.51[ab]	5.21[b]	6.36[a]	0.65
Neutrophils (%)	18.00[ab]	21.16[a]	20.33[a]	13.83[b]	1.02
Lymphocytes (%)	80.16	79.83	79.50	82.50	1.03

[abc] Means in the same row having different superscripts are significantly different ($P<0.05$) PCV = Packed Cell Volume, WBC = White Blood Cell, Hb = Haemoglobin, SEM = Standard Error of Mean;

Table 5: Haematological characteristics of rabbits fed the experimental diets at 8 week.

	Dietary Treatments processed *C. retusa* leaves				
Parameters	Control	Ensiled	Oven dried	shade dried	SEM
PCV (%)	44.33[a]	45.66[a]	41.33[b]	37.91[c]	0.94
WBC ($x10^3$/ mm^3)	8.15	7.30	7.10	9.81	0.44
Hb (g/dl)	15.03[a]	11.70[c]	12.16[c]	16.60[a]	0.20
Total Protein (g/dl)	5.73[b]	7.32[a]	5.80[b]	7.08[a]	0.16
Neutrophils (%)	18.50[b]	22.00[a]	21.16[a]	17.83[b]	0.43
Lymphocytes (%)	80.33	81.16	77.83	80.80	0.57

[abc] Means in the same row having different superscripts are significantly different ($P<0.05$) PCV = Packed Cell Volume, WBC = White Blood Cell, Hb = Haemoglobin, SEM = Standard Error of Mean;

The highest value for total protein (7.32 g/dl) was obtained for rabbits fed the ensiled leaves of *C. retusa* and this is within the normal range reported by [31] which is indicative of availability of high quality protein in the rabbits. Serum neutrophil and lymphocyte values also differed ($p<0.05$) for treatment groups at week 4 and 8. Animals on ensiled *C. retusa* leaves had the highest values of neutrophil 21.16% and 22.00% at week 4 and 8,

respectively. However, these observed values were lower than the normal range of 32.17 – 34.50% reported by [29] for growing rabbits.

Blood lymphocytes were fairly similar ($p>0.05$) in concentration in rabbits fed ensiled and oven dried leaves of *C. retusa* and as well as in the control diet. Animals on ensiled diet had significantly higher ($p<0.05$) concentration of lymphocytes than the control group which however did not differ ($p>0.05$) from the shade dried group. Even though blood neutrophil and lymphocyte concentrations showed variation between treatment groups, the values obtained for all treatment groups were within normal range of 40 – 80% reported by [25]. The observed values for haematological parameters in this study fell within the normal range for growing rabbits. This is an indication that the experimental diet (processed *Crotalaria retusa* leaves) did not have any detrimental effects on haematological parameters during the experimental period.

Conclusion and Application

From the result of this study, it can be concluded that:

1. Processing of *Crotalaria retusa* leaves influenced the proximate composition of the leaves and the diets.
2. Haematological components which consist of PCV, WBC or leucocytes and Hb concentration are valuable parameters in monitoring feed toxicity and were within normal range at all sampling time in this study.
3. Feeding *Crotalaria retusa* to rabbits at 5% has no any deleterious effect.

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