

Effects of diet fortification with *Azadirachta indica* and *Moringa oleifera* leaf meal on parasitaemia, performance, rectal temperature and serum biochemistry of rabbits infected with *Trypanosoma brucei brucei*

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Abstract

Numerous plants are reported to have ethno-therapeutic properties; among these plants are the leaves of *Moringa oleifera* and *Azadirachta indica* used for the treatment of protozoan infections. Forty grower rabbits at approximately 70 days old were used to determine the prophylactic anti-trypanosomal effects of the dry leaf meals of *Moringa oleifera* (MLM) and *Azadirachta indica* (NLM). The rabbits were placed into eight groups of five animals each in a completely randomized design, with mean live weights of 1.2 Kg. The rabbits were allotted to four diets (D1, D2, D3 and D4) and two levels of inoculation (inoculated and uninoculated rabbits) in a 4×2 factorial arrangement of a Completely Randomised Design. Parameters monitored were performance, serum biochemistry and rectal temperature. The parasitaemia were presented as line graph for the infected rabbits. The percent mortality of the groups was evaluated and no mortality was observed for rabbits fed D4. No significant differences ($P>0.05$) were observed between treatments for growth rate, feed intake and feed conversion, except rectal temperature and total protein ($P<0.05$) in serum biochemistry. Rabbits that were uninfected with trypanosomes had ($P<0.05$) lower total protein than the infected animals except T2. It was concluded that only the 1% MLM had prophylactic anti-trypanosomal effect on serum total protein and mortality amelioration of *T. b. brucei* infected rabbits when administered alone and a combination of both MLM and NLM prevented pyrexia and mortality.

Keywords: Fortification, moringa, neem, leaves and antitrypanosomal.

Des Effets de l'enrichissement de régime avec le repas d'*Azadirachta indica* et la feuille de *Moringa oleifera* sur la parasitémie, la performance, la température rectale et la biochimie de sérum des lapins infectés par *Trypanosoma brucei brucei*



Résumé

De nombreuses plantes auraient des propriétés ethno-thérapeutiques ; parmi ces plantes figurent les feuilles de *Moringa oleifera* et d'*Azadirachta indica* utilisées pour le traitement des infections protozoaires. Quarante lapins de culture à environ 70 jours ont été employés pour déterminer les effets anti-trypanosomal prophylactiques des repas secs de feuille de *Moringa oleifera* (le 'MLM') et d'*Azadirachta indica* (le 'NLM'). Les lapins ont été placés en huit groupes de cinq animaux chacun dans une conception complètement randomisée, avec des poids vivants moyen de 1,2 kg. Les lapins ont été attribués à quatre régimes (D1, D2, D3 et D4) et deux niveaux d'inoculation (lapins inoculés et non inoculés) dans un arrangement factoriel de 4×2 d'un Désigne complètement randomisé. Les paramètres surveillés étaient la

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performance, la biochimie sérique et la température rectale. La parasitémie a été présentée comme graphique linéaire pour les lapins infectés. La mortalité en pourcentage des groupes a été évaluée et aucune mortalité n'a été observée chez les lapins nourris au D4. Aucune différence significative ($P>0,05$) n'a été observée entre les traitements pour le taux de croissance, l'apport alimentaire et la conversion des aliments pour animaux, à l'exception de la température rectale et de la protéine totale ($P<0,05$) dans la biochimie sérique. Les lapins qui n'étaient pas infectés par les trypanosomes avaient ($P<0,05$) moins de protéines totales que les animaux infectés, à l'exception du T2. On l'a conclu que seulement le MLM de 1% a eu l'effet anti-trypanosomal prophylactique sur la protéine totale de sérum et l'amélioration de mortalité des lapins infectés de *T.b. brucei* une fois administrés seuls et une combinaison de MLM et de NLM a empêché la pyrexie et la mortalité.

Mots-clés: Fortification, moringa, neem, feuilles et antitrypanosomique.

