Effects Of Dietary Supplementation Of Vitamin E And Selenium On Blood Parameters Of Trypanosome-Infected Rats (*Rattus rattus*)

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Abstract

This study was carried out to evaluate the combined effects of dietary supplementation of vitamin E (Vit E) and selenium (Se) on total white blood cells, mononucleated cells, polymorphonucleated cells and packed cell volume of *Trypanosoma congolense*-infected rats *(Rattus rattus).* Ninety adult *R. rattus* of pure breed of whiskers were used. The rats were randomly assigned to six treatments groups (A - F) of six cages with five rats in each cage. Each treatment was replicated three times. The rats were fed chicks' mash-based diets (Top Feed Industries) containing varied quantities of vitamin E and selenium. In Treatment A, the control, neither vitamin E nor selenium was included in the diet while in other treatments (B - F) the chicks' mash contained different levels of inclusion of vitamin E and selenium, as follows: Diet B, 0.1 mg Se plus 60 mg Vit E, Diet C, 0.3 mg Se plus 80 mg Vit E, Diet D, 0.5 mg Se plus 100 mg Vit E, Diet E, 0.3 mg Se plus 0.0 mg Vit E and Diet F, 0.0 mg Se and 80 mg Vit E respectively. Blood samples of the rats from each treatment were taken on weekly basis and the blood parameters determined. The experiment lasted five weeks at the end of which it was found that 0.3 mg Se plus 80mg Vit E (Diet C) significantly ($p < 0.05$) enhanced the resistance of the *R. rattus* studied against trypanosomiasis, the rats lived beyond the usual 10 - 20 days after being infected with *T. congolense*.

Key words: Vitamin E, Selenium, Blood, Trypanosome, *Rattus rattus*

Introduction

Trypanosomiasis is one of the most important livestock diseases in sub-Saharan Africa. The protozoan parasite that causes it is *Trypanosoma* species transmitted by tse tse flies (*Glossina* species). In livestock, this disease known as *"nagana"* can be caused by either *Trypanosoma brucei*, *Trypanosoma congolense* or *Trypanosoma rhodensiense*. Human and animal trypanosomiasis is among the first ten major health problems facing mankind along with malaria, cancer and heart diseases (Eisler et al., 2001).

Trypanosoma congolense usually inhabits the plasma where it causes damage to tissues. The mechanism of tissue damage involves the utilization of metabolites, excretion of toxic substances and immune-mediated injuries. The first wave of parasitemia in *T. congolense* is accompanied by depressed packed cell volume, neutropenia and thrombocytopenia (Krampitz, 1970).

Clinical manifestations / symptoms may be greatly influenced by factors such as, state of nutrition, infection by bacteria, virus or helminth and stress (Stephen, 1970). Relatively high virulence has been observed in highly susceptible strains of mice, infected with *T. congolense*. This

leads to a high percentage of death between days 10 and 20 (Jackson, 1979). The animals which do not die during this period, however, survive between days 20 and 40. Thus, giving a non poison type distribution for survival within the group (Jackson, 1979). The immune system (humoral immunity and cell mediated immunity) produces substances that help the body to resist disease (Crowther, 1995). Lymphocytes, the main factors in humoral immunity produce specific antibodies against invading pathogens (Beaton et al. 1993) and selenium (Se) is known to enhance humoral resistance (Sidhu et al., 1993).

It has been reported that some animals are tolerant to effects of *Trypanosoma* infection (Osaer *et al.*, 1994; Rowlands et al., 2001). The ability to tolerate trypanosomiasis (trypanotolerance) is also found to interact with nutritional and environmental factors. Van Dan (1996) suggested that substantial interaction exists between tolerance and nutrition, and that strategic feed supplementation might increase an animal's potential for production of trypanotolerant breeds. Laboratory evidences demonstrated that vitamin E (Vit E) (tocopherol) and selenium individually supplemented in nutritionally balanced animal diet, enhanced resistance to trypanosomiasis (Van Vleet and Watson, 1984). Enhanced immunity had been reported for dietary supplementation of selenium (Sidhu, et al., 1993) and for vitamin E (Shukla et al., 1988). Vitamin E and selenium appear to participate in similar nutritional and biochemical pathways which enhance humoral immunity (Spallholz, 1980). In this respect, both vitamin E and selenium are regarded as immunopotentiators. Much research has not been reported on the combined effects of these nutrients.

