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IMPACT OF BOILED MORINGA (*Moringa oleifera)* SEEDS ON GROWTH PERFORMANCE AND HAEMATOLOGY OF RABBITS INFECTED WITH *Trypanosoma brucei brucei*

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ABSTRACT

The study was aimed at evaluating the inhibitory potentials of boiled Moringa oleifera seeds against Trypanosoma brucei brucei. Growth performance, parasitaemia, haematology and Red Blood Cell morphology of T. brucei brucei infected rabbits were determined. 30 mixed sex and breeds of rabbits approximately 90 days old were distributed into 5 treatments with 6 replicates each in a Completely Randomized Design (CRD). Each rabbit was infected with 0.2 mL inoculums and treatments 1 to 5 were fed with diets containing; diet 1 (0 % boiled Moringa seeds), diet 2 (1.5 % boiled Moringa seeds), diet 3 (3 % boiled Moringa seeds), diet 4 (4.5 % boiled Moringa seeds) and diet 5 (6 % boiled Moringa seeds) respectively . Descriptive statistics, One way ANOVA and DMRT were used for data analysis. Results revealed that only T. brucei brucei infected rabbits fed 3 % boiled Moringa seeds recorded weight gain. Blood parameters from T. brucei brucei infected rabbits revealed failure of the boiled Moringa seeds inclusion (up to 6 %) to prevent infection as values of blood parameters in all treatments were either below or above reference range except lymphocyte (40-80 %) where only the control (38.67 %) was below reference range. Anisocytosis (+), Hypochromatic cells (++), Polychromatic cells (+) and Toxic Granulation of the Neutrophils (+) were observed in all infected rabbits and only rabbit fed diet 3 recorded Normocytic and Normochromic Red Blood Cells. In conclusion, graded levels of boiled Moringa seeds up to 6 % failed to prevent the Trypanosomosis infection. The study recommends 3 % inclusion of boiled Moringa oleifera seeds in feed for optimal growth performance to ameliorate emaciation in Trypanosomosis.

Key words: Boiled, Trypanosomosis, inhibition, Moringa-seeds, growth-performance and haematology.

1.0 INTRODUCTION

Trypanosoma brucei brucei is an extra cellular protozoan blood parasite belonging to the subgenus *Trypanozoon* (Adamu *et al*., 2009). The trypanosome causes trypanosomosis in pets, livestock and wild animals and the disease is prevalent across the sub saharan region of Africa (Eyob and Batisa, 2015). The trypanosome is mainly transmitted by the vector Tsetse flies (*Glossina species*) (Gibson, 2015). Its pathogenic effects ranges from reduced parturition rate, abortion, loss of condition, high mortality rate, low meat and milk yield (Bekele and Begejo, 2015). Normal Red Blood Cells are usually the same size. Anosocytosis describes Red Blood Cells that are different in sizes (Christopher, 2004). This is a sign of anemia, causing symptoms like weakness (fatigue) and shortness of breath (dypsnea) observed in Trypanosomosis. Red Blood Cell morphology is altered due to alterations in lipids, iron, oxidative metabolism, immune mediated disease, mechanical fragmentation, electrolyte or metabolic abnormalities (Christopher, 2004). Polychromatic cells are light blue colour of immature Red Blood Cells (caused by residual RNA). This is an indicator of responsiveness to anemia. Hypochromic cells are pale Red Blood Cells with a wide pallor (Christopher, 2004).

There are limited veterinary trypanocidal drugs (Diminazene aceturate, Isometamidium chloride and Homidium salts) with several reports on their lapses such as toxicity, relapse due to resistant trypanosome strains and high cost making it unaffordable for poor rural livestock farmers. These have necessitated the screening of several herbal plants. The use of *M. oleifera* plant parts such as leaves, stem bark and seeds have been reported to have some level of inhibitory antitrypanosomal properties (Shaba *et al*., 2014; Hassan *et al*., 2015; Meireles *et al*., 2020; Ujah *et al*., 2020 and Salifu *et al*., 2021) when used as a herbal treatment for Trypanosomosis. *M. oleifera* has been used to support immune system and enrich anemic blood condition (Ujah *et al*., 2020) however; none of these research findings can stand as a replacement for available trypanocidal drugs in the market. There are reports of raw *M. oleifera* containing antinutrients that interfere with digestion and utilization (Afolayan *et al*., 2020) but heat processing of seeds has been reported to improve nutrient utilization of the seeds by animals (Tuleun and Patrick, 2007). Boiling of *M. oleifera* seeds may proffer better antitrypanosomal potential in the treatment of Trypanosomosis. Information on trypanocidal potentials of boiled *M. oleifera* seeds are scarce and this study is aimed at assessing