Introduction

Animal African Trypanosomosis (AAT) otherwise known as Nagana is a vector borne disease affecting most domestic and wild animals. It is caused by microscopic unicellular protozoan blood parasites of the genus *Trypanosoma* and mainly transmitted cyclically by tsetse fly belonging to the genus *Glossina* (Namangala, 2011; Sumbria *et al.*, 2015). Severe weight loss, pyrexia and aberrations in serum biochemistry are characteristics of trypanosomosis and the severity are often determined by the strain of the infecting trypanosomes and the host (Takeet and Fagbemi, 2009). Over 572 million livestock (cattle, sheep, goats and pigs) are continuously exposed to the risk of contracting trypanosomes in all the agro-ecological zones of Nigeria (Martins, 2013). Annual loss in cattle production due to trypanosomosis is between \$1-1.2 billion (Ilemobade, 2009). The control of trypanosomosis relies principally on old chemotherapy and chemoprophylaxis (isometamidium chloride, homidium bromide and chloride, and diminazene aceturate) for over 40 years which are reported to develop drug resistance in pathogenic trypanosomes (Geerts *et al.*, 2001). Several research findings on natural medicinal plants with potential activity against trypanosomosis are ongoing to get better antitrypanosomal treatments.

Azadirachta indica (Neem) is a tree in the mahogany family Maliaceae that grows in tropical and semi-tropical regions such as India and Nigeria. At maturity, the plants height reaches 24m with a dense crown of leaves (Ogbuewu *et al.*, 2008). The shrub *Moringa oleifera* is a pan-tropical species that can grow up to 10-12m in height and it is reputed to possess a number of nutritional and medicinal properties (Olasehinde *et al.*, 2012). Moringa leaf is known to have high amounts of essential nutrients; vitamins, minerals, fatty acids and fibre (Gafar and Itodo, 2011).

Neem and moringa have been reported to be the basis of traditional treatment for various types of ailments (Patel *et al.*, 2010 and Ugwu *et al.*, 2013). Whole neem leaves have been reported to be used in the management of bovine trypanosomosis in Taraba State Nigeria (Salihu *et al.*, 2014). Neem has been highly valued in India for over 4,000 years, and it is referred to as India's miraculous healing plant. Consumption of Moringa leaf is reported to boost immune systems (Olugbemi *et al.*, 2010). Several parts of *Moringa and Neem extracts* were reported to have antitrypanosomal properties (Atawodi and Shehu, 2010; Temidayo *et al.*, 2015; Bulus *et al.*, 2016). The use of plant leaves as feed or feed additive to combat infectious diseases or boost immune system makes Neem and Moringa possible option to

chemoprophylaxis. Domestic rabbits (*Oryctolagus cuniculus*) were used as model laboratory animals to represent animals naturally infected with *Trypanosoma brucei brucei*. Standard rectal temperature and serum biochemistry ranges for rabbits are; 37°C-39°C (Wokem *et al.*, 2015), total protein 54-73g/dL, AST 10-120µ/L, ALT 10-45µ/L and total bilirubin 0-10mg/dL (Matt *et al.*, 2002). This study was designed to determine the anti-trypanosomal potentials of neem and moringa leaf meals fed to rabbits experimentally infected with *Trypanosoma brucei brucei*.

Materials and methods

Experimental site

This study was conducted at the Nigerian Institute for Trypanosomiasis Research (NITR) Vom in Plateau State, Nigeria with geographical Coordinates between Latitude 9.2422° N- 10.1153° N and Longitude 8.6957° E- 9.5210° E (Majekodunmi *et al.*, 2013).

Preparation of Moringa and Neem leaf meals

Fresh moringa and neem leaves were harvested from residential houses in Hudco quarters north bank, Makurdi, Benue state. The leaves were air dried on clean surfaces in the laboratory indoors until crispy. The dried leaves were then pulverized and stored in sealed polythene bags until they were used in feed preparation.

