Nigerian J. Anim. Sci. 2021, 23 (2): 161-169

Performance and physiological observations of West African Dwarf goats challenged with *Trypanosoma evansi* and treated with Artemether-Lumefantrine

[*]Salifu, A. O[1]., Bot, M. H[3], Olaolu, O. S[1], Uzoigwe, N[1], Alanza, A. J[4]., Panshak, L. A[2] and Abongaby, G. C[1].

[1]*Nigerian Institute for Trypanosomiasis Research (NITR) Vom*
[2]*Nigerian Institute for Trypanosomiasis Research (NITR) Ibadan.*
[3]*Federal College of Animal Health and Production Technology, Vom.*
[4]*National Veterinary Research Insitute, Vom.*

[*]***Corresponding Author:*** salifu215@gmail.com; ***Phone Number:*** *+2347031151079*

Target Audience: *Livestock farmers, Veterinarians, Animal technologists and Animal scientists*

Abstract

Therapeutic trial of Artemether-Lumefantrine, a non trypanocide was investigated in West African Dwarf (WAD) goats challenged with 10^6trypanosomes/ml intravenously. Twelve male WAD goats with initial average weight of 7kg were used to determine the antitrypanosomal potentials of Artemether. They were randomly divided into 3 groups of 4 animals each in a Completely Randomized Design. Groups 1, 2 and 3 were Artemether treatment group, Diminazene treatment group and untreated group respectively. The animals were acclimatized for 14 days. 'Parameters evaluated were; parasitaemia, performance and physiology of the goats inoculated with T. evansi. At prepatency, groups 1 and 2 goats were treated with Artemether (20mg)-Lumefantrine (120mg) (140mg/5-14kg orally for 3 days) and Diminazene diaceturate (7mg/kg i.m once) respectively while group 3 were untreated. Data were subjected to one way ANOVA and Duncan using SPSS. Line graphs were used to illustrate variations. Results revealed prepatency of 21 to 24 days. Parasitaemia were mostly undetected. Parameters were similar ($P>0.05$) except feed intake, where Artemether group 1($P<0.05$) had the highest value (199g/day). However, weights, survival of Artemether treated group 1 were highest and remained constant across the weeks/days while Diminazene group 2 and Untreated Control group 3 declined. Group 3 had the highest rectal temperature of 38°C at week 2; consequently other groups also attained same temperature at week 4. These observations strongly support the use of Artemether to combat 'Surra'.

Keywords: *Artemether-Lumefantrine; 'Surra'; treatment; WAD goats; parasitaemia; performance; physiology.*

Description of Problem

Surra is a major parasitic disease which affects a variety of mammals such as cattle, sheep, goats, equines, dogs and dromedaries (1). It is caused by *T. evansi* and mainly transmitted mechanically by haematophagus flies (2). These are essentially, Tabanids and Stomoxys, although sucking bats may serve as vectors. *T. evansi,* a salivarian trypanosome of African origin, is a derivative of *T. brucei.* However, unlike its ancestor, it is unable to develop cyclically in Tsetse flies as a result of the absence of maxicircle kinetoplastic DNA (3). Surra may be acute, sub acute, chronic and in apparent, and is characterized by fever, anaemia, weight loss and death, if untreated (4). The disease is managed with therapeutic and

prophylactic trypanocide drugs, without existing vaccine treatment, although such drugs are old, toxic and becoming less effective due to resistance (5 and 3). *Surra* is consequently of high economic significance with major economic losses owing to morbidity and mortality of the infected animals (6, 7 and 5). The populations decline as fertility rates decrease, as well as production losses in milk, meat, draught capabilities and manure. Indirect losses are incurred mainly from prophylactic treatment of susceptible hosts, and surveillance (7). These have necessitated the test for alternative treatment measures such as Artemeter- Lumefantrine, an artemisinin derivate of antimalarial drug which has been reported to exhibit trypanocidal activity on *T. evansi*, *in vitro* and *In vivo* (8, 9, 10). The objective of the present study was to investigate the susceptibility of this trypanosome species and the subsequent trypanocidal potentials of Artemether-Lumefantrine in WAD goats.

Materials and Methods

Study location

The study was carried out in the Livestock division of Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Jos-South Local Government Area (LGA), Plateau State, Nigeria, with geographical location of Latitude 9°44' N and Longitude 8°45'E, on an altitude of 4,200 feet above sea level, relative humidity range of 22% in January to 78% in July/August, with daily mean environmental temperature range of 17 °C to 28.8 °C (11).