Since trypanosomiasis retards agricultural development and the drugs used in the control of the disease are not only costly but may produce some side effects, an alternative and less expensive approach on the control of the disease is not only necessary but imperative. The aim of this study is to differentiate the effect of combined dietary supplementation of vitamin E and selenium, on the resistance of rats infected with trypanosomes. The study was also to ascertain the effects of dietary supplementation of vitamin E and selenium on packed cell volume (PVC) and leucocyte counts of trypanosome-infected rats, since blood cells are affected by trypanosomiasis.

Materials and Methods

Procurement and management of experimental animals: 90 adult male rats (*Rattus rattus*, whiskers breed) were purchased from Laboratory Animal Unit of the Department of Physiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were held in stainless wire-rat-cages equipped with drinkers and faecal collecting trays, in a clean fly proof experimental animal house, and were fed *ad libitum* with 25% crude protein commercial chicks' mash diet (Top Feed Nigeria Ltd). The rats were given access to unlimited supply of clean water using animal drinkers. The faecal droppings in the tray were removed daily.

The rats were weighed using a Mettler PC 2000 electronic balance, randomized into six cages at five rats per cage and the rats were differentially marked for identification. Each set of rats was used for one of six treatments labelled A to F, and each experimental set-up was replicated three times.

Procurement and management of *Trypanosoma congolense:* *Trypanosoma congolense* was collected from the Parasitology Unit, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. One rat was first inoculated with the *T. congolense*. After 14 days, a cut was given at a sterilized tip of infected rat's tail with a clean sharp scissors and blood was collected into a vial containing 1 ml of normal saline. The parasites were isolated from the animal. The level of parasitemia was determined using a matching chart (Herbert and Lumbsden, 1976) and 0.1 ml of the blood of that infected rat which contained about 800,000 *T. congolense*/ml was used to inoculate other rats. Thereafter, the infected rats were isolated in cages.

Preparation of experimental diets and feeding: The diets to be used for feeding the rats were made by weighing out six sets of 1 kg 25 % crude protein chicks' mash (Top Feed, Nigeria Ltd.) into six clean containers. These were labeled A, B, C, D, E, and F corresponding to the different treatments. Skipping the first 1 kg chicks' mash to which no selenium and vitamin E were added (control), varied quantities of vitamin E and selenium were added to the others and homogeneously mixed such that the resulting diets were: Diet A, (control) 0.0 mg of Se plus 0.0 mg Vit E; Diet B, 0.1 mg Se plus 60 mg Vit E; Diet C, 0.3 mg Se plus 80 mg Vit E; Diet D 0.5 mg Se plus 100 mg Vit E; Diet E 0.3 mg Se plus 0.0 mg Vit E and Diet F, 0.0 mg Se plus 80 mg Vit E. The rats were weighed at the beginning. Diets A, B, C, D, E and F were fed to the rats in the corresponding treatment cages.

Collection of blood samples and estimation of blood parameters: Blood was collected weekly for estimation of packed cell volume, total and differential leucocyte counts. Absolute ethanol in a cotton swab was used to sterilize rats' tail. A pair of sharp scissors was used to cut the sterilized tip of the tail, from which six drops of blood were drained into a vial containing two drops of EDTA in a blood vials labeled according to the identifying number of rats and their cages. This was thoroughly mixed to avoid clotting of the blood.

Packed cell volume was determined using microhaematocrit method. In this method, microhaematocrit capillary tubes were filled with blood. One end of the capillary tube was sealed with plasticine after filling with blood. The tubes were spun at 10,000 revolutions per minute for five minutes with microhaematocrit centrifuge. The results were read in percentage with haematocrit-reader supplied with the centrifuge.