Experimental animals and their management

Forty weaned rabbits were purchased from Dagwom farm in Vom. They were vaccinated against coccidiosis using Coccinor®, treated with long acting oxytetracycline and Ivermectin following manufacturer's directives against mange and gastro-intestinal parasites. The animal house, hutches and all handling equipments were cleaned with detergent and disinfected with 3.5% sodium hypochlorite before

arrival of the rabbits. Each rabbit were housed individually in wire mesh cages; basal feed and water were fed *ad libitum*. At the end of three weeks acclimatization period, the rabbits were grouped into eight treatments of five replicates each in a Completely Randomized Design. The animals were maintained according to the CIOMS guidelines (1985).

Dietary treatments

The four types of diets formulated contain the gross ingredients with nutrients estimated from Aduku (2004) are shown in Table 1. The prepared 1kg each of moringa and neem leaf meals were used to fortify the basal diets as listed below:

Diet 1 (D₁) basal diet.

Diet 2 (D₂) fortified with moringa leaf meal at 1kg inclusion.

Diet 3 (D₃) fortified with neem leaf meal at 1kg inclusion.

Diet 4 (D₄) fortified with 1kg each of moringa and neem leaf meals combined.

Feed intake

Total feed intake of each rabbit was computed as the sum total of feed given daily minus the left over as shown in the formula below:

$$FI(g) = QSR(g) - QLR(g)$$

FI(g) = Feed Intake

QSR(g) = Quantity of supplied ration

QLR(g) = Quantity of left over ration

Body weight gain

The body weight of each rabbit was determined on a weekly basis using a top-loading 10kg capacity weighing scale (Silvano®). The average body weight was determined using the formula:

$$AW(g) = TW(g)/R$$

Where:

AW = Average weight

TW = Total Weight in a given group

R = Number of replicates in a given group

The average body weight gain was determined using the formula below:

$$AWG(g) = AW_c - AW_p$$

Where:

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Table 1: Ingredients, chemical composition and energy content of experimental diets.

Ingredients	%			
	Diet 1	Diet 2	Diet 3	Diet 4
Moringa leaf meal	-	1.00	-	1.00
Neem leaf meal	-	-	1.00	1.00
Maize	35.19	34.56	34.56	33.93
Full fat Soya beans	25.58	25.84	25.84	26.10
Rice Offal	35.19	34.56	34.56	33.93
Vitamin/Mineral Premix*	0.25	0.25	0.25	0.25
Palm Oil	1.00	1.00	1.00	1.00
Bone Meal	2.00	2.00	2.00	2.00
Methionine	0.30	0.30	0.30	0.30
Salt	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated Chemical Composition				
Crude Protein	15.00	15.25	15.16	15.42
Crude Fibre	12.79	12.68	12.74	12.65
Fat	8.89	9.02	9.00	9.05
Calcium	0.91	0.93	0.93	1.96
Phosphorus	0.76	0.76	0.76	0.67
Ash	8.36	8.29	8.27	8.28
Lysine	0.76	0.76	0.76	0.77
Methionine	0.50	0.53	0.53	0.51
Arginine	0.99	0.99	0.99	1.01
M.E. Kcal/kg	2598.00	2594.79	2593.81	2607.98

* Based on the chemical composition of tropical feed ingredients found in Olomu (1995).

Content of premix (Optimix Poultry) in diet/kg vitamin; vit. A 80,000i.u, vit. D3 170,000i.u, vit. E 50mg, vit. K3 1.5mg, vit. B6 1mg, biotin 0.2mg, antioxidant 12.5mg. /kg mineral; cobalt 0.1mg, iodine 1mg, selenium 0.1mg, iron 25mg, manganese 45mg, copper 3mg, zinc 35mg and choline chloride 100mg. M.E Metabolisable Energy.

AWG = Average Weight Gain (g)

AWp = Average Weight of the previous week (g)

AWc = Average Weight of the current week (g) (Djakalia *et al.*, 2011).