the antitrypanosomal efficacy of boiled *M. oleifera* seed meal fed rabbits experimentally infected with *T. brucei brucei* through growth performance and haematology.

2.0 MATERIALS AND METHODS

2.1 The Study Area

The study was carried out at a laboratory facility in Livestock Division of Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Jos South Local Government Area, Nigeria, with geographical location between latitude 9°72"N and longitude 8°79'E on an altitude of 4200 feet (1280 m) above sea level in the Guinea savannah zone of West Africa. The relative humidity ranges from 78 % during the raining season and 22 % within the dry season, with mean daily environmental temperature ranges of 17 °C-28.6 °C (NVRI, 2018; Salifu *et al*., 2021).

2.2 Collection and Processing of Test Ingredient

Mature and dry *Moringa oleifera* pods containing seeds were harvested within Makurdi Local Government Area of Benue State, Nigeria, with geographical location between latitude 7°75"N and longitude 8°56'E (Salifu *et al*., 2021).

A total of 30 kg of raw *Moringa oleifera* seeds was removed from their pods and 200g sample of the seed was drawn and divided into 2 equal portions where the 1st portion in its raw form was air-dried in the laboratory for 3 days after washing with water. The 2nd portion was boiled using pressure pot with 1 L of water for 10 minutes of whistling; which was then drained after cooling, air-dried at room temperature for 3 days. All the seed portions were pulverized separately using an electric blender. The raw and boiled pulverized Moringa seeds were then stored separately in labeled polythene bags until required. The samples of the raw and boiled Moringa seeds were taken to toxicology laboratory in Biochemistry Department of National Veterinary Research Institute (NVRI) for antinutrient analysis. Sixteen (16) kg of the Moringa seeds were kept to be processed by boiling under pressure for the diet formulation.

2.3 Experimental Design and Procedure

A total of 30 healthy mixed sex crosses of New Zealand White, Chinchila and Gotland rabbit breeds weighing between 0.6-1.7Kg were bred in a rabbitry housing facility at the Livestock Division of NITR Vom, from rabbits procured from rabbit breeders within Vom, Plateau State. Each rabbit

was housed individually in each 30 separate partitions of metallic constructed rabbit cages fitted with feeders and drinkers. The hutches were placed on racks approximately 100 cm above the ground for easy cleaning and proper ventilation. The grower rabbits were weighed and distributed into 5 treatments with 6 rabbits (4 males and 2 females) with 1 rabbit per replicate each in a Completely Randomized Design (CRD). Each rabbit from treatments 1 to 5 were infected subcutaneously with 0.2ml inoculum containing approximately 6.32×10^{6} (8 trypanosomes per field) of *T. brucei brucei* parasitized blood using a rapid matching method (Herbert and Lumsden, 1976) obtained from a donor mouse.

2.3.1 Experimental diets

Five (5) diets were formulated and compounded using the method described by Shaahu and Tiough (2019) to feed the experimental rabbits where the 1st diet was the 0 % control diet without Moringa seeds (D1), the 2nd (D2), 3rd (D3), 4th (D4) and 5th (D5) diets contained graded levels of 1.5, 3, 4.5 and 6 % of the boiled Moringa seeds respectively (Table 1).

2.3.2 Growth performance parameters

The treatments were denoted as T1, T2, T3, T4 and T5. Diet 1 (D1) was served to T1 (control), diets D2, D3, D4 and D5 were served to rabbits in T2, T3, T4 and T5 respectively post inoculation. Daily feed intake was calculated by subtracting the left over from each daily ration served. Body weight measurement was taken weekly using a 10 kg capacity top loading scale (Camry®) and growth was calculated by subtracting the previous weight from the current weight of each rabbit. Feed Conversion Ratio (FCR) was calculated as the ratio of total feed intake to total weight gain; FCR=Total feed intake/Total weight gain.

