

EFFECT OF ORAL COPPER SULPHATE SUPPLEMENTATION ON THE SEVERITY OF *TRYPANOSOMA BRUCEI* AND *TRYPANOSOMA CONGOLENSE* INFECTIONS IN RATS

EGBE-NWIYI¹, T. N., IGWE¹, N.C., IGBOKWE¹, I.O. and NWOSU*², C. O.

¹*Department of Veterinary Pathology, ²Department of Veterinary Microbiology & Parasitology
University of Maiduguri, P. M. B. 1069 Maiduguri,
Borno State, Nigeria*

***Current address for correspondence:**
Dept. of Veterinary Parasitology & Entomology,
University of Nigeria, Nsukka, Enugu State.
E-mail: abiamaiduguri@yahoo.com

SUMMARY

Eighty healthy adult albino rats of both sexes weighing 150-200 grams were used to assess the effect of oral copper sulphate (CuSO₄) supplementation on *T. brucei brucei* (Wamba strain) or *T. congolense* (Karu strain) infections. Two studies were carried out and the rats were divided into 4 groups of 10 rats each (per study) namely; Group A (uninfected un-supplemented control), Group B (uninfected supplemented control), Group C (*T. b. brucei* infected, 1 x 10⁶ trypanosomes; or *T. congolense* infected, 1 x 10⁶ trypanosomes) and Group D (*T. b. brucei* infected, 1 x 10⁶ trypanosomes or *T. congolense* infected, 1 x 10⁶ trypanosomes and supplemented). Each rat in groups B and D received copper sulphate solution (at 50 mg/kg diluted in distilled water) by intra-gastric infusion daily for 10 days before and during the course of the infection. The prepatent periods and survival times were shorter (P<0.05), the level of parasitaemia higher (P<0.05) and anaemia more severe (P<0.05) in *T. b. brucei* or *T. congolense* infected un-supplemented rats than the infected but supplemented counterparts. Copper sulphate supplementation appeared to reduce the severity of *T. b. brucei* or *T. congolense* infections in rats. Subsequent studies are expected to focus on the effect of copper sulphate supplementation alone and in combination with other known trypanocides (such as diminazene aceturate) in domestic animals.

KEYWORDS: Copper sulphate, supplementation, *Trypanosoma brucei*, *Trypanosoma congolense*, infection, severity, rats.

INTRODUCTION

Many haemoparasitic diseases including anaplasmosis, babesiosis and trypanosomosis are present and affect livestock production in Nigeria (Losos, 1986; Egbe-Nwiyi and Chaudhry, 1994).

Livestock trypanosomosis represents considerable economic problem associated with poor milk production, emaciation, anaemia and sometimes death (Radostits *et al.*, 1994). The

major species of trypanosomes responsible for livestock trypanosomosis are

Trypanosoma vivax, *T. congolense* and *T. brucei*. Sleeping sickness in man is caused by *T. brucei rhodesiense* and *T. b. gambiense* (Losos, 1986).

Drugs, such as diminazene aceturate (Berenil®), used for the treatment of livestock trypanosomosis are not usually available to the rural livestock farmers in Nigeria due to scarcity and high cost. In addition, the relapse of infection in infected animals that were treated with diminazene aceturate or other known trypanocides had been reported (Losos, 1986; Onyeyili and Onwualu, 1991; Onyeyili and Egwu, 1995; Egbe-Nwiyi and Antia, 1996). Consequently, attention has been directed at the development of local herbal trypanocides and the supplementation of the nutrition to enhance resistance and tolerance. For instance, the level of parasitaemia in *T. brucei* infected mice was suppressed by the administration of ethanolic extract of root bark of *Nauclea Latifolia* (Madubunyi, 1995) while the level of parasitaemia was suppressed and the survival time of rats infected with *T. brucei* was prolonged by stem bark extract of *Butyrospermum paradoxum* (Rabo, 1998). Similarly, nutrition had been reported to ameliorate the severity of trypanosome infections in animals (Otesile *et al.*, 1991; Igbokwe, 1995) while rats infected with various species of trypanosomes but whose diets were supplemented with oral magnesium chloride (at 100 mg/kg body weight) were shown to tolerate the effects of the infections better than the infected but un-supplemented group (Egbe-Nwiyi *et al.*, 2003). Sheep infected with *T. congolense* and supplemented with high protein diet withstood the effect of the infection better than those infected but received low protein diet (Katunguka – Rwakishaya, 1997).