Feed conversion ratio (FCR)

This is the ratio between the total average feed intake and the average weight gain of rabbits per group as shown in the formula below:

$$FCR = \text{Feed intake/Weight gain.}$$

Collection of blood samples

Trypanosomes were monitored daily using the wet film parasitological method and detected using a rapid approximation method (Herbert and Lumsden, 1976).

Rectal temperature

Rectal temperature of the animals were taken weekly with a 10 seconds fast reading digital centigrade thermometer (Shenzhen Soundicare®), between 8:00 – 11:00 am.

The average rectal temperature was determined using the formula below:

$$ART^{\circ}C = TRT/R$$

Where:

ART °C = Average Rectal Temperature

TRT = Total rabbits in the group Rectal Temperature

R = Number of replicates per group

Mortality (%)

The percent mortality is computed as the ratio between the number of dead rabbits and the total number of rabbits multiplied by hundred as shown in the formula below:

$$\text{Mortality (\%)} = \text{number of dead rabbits/total number of rabbits} \times 100$$

Serum biochemistry

At the end of the experiment, 3mLs of blood was collected from three sampled animals per treatment into empty blood sample bottles for serum biochemistry. Serum biochemical indices measured were total

protein, Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST), and bilirubin.

Experimental design

Forty of the rabbits were assigned to eight treatments of T1, T2, T3, T4, T5, T6, T7 and T8. Treatments T1, T2, T3 and T4 were UnInfected (UI) with *T. b. brucei*, while treatments T5, T6, T7 and T8 were Infected (I) with *T. b. brucei*. The UI and I groups of rabbits were fed with diets D1, D2, D3 and D4 (Table 1) respectively.

- T1 Uninfected rabbits fed D1
- T2 Uninfected rabbits fed D2
- T3 Uninfected rabbits fed D3
- T4 Uninfected rabbits fed D4
- T5 Infected *T. b. brucei* rabbits fed D1
- T6 Infected *T. b. brucei* rabbits fed D2
- T7 Infected *T. b. brucei* rabbits fed D3
- T8 Infected *T. b. brucei* rabbits fed D4

Statistical analysis

Data generated from parameters of treatments T1, T2, T3, T4, T5, T6, T7 and

T8 were subjected to 4×2 two-way ANOVA using the macro analysis tool pak enabled excel software of windows 7. Duncan new Multiple Range (DMRT) test was used to separate the means where there were significant means for each parameter. Line graph was also plotted using the excel 2007 application.

Results and discussion

Parasitaemia

The rabbits infected with *T. brucei brucei* had their mean prepatency within 7 days post inoculation with intermittent fluctuations of parasitaemia up to the 49th day when all the infected rabbit's parasitaemia were undetected until the end of the experiment (Figure 1). The highest peak parasitaemia was observed at day 17. The seven days prepatency agrees with the report of Takeet and Fagbemi (2009) but their highest peak at day 10 disagrees with this study.

