# EFFECT OF AGE ON IMMUNE RESPONSE OF TRYPANOSOME-INFECTED RATS (*Rattus rattus*) FED DIETARY VITAMIN E AND SELENIUM

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**ABSTRACT**

***This study was done to determine the combined effects of dietary supplementation of vitamin E and selenium on age-dependent immune response of Trypanosoma congolense-infected white rats (Rattus rattus, whiskers breed). Sixty rats were used in the study, 30 20-day old (newly weaned) rats and 30 90-day old (adult) rats. Four groups of rats with five rats of identical age per group were kept in wire-rat-cages. The cages were labeled G to J. Cage G contained adult rats (Control 1), while cage H contained newly weaned rats (Control 2). Cage I contained adult rats fed diet containing selenium and vitamin E (nutrient), while cage J contained newly weaned rats also fed diets containing selenium and vitamin E. Each treatment was replicated three times. Longevity (days of survival) and differential leucocyte counts which are functions of immune response of the rats upon infection with T. congolense were determined. At the end of the study, the longevity and differential leucocyte counts were analysed for significant differences using analysis of variance (ANOVA) and any differences were partitioned with the least significant difference (LSD) and the Duncan's Multiple Range Test (DMRT). The results revealed that there was no significant difference in longevity (P > 0.05) between the two control groups (newly weaned and adult rats) but there were significant differences between the longevity of each control group and the longevities of the rats given combined dietary supplementation of the nutrients. Longevity of newly weaned and adult rats given dietary supplementation of selenium and vitamin E were not different (P < 0.05). These results implied that age of the rats was not a contributory factor in improved immune response of the trypanosome-infected rats fed the combined dietary supplementation of selenium and vitamin E.***

**Keywords:** Immune response, Newly weaned rats, Longevity, *Trypanosoma congolense, Rattus rattus*

## INTRODUCTION

Food supplemented with vitamins and minerals had been suggested for control of trypanosomiasis (Van Dan, 1996; Bass, 1999) ranked among the first 10 diseases of man (Eisler *et al.*, 2001). Earlier researches demonstrated that supplemental vitamin E enhanced animals' immune response (Tengerdy and Brown, 1977; Hutchinson, 1999). Also, similar results had been reported for dietary supplement of selenium (Teige *et al.*, 1982; Nockel, 1986). The enhancement of animal humoral immunity by Vitamin E and by selenium may be due to the participation of both selenium and Vitamin E (nutrients) in similar nutritional and biochemical pathways (Spallholz, 1980).

Feed supplements of 0.1 mg - 0.3 mg Selenium had been recommended for animals (FDA, 1987). The effect of each nutrient had been individually studied (Shukla *et al.*, 1988; Sidhu, *et al.*, 1993; Van Dan, 1996). Mgbenka and Ufele (2004) found that the combined dietary supplementation with 0.3 mg Selenium and 80 mg Vitamin E for adult white rats (*Rattus rattus*) significantly ($P < 0.05$) enhanced the resistance of *R. rattus* to trypanosome infection but not much is known on the effect of age on this resistance. Age-dependent effects on rats of disease-causing agent's challenge and of hormonal treatment had been reported (Antonini *et al.*, 2001; Jacobson, 2004).

In this study, age of individual rats is being checked as a factor in eradicating the disease using Vitamin E and Selenium as combined supplements. The specific objectives were to ascertain if there are synergetic effects of dietary supplementation of Vitamin E and Selenium on packed cell volume (PCV), differential leucocyte counts and longevity of trypanosome-infected white rats.

## MATERIALS AND METHODS

**Procurement and Management of *Rattus rattus* and *Trypanosoma congolense***: Male rats (20- and 90-day old) were purchased from the Animal Unit of the Department of Physiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The rats were held in stainless wire-rat-cages which were kept in the animal house. They were fed *ad libitum* with 25% crude protein chicks' mash diet (Top Feed Nigeria Ltd). The rats were given access to unlimited supply of water using drinkers. The faecal droppings in the tray were removed daily.

