EFFECT OF FOOD SUPPLEMENTATION ON THE WHITE BLOOD CELLS COUNT AND DIFFERENTIAL LEUCOCYTES COUNT OF TRYPANOSOME-INFECTED PREGNANT RATS

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ABSTRACT

Trypanosomiasis is an important livestock disease in sub-Saharan Africa. Improvement on host's nutrition is important in moderating the severity of pathophysiological effect of trypanosomiasis and it also influences the rate of recovery. Earlier researchers demonstrated that dietary supplement of selenium and vitamin E enhanced immune response in white rats. It has also been reported that during pregnancy, immune response is depressed. Leucocytes count has been recognized as a measure of immune response. This research was therefore conducted using chicks' marsh fortified with 80 mg of vitamin E and 0.3 mg of selenium as control (Diet 1) to determine the effect of dietary supplementation of moderate protein (combination of 250 g of corn meal, 240 g soyabean meal and 10 g of crayfish) in the chicks' mash (Diet 2), high dietary protein (combination of 400 g of caseinogen and 300 g of soyabean meal) (Diet 3), and high dietary carbohydrate (combination of 400 g dextrose and 300 g cornmeal) (Diet 4) in the chicks' mash on the white blood cells count and differential leucocytes count of trypanosome-infected pregnant rats. Dies 1 – 4 were given to rats in Cage A – D, respectively. The rats were infected with trypanosomes within 10th to 14th day of pregnancy. Each experimental set up was replicated three times. On comparing the total white blood cell counts of all the rats fed different diets, there was no significant difference ($P > 0.05$) between the rats in Cage A (fed Diet 1) and rats in Cage B (fed Diet 2), and similarly between rats in Cage C (fed Diet 3) and rats in Cage D (fed Diet 4). There was, however, significant difference ($P < 0.05$) between rats in Cage A and rats in Cages C and D, and also between rats in Cage B and those in Cages C and D. Diet 2 with a moderate (20.01%) protein level and a balance of other nutrients produced the highest leucocytes count. Diet 2 therefore produced the highest immune response in the pregnant trypanosome-infected rats.

Keywords: Trypanosomiasis, Nutrition, Leucocytes count, Immune response

INTRODUCTION

Phenotypically, every organism is a product of its genes and environment. The most critical period for this interaction and one which has the most profound implications for life long health and well being occur before birth (Haggarty, 2002). There is evidence that natural variation in genetic make up has a direct effect on an organism's development, and that genotype interacts with nutrient intake and their effect can be modulated by nutritional status (Haggarty, 2002). Trypanosomiasis is one of the most important livestock diseases in sub-Saharan Africa (Morrison *et al.*, 1981). Improvement on host's nutrition is important in moderating the severity of pathophysiological effect of trypanosomiasis and it also influences the rate of recovery (Katungka-Rwakishaya, 1996).

Nutrition and disease are the major factors that affect reproduction and also determine the health condition of pregnant animal and the feotus. Many outcomes of pregnancy are affected by the balance of different nutrients. Nutrient may have an effect on different critical development and it may also be simultaneously beneficial for one outcome and detrimental for another (Haggarty, 2002). Physiological changes in pregnancy calls for extra nutrients and energy to meet demands of an expanding blood supply, the growth and development of maternal tissues before birth and preparation for lactation (Ladipo, 2000). Good maternal nutrition is vital for the health and reproductive performance of women and the health, development and survival of their children (Mora and Nestel, 2000). It has been suggested that a brief period of malnutrition may result in permanent alterations in development of organs that may be translated into pathology in later life (Barker, 1995). Leucocytes count during stress and infectious disease is a measure of immune response (Hardie *et al.*, 1991; Dufva and Allander, 1995). It has been reported that during pregnancy, immune response is depressed (Purtilo *et al.*, 1972) involving reduced leucocytes count. Earlier researchers demonstrated that supplemental vitamin E enhances animal immune response (Haeger, 1974; Tengerdy and Brown, 1977). Also, similar results had been reported on dietary supplement of selenium (Nockel, 1986). Mgbenka and Ufele (2004) showed that combined supplementary selenium and vitamin E enhances trypanotolerance in rats.

In line with this background, using chicks' marsh fortified with 0.3 mg of selenium and 80 mg of vitamin E as control, this research was conducted to determine the effect of dietary supplementation of moderate dietary protein (combination of 250 g of corn meal, 240 g soyabean meal and 10 g of crayfish) in the fortified chicks' mash, high dietary protein (combination of 400 g of caseinogen and 300 g of soyabean meal) on one hand and high dietary carbohydrate (combination of 400 g dextrose and 300 g cornmeal) in the fortified chicks' mash on the other hand on the white blood cell and differential leucocyte counts of trypanosome-infected pregnant rats.