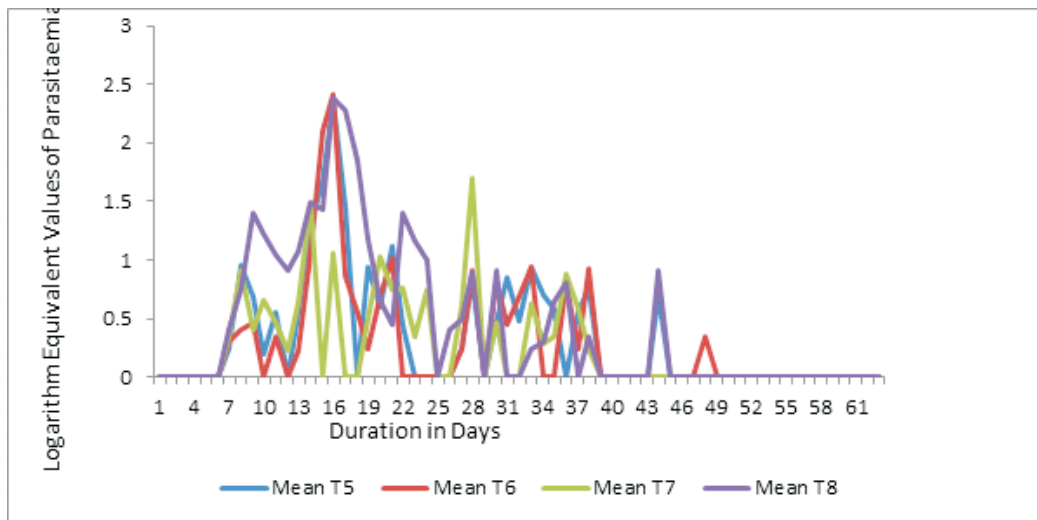


Figure 1: Trend in Parasitaemia of grower rabbits infected with *T. brucei brucei* and the control at the 4th to 12th week of the experiment (0-63 days Post Infection) showing prepatent period of 7 days.

T5 infected rabbits fed D₁ basal diet (control), T6 infected rabbits fed D₂ basal + 1% Moringa leaf meal diet, T7 infected rabbits fed D₃ basal + 1% Neem leaf meal diet and T8 infected rabbits fed D₄ basal + 1% Moringa leaf meal + 1% Neem leaf meal diet.

Evaluation of performance, water intake and rectal temperature

There were no significant ($P>0.05$) differences in performance and water intake of the rabbits across diets. Rectal temperature of rabbits in group 3 (T3) was significantly ($P<0.05$) lower than all other groups. Only T7 and T8 had ($P<0.05$) higher rectal temperatures than their corresponding uninfected T3 and T4 rabbits (Table 2). Although the higher rectal temperatures in this study were within standard range for rabbits reported by Wokem *et al.* (2015), only the uninfected rabbits in this study had lower rectal temperatures below the standard rectal temperatures. This may have occurred due to the low ambient temperature in Vom at the time of the experiment (harmattan season) which may have hypothermic effect on the rectal temperatures of the rabbits. The performance parameter in this study agreed with the report of Ayo-Ajasa *et al.* (2020) on an insignificant ($P>0.05$) effect of NLM diets fed to rabbits on performance. This may suggest that the *T. brucei brucei* used in this study had no adverse health effects on the performance of the experimental rabbits. No mortality occurred in T6 and T8 *T. brucei* infected rabbits containing 1% MLM and both MLM and NLM at 1% inclusion each. Out of 5 rabbits per treatment, 1 mortality (20%) occurred in T5 (infected control) and T7 (rabbits infected with *T. brucei*) that may have resulted from the *T. brucei* infection. The cause of a mortality in T2 (uninfected rabbits fed with D2 containing 1% MLM) was not known because its investigation was beyond the scope of this study. Intermittent pyrexia has been reported (Takeet and Fagbemi, 2009) to be among the cardinal signs of trypanosomosis which has also occurred in rabbits as observed in

this study and only those infected rabbits fed with diets containing 1% MLM and NLM had normal ($P<0.05$) rectal temperature values than the uninfected rabbits exhibiting hypothermia, which were fed with the same diets. This may suggest that both leaf meals contain properties that inhibit pyrexia as the rectal temperatures of the infected rabbits were not beyond normal standard.