The rats were weighed before and after the experiment using a Mettler balance (electronic PC 2000). After initial weighing, each rat was differentially marked and kept five rats in each of four cages labelled G to J corresponding to four treatments. Each treatment set-up was replicated three times. One rat was first inoculated with *Trypanosoma congolense* isolated from other animals in NITR Veterinary Medicine Faculty. After 14 days, the level of parasitemia was determined to be 80,000 *Trypanosoma congolense* using a matching chart (Herbert and Lumbsden, 1979). Tip of infected rat's tail was sterilised and a cut given to it using a sharp scissors. The blood of the infected rats used for inoculation was collected from the tip of the tail into a vial containing 1 ml of normal saline. This infected blood was used to inoculate other rats. Each experimental rat was given 0.1 ml of infected blood. Once infected, the rats were isolated and kept in cages.

**Preparation of Diets:** One kilogram of 25% protein chicks' mash was weighed into each of four clean containers labelled G – J to be fed to corresponding to Treatments G, H, I and J. Similarly, 0.3 mg Selenium and 80 mg Vitamin E were each weighed into containers labelled I and J, and the nutrients thoroughly mixed into the mash. In diet G (Control 1) and H (Control 2) Selenium and Vitamin E were not weighed in and mixed into 1 kg of chicks' mash. Treatment G and I contained adult white rats while treatment H and J contained newly weaned rats. Each treatment was fed the corresponding diets for five weeks.

**Estimation of Blood Parameters**: Blood was collected weekly for estimation of total and differential leucocytes counts, and packed cell volume. For this purpose, absolute ethanol was used to sterilize rats' tails, sharp scissors was used to cut the tip of the tail, from which six drops of blood were drained into a vial containing two drops of EDTA. This was thoroughly mixed to avoid clotting. Each vial was labelled according to the number of animals and cages they belong to. Packed cell volume was determined using microhaematocrit method. In this method, microhaematocrit capillary tubes were ⅔ filled with blood. One end of the capillary tube was sealed with plasticine after filling with blood. The tubes were spun at 10,000 rounds per minute for five minutes with microhaematocrit centrifuge. The results were read in percentage with haematocrit reader which was supplied with the centrifuge.

Total white blood cell count was determined using haemocytometer. Blood was drawn to 0.5 mark of the white cell pipette from haemocytometer and was used to mix 0.3 ml of diluting fluid of 1% glacial acetic acid mixed with a pinch of gentian violet. A firm pressure was used to slide in a cover glass into position on the counting chamber. The counting chamber was filled with mixed blood by holding a dropper which contained the mixture at an angle of 45° and lightly touching the tip against the edge of the cover glass. The chamber was placed on a microscope for five minutes for the cells to settle. The objective was focussed on each of the cover square millimetres and cells contained in them were counted. Cells touching the border lines on the top and right hand side of each square were included in the count, while those touching the border lines on the bottom and left hand side were disregarded. The final result was expressed as the number of cells per $mm^3$ of blood. The diluting fluid helped to kill the red cells so that it was only the white blood cells that were seen and counted. The total white blood cells were calculated as follows:

If N = number of cells counted in square mm, then N/4 = number of cells in square mm. The volume of each square mm = 1 x 1 x 10 $mm^3$.