Economic cost of production and profitability or losses were calculated using the methods of Carew *et al*. (2020) as follows:

I. Cost of diet (₦/kg) = sum of price of each ingredient multiplied by the proportion of the ingredient in the diet and divided by the total quantity of ingredients in the diet.
II. Cost of Diet Consumed (₦/kg) = Cost of diet × Feed Intake.
III. Cost of Diet/kg weight (₦/kg) = Cost of Diet Consumed/Final weight (Final weight was used in place of Feed Conversion Ratio due to calculation complications encountered when loss of weight were observed).

IV. The market prices per weaned and grower rabbit were ₦6,000 and ₦10,000 respectively.

V. Total Production Cost (TPC) = Cost of Diet/kg weight + Cost of Labour + Cost of Housing repairs and fumigation + Cost of weaned rabbit + Cost of Transportation.

VI. Gross Profit or Loss = Market price of grower rabbit – TPC.

2.3.3 Parasitaemia

Parasitaemia was monitored daily via the collection of a drop of blood by shaving off hairs of the marginal ear vein of the rabbits and piercing it using a sterile lancet and subsequently preparing a wet film mount using microscopic slides and cover slip. Parasitaemia values were converted to Logarithm Equivalent Value (LEV). The LEV was calculated using the following formulae:
Where parasites were seen in the first field; LEV = Log number of parasites seen × 1000/1
Where parasites were seen beyond one field of view; LEV = Log number of parasites seen × 1000/ number of fields viewed (Ajayi *et al*., 2013).

2.3.4 Determination of haematology and Red Blood Cell (RBC) morphology

At 60th day Post Infection (PI), 3 rabbits having weights closest to the mean live weight of each group were selected and 5 mL of blood **(**Saka *et al*., 2019) was aspirated from each rabbit directly from the ear veins using a 5 mL syringe and dispensed into a set of blood collection tubes at 2.5 mL each containing the anti-coagulant Ethyl Diamine Tetra Acetic acid (EDTA). The blood was used to determine the following haematological parameters; Red Blood Cell count, White Blood Cell (WBC) count, Packed Cell Volume (PCV), Haemoglobin (Hb) and differential WBC counts (Monocytes, Basophils, Eosinophils, Lymphocytes and Neutrophils). The Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC) and Mean Cell Volume (MCV) were calculated using the following formula as described by Ibitoye *et al*. (2020): MCV = PCV×10/RBC (10^{-15} L) , MCH = Hb×10/RBC (10^{-2} g) , MCHC = RBC×100/PCV (g/dL). Red Blood Cell morphology was observed under an oil immersion × 100 compound microscope for aberrations in cell size, shape and colour. Red Blood Cell (RBC) morphology was assessed on a thin film microscopic slide blood smear. Abnormalities were marked as 1+ to 4+ (Christopher, 2004).

Table 1: Ingredients, Chemical Composition and Energy Content of Graded Replacement Levels of Rice offal with boiled *Moringa oleifera* seed meal Experimental Diets for Grower Rabbits infected with *Trypanosoma brucei brucei*

Ingredients (Kg)	**Diets**				
	D1	**D2**	**D3**	**D4**	**D5**
Full Fat Soya Beans	38.00	38.00	38.00	38.00	38.00
Rice Offal	25.00	23.50	22.00	20.50	19.00
Maize Offal	15.23	15.23	15.23	15.23	15.23
Maize	9.34	9.34	9.34	9.34	9.34
Sweet potato vine meal	7.68	7.68	7.68	7.68	7.68
Boiled *Moringa* Seed meal	0.00	1.50	3.00	4.50	6.00
Palm Oil	3.15	3.15	3.15	3.15	3.15
Bone Ash	1.20	1.20	1.20	1.20	1.20
Table Salt	0.20	0.20	0.20	0.20	0.20
Methionine	0.10	0.10	0.10	0.10	0.10
Grower Vitamin and Mineral Premix*	0.10	0.10	0.10	0.10	0.10
Total	**100**	**100**	**100**	**100**	**100**
Calculated Chemical Composition (%)					
Crude Protein	18.30	18.56	18.83	19.09	19.36
Crude Fibre	15.77	15.32	14.12	14.43	13.98
Ether Extract	8.87	9.04	9.20	9.37	10.29
Ash	10.86	10.04	9.64	9.22	8.81
Moisture	8.54	8.51	8.63	8.46	8.52
M. E. Kcal/Kg	2568.28	2557.26	2546.24	2535.22	2524.20

