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Effects of mistletoe leaf (*Viscum album*) aqueous extracts on performance, hematology and serum biochemistry of rabbits in a humid tropical environment

Iso Ekong Iso [1,*] and Ukam Victor Efut [2]

[1] *Department of Animal Science, University of Cross River State, Nigeria.*
[2] *Department of Agricultural Education, Cross River State College of Education, Akamkpa-Nigeria.*

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Abstract

The study examined the physiological effects of mistletoe leaves Aqueous Extracts (MLAE) on the performance, haematology and some serum biochemistry of rabbits. Thirty New Zealand white x Chinchilla weaned rabbits of 28days old were matched for weight, sex and randomly assigned to five groups (n=6 per-group), sub-divided into two replicates of three rabbits each. The basal diet was forages *Tridax procumbens* and *Ipomea batata* leaves 20 g morning and evening daily as common rabbit feed available in the environment. The rabbits were served twice daily with mistletoe leaves aqueous extracts 200mls of 20g dried leaves from four host trees and water 200 ml twice daily as control. All rabbits were fed *ad libitum* throughout the ten weeks period of the study. The proximate results revealed that mistletoe leaves from the four host trees were rich in crude protein, crude fibre and ash, which provided protein and minerals supplement for rabbits' performance. Data obtained were subjected to one-way ANOVA and significant means separated using Turkey's Comparision Test. Results on performances showed significant($P<0.05$) difference between treatments indicated with the following ranges: final body weight (1220.00–1490.00g), daily weight gain (9.86-11.40g/d). Feed conversion ratio (0.11-0.86), total feed intake (4122.00–4392.00), total water and mistletoe aqueous extracts (8974.00-11627.00mls), Heamatology and serum biochemistry analysis results revealed significant ($\rho<0.05$) difference between treatments compared to the control. The differences between the treatments could be attributed to mistletoe and their host trees, since mistletoe are hemi-parasites, depend partly on their host for nourishment. Extracts yielded good rabbit performance and blood physiological response, hence it could be used for forage–concentrate feed supplement for rabbits during dry season scarcity of forages.

Keywords: Performance; Haematology; Weaned rabbits; Mistletoe leaves; Aqueous extracts; Host trees

1. Introduction

Tropical countries are blessed with diversity of food stuffs and plants vegetables which play a basic role in human nutrition and live stocks. Some of the plants has been traditionally noted for their medicinal values based on their antibacterial and antifungal properties they possessed. However, their properties lie in some of the chemical substances present in them such as: alkaloids Lectins, tannins, saponins, flavonoids and in addition protein, vitamins and minerals, which produces physiological actions in the animal body, (Sofowora, 1993), (Edmonds et.al, 2001) and (Agrios, 1997). Studies have shown that green vegetables, browse plants are the cheapest and most abundant sources of feed stuff for livestocks, (Olawoyin, 2007), (Abdel et.al, 2014) and (Dalle et.al, 2016).

One of such is the mistletoe leaf or broad leaf mistletoe (*Viscum album* L). It is a peculiar partial parasitic plant bearing evergreen leaves that photosynthesized and on the host mainly for minerals from the soil. They are found on varieties

* Corresponding author: Iso Ekong Iso

of tree plants such as Pear, Rubber, Citrus, Kola nuts, Apple, Oak, Apple and Pine etc., (Roseville, 1995) and (Osadebe *et.al*, 2004).

A broad array of animals depends on mistletoe for food, consuming the leaves and young shoots, (Agrios, 1997) and (FAO, 2011). The practice of feeding rabbits as pseudo-ruminants involved sole forage, forage-concentrates and sole concentrate feeding. Studies revealed that animals can equally be served with aqueous extracts forms of some vegetables for nutrients, mineral supplementation to improve on performances and other physiological processes (Adelapo et.al, 2002) and (Cheeke, 1984). Hence the objectives of this study were to evaluate the effects of aqueous extracts of mistletoe (*Viscum album*) leaves on performance, hematology and serum biochemistry of weaned rabbits in a humid tropical environment.

2. Materials and method

A ten (10) weeks study period were carried out in the late dry season (January - March) in the Cross River University of Technology Research farm. The farm lies in the Central Agro Ecological zone. The mean annual rainfall was 1520-2009mm, with a mean monthly temperature of 27 °C and relative humidity of 69% within the months of the study. A total of 30 weaned rabbits (New Zealand white x Chinchilla) of both sexes averagely weighing 512.40g were procured.

The rabbits were allowed one-week preconditioning period during which they were fed growers diet. They were matched for sex, weight and distributed to five treatments in a Completely Randomized Design (CRD), each treatment had two cells with three rabbits per cell in a hutch.