Total white blood cell count was determined using haemocytometer. Blood was drawn to 0.5 mark of the white cell haemocytometer pipette. The blood was mixed with 0.3 ml of the diluting fluid comprising 1% glacial acetic acid and 0.05 g gentian violet. The diluting fluid helped to kill the red cells so that it was only the white blood cells that were counted. The counting chamber filled with the mixed blood was placed on a microscope for 5 minutes for the cells to settle. The objective was focused on each of the cover square millimeters and cells contained in them were counted and expressed as the number of cells per mm^3 of blood. The total white blood cells were calculated as follows: If N was the number of cells counted/mm^2, N/4 would be the number of cells/mm^2 and the volume of each square mm

was 1 x 1 x 10 mm^3. Therefore, the number of cells in 1 mm^3 was N/4 x 10. The blood was diluted 1 in 20; the number of cells/mm^3 of undiluted blood was 50N.

Procedure for differential leucocyte count was by blood smear using Leishman stain technique. Blood film was dried and stained immediately by adding 10 drops of Leishman stain and 20 drops of distilled water at 6.8 pH to the dried smear. These were mixed by rocking the slide gently and allowed to stand for 10 to 15 minutes. The stain was washed first with distilled water and thereafter flooded with tap water for 1 to 2 minutes. Later, the slide was allowed to dry. The slide was examined, the different cell types counted under oil immersion objective, at x 100. The polymorphonuclear cells and mononuclear cells were counted separately.

The cells were counted in one complete longitudinal strip of the film. The different types of leucocytes observed were recorded. The results for each group of the leucocytes were expressed as a percentage of the total white blood cell count. For instance, if mononuclear cells of cage A1 is 80 % and polymorphous cells of the same animal is 20%, the actual number of the mononuclear cells was 80/100 x total white blood cell count. For polymorphous cells it was 20/100 x total white blood cell count.

Statistical analysis: Multiple comparisons were done for the different blood parameters of the rats from different treatments and analyzed for significant differences by analysis of variance (ANOVA). Any significant differences found were partitioned with the Least Significant Difference (LSD) using the Statistical Package Statistical Sciences (SPSS) computer package. Plots showing weekly means of various blood parameters of rats fed different dietary levels of selenium and vitamin E were produced from the results.

Results

Total white blood cells: Figure 1 shows the mean weekly total white blood cells of *T. congolense*-infected *Rattus rattus* fed diets containing different levels of selenium and vitamin E. The mean of all treatments increased and peaked at week 2, it then declined until week 4 when most of the animals died, except few in treatments B, C, and D. In week 0 there was no significant difference ($P > 0.05$) between the treatments in the mean total white blood cell count (Fig. 1). However, weeks 1 to 4 showed highly significant difference ($P < 0.01$) in white blood cell count between the treatments.

Significant differences exist ($p > 0.05$) in total white blood cell count in week 1 when all other treatments were compared with the Control. Also in week 1, Treatments B and C, E and F had no significant differences ($P > 0.05$), but there was significant difference ($P < 0.05$) between Treatment D and treatments B and C, E and F. From week 2 the significant differences ($P < 0.05$) of the other treatments with the control became more apparent (Fig. 1).

By week 3, there were significant differences ($P < 0.05$) in all the treatments except in Treatments B and C and E and F which showed no significant differences ($P > 0.05$). By week 4, only rats in Treatments B, C, and D survived, with treatment C having the highest survivors.

Polymorphonucleated cells: Figure 2 shows the mean weekly polymorphonucleated cells of *T. congolense*-infected *R. rattus* fed diets