Copper (Cu) is one of the most available minerals in animals and it is necessary for normal metabolism in the body (Kaneko, 1989). The deficiency of this important trace element had been reported to result in nutritional anaemia because of its involvement in haemoglobin synthesis and poor immune responses in both man and animals (Schalm *et al.*, 1975; Kaneko, 1989; Suttle and Jones, 1989) while higher traces of it were present in the trypanotolerant Keteku than the trypanosusceptible White Fulani cattle (Awolaja *et al.*, 1997). On the other hand, large oral dose of copper (Cu) caused renal damage (Kaneko, 1989; Radostits *et al.*, 1994).

This study was therefore designed to determine the effect of low oral copper sulphate supplementation on the severity of trypanosome infections in rats.

MATERIALS AND METHODS

Experimental animals

Eighty healthy adult albino rats of both sexes weighing between 150-200 grams obtained from the Department of Human Pharmacology, University of Maiduguri, Nigeria, were used for the study. The rats were maintained on a standard diet (ECWA Feeds LTD, Jos, Nigeria) and housed in clean plastic cages maintained at room temperature (30-35°C). Clean water was provided *ad libitum*.

Trypanosomes

Trypanosoma congolense (Karu strain) and *T. brucei brucei* (Wamba strain) were obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom, Nigeria. Each species was passaged serially in donor rats.

Trypanosome infection

The experimental rats were infected with tail blood from the donor rats containing 1×10^6 *T. congolense* or *T. b. brucei*. Blood samples were diluted with cold normal saline (pH 7.4). Forty rats (20 for *T. congolense* and 20 for *T. b. brucei*) were infected with the parasites by the intraperitoneal route while the remaining forty rats served as uninfected un-supplemented or supplemented controls. Parasitaemia was first detected by the wet mount method while the level of parasitaemia was estimated using the haemocytometer method (Schalm *et al.*, 1975).

Copper sulphate

Five grams of copper sulphate (BDH Chemicals Ltd., Poole England) were weighed, dissolved in 100ml of distilled water and administered to the rats intragastrically (Waynforth and Flecknell, 1992). Each rat in groups B and D received 0.1ml (5 mg/rat) of the CuSO_4 daily for 10 days before and during the course of the infection.

Experimental design

Two studies were carried out and the eighty rats (40 per study) used were divided as follows into 4 groups of 10 rats each: Group A, uninfected un-supplemented; Group B, uninfected supplemented; Group C, infected with *T. b. brucei* (1×10^6 trypanosomes) or *T. congolense* (1×10^6 trypanosomes) and un-supplemented; Group D, infected with *T. b. brucei* (1×10^6 trypanosomes) or *T. congolense* (1×10^6 trypanosomes) and supplemented.

Determination of Packed Cell Volume

The packed cell volume (PCV) of the tail blood of the rats was determined every 4 days by the microhaematocrit method (Schalm *et al.*, 1975).

Post mortem examination of carcasses

The infected rats that died from the disease and the uninfected rats humanely sacrificed at the end of the experiment were subjected to necropsy (Igbokwe, 1989). The liver and spleen were carefully removed, washed, weighed and gross lesions (if any) were recorded. The survival time of each rat from infection to death was noted.

Statistics

The data obtained were summarized as mean and standard deviations and analysis of variance (ANOVA) and students' t-test were used to analyse the data (Chatfield, 1983).

RESULTS

The mean prepatent periods and survival times were shorter ($P < 0.05$) in both *T. b. brucei* and *T. congolense* infected un-supplemented rats (group C) than in the infected but supplemented group D (Table I). The onset of parasitaemia was earlier and progressively higher ($P < 0.05$) in

T. b. brucei or *T. congolense* infected un-supplemented animals (group C) than in the infected but supplemented group D (Figs. 1 and 2). Mortality was also earlier in both *T. b. brucei* and *T. congolense* infected un-supplemented rats. The *T. b. brucei* infected un-supplemented and *T. congolense* infected un-supplemented rats could not go beyond days 16 and 38 post infection (P1) respectively.