**Table 1: Initial weight (g), final weight (g) ± standard error of mean and longevity of white rats (*Rattus rattus*) fed combined dietary supplementation of 0.3 mg selenium (Se) and 80 mg of vitamin E in 5 weeks[1].**

| Treatment[2] | Weight of rats (g) | |
|---|---|---|
| | Initial | Final |
| G | 267.93 ± 6.44 a | 224.07 ± 6.83 b |
| H | 111.93 ± 2.81 a | 77.73 ± 2.29 b |
| I | 266.80 ± 7.69 a | 225.27 ± 6.73 b |
| J | 110.33 ± 2.52 a | 76.67 ± 2.41 b |

*[1]Means ± SEM in a row with different letters are significantly different (P < 0.05).[2]G, Adult white rats (Control 1); H, Newly weaned white rats (Control 2); I, Adult white rats fed 80 mg Vitamin E and 0.3 mg selenium supplemental diet; J, Newly weaned white rats fed 80 mg Vitamin E and 0.3 mg selenium supplemental diet.*

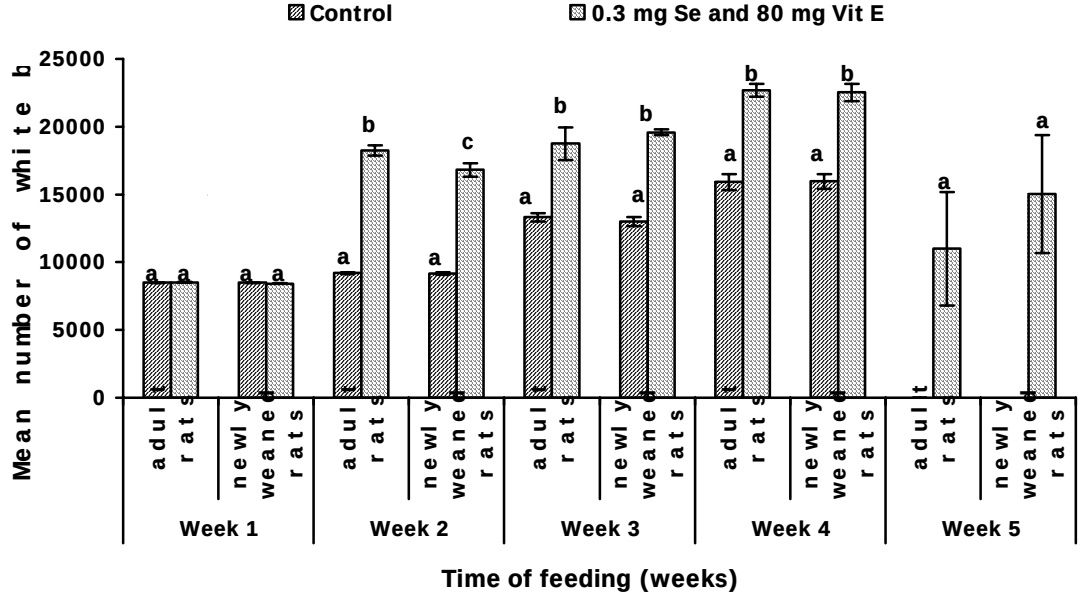


**Figure 1: Weekly total white blood cells (mean ± standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed dietary 0.3 mg selenium (Se) and 80 mg vitamin E (Vit E) for 5 weeks. Columns with different letters on top in a week are significantly different (P< 0.05).**

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

Procedure for differential leucocytes count was by dropping a fresh blood onto one end of clean, grease-free slide placed on a horizontal surface. Using a spreader, a little narrower than the slide, the drop was spread along the slide until the blood was smeared. When the blood film was made, drying was hastened by waving in air. It was stained immediately by Leishman technique, by adding 10 drops of Leishman stain on the dried smear and 20 drops of distilled water at pH 6.8. This was mixed by rocking the slide gently or with Pasteur pipette and allowed to stand for 10 to 15 minutes. The stain was washed with distilled water and flooded with tap water for 1 to 2 minutes. The slide was allowed to dry. The slide was later examined and 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 noted by scoring system. The cells counted were the polymorphonucleated cells comprising of neutrophils, basophils and eosinophils, and the mononucleated cells comprising of lymphocytes and monocytes. The results for each group of the leucocytes were expressed as a percentage of the total white blood cell count.