MATERIALS AND METHODS

Twenty 120-day-old female rats of were used for this experiment. The rats were marked for identification and held in stainless wire-rats-cages in clean experimental animal house. The rats were placed five per cage. The cages were labeled A to D, corresponding to four diets given to the different groups of rats. Diet 1 was given to rats in Cage A (Treatment 1). Diet 2 was given to rats in Cage B (Treatment 2). Diet 3 was given to rats in Cage C (Treatment 3) and Diet 4 was given to rats in Cage D (Treatment 4). Each experimental set up was replicated three times. The rats had unlimited supply of clean water.

The rats were fed with diets containing different levels of protein and carbohydrate and constant levels of vitamin E and selenium. The diets were: Diet 1 (control), Diet 2, Diet 3 and Diet 4. The ingredient and proximate composition of the diets are shown in Table 1. Male and female reproducing rats were paired. The female rats were naturally impregnated by male rats. The pregnancy was detected by the presence of pan plug at the feacal pan which was released when pregnancy occurred after natural mating (Cukierski, *et al.*, 1991). The pregnant rats were infected with 8000 trypanosomes per ml of blood within 10th to 14th day of pregnancy.

Since the white blood cells count is a measure of immune response of the rats to the trypanosomes (Dufva, R. and Allander, K., 1995), at the end of the experiment, the total white blood cells count and the differential leucocytes count were taken following the method Mgbenka and Ufele (2004). The data were analysed for significant differences by descriptive statistics and analysis of variance (ANOVA) using SPSS computer package. Multiple comparisons of significant differences were done using the least significant difference (LSD) and the Duncan's Multiple Range Test post hoc tests.

RESULTS

There were significant differences (P < 0.05) among all the groups of rats in their total white blood cell count, polymorphonucleated cells and mononucleated cells (Figure 1).

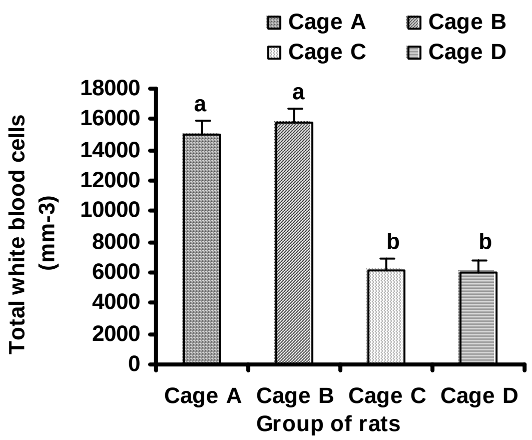

Figure 1: Mean of total white blood cells. Bars with the same letters on top are not significantly different (P > 0.05).

On comparing the total white blood cell counts of all the rats fed different diets, there was no significant difference (P > 0.05) between the rats in Cage B (fed with Diet 2) and rats in Cage A (fed with Diet 1), and also between rats in Cage C (fed with diet three) and rats in Cage D (fed with diet four). There was, however, significant difference (P < 0.05) between rats in Cage A and rats in Cages C and D and also between rats in Cage B and those in Cages C and D. Comparing the polymorphonucleated cells, there was no significant difference (P > 0.05) between the polymorphonucleated cells of rats in Cage A and the rats in Cage B, and also between rats in Cage C and that in Cage D (Figure 2).

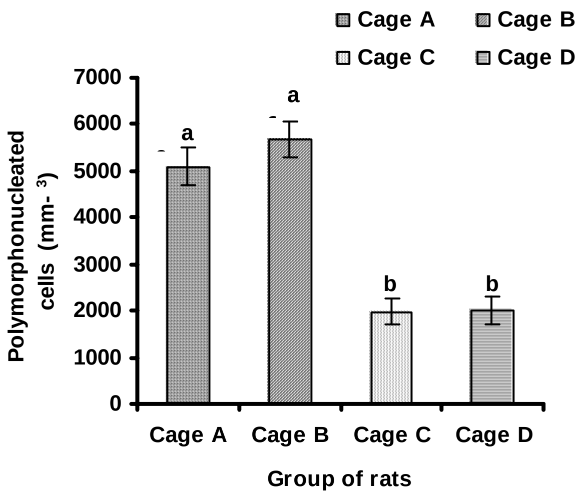

Figure 2: Mean of polymorphnucleated cells. Bars with the same letters on top are not significantly different (P > 0.05)

On the other hand, there was significant difference (P < 0.05) between the rats in Cage A and those in Cages C and D, and also between the rats in Cage B and those in cages C and D. Comparing mononucleated cells, there was no significant difference (P > 0.05) between the mononucleated cells of rats in Cage A and the rats in Cage B, and between rats in Cage C and that in Cage D (Figure