TABLE I: Mean (\pm S.D.) prepatent periods and survival times of rats infected with *Trypanosoma brucei brucei* and *T. congolense* with or without copper sulphate supplementation

Treatment group	Prepatent period (days)	Survival time (days)
T.b. brucei (infected/supplemented)	7.0 \pm 0.9	21.2 \pm 2.9
T. congolense (infected/supplemented)	12.3 \pm 1.5	55.2 \pm 3.2
T.b. brucei (infected/unsupplemented)	4.1 \pm 0.9	13.4 \pm 1.5
T. congolense (infected/unsupplemented)	8.1 \pm 0.7	38.4 \pm 6.0

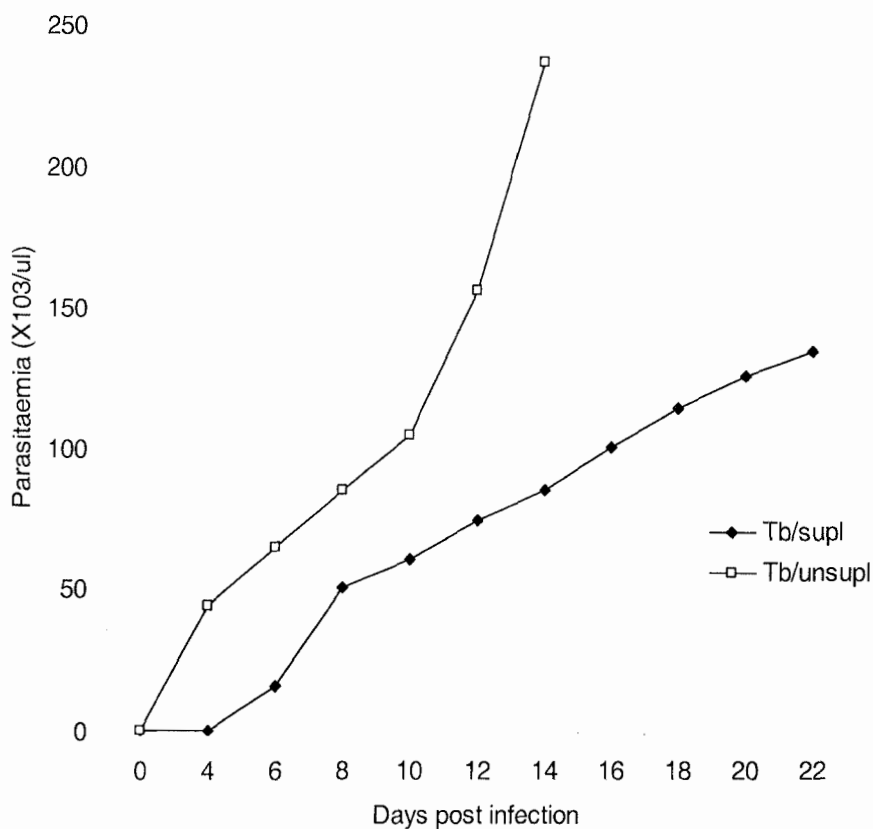


Fig. 1. Mean parasitaemia level of *T.b. brucei* infected rats with or without oral copper sulphate supplementation