Blood parameters for each experiment were analysed for significant differences by descriptive statistics and by the one way analysis of variance (ANOVA) using SPSS computer package. Multiple comparisons on any detected significant differences were partitioned with the least significant difference (LSD) and the Duncan's Multiple Range Test.

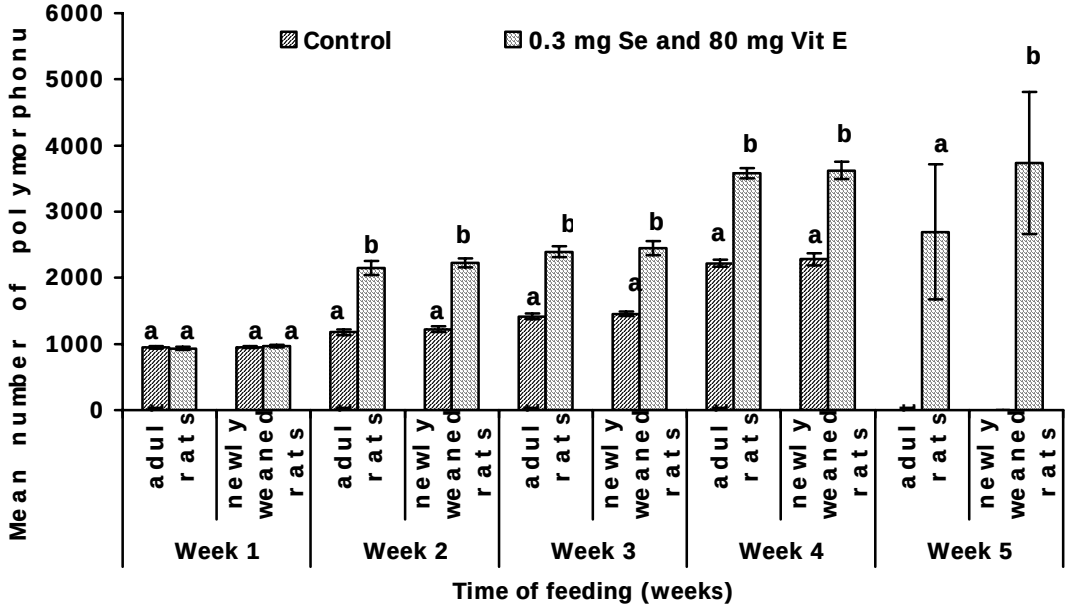


**Figure 2: Weekly polymorphonucleated cells (mean ± standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed dietary 0.3 mg selenium (Se) and vitamin E (Vit E) for 5 weeks. Colomns with different letters on top in a week are significantly different (P< 0.05)**