D1 0 % *Moringa oleifera* Seed diet, D2 1.5 % *Moringa oleifera* Seed diet, D3 3 % *Moringa oleifera* Seed diet, D4 4.5 % *Moringa oleifera* Seed diet, D5 6 % *Moringa oleifera* Seed diet.* Content of premix (Optimix Poultry) in diet/kg vitamin; vit. A 80,000 i.u, vit. D3 170,000 i.u, vit. E 50 mg, vit. K3 1.5 mg, vit. B6 1 mg, biotin 0.2 mg, antioxidant 12.5 mg./kg mineral; cobalt 0.1 mg, iodine 1 mg, selenium 0.1 mg, iron 25 mg, manganese 45 mg, copper 3 mg, zinc 35 mg and choline chloride 100 mg. M.E Metabolisable Energy. Calculated diet compositions were formulated from the following sources 1. National listing of approved feed ingredients **(**NIAS, 2020). Contents of the *Moringa* seed included as proximate values of *Moringa oleifera* fermented seed flou r (CP 21.15, CF 6.17, Ash 4.50, EE 14.00, NFE 61.07, Energy 465.32, Moisture 8.13) was from 2. Ijarotimi *et al*., 2013. Sweet potato vines proximate values (CP 17.43, CF 33.42, Ash 5.84, EE 6.22, NFE 36.40, Energy 2245.4, Moisture 6.64) was sourced from 3. Baba *et al*., 2018.

2.4 Ethical clearance

Approval on the use of laboratory animals to be infected with *T. brucei brucei* was obtained from the Animal Care and Use Ethical Committee of the College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi with reference number: **JOSTUM/CVM/ETHICS/2023/8** before commencement of the study.

2.5 Experimental Animals and Management

The concrete floor of the rabbitry was swept with broom and washed with detergent (Viva plus®) and disinfectant (Izal®) diluted in appropriate volume of water on a weekly basis. The rabbits were kept to acclimatize for 1 week before commencement of the experiment. During this period the rabbits were given prophylactic treatment against infectious bacteria, coccidiosis, endo and ecto parasites using their appropriate drugs respectively and following the manufacturer's instructions. The rabbits were fed with the control diet and given potable drinking water daily until the commencement of the experiment. All equipments used in the animal house were washed, disinfected and cleaned weekly.

2.6 Data analysis

Data generated from the experiment were subjected to descriptive statistics and analyzed with One-way Analysis of Variance (ANOVA) at $p<0.05$ and $p<0.01$ using the Statistical Package for Social Sciences (SPSS Version 24). Duncan's Multiple Range Test (DMRT) was used to compare the significant treatment means using same SPSS

3.0 RESULTS AND DISCUSSION

3.1 Effect of Boiling on Antinutritional Factors of *Moringa oleifera* Seed Meal

The effect of processing of *Moringa* seeds by boiling revealed that boiling increased tannin and saponin when compared to the raw *Moringa* seeds. Boiling reduced oxalate and increased alkaloid (Table 2). Tuleun and Patrick (2007) reported contrary finding in 29.70 % reduction of tannin. The boiled *Moringa* seeds which had increased values in all the antinutrients analysis (tannin = 32.40 % increment, saponin = 14.93 % increment and alkaloid = 41.12 % increment) except oxalate having a reduction of 4.50 % agrees with the report of Tuleun and Patrick (2007) for anti-nutrient values of saponins and oxalate in boiled *Mucuna utilis* seeds. The high pressured boiling of the seeds in this study may have released these bound antinutrients from cellular structures to the surface coat of the seeds, activated enzymes that converts inactive precursors into active antinutrients, concentrate antinutrients through water loss and transform other compounds into antinutrients thereby increasing their quantities. The presence of tannin in *M. oleifera* seeds in this

study disagrees with the report of Amuga *et al*. (2012) where absence of tannin was reported in *M. oleifera* seeds. This may have been due to difference in location of the tree, age at harvest of seeds or the difference in laboratory phytochemical analysis. This increase in antinutrients may have played a role in improving the antitrypanosomal properties of boiled *Moringa oleifera* seeds.