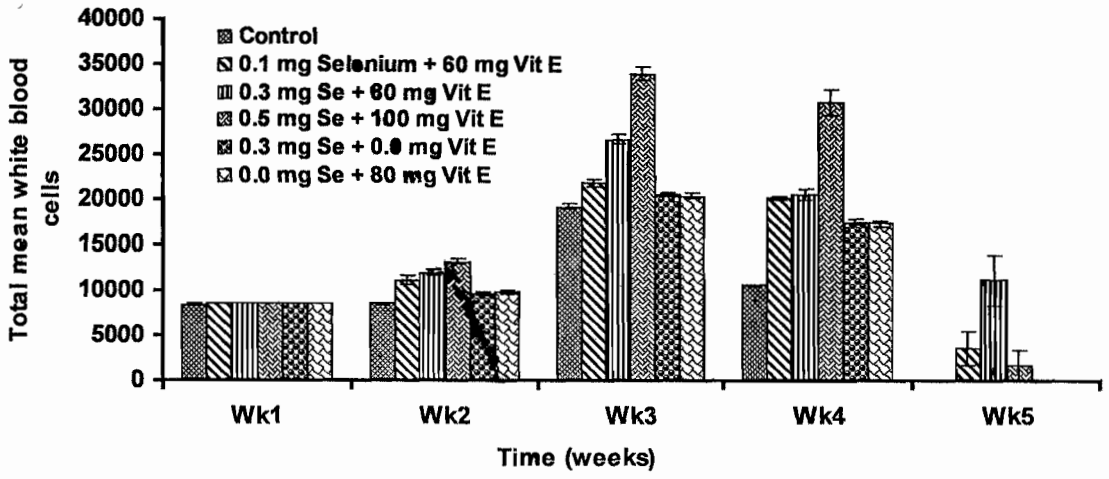

Fig. 1 Mean weekly total white blood cells of Trypanosoma congolense-infected Rattus rattus fed diets containing different levels of selenium and vitamin E.

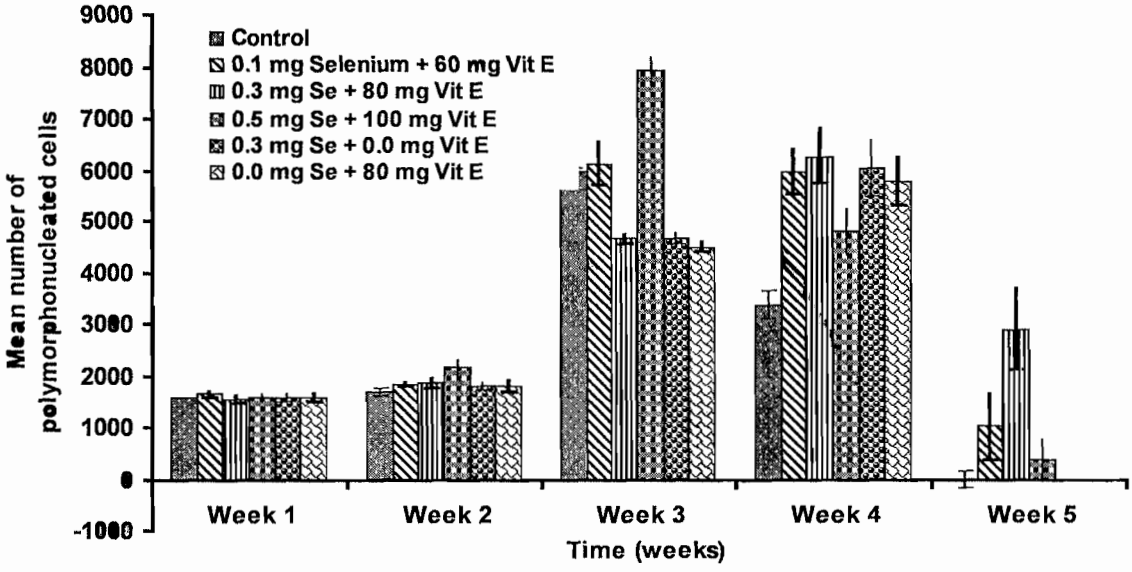

Fig. 2. Mean weekly polymorphonucleated cells of Trypanosoma congolense-infected Rattus rattus fed diets containing different levels of selenium and vitamin E.

containing different levels of selenium and vitamin E. In Treatments C, E and F the polymorphs reached the highest peak at week 3, where as in other Treatments (A, B and D) the polymorphs reached the highest peak at week 2. In week 4, Treatment C had the highest number of polymorphs in line with total white blood cell count (Fig. 1). Analysis of variance (ANOVA) showed that in week 0, there was no significant difference ($P > 0.05$) between the treatments. But in weeks 1 to 4 there was highly significant difference ($P < 0.01$) between the treatments.