2.1. Preparation of the leaves and aqueous extracts

Fresh leaves of mistletoe (*Viscum album*. L) were harvested from four host trees–Pear, Sweet Orange (citrus), Rubber and kolanut in Calabar, Southern Zone of Cross River State, Nigeria.

The leaves were air dried in a shady environment for a period of 14days. The dried leaves were milled into smaller particles of about 2mm size using an electric-blender and stored in an airtight container prior to extraction.

The aqueous extracts of mistletoe leaves from four host trees were prepared daily by soaking 20g each of mistletoe leaf in 200ml of water for 22hours.The extracts were filtered using Whatman filter paper1.The weight of dry residues were recorded and the percentage extraction yield was calculated thus:

$$\% \text{ Extract yield} = \frac{Wt.of\ dried\ mistletoe\ leaf - weight\ of\ dried\ residue}{Wt.of\ dried\ mistletoe\ leaf} \times \frac{100}{1}$$

2.2. Treatments application and analysis

The rabbits were fed the same forage (Tridax procumbens and Ipomea batata) at the rate of 100g morning and evening per rabbit per day. The four aqueous extracts of mistletoe leaves treatments of 200ml and 200ml water (Control) were served twice per cell per day through out the 70days period of the experiment.

Data on feed intake, mistletoe aqueous extracts and water intake were recorded daily, while weight gain was determined on weekly basis.

2.3. Blood collection and analysis

At the end of the 70days experiment, three rabbits were bled between 9.00am and 10.30am from a punctured ear vein per treatment. 12ml of blood samples were collected from three rabbits per experimental group. 3mls of each blood samples were discarded into labelled bijour bottles which contain Ethylene diamine tetra-acetic acid (EDTA), while the other set was without EDTA. The two samples were processed for haematology and Serum biochemistry respectively. Packed Cell Volume (PCV) was determined by microhaematocrit method, (Mitrika, et.al, 1972). Haemoglobin (Hb) concentration was measured Spectro-photometrically by Cyanomethaemoglobin method, (Jain, 1986). Using sp6-500uv Sspectrophotometer (Pyeunicom, England). The Red Blood Cell (RBC) and White blood cell (WBC) counts were estimated using haemocytometer, (Raharjo et.al, 1986). The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were calculated from Hb, PCV and RBC (SPSS Inc., 2011).

2.4. Statistical analysis

Data obtained were subjected to the one-way (ANOVA) in a Completely Randomized Design. The analysis were perform using SPSS (Santleben et.al 1999) software and errors were calculated as Standard Errors of Mean (SEM). Significant treatment means were compared using Duncan multiple range test at 5% Significant level.

3. Results and discussion

The proximate chemical composition of the mistletoe leaves in (Table 1) indicated that the leaves are rich in CP in different host trees with a ranged of 17.86%-21.64%, CF 11.70-13.68%, and Ash 23.40-25.77% respectively. Hence, mistletoe leaves could serve as valuable feed supplement for rabbits, most especially during dry season when there is scarcity of forage materials.

Table 1 Proximate composition of four host Mistletoes Leaves and Aqueous Extracts (MLAE)

Treatment									
		A		**B**		**C**		**D**	
Fraction	**PML**	**PMLAE**	**CML**	**CMLAE**	**RML**	**RMLEA**	**KML**	**KMLAE**	**SEM**
Dry matter	92.88		92.16		92.74		93.22		0.21
Moisture (%)	7.12^{c}	9.85^{a}	7.84^{c}	8.65^{b}	7.26^{c}	8.73^{b}	6.68^{c}	9.41^{a}	0.05
Crude protein (%)	18.36^{c}	22.78^{b}	17.86^{c}	24.12^{a}	18.62^{c}	25.63^{a}	21.64^{b}	27.21^{a}	0.74
Crude fibre (5)	12.86^{a}	11.25^{b}	12.75^{a}	10.86^{b}	11.70^{b}	9.45^{c}	13.68^{a}	12.14^{a}	0.35
Ether Extract (%)	1.54^{a}	0.81^{c}	1.62^{a}	0.67^{c}	1.25^{b}	0.97^{c}	1.16^{b}	0.63	0.09
Ash (%)	24.76^{a}	25.14^{a}	23.40^{b}	24.21^{ab}	25.77^{a}	24.63^{ab}	24.36^{ab}	23.41^{a}	0.85
Carbohydrate (%)	35.36^{a}	29.45^{bc}	36.53^{a}	30.21^{b}	35.40^{a}	29.72^{b}	32.48^{b}	27.41^{c}	0.75
Energy(kg/kcal)	2030.57	1954.60	2090.00	2021.12	2047.89	2082.72	2048.62	2031.36	6.35

a,b,c: means with different superscripts on the same horizontal row differ significantly($\rho < 0.05$); SEM - Standard Error of Mean; PML - Pear Mistletoe Leaf; PMLAE -Pear Mistletoe Leaf Aqueous Extract; CML -Citrus Mistletoe Leaf; CMLAE - Citrus Mistletoe Leaf Aqueous Extract; RML- Rubber Mistletoe Leaf; RMLAE - Rubber Mistletoe Leaf Aqueous Extract; KML - Kola Mistletoe Leaf; KMLAE - Kola Mistletoe Leaf Aqueous Extract