Table 2: Effect of boiling on antinutritional factors of *Moringa oleifera* seed meal

Antinutrients	Processed method	
	Raw	**Boiled**
Tannin (mg/100g)	12.56	18.58
Processing effect on Tannin (%)	-	32.40 (Increment)
Oxalate (mg/100g)	55.00	52.50
Processing effect on Oxalate (%)	-	4.50 (Reduction)
Saponin (%)	1.14	1.34
Processing effect on Saponin (%)	-	14.93 (Increment)
Alkaloid (%)	5.90	10.02
Processing effect on Alkaloid (%)	-	41.12 (Increment)

Calculations on the % processing effect on antinutrients were adopted from Tuleun and Patrick (2007). Where Level of Increment/Reduction (%) = Difference in antinutrient values of raw and processed seed/Higher antinutrient value ×100.

3.2 Growth Performance, Survival, Mortality, Parasitaemia and Economics of *T. brucei brucei* Infected Rabbits Fed Graded Levels of Boiled *Moringa oleifera* Seed Meal

Table 3 showed that only the treatment 3 (rabbits fed 3 % boiled *Moringa* seed meal) had weight gain and higher ($p<0.05$) survival time than treatment 1 (control). Only treatments 3 and 5 had lower mortality than the control. Feed intake, feed conversion ratio and parasitaemia were insignificant ($p>0.05$).

Table 4 revealed economic losses in all the treatments due to the *T. brucei brucei* infection in the rabbits. The report of Salifu *et al*. (2021) disagrees with the weight loss in the treatments as only weight gain was observed in all the *T. brucei brucei* infected rabbits treated with single and combined *Moringa* (*M. oleifera*) and Neem (*Azadirachta indica*) leaves. Hassan *et al*. (2015) report on weight loss of control rabbits infected with *T. congolense* agrees with the findings in this study

and they also observed weight gain in the infected rabbits treated with *M. oleifera* leaves. This may have proven that *M. oleifera* and *Azadirachta indica* leaves have better potent antitrypanosomal properties than the boiled *M. oleifera* seeds. Saka *et al*. (2019) reported weight gain in all growing rabbits fed up to 2 % of raw *Moringa oleifera* seed powder. Hence the loss of weights mainly observed in rabbits in this study was due to the *T. brucei brucei* experimentally infected rabbits of which the boiled *M. oleifera* seeds failed to prevent the infection. Amaechi *et al*. (2016) reported emaciation of growing rabbits experimentally infected with *T. brucei brucei* which also corroborates with the weight loss observed in this study.

The high % mortality (83.33 %) observed in this study agrees with the report of Amaechi *et al*. (2016) that had 100 % mortality in growing rabbits experimentally infected with *T. brucei brucei a*nd treated with 3.5 mg/kg of Diminazene aceturate.

The T3 that had ($p<0.05$) weight gain was also observed to have ($p<0.05$) higher survival time when compared to the other treatments. This may suggest that the 3 % boiled *M. oleifera* seed diet had the optimum Trypanosomosis resistance among the infected rabbits.

The parasitaemic trend observed in wet film blood mount was generally low in this study. This low parasitaemia was also observed in the report of Salifu *et al*. (2021) and Salifu *et a*l. (2022) who worked on treatments of *T. brucei brucei* infected rabbits and *T. evansi* infected WAD goats respectively while Jolayemi *et al*. (2020) reported higher parasitaemic values in peripheral blood circulation for *T. brucei brucei* infected wistar rats. The study observed that more economic loss was derived from the treatments T3 and T5 as they consumed more feed due to their longer duration of survival but did not convert the feed to meat due to the emaciation effect caused by the *T. brucei brucei* infection. According to other researchers, a profitable gain of growing rabbits not infected with *T. brucei brucei* was attained which was contrary to the findings in this study (Shaahu and Tiough, 2019; Carew *et al*., 2020 and Amos *et al*., 2021). These economic loss observed in this study testifies to the serious economic losses suffered by livestock farmers in areas endemic to Trypanosomosis and their vectors.