Source of trypanosomes

T. evansi (Maiduguri strain) used for the study was obtained from parasitological division of NITR, Vom, where it is being preserved. Parasitized blood was collected via the tail vein of a donor rat with massive parasitaemia using a 5ml syringe containing normal saline which was used to dilute the blood until 32 parasites per field was observed under a × 40 objective. This represents 12.9×10^6 ml^{-1} of parasitized blood using the method of (12).

Experimental drug

A fixed combination of 14% artemether and 86% lumefantrine (Lokmal®), anti malaria, powdered form was procured from a pharmaceutical store in Jos South LGA of Plateau State. The oral dose administered to the experimental WAD goats was based on the manufacturer's directives for infants.

Experimental animals, ethical clearance, management and design

Twelve male WAD goats weighing 7kg approximately, with age range of 15-24 months were purchased from Vom market in Jos South LGA. They were quarantined for two weeks. Ethical clearance was obtained for the use of WAD goats in the experiment from the University of Jos ethical committee with reference number; UJ/FPS/F17-00379. Each goat was given 200g of formulated concentrate ration daily at morning hours of 8am; potable water and fresh mango tree leaves (which was noticed to be the goat's favourite among other browse tree; gmelina, cashew and guava leaves given during acclimatization) was selected and supplied to the animals *ad libitum*. The animals were vaccinated against Pest de Petits Ruminants (PPR), treated against ecto parasites and worm infestation with Ivermectin and Albendazole respectively at 1mL/50kg. Broad Long Acting (L.A) antibiotic spectrums (Oxytetracycline) were administered as prophylactic treatment. Daily morning routine washing and disinfection of the pens, feeding and watering troughs were adhered to. The feed ingredients were procured in the dried form

except the yam peel which was procured from women selling fried and pounded yam in restaurants within Jos South LGA of Plateau State. It was then sun dried for 3 days during the dry season of January to March before storage until used to formulate the concentrate feed. The feed ingredients, proximate composition and phytoconstituents of mango leaves are shown in Table 1. The goats were allotted into three treatment groups of 1 (Artemether-Lumefantrine), 2 (Diminazene diaceturate, positive control) and 3 (Untreated, negative control) comprising four animals per group in a Completely Randomized Design. All the groups were inoculated with blood containing *Trypanosoma evansi*, collected from a donor mouse and treatment was given at prepatency. The post infection period of the experiment was terminated after having 2 mortalities in group 3 and thereafter all surviving goats were treated with Diminazene diaceturate (Nozomil®) at 7mg/kg being the common conventional trypanocide treatment drug. This was done to prevent the goats from becoming a source of '*surra'* outbreak when allowed to graze outdoors after the experiment within the surrounding community. The intensive system of rearing goats was adopted during the experiment.

Laboratory procedures and data collection

The trypanosome parasites were monitored daily at morning hours via drop of blood collected from the marginal ear vein of each goat using the wet thin film parasitological method and viewed under a ×40 objective. The absolute number of parasites viewed were estimated using the (12) method.

Table 1: Percentage composition of the experimental concentrate feed and phytoconstituents of mango leaves fed to growing goats.

Ingredients	Inclusion (kg)	Phytoconstituents of Mango leaves (mg/100g)	
Yam peel (dried)	38.8	Phenol	0.25
Wheat offal	3.4	Flavonoid	12.05
Rice offal	6.8	Tannin	0.5
Cowpea husk	10.2	Saponin	3.18
Grand Nut Cake	13		
Palm Kernel Cake	25.8		
Molasses	1.3		
Common salt	0.5		
Grower vitamin and mineral premix	0.5		
Total	100		
**Calculated proximate analysis of concentrate Feed :*			
Crude Protein	21.80		
Crude fibre	13.2		
Ether Extract	6.1		
Nitrogen Free Extract (NFE)	52.8		
Ash	6.1		
Energy (Kcal/Kg)	3160.58		

*****Nutrient values Source: Aduku's Tropical Feedstuff Analysis Table and NIAS 2020 National Listing of Approved Feed Ingredients for Feed mills in Nigeria.*Phytoconstituent values Source: (13).
NFE=100-(%CP+%CF+%EE+%Ash).. Energy=37×%CP+81.8+%EE+35+%NFE (Source:14)

The rectal temperatures of the goats were collected weekly using digital centigrade thermometer between 8am and 9am. Respiratory rates (cycles.minute) were collected twice during the experiment; at prepatency week and at terminal week respectively by carefully counting the rhythmic movement of the abdominal cavity of each restrained goat for 15 seconds and the number multiplied by 4. The pulse rate (beats/minutes) were determined using a stethoscope placed at the right side of the abdominal cavity of each restrained goat by counting the number of beats heard for 15 seconds and the number multiplied by 4.