Multiple comparisons showed that there was no significant difference ($P > 0.05$) between the treatments in week 0. In week 1, however, Treatment D was significantly different ($P < 0.05$) from other treatments. In

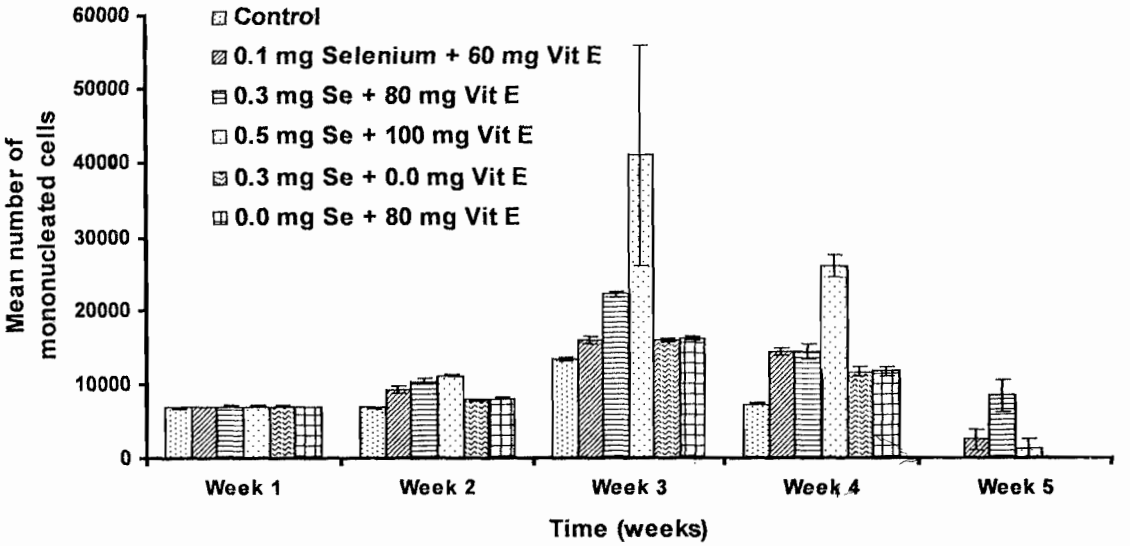

Fig. 3. Mean weekly number of mononucleated cells in Trypanosoma congolense-infected *Rattus rattus* fed diets containing different levels of selenium and vitamin E.