Serum biochemistry

The parameters of serum biochemistry revealed no significant ($P>0.05$) difference on bilirubin, AST and ALT. Total protein however had higher ($P<0.05$) values for infected rabbits (T5, T7 and T8) except T6 fed D2 containing the 1% MLM and was not significantly ($P>0.05$) different from all the uninfected rabbits. This strongly suggests that MLM has properties inhibiting abnormal increase in serum protein than NLM. This superior effect of *Moringa oleifera* over *Azadirachta indica* on *T. brucei brucei* infection corroborates with the report of Bulus *et al.* (2016) that reported a lower *in vitro* IC 90 value of *Moringa oleifera* ($190\mu\text{mL}$) than *Azadirachta indica* ($420\mu\text{mL}$). The AST and ALT results of this study agreed with the findings of Ayo-Ajasa *et al.* (2020) that there was no effects of graded levels of NLM in diets fed to rabbit bucks on AST and ALT but was not in line with serum total protein. Takeet and Fagbemi (2009) however reported a contrary finding for total protein which had significantly ($P<0.05$) higher mean value of 86g/L of *Trypanosoma congolense* infected rabbits which is similar to the highest total protein value of 85.6g/L in this study. This difference in total protein may be due to the different species of trypanosomes involved as they used *T. congolense* as against *T. brucei brucei* in the current study.

Table 2 : Effect of moringa leaf, neem leaf and combined leaf meals diets on performance, water intake and rectal temperature of grower rabbits infected with *T.brucei brucei* and their controls

Parameters (g/animal)	Diets				P-values			SEM
	D ₁	D ₂	D ₃	D ₄	Diet	Infection	Interaction	
Weight Gain	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		1040	820	540	980			
		680	580	600	840	0.17	0.14	0.59
								159.70
Total Feed Intake	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		6273.70	5542.09	5890.90	5488.71			
		5139.07	5922.05	5790.60	5444.80	0.95	0.62	0.68
								636.30
WI* (mL/day)	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		212.08	187.68	198.22	178.12			
		204.34	184.98	180.37	206.03			
FCR	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		6.34	11.25	13.42	6.04			
		8.54	15.07	12.31	6.92	0.12	0.55	0.91
								1.54
Rectal Temp. (°C)	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		36.98 ^{abc}	36.22 ^{bc}	35.13 ^d	36.19 ^c			
		37.19 ^{abc}	37.62 ^{abc}	37.57 ^{ab}	37.49 ^a	0.38	0.00	0.27
								0.49
Mortality* (%)	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		0	20	0	0			
		20	0	20	0			

* Not subjected to ANOVA, different a, b, c and d means superscripts are significant. FCR Feed Conversion Ratio, D₁ basal diet (controls T1&T5); D₂ basal + 1% moringa leaf meal (T2&T6); D₃ basal + 1% neem leaf meal (T3&T7); D₄ basal + 1% moringa leaf meal + 1% neem leaf meal (T4&T8); WI Water Intake; UI Uninfected; I Infected; SEM Standard Error of Means.

Table 3: Effect of moringa leaf, neem leaf and combined leaf meals diets on serum biochemistry of grower rabbits infected with *T. brucei brucei* and their controls

Parameters	Diets				P-values			SEM
	D ₁	D ₂	D ₃	D ₄	Diet	Infection	Interaction	
Total protein (g/L)	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		47.63 ^d	52.47 ^{cd}	53.63 ^{cd}	51.00 ^{cd}	0.15	0.00	0.18
		72.60 ^{ab}	62.60 ^{bc}	82.17 ^a	85.60 ^a			5.2
Bilirubin (conjugated) Mg/dL	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		0.4	0.5	1.2	0.6	0.59	0.97	0.76
		0.6	0.5	0.7	0.9			0.4
AST (GOT) μ/l	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		13.00	13.00	16.33	13.00	0.71	0.55	0.65
		12.67	13.67	15	21.33			4
ALT (GPT) μ/l	UI	T1	T2	T3	T4			
	I	T5	T6	T7	T8			
		10.67	8.00	12.33	11.00	0.55	0.36	0.66
		9.67	12.00	13.33	20.33			4

a, b, c, d means of the same parameter with different superscript are significantly different (P<0.05); D₁ basal diet (controls T1&T5), D₂ basal +1% moringa leaf meal (T2&T6), D₃ basal + 1% neem leaf meal (T3&T4), D₄ basal + 1% moringa leaf meal + 1% neem leaf meal (T4&T8); UI Uninfected; I Infected; SEM Standard Error of Means, AST Aspartate Serum Transferase, GOT Glutamic Oxaloacetate, ALT Alanine Transaminase, GPT Glutamic Pyruvic Transaminase.