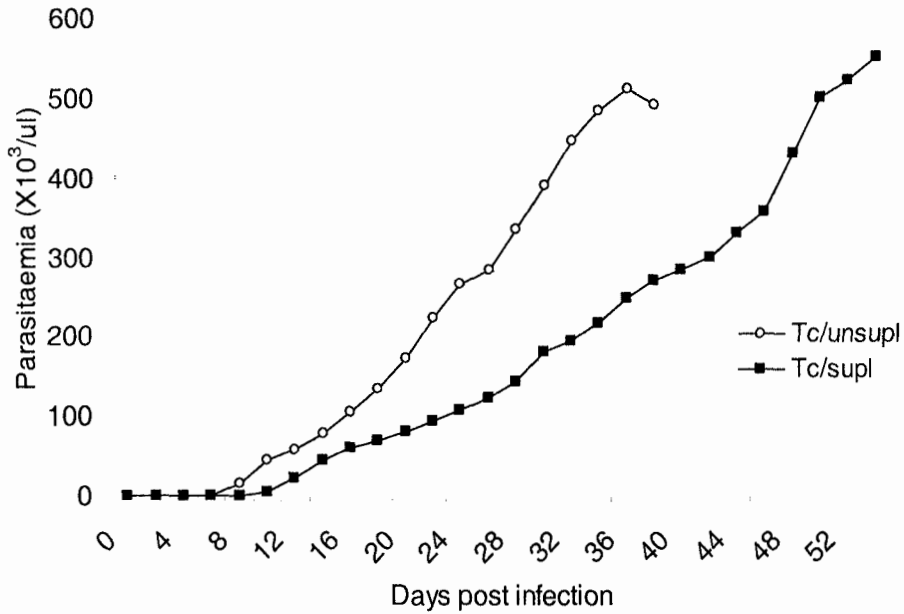


Fig. 2. Mean parasitaemia of *T. congolense* infected rats with or without oral copper sulphate supplementation

The packed cell volume (PCV) of the uninfected controls (groups A and B) did not differ significantly ($P>0.05$) from each other but differed significantly ($P<0.05$) from the *T. b. brucei* and *T. congolense* infected groups (C and D) (Figs. 3 and 4). In the *T. b. brucei* infected un-supplemented and supplemented rats; the PCV started decreasing from days 8 and 12 PI respectively. The PCV value was lower ($P<0.05$) in *T. b. brucei* infected un-supplemented than the infected supplemented rats (Fig. 3). The PCV started decreasing from day 20 PI in *T. congolense* infected groups (Fig. 4) and the value of the infected un-supplemented rats became significantly lower ($P<0.05$) than that of the infected supplemented animals between days 24-36PI.

The copper sulphate supplementation exhibited no appreciable effect on the PCV values of the uninfected supplemented rats (group B).

The liver and spleen weights of the *T. b. brucei* and *T. congolense* infected rats increased significantly when compared with those of the uninfected controls (groups A and B). Therefore, the hepatomegaly and splenomegaly were higher ($P<0.05$) in both *T. b. brucei* and *T. congolense* infected un-supplemented than the infected supplemented rats (Table II).

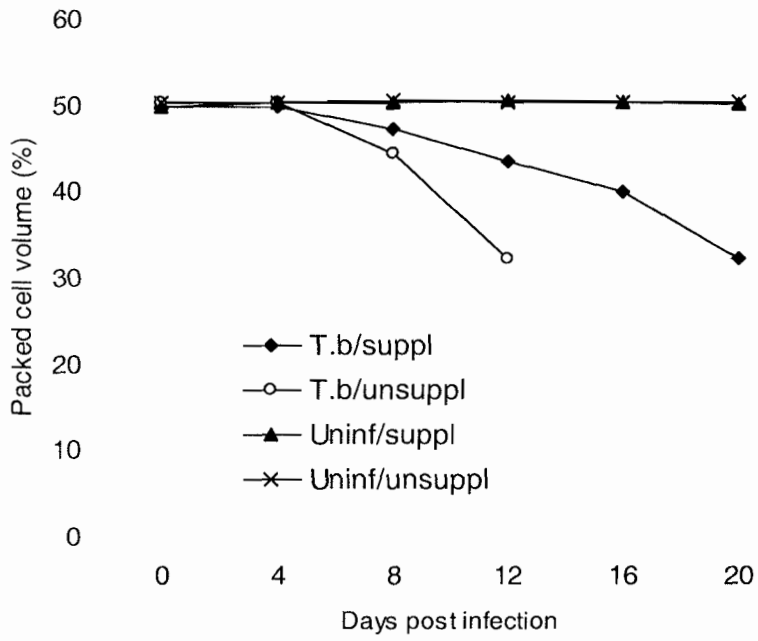


Fig. 3. Mean packed cell volume of rats infected with *T. b. brucei* (with or without oral copper sulphate supplementation) and their controls