Table 3: Growth performance, survival, mortality and parasitaemia of *Trypanosoma brucei brucei* infected rabbits fed diets containing graded levels replacement of rice offal with boiled *Moringa oleifera* seed meal

Parameters	Levels of boiled seed meal inclusion (%)					p-value	SEM
	T1 (0)	T2 (1.5)	T3 (3)	T4 (4.5)	T5 (6)		
Mean Initial weight (g)	1120	1220	1080	1080	1130	0.94	0.05
Mean Final weight (g)	980	1030	1130	1000	1080	0.88	0.05
Weight gain or loss (g)	-140	-190	50	-80	-50	0.31	0.04
Mean Feed Intake (g)	1790	2290	2630	2090	2490	0.58	0.17
FCR	-12.79	-12.05	52.60	-26.13	-49.80	0.99	2.65
Survival period (Days)	40^{b}	48^{ab}	53^{a}	47^{ab}	50^{ab}	0.04^{*}	1.98
No. of rabbits	6	6	6	6	6		
No. Dead (% mortality)	5 (83.33)	5 (83.33)	4 (66.67)	5 (83.33)	3 (50.00)		
Parasitaemia (LEV)	0.59	0.43	0.45	0.46	0.30	0.94	0.1

a and b means in the same row with different superscripts are significant at $p<0.05^{*}$, T1 rabbits fed diet without boiled *Moringa oleifera* seed meal, T2 rabbits fed diets with 1.5 % of boiled *Moringa oleifera* seed meal, T3 rabbits fed diets with 3 % of boiled *Moringa oleifera* seed meal, T4 rabbits fed diets with 4.5 % of boiled *Moringa oleifera* seed meal and T5 rabbits fed diets with 6 % of boiled *Moringa oleifera* seed meal. LEV Logarithm Equivalent Value, SEM Standard Error of Mean, No. Number.

Table 4: Economics of feeding *Tryponasoma brucei brucei* infected rabbits with diets containing graded levels replacement of rice offal with *Moringa oleifera* seed meal

	Levels of boiled seed meal inclusion (%)					p-value	SEM
Parameters	**T1 (0)**	**T2 (1.5)**	**T3 (3)**	**T4 (4.5)**	**T5 (6)**		
Mean Feed Intake (kg)	1.79	2.29	2.63	2.79	2.49	0.58	0.17
Mean Final Weight (kg)	0.98	1.03	1.13	1.00	1.08	0.88	0.05
CFC (₦)	1241.57	1693.36	2060.61	1722.86	2162.22	0.31	146.96
CF/kg (₦)	1234.76	1707.06	1830.39	1774.09	2092.32	0.49	147.17
TPC (₦/Rabbit)	10401.43	10873.73	10997.06	10940.76	11258.99	0.49	147.17
Revenue (₦/kg Rabbit)	4,900	5,150	5,650	5,000	5,400	-	-
Gross Profit or Loss (₦/Rabbit)	-5,501.43	-5,718.73	-5,347.06	-5,940.76	-5858.89	-	-

p>0.05 are not significant, T1 rabbits fed diet without boiled *Moringa oleifera* seed meal, T2 rabbits fed diet with 1.5 % of boiled *Moringa oleifera* seed meal, T3 rabbits fed diet with 3 % of boiled *Moringa oleifera* seed meal, T4 rabbits fed diet with 4.5 % of boiled *Moringa oleifera* seed meal and T5 rabbits fed diet 6 % of boiled *Moringa oleifera* seed meal. CFC Cost of Feed Consumed, CF/kg wt Cost of Feed/kg weight. TPC Total Production Cost, Revenue = Cost/kg rabbit × Final weight (kg), Cost/kg rabbit = ₦ 5,000.