Table 2 Effects of mistletoe leaves Aqueous Extracts (MLAE) on theHaematological and serum chemistry of Rabbits

Treatment						
	A	**B**	**C**	**D**	**E**	**SEM**
Parameters	**(Water200m/control)**	**(200ml PMLAE)**	**(200ml CMLAE)**	**(200ml RMLAE)**	**200ml (KMLAE)**	**SEM**
Packed cell volume (%)	26.00^{c}	25.00^{c}	27.00^{b}	30.00^{a}	26.00	0.92
Red blood cells(x10^{b}L^{-1})	1.64^{cd}	2.18^{c}	2.45^{ab}	2.46^{a}	2.44	0.14
White blood cells (x10^{6}L^{-1})	2.40^{c}	2.49^{c}	2.90^{bc}	8.40^{a}	7.30	0.05
Haemoglobin(g/dl)	13.00^{c}	12.00^{c}	16.00^{a}	16.00^{a}	15.20	0.69
Mean cell volume(fl)	108.54^{a}	114.70^{c}	110.20^{c}	121.95^{b}	116.95	7.65
Mean cell haemoglobin (pg/cell)	50.27^{b}	55.06^{c}	65.31^{b}	65.04^{b}	63.52	3.48
Mean cell haemoglobin concentrations (%)	52.31^{b}	48.90^{c}	59.26^{a}	53.33^{b}	50.67	1.67

Total protein (g/dl)	6.40[b]	4.70[c]	5.80[b]	7.00[a]	8.20	0.52
Albumin(g/dl)	4.50[b]	3.90[c]	4.30[b]	5.50[a]	5.20	0.26
Aspartic Transaminase (AST)iu/L	23.00[c]	47.00[c]	27.00[b]	23.00[c]	27.00	2.02
Globulin(g/dl)	1.90[b]	0.80[c]	1.50	1.50	3.00	0.32
Alkaline Transaminase (ALT)iu/L	8.00[c]	4.00[c]	22.00[a]	17.00[b]	16.00	0.04
Alkaline Phosphate (ALP)iu/L	32.00[b]	26.00[c]	37.00[a]	32.00[b]	30.00[b]	1.59

a,b,c: Means with different super scripts on the same horizontal row differs significantly(ρ >0.05); PMLAE - Pear Mistletoe Leaf Aqueous Extract; CMLAE - Citrus Mistletoe Leaf Aqueous Extract; RMLAE - Rubber Mistletoe Leaf Aqueous Extract; KMLAE - Kola Mistletoe Leaf Aqueous Extract

Table 3 Performance of Rabbits Served Mistletoe Leaves Aqueous Extracts (MLAE)

Treatment						
	A	**B**	**C**	**D**	**E**	**SEM**
Parameters	**(Water200m/ control)**	**(200ml PMLAE)**	**(200ml CMLAE)**	**(200ml RMLAE)**	**200ml (KMLAE)**	**SEM**
Initial live weight(g/rabbit)	809.00c	804.00c	957.00a	870.00b	930.00a	27.67
Final live weight(g/rabbit)	1220.00c	1244.00c	1423.00c	1490.00a	1478.00b	51.86
Average body weight gain(g/rabbit)	685.00c	798.00a	747.00b	728.00bc	739.00b	16.22
Average daily weight gain(g/rabbit)	9.86c	11.40a	10.70b	10.40bc	10.60bc	0.22
Total feeds intake(g/rabbit)	421.6b	4179.00c	4392.00a	412.00c	4233.00b	62.4
Daily feed intake(g/rabbit)	60.26b	59.70c	62.74a	60.17	60.47	18.72
Feed conversion ratio	0.11c	0.24c	0.86a	0.78	0.70a	0.10
Total water and mistletoes Aqueous. Extracts(ml)intake	1219a	8974.00c	11579.40bc	11627.00b	10858.40c	91.5
Feed, Mistletoe leaves aqueous extract ratio	1:2.89	1:2.15	1:2.64	1:2.76	1:2.5	-
Average daily water and aqueous extract intake(ml/rabbit)	116.17c	128.2c	165.42a	166.10a	155.12b	10.01
Survivability (%)	100	100	100	100	100	100

a,b,c: Means with different superscripts on the same horizontal row differs; significantly(ρ <0.05).