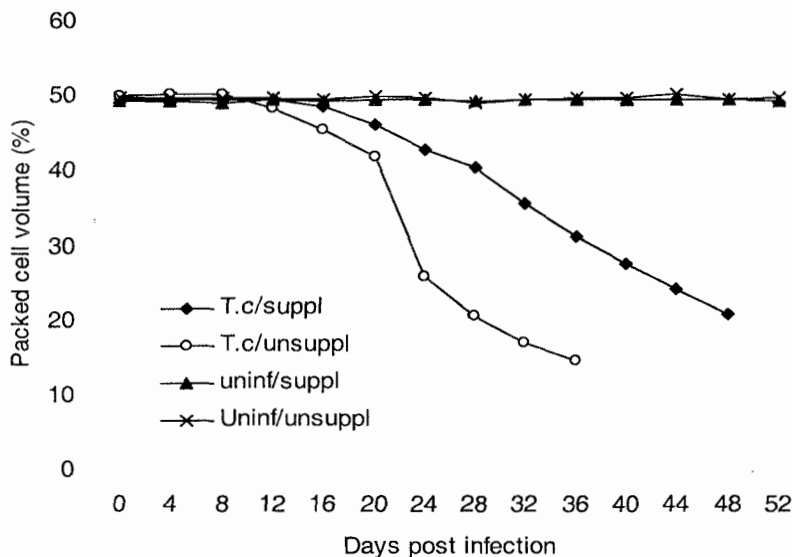


Fig. 4. Mean packed cell volume of rats infected with *T. congolense* (with or without oral copper sulphate supplementation) and their controls

TABLE II: Mean (\pm S.D.) Liver and spleen weights (g/100 g body weight) of control rats and *T. b. brucei* with or *T. congolense* infected rats with or without oral copper sulphate supplementation

Treatment groups	Liver weight (g/100g body weight)	Spleen weight (g/100g body weight)
<i>T. brucei brucei</i> infected		
Supplemented (n=10)	6.6 \pm 0.7 ^b (2.1)*	2.7 \pm 0.3 ^b (1.6)
Unsupplemented (n=10)	10.1 \pm 0.8 ^c (3.3)	4.8 \pm 0.3 ^c (2.8)
<i>T. congolense</i> infected		
Supplemented (n=10)	6.5 \pm 0.4 ^b (2.2)	2.8 \pm 0.2 ^b (2.3)
Unsupplemented (n=10)	9.8 \pm 1.0 ^c (3.3)	4.9 \pm 0.4 ^c (4.1)
Uninfected controls		
Supplemented (n=10)	3.1 \pm 0.2 ^a	1.7 \pm 0.2 ^a
Unsupplemented (n=10)	3.0 \pm 0.2 ^a	1.2 \pm 0.2 ^a

n = number of rats in each group; *Number of times organ weight increased
^{abc}Values in rows with different superscripts are significantly different (P<0.05).

DISCUSSION

This study has shown that prepatent periods and survival times were shorter in *T. b. brucei* or *T. congolense* infected un-supplemented rats when compared with similarly infected rats that received oral copper sulphate supplementation. The reduction of the above two parameters in the infected but un-supplemented groups may not be infective-dose related, as all the infected groups received similar numbers of the parasites. Similar observations were made in previous studies (Egbe-Nwiyi, 2002; Egbe-Nwiyi *et al.*, 2003). In addition, the shorter prepatent periods and survival times may not be attributed to the nutritional status of the host or the virulence of the species of the trypanosomes used but rather to lack of mineral supplementation in them. This is based on the premise that each group of the rats was exposed to the same quantity and quality of feed and water and the same strain of the species of the trypanosome.

Anaemia is a constant phenomenon in African animal trypanosomosis (Anosa, 1988; Murray and Dexter, 1988) and it signals and determines the severity of infection in an infected host (Losos, 1986; Radostits *et al.*, 1994). The anaemia observed in the present study appeared to be more severe in the infected un-supplemented animals and this might have been contributed by the higher levels of parasitaemia encountered in these groups of animals. Murray and Dexter (1988) showed that the height of parasitaemia determine the degree of anaemia in African trypanosomiasis depending on several biological factors including the virulence of the strain of the trypanosomes used, the nutritional status and the age of the host. Therefore, in the present study, the copper sulphate supplementation seemed to have played a key role in the

differences observed between the supplemented and un-supplemented infected groups with respect to anaemia and other vital parameters. For instance, copper plays an important role in the synthesis of haemoglobin (Schalm, *et al.*, 1975).