3.3 Haematology of *T. brucei brucei* Infected Rabbits Fed Diets Containing Graded Levels of Boiled *Moringa oleifera* Seed Meal

The Packed Cell Volume and Haemoglobin (Table 5) in this study that were below the reference range except for T5 having Haemoglobin (11.13 g/dl) within the reference range agrees with the finding of Hassan *et al*. (2015) Packed Cell Volume and Haemoglobin of control rabbits infected with *Trypanosoma congolense*. Although the *T. congolense* infected rabbits treated with their test material (*M. oleifera* leaves) which significantly ($p<0.05$) increased Packed Cell Volume (41.81 %) and Haemoglobin (14.29 g/dl) was contrary to the observation in this study. Abenga *et al*. (2016) agrees with the findings in this study as they observed decline in Packed Cell Volume and Red Blood Cell values in Nigerian dogs infected with *T.brucei brucei*. Opaluwa-Kuzayed *et al*.

(2022) reported Packed Cell Volume (36.42 %) and Haemoglobin (12.21 g/dl) of rabbits infected with *T. brucei brucei* which were within reference range values but had significant ($p<0.05$) reductions when compared to their uninfected controls. The White Blood Cells in this study which were insignificant ($p>0.05$) with only T2 having White Blood Cells (4.77 10^9/L) within reference range agrees with the report of Saka *et al.* (2019) with insignificant ($p>0.05$) White Blood Cell (3.74 to 6.88 10^9/L) values in healthy rabbits fed graded levels of *Moringa oleifera* seed powder. The report of Takeet and Fagbemi (2009) on decreasing ($p<0.05$) White Blood Cells in *Trypanosoma congolense* infected rabbits disagrees with the White Blood Cells observation in this study. These variations in increase and decrease of White Blood Cells may have been due to differences in immune response of Trypanosomosis in the rabbits. Lymphocytes in this study which were insignificant ($p>0.05$) and within reference range except the control had contrary findings with the report of Takeet and Fagbemi (2009) that observed lymphocytosis in rabbits infected with *T. congolense*. These suggest that all the graded levels of boiled *M. oleifera* seeds fed the rabbits must have prevented Lymphocytosis. The Red Blood Cells (3.67-5.57 10^9/L) in this study is similar to Red Blood Cells of 4.37-6.23 10^9/L reported by Gbenge *et al*. (2022) that fed healthy growing rabbits yam-cassava peel composite meal as replacement for maize. The haematopoitetic activities of blood parameters that were below the reference range (all Packed Cell Volume treatments; T1 to T4 Haemoglobin; T1, T4 and T5 White Blood Cells; T1, T2 and T4 Red Blood Cells; T2 to T5 Monocytes; Basophils; Mean Corpuscular Haemoglobin Concentration; Mean Corpuscular Volume and T1 Lymphocytes) must have been as a result of the pathogenic effects of the *T. brucei brucei* on the infected rabbits. While other blood parameters within reference range (T5 Haemoglobin; T2 and T3 White Blood Cells; T3 and T5 Red Blood Cells; T2 to T5 Lymphocytes and Eosinophils) must have attained enhancement due to the inclusion of boiled *M. oleifera* seeds in diets of the *T. brucei brucei* infected rabbits. All the Neutrophil values of the treatments in this study which were above reference range were reported to signify infectious and inflamed conditions (Toth and Krueger, 1989) in Trypanosomosis infections. Rabbits in T4 had more of the rabbits scratching off their feed from the feeder than rabbits in other treatments.

This may have led to the lower antitrypanosomal efficacy noticed in T4 when compared to T3 and T5 as this treatment was observed to have consumed more feed containing lesser quantity (3 %)

of the boiled *M. oleifera* seeds than T4 rabbits that consumed less of the boiled *M. oleifera* 4.5 % fortified experimental feed. Higher observations of Anisocytosis, Polychromatic and Hypochromic Red Blood Cells in rabbits (Table 6) connote rapid depletion of Red Blood Cells and there was early feedback response mechanism of replacing the lost Red Blood Cells with immature Red Blood Cells into blood circulation which may not function optimally as the mature Red Blood Cells. More of hypochromic cells (++) observed in all the treatments in this study confirms the higher presence of immature Red Blood Cells in *T. brucei brucei* infected rabbits. The blood parameters below or above reference range and abnormal Red Blood Cells morphology reported in this study are indications of persistence of Trypanosomosis in the rabbits. This may have been due to the ability of trypanosomes to evade immune response by changing its antigenic Variant Surface Glycoproteins (VSG) (Meireles *et al*., 2020).