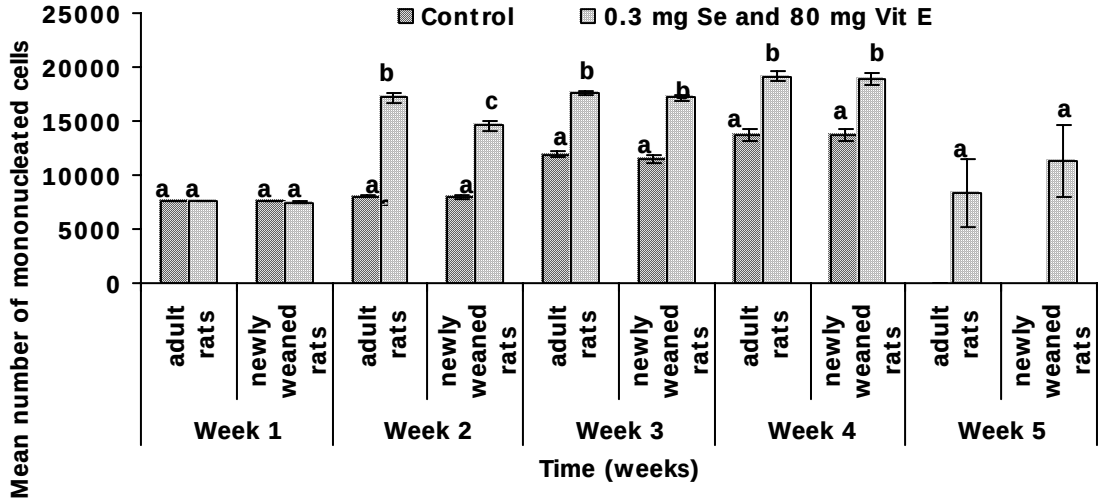


**Figure 3: Weekly mononucleated cells (mean ± standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed 0.3 mg selenium (Se) and 80 mg vitamin E (vit E) for 5 weeks. Columns in a week with different letters on top of columns are significantly different (p < 0.05).**

Means and standard error of means (SEM) from results of descriptive statistics analyses were used for production of figures and tables of the various blood parameters.

## RESULTS

**Total White Blood Cells:** The mean weekly differential total white blood cells and packed cell volume of *Trypanosoma congolense*-infected *R. rattus* of different ages fed diets containing the same level of Selenium and Vitamin E is shown in Figure 1. Total white blood cells were highest in Week 4 but declined rapidly thereafter. The rats died in the control groups by Week 5. Treatment J (newly weaned, nutrients-supplemented diet rats) had the highest though not significantly different (P < 0.05) total white blood cells. Figure 1 shows that there were no significant differences (P >

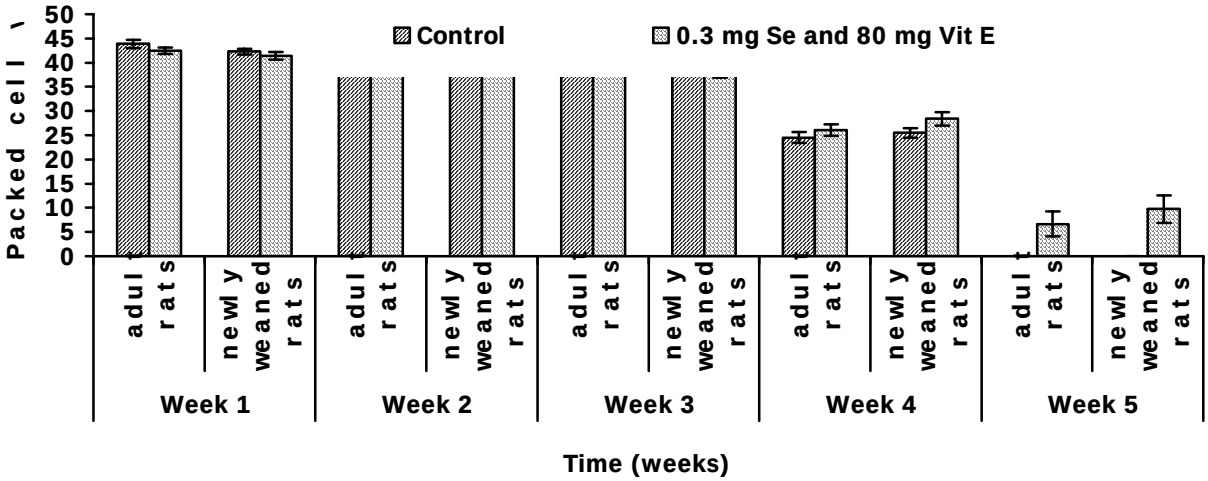


**Figure 4: Weekly packed cell volume (mean ± standard error of mean) of newly weaned (NW) and adult (AD) *Trypanosoma congolense*-infected *Rattus rattus* fed 0.3 mg dietary selenium (Se) and 80 mg vitamin E (Vit E) for 5 weeks. Columns with different letter on top in a week are significantly different (P < 0.05).**