The liver and spleen were enlarged in both *T. b. brucei* and *T. congolense* infected supplemented and un-supplemented animals and these observations agree with Anosa (1988). However, the two organs were more enlarged in *T. b. brucei* or *T. congolense* infected un-supplemented rats and this is in consonance with the findings of Egbe-Nwiyi *et al.* (2003). The greater degree of enlargement of these organs in the *T. b. brucei* or *T. congolense* infected un-supplemented rats might have contributed to the more severe anaemia experienced by these groups than their infected but supplemented counterparts. Enlarged liver and spleen have been reported to contribute significantly to the level of anaemia in African animal trypanosomosis through increased erythrophagocytosis in the spleen and liver (Anosa, 1988). The phagocytic activity of the organs especially the spleen is increased during trypanosomiasis and Murray and Dexter (1988) have shown that the enlarged spleen traps and destroys normal and abnormal red cells. Although, the spleen increased many times in the present study, the increase is less than the 12 times in *T. brucei* infected rats, 11 times in *T. congolense* infected rats (Brown and Losos, 1977), 3 times in *T. brucei* infected rats, 5 times in *T. congolense* infected rats (Igbokwe and Nwosu, 1997) and 25.9 times in *T. brucei* infected mice (Anosa and Kaneko, 1984) reported in previous studies. The difference in splenic enlargement observed between this and previous studies may be related to the dose and virulence of the

strains of trypanosomes used as well as the nutritional status of the hosts.

Copper sulphate supplementation did not exhibit any significant influence on the PCV of the uninfected supplemented rats throughout the observation period. Consequently, copper sulphate supplementation appears to reduce the pathogenicity of trypanosome infections in rats probably through its role in generation of immune response (Suttle and Jones, 1989). The positive effect of copper sulphate supplementation in reducing the severity of trypanosome infection in the present study may be corroborated with the fact that copper is one of the trace elements and higher levels of trace elements were reported in the trypanotolerant Keteku than the trypanosusceptible White Fulani cattle (Awolaja *et al.*, 1997; Oladele and Antia, 2000). Therefore, in an apparent absence of any of the conventional trypanocides, mineral supplementation (especially with copper sulphate) may be of assistance in trypanosusceptible animals likely to be exposed to trypanosome infection or tsetse challenge.

In conclusion, oral copper sulphate supplementation has no significant effect on the PCV but seems to reduce the severity of trypanosome infections in rats as evidenced by the shorter prepatent periods and survival times, higher level of parasitaemia, more severe anaemia and higher degrees of splenomegaly and hepatomegaly in the trypanosome infected un-supplemented rats.

ACKNOWLEDGEMENTS

The authors are grateful to Messrs J. Jubril, N.D. Nwagbara, A. Ibrahim and S. Amali for technical assistance and to Mr.

Patrick Chuhwak Peter for Secretarial services.

REFERENCES

- ANOSA, V.O. (1988). Haematological and biochemical changes in human and animal trypanosomiasis. Parts I and II. *Rev. Elev. Med., Vet. Pays. Trop.* **41**: 65-78; 151-164.
- ANOSA, V.O. and KANEKO, J.J. (1984). Pathogenesis of *Trypanosoma brucei* infection in deer mice (*Peromyscus maniculatus*). Ultrastructural pathology of the spleen, liver, heart and Kidneys. *Vet. Path.* **21**: 229-237.
- AWOLAJA, O.A, ANTIA, R.E. and OYEJIDE, A. (1997). Trace element levels in plasma/serum and erythrocytes of Keteku and white Fulani cattle. *Trop. Anim. Hlth Prod.* **29**: 2-6.
- BROWN, L.A. and LOSOS, G.J. (1977). A comparative study of the response of the thymus, spleen, lymph nodes and bone marrow of the albino rat to infection with *Trypanosoma congolense* and *Trypanosoma brucei*. *Res. Vet. Sci.*, **23**: 196-203.
- CHATFIELD, C. (1983): Statistics for Technology, A course in Applied Statistics, 3rd edn., Chapman and Hall, London, Pp. 134-148.
- EGBE-NWIYI, T.N. (2002). Studies on some factors affecting the pathogenicity of trypanosome infections in rats. Ph.D Thesis, University of Maiduguri, Nigeria.
- EGBE-NWIYI, T.N. and CHAUDHRY, S.U.R. (1994). Trypanosomosis: Prevalence and pathology of camel of Arid Zone of North Eastern Nigeria. *Pakistan Vet. J.* **14**: 24-27.
- EGBE-NWIYI, T.N. NWOSU, S.C. and TSUYA, R.D. (2003). The effect of high oral magnesium chloride supplementation on the pathogenicity of *Trypanosoma brucei brucei* and *T. congolense* infections in rats. *Trop. Vet.* **21**: 152-159