3). There was, however, significant difference ($P < 0.05$) between rats in Cage A and those in Cages C and D. Also, there were significant differences ($P < 0.05$) between rats in Cage B and those in Cages C and D. Figure 1 showed that rats fed with Diet 2 (Cage B) had the highest total white blood cells (15826 ± 882 mm^3), followed by rats fed with Diet 1 (Cage A) (15014 ± 894 though the values are not significantly different (0.05), while rats fed with Diets 3 and 4 (Cages C and D) were on the same range (6105 ± 750 mm^3 and 6057 ± 762 mm^3) respectively.

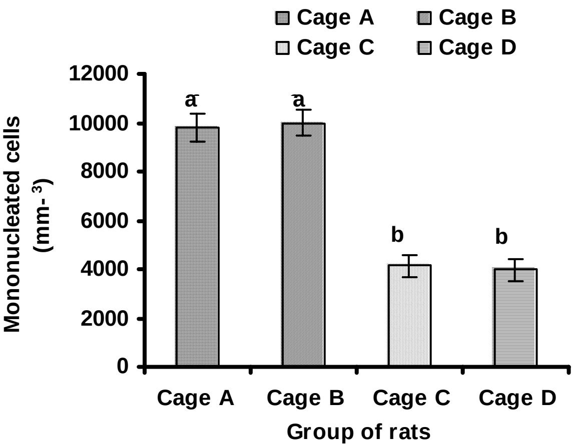

Figure 3: Mean of mononucleated cells. Bars with the same letters on top are not significantly different ($P > 0.05$).

It was observed that rats fed with Diet 2 (Cage B) had the highest polymorphonucleated cells (5682 ± 397 mm^3), though not significantly different from the value of the rats fed with Diet 1 (5100 ± 386 mm^3), while rats fed with Diets 3 and 4 (Cages C and D) were on the same range (1981 ± 286 mm^3 and 1997 ± 296 mm^3) (Figure 2). It was observed that rats fed with Diet 2 (Cage B) (9994 ± 510 mm^3) had the highest though not significantly different ($P > 0.05$) mononucleated cells to the value of the rats fed with Diet 1 (Cage A) (9813 ± 549 mm^3) (Figure 3). The rats fed with Diets 3 and 4 (Cages C and D) were on the same range (4124 ± 474 mm^3 and 3980 ± 466 mm^3) respectively.

DISCUSSION

From the above results, it was observed that rats fed with Diet 2 had the highest total white blood cell count, polymorphonucleated cells and mononucleated cells, when compared with Diet 1, Diet 3 and Diet 4. Our results of the experiments indicated that adequate nutrition enhanced trypanotolerance. Katungka-Rwakishaya (1996) observed that improvement on host's nutrition was important in modulating the severity of patho-physiological effect of trypanosomiasis and also influences the rate of recovery. It has been reported that during pregnancy, immune response are depressed (Purtilo, *et al*., 1972). Our results indicated that balanced diet enhances the total white blood cell count, differential leucocytes count and hence immune response of pregnant rats. Furthermore it was observed that good maternal nutrition was vital to health and reproductive performance of pregnant rats and the health, survival and development of their offsprings (Mora and Nestel, 2000).

The high mean values of total white blood cell count, mononucleated cells and polymorphonucleated cells in rats fed with Diet 2, indicate that balanced diet with moderate protein level (20.01%) has positive influence on the immune response of trypanosome-infected rats. This agrees with the suggestion that the degree of trypanotolerance is greatly affected by the nutritional status of the host animal (Murray, 1988; Agymang *et al*., 1990) and that supplementary diet enhances trypanotolerance in rats (Mgbenka and Ufele, 2004). The nutritional status of animals including rats influences trypanotolerance and reproduction. Since it is well known that the number and proportions of different types of leucocytes reflect the health status of individuals, as these cells quickly respond to stress and infectious diseases, leucocytes count, is a measure of immune response (Hardie *et al*., 1991; Dufva and Allander, 1995). Vitamin E and selenium, together with dietary protein and carbohydrate, reduce depression of immune system in the pregnant trypanosome-infected rats. Agnew *et al*. (2005) found that *Echinacea* intake induced an immune response through altered expression of leucocyte hsp70, increased white cell counts and improved erythrocyte antioxidant. It is therefore inferred that Diet 2, a balanced diet with intermediate level of protein (20.01 %) produced the highest (though not significantly different from Diet 1) leucocytes counts, the best immune response to trypanosomes in the pregnant trypanosome-infected rats**.**

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