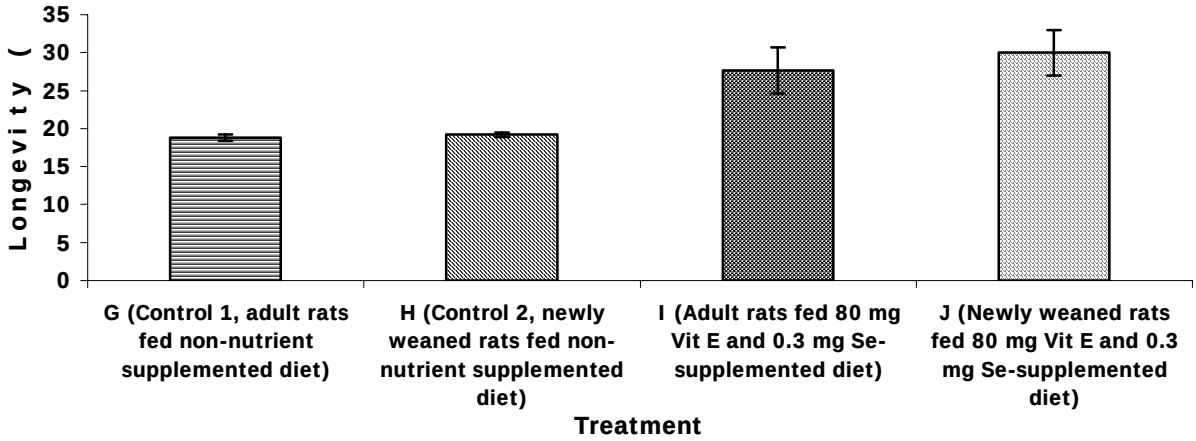


**Figure 5: Longetivity ± standard error of mean (days) of white rats (*Rattus rattus*) fed dietary supplementation of 0.3 mg selenium (Se) and 80 mg vitamin E (Vit E) for 5 weeks.**

0.05) among the treatments in total white blood cells by Week 1. From Weeks 2 to 5, there were significant differences (P < 0.05) in total white blood cells between the control treatments and nutrients-supplemented treatments. But for Week 2, there were no significant differences (P < 0.01) between Treatments G and H, and Treatments I and J in each week.

**Polymorphonucleated Cells:** The mean weekly polymorphonucleated cells of *Trypanosoma congolense*-infected *R. rattus* of different ages fed diets containing the same level of combined dietary selenium and Vitamin E was highest in Week 4 (Figure 2). There was no significant difference (P > 0.05) between the treatments (groups) at Week 1, while from Weeks 2 to 5, there were significant differences among treatments in polymorphonucleated cells (P > 0.05). Comparison of the polymorphonucleated cells among the treatments through the weeks revealed that it had the same pattern as the total white blood cells. Namely, there were no significant differences in polymorphonucleated cells of rats of both ages among the control treatments and among nutrients-supplemented treatments but highly significant differences (P < 0.001) were observed when the controls were compared with the treatment group.

**Mononucleated Cells:** Figure 3 shows that mean weekly mononucleated cells of a *Trypanosoma congolense*-infected *R. rattus* of both ages fed diets containing the same level of selenium and Vitamin E was highest at Week4.

Treatment I had the highest number of mononucleated cells at Week 5.

There was no significant difference ($P > 0.05$) between the treatments at Week1 with respect to the mononucleated cells. From Weeks 2 to 5 there were highly significant differences ($P < 0.01$) in the number of mononucleated cells among the treatments. Figure 5 shows that there was no significant difference in mononucleated cells ($P > 0.05$) in Week1 among the groups except in Treatments I and J (nutrients-supplemented groups) where significant difference ($P < 0.05$) existed.

**Packed Cell Volume (PCV):** Figure 4 shows that mean weekly packed cell volume of *Trypanosoma congolense*-infected *R. rattus* of different age fed diets containing the same level of selenium and vitamin E declined as the weeks progressed. By Week 4 the value of packed cell volume declined sharply due to death of the rats. There were significant differences ($P > 0.01$) in packed cell volume between nutrient supplemented groups and control groups from Weeks 2 – 5.