The feed intakes per goat were determined by deducting the left over from the total feed given daily. The body weight of each goat were determined on a weekly basis using a 50kg spring balance (pocket scale®), where the live goat restrained in 50 kg woven sack was hung and weight read and thereafter, weight gain or loss was deduced using the formula: Weight gain or loss (Growth) = weight of current week – weight of previous week. The average weight per group were determined using the formula: Average weight (g) = Total weight/ number of replicates. The feed conversion was calculated using the formula: FCR = feed intake/weight gain. The duration of survival up to termination of the experiment for each goat were computed as the survival period.

Statistical analysis

Data generated were subjected to one-way analysis of variance at 5% difference of significance using the Statistical Package for Social Science version 24 and significant values were subjected to Duncan Multiple Range Test (DMRT).

Results and Discussion

The nutrient composition (Table 1) of the concentrate diet for the WAD goats conforms with the recommended % CP and energy requirements described by (15). The phytoconstituents of the mango leaves cut fresh and given to the goats *ad libitum* was tolerable, although slight bloat occurred on few of the experimental goats without adverse effects. This observation corroborates with the report of (13) that mango leaf supplements had no adverse effects in rabbits, which are not known to be natural browsers of tree leaves like goats (16). There was insignificant differences ($P>0.05$) in performace and physiological parameters (Tables 2 and 3) except for the feed intake of which the artemether treated group was significantly higher ($P<0.05$) than the untreated control group. The parasitaemia observed in the study using the wet thin film parasitological method were very low, where parasites at most of the days (figure 1) were undetected (0). This findings were similar to the reports of (17) that had only 4% positive cases out of 1054 WAD goats screened for trypanosomosis. This may either suggest that the wet film diagnosis is not sensitive in detecting trypanosomes in blood of WAD goats or the trypanosmes being in the trypanozoon subgenus must have migrated into the tissues of the animals. (18) Suggest that Apolipoproteins in blood of humans clears *T. evansi* infections. Therefore a presence of trypanolytic lipoproteins in WAD goats could also be a reason for low parasitaemia in this study. Prepatency occurred within 21 to 24 days in this study which was similar to prepatency of experimental *T. evansi* infection in Sheep (19). The significantly higher ($P<0.05$) feed intake and wellness of the artemether treated group compared to the untreated group in this study strongly supports the trypanocidal potentials of Artemesinin derivatives treatment against trypanosomosis as also

reported by other workers (8, 9 and 10) who used rodents in their study.The weights and survival of Artemether treated group 1 were highest and remained constant across the weeks/days while Diminazene group 2 and Untreated Control group 3 declined (Figures 2 and 3). Group 3 had the highest rectal temperature of approximately 38°C at week 2; consequently other groups also attained same temperature at week 4 which were still within the normal range of rectal temperatures for goats. Despite the contrary clinical signs observed for trypanosomosis in this caprine species, acute fatality was observed in groups 2 and 3 as they are seen to stay off feed for a few (1-3) days and exhibited signs of respiratory infection (coughing and gasping for breath) in this study. The symptoms of *T. evansi* infection in this study are similar to *T. simiae* which causes fatal hyper acute infections in pigs (20). The artemisinin derivatives mechanism of trypanocidal activities may be that the lipophilic sesquiterpene lactones present in its extract can increase fluidity of the parasite's membrane leading to continuous flow of ions and metabolites resulting to the parasite's death (21).

Table 2. Performance and cost of feed concentrate used to feed WAD Goats challenged with *T. evansi* and treated with Artemether-Lumefantrine.