Conclusion

The study showed that 1% supplementation of moringa leaf meal diet had prophylactic anti-trypanosomal activity effects on serum total protein and mortality amelioration of *Trypanosoma brucei brucei* infected rabbits when administered alone and a combination of both moringa leaf meal and neem leaf meal at 1% diet inclusion prevented pyrexia and mortality. It is recommended that 1% moringa leaf meal alone and a combination of 1% each of moringa and neem leaf meals in diets should be selected to ameliorate trypanosomosis disease over 1% neem leaf meal diet alone.

References

- Aduku, A. O. 2004.** Animal Nutrition in the Tropics. Feed and Feeding, Pasture Management, Monogastric and Ruminant Nutrition. PP. 60.
- Aduku, A.O. and Olukosi, J.O. 2000.** Animal Products: processing and Handling in the tropics. GU publications Abuja FCT, Nigeria PP.11
- Atawodi, S. E. and Shehu, H. 2010.** Antitrypanosomal property of some extracts of different parts of *Moringa oleifera* LAM. *Electronic Journal of Biology*, 6 (1): 19-23.
- Ajayi, O. O., Toshak, E. L., Obaloto, O. B., Iliyasu, B., Igweh, A. C., Dadah, A. J. and Idehen, C. O. 2013.** Comparative efficacy of Berenil and Samorin in albino rats experimentally infected with current field isolates of *Trypanosoma brucei brucei*. *International Journal of Biological and Chemical Sciences*. 10 (10): 20-40.
- Ayo-Ajasa, O. Y., Egbeyale, L. T., Abiona, J. A., Ekunseitan, D. A., Ayoola, A. A., Atoyebi, J. F. and Aremu, O. M. 2020.** Growth performance, carcass characteristics, haematology and serum biochemistry of rabbit bucks fed diets containing graded levels of neem (*Azadirachta indica*) leaf meal. *Nigerian Journal of Animal Science*, 22 (1): 270-278.
- Bulus, T., Ahmed, A. B., Aboi, T. Y. and Danbaki, D. A. 2016.** Determination of IC₅₀ and IC₉₀ values of ethanolic extracts of some medicinal plants against *Trypanosoma brucei brucei*. *Archives of clinical microbiology*, 7: 3. <http://www.acmicob.com/>
- CIOMS 1985.** Council for International Organizations of Medical Sciences International guiding principles for biomedical research involving animals.
- Djakalia, B., Guichard, B. L. and Soumalia, D. 2011.** Effect of *Moringa oleifera* on Growth Performance and health Status of Young Post-Weaning Rabbits. *Research Journal of Poultry Sciences*. 4 (1): 7-13.
- Gafar, M. K. and Itodo, A. U. 2011.** Proximate and Mineral Composition of Hairy Indigo Leaves. In *Electronic journal of Environmental, Agricultural and Food Chemistry (EJEAChe)*. 10 (3): 2007-2018.
- Geerts, S., Holmes, P. H., Eisler, M. C. and Diall, O. 2001.** African bovine trypanosomiasis: The problem of drug resistance. *Parasitology Today*, 17(1): 25-28.
- Namangala, B. 2011.** How the African trypanosomes evade host immune killing. *Parasite Immunology*, 33: 430-437.
- Ilemobade, A. A. 2009.** Tsetse and trypanosomes in Africa: The challenges, the opportunities. *Onderst. Journal of Veterinary Research*, 76(1), 35-40.