Table 5: Haematological parameters of *Trypanosoma brucei brucei* infected rabbits fed diets containing graded levels replacement of rice offal with boiled *Moringa oleifera* seed meal

Parameters (Kg)	**Levels of boiled seed meal inclusion (%)**						**p-value**	**SEM**
	T1 (0)	**T2 (1.5)**	**T3 (3)**	**T4 (4.5)**	**T5 (6)**	**Ref. Ranges**		
PCV (%)	25.33	25.00	29.67	26.67	29.00	30-50	0.13	0.72
Hb (g/dl)	8.43	8.83	9.70	9.77	11.13	10-15	0.22	0.39
WBC (10^3mm)	4.00	4.77	4.70	4.20	4.46	4.5-11	0.75	194.56
RBC (10^3mm)	4.40^{c}	3.67^{c}	5.23^{ab}	4.87^{ab}	5.57^{a}	5-8	0.01^{**}	0.21
Lymphocytes (%)	38.67	65.67	60.00	51.67	45.33	40-80	0.41	4.72
Monocytes (%)	1.00	0	0.67	0	0	1-4	-	-
Eosinophils (%)	0	1.67	0	0.3	0.3	0-4	-	-
Basophils (%)	0	0	0	0.3	0	1-7	-	-
Neutrophils (%)	60.33	38.33	39.67	47.33	54.33	22-38	0.47	4.28
MCH	19.13	24.27	18.55	20.05	21.61	19-22	0.22	0.86
MCHC	17.44^{a}	14.71^{b}	17.61^{a}	18.24^{a}	17.90^{a}	30-35	0.03^{*}	0.43
MCV	57.41^{b}	68.51^{a}	56.99^{b}	54.85^{b}	56.10^{b}	78-95	0.02^{*}	1.63

a, b and c means in the same row with different superscripts are significant at $p<0.01^{**}$and $p<0.05^{*}$, T1 rabbits fed diet without boiled *Moringa oleifera* seed meal, T2 rabbits fed diets with 1.5 % of boiled *Moringa oleifera* seed meal, T3 rabbits fed diets with 3 % of boiled *Moringa oleifera* seed meal, T4 rabbits fed diets with 4.5 % of boiled *Moringa oleifera* seed meal and T5 rabbits fed diets with 6 % of boiled *Moringa oleifera* seed meal. Reference range source: Ibitoye *et al*. (2020).

Table 6: Red blood cell morphology of *Trypanosoma brucei brucei* infected rabbits fed diets containing graded levels replacement of rice offal with boiled *Moringa oleifera* seed meal

Treatments	Red blood cell morphology
T1	Anisocytosis (+), Polychromatic (+)
	Hypo (+) Anisocytosis (++) Creanitic (+) Polychromatic (+) cells.
T2	Hypochromic cells (++), toxic granulation of
	Neutrophils (+), Anisocytosis (+).
T3	Hypochromic cells (++), Anisocytosis (+)
	Anisocytosis (+), Polychromatic (+)
	Normocytic, Normochromic cells.
T4	Anisocytosis (+), Polychromatic (+)
	Hypochromic cells (++)
	Hypochromic cells (+).
T5	Hypochromic cells (++)
	Hypochromic cells (+), Normocytic cells.

4.0 CONCLUSION AND RECOMMENDATION

4.1 Conclusion

Boiled *Moringa oleifera* seeds as a prophylactic treatment failed to prevent Trypanosomosis in *Trypanosoma brucei brucei* infected rabbits. However, rabbits fed 3 % boiled Moringa seeds showed significant weight gain and survival time, indicating mild antitrypanosomal activity. Despite this, the treatment didn't improve overall growth performance, increasing economic costs. Haematological profiles revealed abnormalities, although some parameters were within reference range for some treatments. Overall, the study suggests that boiled *Moringa oleifera* seeds recorded limited potential as a prophylactic treatment in feed for Trypanosomosis in rabbits.

4.2 Recommendations

I. 3% boiled *Moringa oleifera* seeds could be recommended as feed additive to ameliorate emaciation and anemia in Trypanosomosis.

II. Further research using other treatment methods for *Moringa oleifera* seeds is suggested.

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