Parameters	Group 1 (Artemether) 140mg/5-14kg	Group 2 (Diminazene) 7mg/kg	Group 3 (Untreated)	P-value	SEM
Final Weight (g)	8000	7666.7	8133.3	0.51	15.5
Initial Weight (g)	7166.7	6833.3	6833.3	0.55	13
Growth (g)	833.33	833.33	1300.00	0.56	182.9
Feed Intake/day (g)	199.33^{a}	197.67^{ab}	183^{b}	0.04	3.4
Total Feed Intake (g)	6773.33	6720.00	5986.67	0.117	177.57
*Cost of Feed/Kg (₦)	131.88	131.88	131.88		
Total cost of Feed (₦)	893.27	886.24	789.43	0.116	23.42
FCR	6	9	10	0.502	1.44
Survival period (days)	52	51	41	0.31	3.1

Different a, b means are significant (P<0.05), FCR Feed Conversion Ratio. Cost of feed/Kg not subjected to ANOVA.

Table 3. Physiological indices of WAD Goats challenged with *T. evansi* and treated with Artemether-Lumefantrine.

Parameters	Normal Range	Group 1 (Artemether) 140mg/5-14kg	Group 2 (Diminazene) 7mg/kg	Group 3 (Untreated)	P-value	SEM
Rectal Temperature (°C)	38-39	37.79	37.58	37.76	0.88	0.2
Respiratory Rate (Cycles.Minute)	12-20	26.13	28.5	29.75	0.15	0.8
Pulse Rate (Beats/Minute)	70-90	103	95	97	0.28	2.2

Normal Range of rectal temperature, respiratory rate and pulse rate Sources; (22), (23) and (24) .

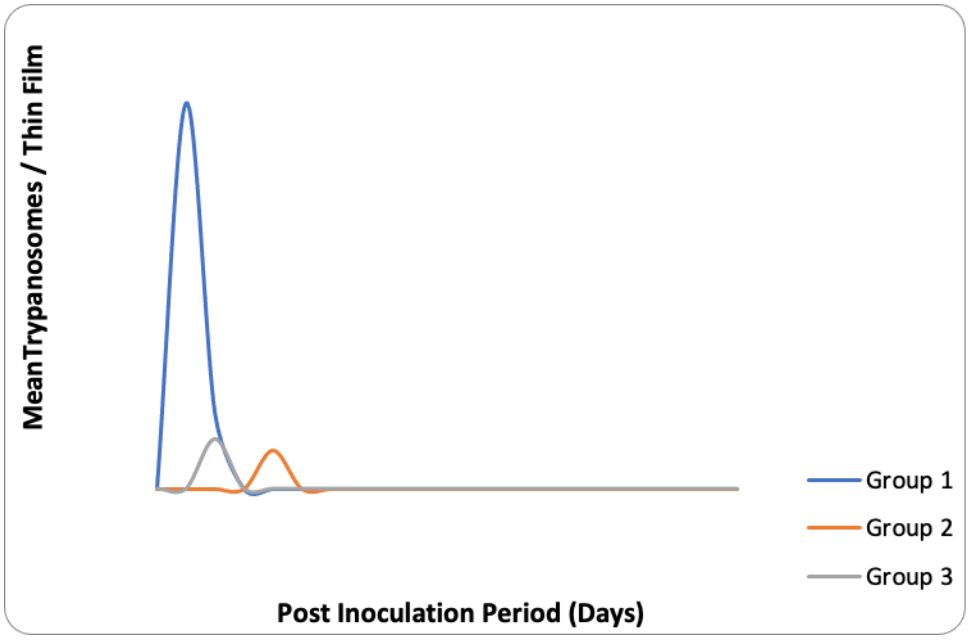

Figure 1: Parasitaemic trends of WAD goats infected with *T. evansi* and treated with Artemether-Lumefantrine using 140mg/5 to 14 kg for 3 days (Group 1),
Diminazene diaceturate using 7mg/kg once (Group 2) and untreated Control
(group 3) across week 1 to 3.