- EGBE-NWIYI, T.N. and ANTIA, R.E. (1996). Relapses in experimental canine trypanosomosis with special reference to the effect of splenectomy. *Israel J. Vet. Med.* **51**: 37-41.
- IGBOKWE, I.O. (1989). Post-mortem examination of carcasses. *Zariya Vet.* **4**: 145-152.
- IGBOKWE, I.O. (1995). Nutrition in the pathogenesis of African trypanosomosis. Protozoological Abstracts, CAB International, **19**: 797-807.
- IGBOKWE, I.O. and NWOSU, C.O. (1997). Lack of Correlation of anaemia with splenomegaly and hepatomegaly in *Trypanosoma brucei* and *Trypanosoma congolense* infections in rats. *J. Comp. Path.* **117**: 261-265.
- KANEKO, J.J. (1989). Clinical biochemistry of domestic animals, 4th edn., Academic Press, Philadelphia, USA, Pp. 360-389.
- KATUNGUKA-RWAKISHAYA, E. (1997). Influence of dietary protein on some blood biochemical parameters in Scottish Blackface sheep experimentally infected with *Trypanosoma congolense*. *Vet. Parasitol.* **68**: 227-240.
- LOSOS, G.J. (1986). Infectious Tropical diseases of domestic animals, Churchill Livingstone Inc., New York, Pp. 183-231.
- MURRAY, M. and DEXTER, T.M. (1988). Anaemia in bovine African trypanosomosis. *Acta Trop.* **45**: 389-432.
- MADUBUNYI, I.I. (1995). Antihepatotoxic and trypanocidal activities of the ethanolic extract of *Nauclea latifolia* root bark. *J. Herbs, spices and Medicinal plants*, **3**: 23-35.
- OLADELE, O.A. and ANTIA, R.E. (2000). Tissue minerals and trypanotolerance in Nigerian cattle. *Nig. Vet. J.* **21**: 51-60.
- ONYEYILI, P.A. and EGWU, G.O. (1995). Chemotherapy of African trypanosomosis. A historical perspective. Protozoological Abstracts, CAB International, **19**: 230-241.
- ONYEYILI, P.A. and ONWUALU, J.E. (1991). Efficacy of combination of DFMO and diminazene aceturate in the treatment of late stage *Trypanosoma brucei brucei* infection in rats. *Trop. Med. Parasitol.*, **42**: 143-145.
- OTESILE, E.B., FAGBEMI, B.O. and ADEYEMO, O. (1991). The effect of *Trypanosoma brucei* infection on serum biochemical parameters in boars on different planes of dietary energy. *Vet. Parasitol.*, **40**: 207-216.
- RABO, J.S. (1998). Toxicity studies and Trypanosuppressive effects of stem bark extract of *Butyrospermum paradoxum* in laboratory animals. Ph.D Thesis, University of Maiduguri, Nigeria.
- RADOSTITS, O.M., BLOOD, D.C. and GAY, C.C. (1994). Veterinary Medicine, 8th edn., Bailliere Tindall, London, Pp. 1209-1220.
- SCHALM, O.W. JAIN, N.C. and CARROLL, E.J. (1975). Veterinary Haematology, 3rd edn., Philadelphia, Lea and Febiger, P. 20-280.
- SUTTLE, N.F. and JONES, J.D. (1989). Recent developments in trace elements metabolism and function. Trace elements, disease resistance and immune responsive in ruminants. *J. Nut.* **119**: 1055.
- WAYNFORTH, H.B. and FLECKNELL, P.A. (1992). Experimental and surgical techniques in the rat. 2nd edn., Academic Press Ltd, London and New York, Pp. 342.