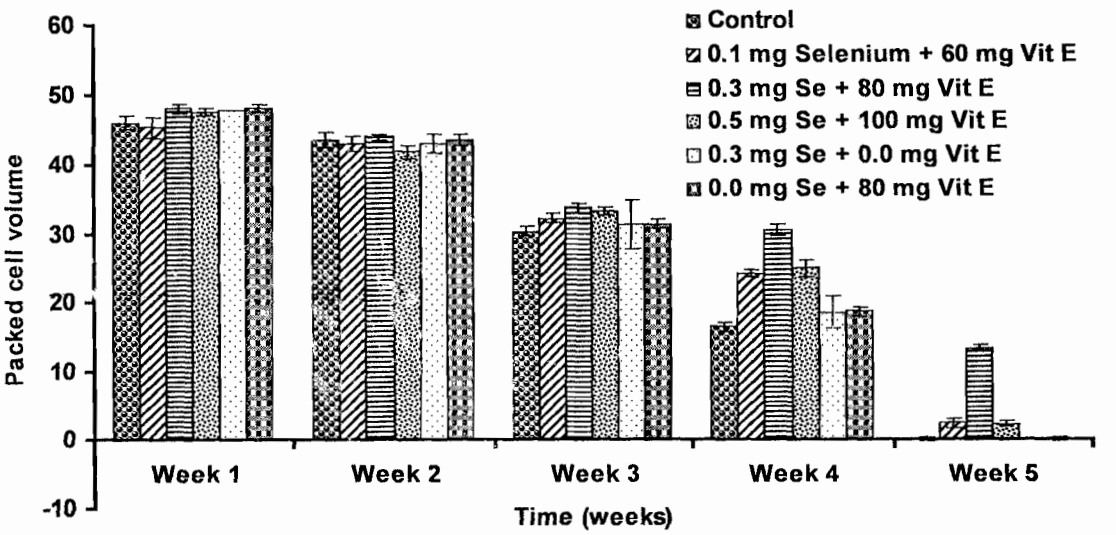

Fig. 4 Packed cell volume of Trypanosoma congolense-infected Rattus rattus fed with diets containing different levels of selenium and vitamin E.

week 2, there was no significant difference ($P > 0.05$) between Treatments A and B, both of which showed highly significant difference ($P < 0.01$) from other treatments in week 2. Also, there was no significant difference ($P > 0.05$) between Treatments C, E and F (Fig 2).

In week 3 there was significant difference ($P < 0.05$) between other treatments compared with Treatment A (Control). Treatment C was significantly different ($P < 0.05$) from Treatments A and D, but not significantly difference ($P > 0.05$) from other treatments (B, E, and F). In week 4, the rats survived only in Treatments B, C, and D. Treatment C showed a significant difference ($P > 0.05$) with Treatments B and D which it

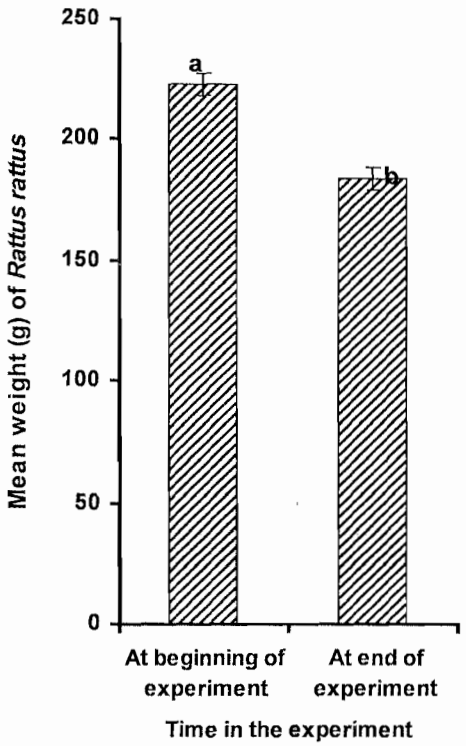

Fig. 5 Mean initial and final weights (g) of 150 Trypanosoma congolense-infected *Rattus rattus* fed diets containing different levels of selenium and vitamin E. Figure marked with different letters are significantly different (P < 0.01)

had the highest survivors.

Mononucleated cells: Figure 3 shows the mean weekly mononucleated cells of *T. congolense*-infected *R. rattus* fed diets containing different levels of selenium and vitamin E. The mean number of mononucleated cells in the treatments reached its peak at week 2, then declined progressively to week 4. All the rats died in Treatments A, E and F in week 4, except those in Treatments B, C and D which survived to week 4 with rats in Treatment C showing the highest number of mononucleated cell, that is, the highest trypanotolerance.

From analysis of variance (ANOVA) there was no significant difference ($P > 0.05$) between all the treatments at week 0, whereas there were significant differences ($P < 0.05$) between the treatments from weeks 1 to 4. In week 2, there was no significant difference ($P > 0.05$) between the treatments except in Treatment D which was different from the rest ($P > 0.05$). In week 3, on the other hand there was highly significant difference ($P < 0.01$) between treatment A (Control) and other treatments. However, no significant difference ($P > 0.05$) existed between Treatments B and C, and between Treatments E and F, respectively.

In week 4, there was highly significant difference ($P < 0.01$) between mononucleated cells count in Treatment C and the mononucleated cell counts of other two surviving treatments. The number of mononucleated cells did not however differ between these latter two treatments ($P > 0.05$).