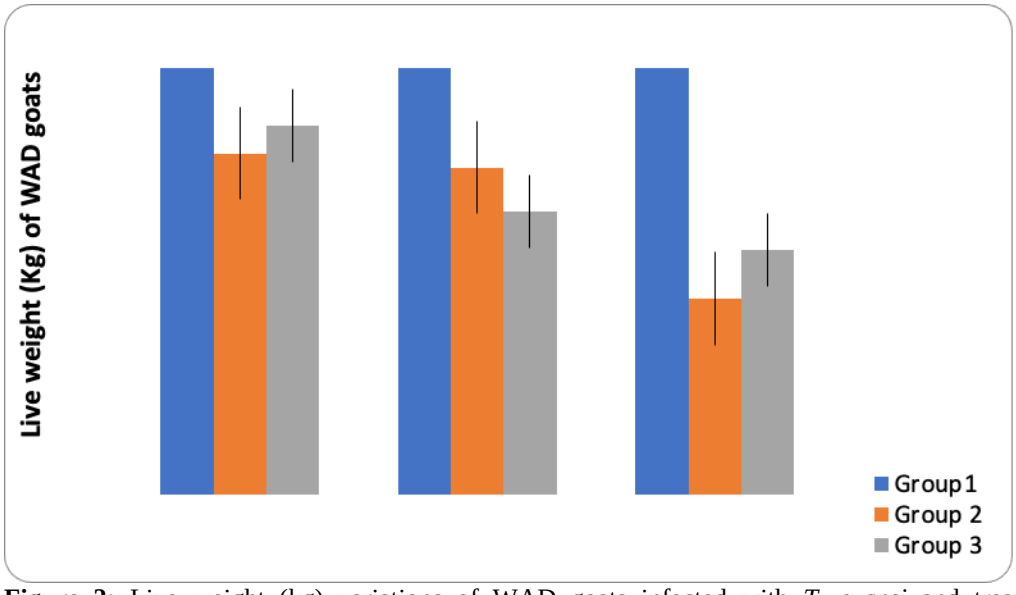

Figure 2: Live weight (kg) variations of WAD goats infected with *T. evansi* and treated with Artemether-Lumefantrine using 140mg/5 to 14 kg for 3 days (Group 1),
Diminazene diaceturate using 7mg/kg once (Group 2) and untreated Control
(group 3) across week 1 to 3.

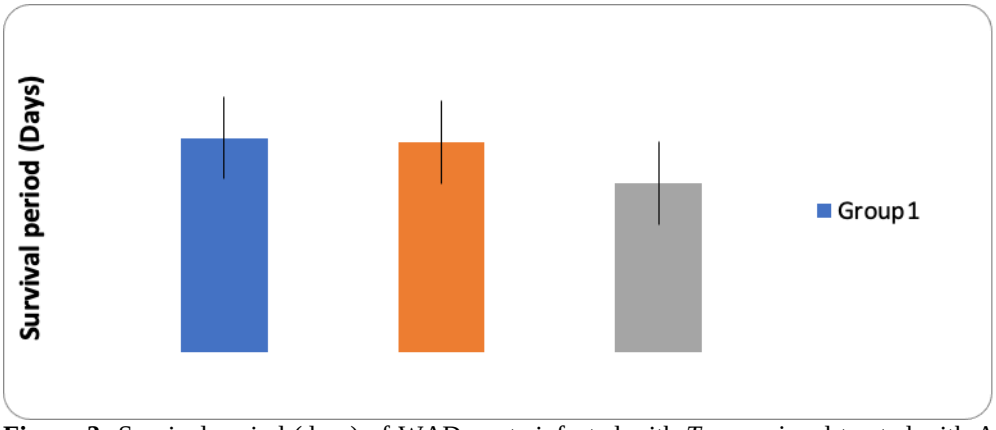

Figure 3: Survival period (days) of WAD goats infected with *T. evansi* and treated with Artemether-Lumefantrine using 140mg/5 to 14 kg for 3 days (Group 1), Diminazene diaceturate using 7mg/kg once (Group 2) and untreated Control (group 3).