Results of haematological, serum biochemistry concentrations of rabbits served mistletoe leaves aqueous extracts are presented in Table 2, the Haematologies showed significant ($p<0.05$) difference among the treatments compared with the control. The PCV, RBC, WBC, Hb, MCV, MCH and MCHC values ranges were 25.00-30%, 1.64-2.46x106l-1, 1.40-8.40(x106L-1), and 13.00-16.00gldl, 110.20-158.54fl, 55.06–79.27pg/cell and 48.90–59.26% respectively.The general increased in haematological indices of the rabbits are an indication of physiological response of rabbits to the extracts being rich in protein, minerals and vitamins which aided blood and helped in boosting the immune system of the rabbits against disease (Schalm *et.al*, 1975).

The values of WBC (2.40-8.40x106L-1) are within the range of 2.0x106–15.0x106L-1 reported by (Tuffery, 1995). The values of PCV (25.00-30.00%) reported here are lower than the value given by (MVM, 1986) (35-45%), but are within the range of (27.0-35.5%) reported by (RAR, 2009), (28.75-35.50%), (MVM, 1986) and (Sokunbi and Egbunike, 2000).

The RBC in the present study (1.64-2.46x106L-1) is lower than (5.0x106-7.0x106L-1) reported by MVM (1986). However, the Hb in this study (13-16g/dl) is higher than the values reported by Sokunbi and Egbunike (2000) (9.53-11.80g/100ml). The values of MCV, MCH and MCHC in this study range (110.20-121.95fl), (50.27-65.31pg/cell), and (48.90–59.26%) are higher than the values reported by of (Adewumi, et al., 2004), 95.49–106.16fl. 11.60-13.03pg/cell and 11.23-25.78%, respectively. Haematological indices differences in this study could be attributed to treatments concentrations of diets fractions like age of the rabbits. The serum albumen, globulin, total protein showed significant ($p<0.05$) difference between the treatment, this could be attributed to mistletoe leaf extracts for the physiological changes. The Aspartate amino transferase (AST) activity showed significant ($p<0.05$) increased within the range 23.00–47.00iu/l in the study, higher than reported values (1-12iu/l) by (CCAC, 1980).

In the same vein, results in Alanine Amino-Tranferase (ALT) and Alkaline Phosphate (ALP) showed significant differences ($p<0.05$). The values for (ALT) ranges between (4.00-22.00iu/l) and was lower than the reported physiological range (48.5-78.9iu/l) by (CCAC, 1980). This study showed decreased in Alanine Amino Transferase activity across treatments, an indication of normal functioning of the livers of the rabbits, based on normal physiological colour as well as other organs such as hearts and kidneys respectively.

Values of Alkaline Phosphate (ALP) in this study showed significant difference ($\rho<0.05$) between treatments, with a range of 26.00-37.00iu/l. Hence, the values were higher than values (4.10-16.2iu/l) reported by (CCAC, 1980). Performance of the rabbits served mistletoe leaves aqueous extracts (MLAE) is presented in Table3.The rabbits significantly ($\rho<0.05$) responded to the (forage diet mixed feeding) and mistletoe leaves aqueous extracts (MLAE) differently in its performance characteristics. These significance differences ($\rho <0.05$) observed in the performance indices could be attributed to the feed and nutritive values of (MLAE) which seem to be rich in protein and minerals, as the rabbits were fed the same diets and served difference aqueous extracts of mistletoe leaves. The observed differences in these treatments could be attributed to better digestion, absorption and utilization of the feed by the rabbits served with mistletoe leaves extracts which was rich in protein, minerals and could serve as supplemented forage for rabbits feeding.

Average daily water and mistletoe aqueous extract intake differed significantly ($p<0.05$) with extracts intake ranges between 128.20-166.10ml/ rabbit less than the control 116.17ml. The survivability in all the treatments was 100%.

4. Conclusion

The aqueous extracts of mistletoe leaves yielded positive effects on the performance and physiological response of the rabbits. Values of haematological indices, serum biochemistry were influenced positively by the varying mistletoe leaves aqueous extracts (MLAE) treatments.

Significant difference ($\rho <0.05$) were found between the parameters and treatments. The PCV, Hb, RBC, ALT, AST, Extracts intake and weight gain (W.G) in rabbits served rubber mistletoe leaf aqueous were significantly ($\rho <0.05$) different, followed by kolanut, citrus and pear compared to control. These differences may be due to their compositions, the host plants, since they gets nutrients from the host and precipitation from the atmospheres.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Approval was granted appropriately and the research was conducted in line with best practices for animal research in the humid tropical environment. Additionally, all experimental procedures involving rabbits adheres to humane treatment standards, minimizing suffering and distress.

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