**Longevity (Days) of Rats:** Figure 5 shows the longevity of the rats. From Figure 5, Treatment J (newly weaned, nutrients-supplemented diet rats) had the highest mean longevity (days), followed by Treatment I (adult rats, nutrient-supplemented). There was highly significant difference in longevity ($P < 0.01$) between the groups fed nutrient supplemented diets and the control groups.

**Weight (g) of Rats:** Table 1 shows the weight difference of *R. rattus*. From this table, it was observed that there was significant difference ($P < 0.01$) between the initial and final weights of rats in each age category.

## DISCUSSION

The significantly higher ($P < 0.05$) total white blood cells in the nutrient-supplemented diet groups beyond Week 1 in this study (Figure 1) implies that immune response whereby different types of white blood cells multiplied in numbers was elicited by infection of the rats with *T. congolense*. That Treatments I and J (adult and newly weaned rats fed nutrients-supplemented diets) had significantly higher ($P < 0.05$) numbers of polymorphonucleated cells compared to controls of both ages from Weeks 2 – 5 implies that the polymorphonucleated cells were secreted more as the infection intensified and that the nutrients effected the immune response of the rats irrespective of their age groups. This agrees with the finding of Tengerdy and Brown (1977) with supplemental Vitamin E and that of Nockel *et al.* (1986) with supplemental selenium that each nutrient used singly boosts immune response. Using polymorphonucleated cells as a parameter, the non-significant difference ($P > 0.05$) among the treatments in Week 1 shows that the rats had the same immune response at the beginning of the study.

The non-significant difference in mononucleated cells ($P > 0.05$) in Week 1 among the groups while significant differences ($P < 0.01$) existed in later weeks indicated that under normal circumstances there is no age-dependent difference in the number of mononucleated cells. The non-significant difference in PCV ($P > 0.05$) between treatments G and H and between treatments I and J, while in other comparisons there were significant differences among the treatments ($P < 0.05$) implies that treatments of identical nutrient supplementation had the similar physiological depression.

The highly significant difference ($P < 0.01$) in the weight of animals before and after the experiment implies that there was weight loss due to trypanosomiasis. Despite the improvement of the immune response with 0.3 mg selenium and 80 mg Vitamin E dietary supplementation, as is evidenced by the significant increases ($P < 0.05$) in leucocyte counts in *T. congolense*-infected rats, trypanosomiasis caused the weight loss. Also, age of the rats did not have any effect on the weight loss. It was concluded that there was loss of weight associated with the trypanosome infection irrespective of the age of the rats. Physiological stress has been incriminated for weight loss during trypanosome infection (Tizard, 1985; Ogwu *et al*., 1986; Stephen, 1986).

In summary, the lack of significant differences ($P > 0.05$) in mean weekly total white blood cells, mononucleated cells and polymorphonucleated cells among rats of different age groups treated alike in this study implies that unlike in the studies of Antonini *et al.* (2001) who recorded age-dependent respiratory defense mechanism in bacteria-infected rats and Jacobson and Ansari (2004) who found age-depended reaction of rats to leuteinising hormone releasing hormone**,** age of *R. rattus* did not hinder or aid the effects of trypanosomes in *R. rattus*. Furthermore, the

combined dietary supplementation of the nutrients in this study enhanced the immune system of the rats in resistance to the trypanosomiasis, irrespective of age, as evidenced by the fact that some trypanosome-infected rats lived more than the mandatory 20 days (Figure 5). This is in agreement with earlier finding of Mgbenka and Ufele (2004) that combined dietary supplementation with 0.3 mg selenium and 80 mg Vitamin E enhanced immune response of *Trypanosoma Congolese*-infected *R. rattus* leading to living beyond 20 days

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