- Majekodunmi, A. O., Fajinmi, A., Dongkum, C., Picozzi, K., Thrusfield, M. V. and Welburn, S. C. 2013.** A longitudinal Survey of African Animal Trypanosomiasis in domestic cattle on the Jos Plateau, Nigeria: Prevalence, Distribution and risk factors. *Parasites and Vectors*. 6: 239.
- Matt, J., Karen, R. and Cailin, H. 2002.** Standard Hematology and Blood Chemistry Ranges for Rabbits. University of Pennsylvania, School of Veterinary Medicine.
- Martins 2013.** A Business Plan Animal/Livestock Production (example sheep and pigs) Feasibility Study. Retrieved from Martinslibrary.blogspot.com>Home>Agri culture>Feasibility Study.
- Ogbuewu, I. P., Okoli, I. C. and Iloeje, M. U. 2008.** Serum Biochemical Evaluation and organ weight characteristics of Buck Rabbits fed Graded levels of Neem (*Azadirachta indica*) leaf meals Diets. www.priory.com/neem_rabbits.
- Olasehinde, G. I., Ayanda, O. I., Ajayi, A. A. and Nwabueze, A. P. 2012.** *In-vivo* antiplasmodial activity of crude n-hexane and ethanolic extracts of *Moringa oleifera* (LAM.) seeds on *Plasmodium berghei*. *International Scholars Journals*, 1(5): 50 - 54. www.internationalscholarsjournals.org
- Olomu. J. M. 1995.** *Monogastric Animal Nutrition. Principles and Practices.* Jachem Publishers Benin, Nigeria. Pp. 67-159.
- Olugbemi, T.S., George, P. and Dauda, O.M. 2010.** Anti-trypanocidal potentials of aqueous and ethanolic leaf extracts of *Moringa oleifera* in wistar stock albino rats. www.ajol.info/journal/home 8: (2).
- Patel, J. P., Gami, B. and Patel, K. 2010.** Evaluation of *in vitro* schizonticidal properties of acetone extract of some Indian medicinal plants. *Advances in Biological Research*. 4(5): 253-258.
- Salihu, T., Ameen, S. A., Mbaaji, C. O., Anoruo-Dibia, C. A. and Arowolo, R. O. A. 2014.** Management of Bovine Trypanosomiasis with Medicinal Plants in Taraba State, Nigeria. *International Journal of Health and Medical Information*. 3 (25): 2350-2150.
- Sumbria, D., Singla, L. D., Sharma, A., Bal, M. S. and Kumar, S. 2015.** Multiplex PCR for detection of *Trypanosoma evansi* and *Theileria equi* in equids of Punjab, India. *Veterinary Parasitology*, 211(3-4): 293-299.
- Takeet, M. I. and Fagbemi, B. O. 2009.** Haematological, Pathological and Plasma Biochemical changes in Rabbits experimentally infected with *Trypanosoma congolense*. *Science World Journal*, 4(2):1597-6343.
- Temidayo, O. O., Ademola, A. O., Oyefunbi, A. O. and Olumuyiwa, A. A. 2015.** Chemopreventive effect of methanolic extract of *Azadirachta indica* on experimental *Trypanosoma brucei* induced oxidative stress in dogs. <https://www.phcogres.com/>
- Ugwu, O. P. C., Nwodo, O. F. C., Joshua, P. E., Odo, C. E., Bawa, A., Ossai, E. C. and Adonu, C. C. 2013.** Anti-malaria and haematological analyses of ethanol leaf extract of *M. oleifera* on malaria infected mice. *International journal of*

Effects of diet fortification with Azadirachta indica and Moringa oleifera leaf meal

Pharmacy and Biological sciences.
3(1): 360-371.

Wokem, G. N., Oyero, S. and Nwachukwu, C. B. 2015. Effect of Methanolic Extract of Mexican Sunflower (*Tithonia diversifolia*) leaves on the histology and iron metabolism of the liver of Rabbits. *Nigerian Journal of Parasitology.* 36(2): 132-136.

Received: 14th October, 2020

Accepted: 5th February, 2021