Packed cell volume (PCV): The mean weekly of packed cell volume (PCV) of the *T. congolense*-infected *R. rattus* declined progressively from week 1 to 4 (Figure 4). Results from the ANOVA, showed that there was no significant differences ($P < 0.05$) in the parked cell volume of the treatments in weeks 0 and week 1 though the level of PCV was lower in week 1 compared to week 0. From week 2 to four, however, there was highly significant difference between the PCV of some of the treatments ($P < 0.01$). There was no significant difference ($P > 0.05$) between Treatments A, E and F which trend continued into week 3. The non-significant difference ($P > 0.05$) between Treatments A, E and F, shows equal depression of PCV in them for the two weeks. The rats died in week 4. Treatments B, C, and D though not significantly different ($P <$

0.05) in week 2 but of higher PCV than Treatments A, E and F showed significant differences by week 3 with Treatment C giving the highest PCV. This high value and significant difference ($P < 0.01$) of Treatment C compared with the other two treatments was carried into week 4. Treatment B and D were not significantly different ($P > 0.05$) in weeks 3 and 4.

Weight of the *T. congolense*-infected rats: Figure 5 shows the weight difference of the *R. rattus*. It as observed that there was highly significant difference ($P < 0.01$) when the initial weight of animals were compared with final weight at the end of experiment.

Discussion

The increase in mean weekly total white blood cells of rats in Treatments B, C, D, E and F than Treatment A (control) indicates that selenium and vitamin E had impact on the immune system of the rats. It does appear therefore, that selenium and vitamin E are immunopotentiators in rats as they improve the strength of the immune system under disease condition (Van Vleet and Watson, 1984; Spallholz, 1980). Treatment C had the highest mean of white blood cell count (11216), indicative of the rats in this treatment having the highest trypanotolerance. Also, that beyond week 1 the significant differences ($P < 0.05$) of the other treatments with the control and among rats treated differently became more apparent (Fig 1) implies that the different levels of selenium and vitamin E had effect on the *T. congolense*-infected *R. rattus* resistance to the parasite.

Mononucleated cells and polymorphonucleated cells showed similar results as total white blood cells, increasing more in other treatments than in the control. It seemed that selenium and vitamin E influenced nutritional status of the rats, which in turn led to the increase in total white blood cells, mononucleated cells and polymorphonucleated cells despite the trypanosome load. The *T. congolense*-infected rats fed with selenium and vitamin E, exhibited more trypanotolerance than rats in Treatment A not fed with selenium and vitamin E. That Treatment C had the highest number of polymorphs in line with total white blood cell count (Fig. 1), ascertains that Treatment C had the highest trypanotolerance among all the treatments. The fact that there was no significant difference ($P > 0.05$) between all the treatments at week 0, while there was significant differences ($P < 0.05$) between the treatments from weeks 1 to 4, indicates that the various levels of the combined nutrients fed to the rats had varied impacts on the rats' immune system. Agyenmang *et al.* (1990) showed that nutritional state of an animal can influence its tolerance. Treatment C yielded the best result in these treatments, showing that there is a standard threshold value below or above which positive reactions become minimal or cannot be observed (National Research Council, 1980). Treatment C also showed the best resistance to *T. Congolense* since a rat in that treatment lived longer than the 20 days stipulated life span in trypanosomiasis (Jackson, 1979). Thus, the level of selenium and vitamin E in Treatment C based on this experiment would appear to be the recommended inclusion rate in the diet of rats that have trypanosomiasis to enhance trypanotolerance.

The continuous depression of packed cell volume and weight of the rats (Fig. 5) in all the Treatments B through F despite the ameliorating

effect of the dietary selenium and vitamin E showed that the rats were actually infected with *T. congolense* (Krampitz, 1970). Depression of packed cell volume and the death of the rats which in the present study death commenced from week 4 has previously been noted in *T. congolense* infection (Mwangi *et al.*, 1990). The reduction in weight at the end of the experiment implies that there was weight loss due to trypanosomiasis. The level of nutrients (selenium and vitamin E) did not prevent weight loss due to physiological stress caused by trypanosomiasis.

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