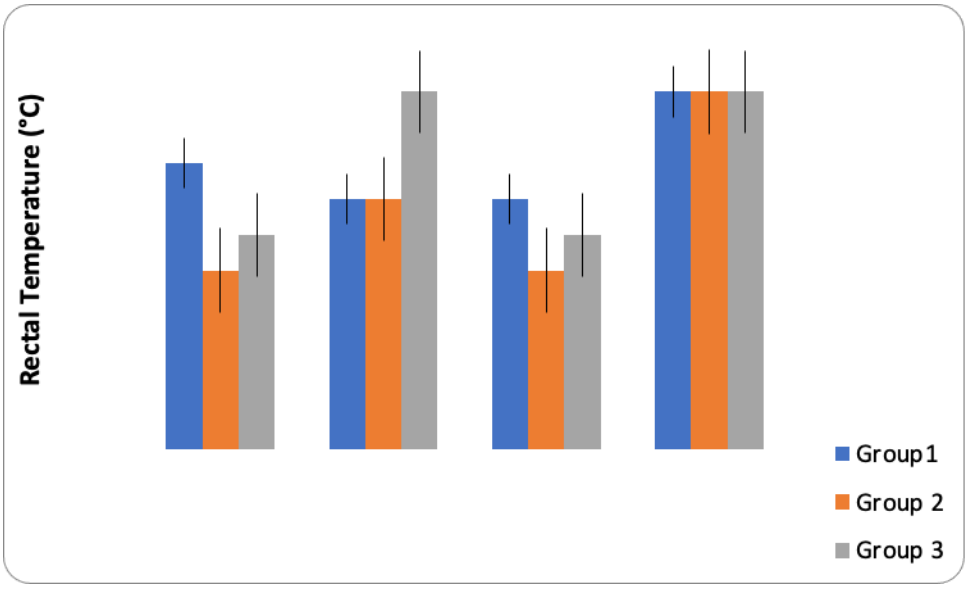

Figure 4: Rectal Temperature (°C) of WAD goats infected with *T. evansi* and treated with Artemether- Lumefantrine using 140mg/5 to 14 kg for 3 days (Group 1), Diminazene diaceturate using 7mg/kg once (Group 2) and untreated Control (group 3).

Conclusion and Applications

1. Results of this study revealed that WAD goats are susceptible to *Trypanosoma evansi* infection.
2. The wet thin film parasitological method of diagnosis is not sensitive to the detection of *T. evansi* in WAD goats when compared to laboratory rats and mice.
3. Artemether-Lumefantrine exhibited trypanocidal potentials within the 38 days post infection trial on the WAD male goats.
4. Positive outcome of the trials could

make Artemether-Lumefantrine also listed officially as a trypanocide.

5. A comprehensive study of Artemether-Lumefantrine use as treatment in various *Trypanosoma species* on different susceptible livestock is highly recommended.

References

1. Chandu, A.G., Sengupta, P.P., Jacob, S.S., Borthakur, S., Patra, G., and amp; Roy, P. (2021). Mining the pervasiveness of surra in different animal species of Northeastern states of India: Assam, Mizoram and Tripura. *Journal of Parasitic Diseases*, 45, 330 - 335.
2. Birhanu, H., Rogé, S., Simon, T., Baelmans, R., Gebrehiwot, T., Goddeeris, B. M., and Büscher, P. (2015).Surra Sero K-SeT, a new immunochromatographic test for sero diagnosis of *Trypanosoma evansi* infection in domestic animals. *Veterinary parasitology*, 211(3-4), 153–157.
3. Desquesnes, M., Holzmuller, P., Lai, D. H., Dargantes, A., Lun, Z. R., and Jittaplapong, S. (2013).*Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomedical research international*, 19:41-76.
4. Eyob, E., and Matios, L. (2013). Review on camel trypanosomosis (surra) due to *Trypanosoma evansi*: Epidemiology and host response. *Journal of veterinary medicine and animal health* 5(12), 334-343.
5. Aregawi, W.G., Agga, G.E., Abdi, R.D. and Buscher, P. (2019).Systematic review and meta-analysis on the global distribution, host range, and prevalence of *Trypanosoma evansi*. *Parasites Vectors* 12, 67.
6. Assefa, S. and Shibeshi, W. (2018). Drug resistance in African animal trypanosomes: A review. *African Journal of Microbiology Research*, 12(17), 380-386.
7. Dewi R. S, Damajanti, R., Wardhana, A.H., Mulatsih, S., Poetri, O.N., Steeneveld, W., and Hogeveen H. (2020). The Economic Losses of Surra Outbreak in Sumba Timur, Nusa Tenggara Timur- Indonesia. *Tropical Animal Science Journal*, 43(1):77-85
8. Mbaya, A.W., Ahmed, M.I., Adamu, M. and Gyang, S.N. (2018). Toxicity and combined therapeutic activity of artemether and lumefantrine in *Trypanosoma-brucei*-infected rats. *Nigerian Journal of Parasitology· July 2009* https://www.researchgate.net/publication/272694727
9. Salifu, A. O., Abongaby, G., Aluma, L. A. and Alanza, A. J. (2020). Sensitivity of *Trypanosoma evansi In vitro* to Artemether-Lumefantrine and its bioinfectivity trial in mice. *Tropical Journal of Agricultural Research*, 2(2): 1-8.
10. Jolayemi, K. O., Mamman, M., Sani, D., Okoronkwo, M. O. and Ameje, J. (2020). *In vivo* and *in vitro* changes observed in Trypanosoma brucei brucei infected rats treated with artesunate and/or diminazene aceturate. *Sokoto Journal of Veterinary Sciences*, 18(4): 211-220.
11. National Veterinary Research Institute (NVRI). (2018). Meteorological unit, Vom.
12. Herbert, N.J. and Lumsden, W.H. (1976). *Trypanosoma brucei*: A rapid ―Matching‖ Method for Estimating the Host‘s parasitaemia. *Experimental Parasitology* 40: 429-431.
13. Ogunsipe, M. H. and Ibidapo, I. (2021).

Supplemental value of Mango leaf meal (*Mangifera indica* L) on Growth performance and Haemo-Biochemical properties of Rabbits. NSAP 46th Annual Conference-Dutsin-Ma book of proceedings. P. 316.

14. Pauzenga, U. (1985). Feeding parent stock. *Zootenicia International*. December, 1985. P. 22-24.
15. Aduku, A. O. (2004). Animal Nutrition in the Tropics. Feed and Feeding, Pasture Management, Monogastric and Ruminant Nutrition. P. 133.
16. Gaddafi, S., Garba, M. G., Bature, I., Babba, H. U. and Alkali, M. M. (2021). An overview of goats natural browsing potentials: Short communications. NSAP 46th annual conference-Dutsin-Ma book of proceedings. P. 504.
17. Behnke, J. M., Chiejina, S. N., Musongong, G. A., Nnadi, P. A., Ngongeh, L. A., Abonyi, F. O. and Fakae, B. B. (2010). Resistance and resilience of traditionally managed West African Dwarf goats from the savanna zone of northern Nigeria to naturally acquired trypanosome and gastrointestinal nematode infections. *Journal of Helminthology*, 85 (1).
18. Van Vinh, C. N., Buu, C. L., Desquesnes, M., Herder, S., Phu Huong, L. N., Campbell, J. I., Van Cuong, N., Yimming, B., Chalermwong, P., Jittapalapong, S., Ramon, F. J., Tri Tue, N., Rabaa, M. A., Carrique-Mas, J., Pham Thi Thanh, T., Tran Vu, T. N., Berto, A., Thi Hoa, N., Van Minh Hoang, N., Canh Tu, N., Khac Chuyen, N., Wills, B., Tinh Hien, T., Thwaites, G. E., Yacoub, S. and Baker, S. (2016). A clinical and epidemiological investigation of the first reported human infection with the zoonotic parasite *Trypanosoma evansi* in Southeast Asia. *Clinical Research in Infectious Diseases* 62, 1002–1008.
19. Adeyeye, A. A., Ate, I. U., Lawal, A. I. and Adamu, S. (2017). Leukocyte changes in pregnant Yankassa Ewes Experimentally infected with *Trypanosoma evansi*. *Nigerian Veterinary Journal*, 38(2).
20. Leach, T. M. and Roberts, C. J. (1981). Present status of chemotherapy and chemoprophylaxis of animal trypanosomiasis in the Eastern hemisphere. *Pharmacology and Therapeutics* 13, 91–147.
21. Nibret, E. and Wink, M. (2010). Volatile components of four Ethiopian Artemisia species extracts and their *in vitro* antitrypanosomal and cytotoxic activities. *Phytomedicine*, 17(5):369–374.
22. Sanusi, A. O., Peter, S. O., Sonibere, A. O. and Ozojie, M. O. (2011). Effect of coat colour on heat stress among West African Dwarf sheep. *Nigerian Journal of Animal Production*, 38(1): 28-36.
23. Takuji, H. and Kazuo, K. (2004). Effect of heat exposure and restricted feeding on behaviour, digestibility and growth hormone secretion in goats. *Asian-Australian Journal of Animal Science*. 17(5): 655–658.
24. Saka, A. A, Adedeji, O. Y. and Jinadu, K. B. (2021). Influence of diets containing graded levels of raw and fermented malted sorghum sprout on the thermo-physiological parameters of West African Dwarf goats. NSAP 46th Annual Conference book of Proceedings. P. 517.