
EFFECT OF DIETARY VITAMIN A SUPPLEMENT ON SERUM PROTEIN OF RATS INFECTED WITH *Trypanosoma brucei*

EDOGA, C. O., NJOKU, O. O., UFELE, A. N. and EBENEKE, C. I.

Department of Zoology, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria.

Corresponding Author: Ufele, A. N. Department of Zoology, Nnamdi Azikiwe University, PMB 5025, Awka, Anambra State, Nigeria. **Email:** ufeleangel@yahoo.com **Phone:** ±234 8038989944

ABSTRACT

The role of dietary vitamin A supplement on the serum protein of trypanosome-infected rats was studied. The rats were inoculated with trypanosomes intraperitoneally and samples were collected on fourth, eighth, twelfth and sixteenth days post infection. The experiment was carried out at the Department of Biochemistry, Nnamdi Azikiwe University Awka. Sixty parasite free-albino rats were used, which were divided into four groups. Group A (control) was left uninfected with trypanosomes, group B and C were infected with trypanosomes and treated with 50mls and 100mls of vitamin A per kg of feed respectively and group D was infected and left untreated with vitamin A. Analyses of the sera using Bradford method and cellulose acetate electrophoresis showed that vitamin A influenced the state of hypoproteinaemia in the trypanosome-infected rats. This was manifested by a positive increase in the level of total serum protein concentration, albumin and beta-globulin. Vitamin A also delayed the proliferation of the parasites associated with trypanosomiasis.

Key words: Vitamin A, Dietary supplement, *Trypanosoma brucei*, Serum protein, *Rattus novегicus*

INTRODUCTION

For several decades, trypanosomiasis has continued to contribute adversely to the economic well being of sub-Sahara Africa. Trypanosomiasis is one of the most important livestock diseases in sub-Saharan Africa (Morrison *et al.*, 1981). This scourge remains a pressing challenge especially to African scientists in formulating possible action plan that would eradicate trypanosomiasis from the African continent. The articulation of such plan would include both preventive measures and treatment modalities.

Trypanosome is known to attack red blood cells and vascular endothelium. It concentrates more in the peripheral circulation (Jackson, 1979). These trypanosomes exhibit remarkable antigenic variation of their surface glycoprotein with hundreds of antigenic type.

One antigenic type will coat the surface of the parasites for approximately 10 days followed by other types in sequences in the new progeny. This variation is due to sequential movement of the glycoprotein genes to a preferential location of the chromosome were only that specific gene is transcribed into messenger RNA. These antigenic variations allow the organism to continually invade the host immune response. The first wave of parasitemia is accompanied by depressed packed cell volume, neutropenia and thrombocytopenia (Krampitz, 1970).

Trypanosomiasis in rats is associated with a decreased serum protein as infection progressed. Improvement on host's nutrition is important in moderating the severity of pathophysiological effect of trypanosomiasis and also influences the rate of recovery (Katungka-Rwakishaya, 1996).

It was also discovered that supplementary feeding significantly reduces the severity of trypanosomiasis (Agyemang *et al.*, 1990; Little *et al.*, 1990). Vitamin A is essential for the development of bursa of fabricius, thymus and immunity in chickens (Raza *et al.*, 1997). Vitamin A supplements strongly increase serum protein and glycoprotein synthesis which maintain synthesis of surface coating mucopolysaccharides. Hence increase mucosal immune system, together with the epithelial rebuilding character of vitamin A raise macrophage activity and increase humoral and cell mediated response of vitamin A sufficient chicken; strongly suggest a much high resistance to infection of higher animals (Sijtsma *et al.*, 1991; Johannsen *et al.*, 1998).

Over the years, vitamin A has been used to tackle all kinds of infections and it is very popular both among the low, middle and higher socio-economic class (Benynen *et al.* 1989; Bang *et al.*, 1995). This study was design to evaluate to what extent this vitamin A can influence the state of hypoproteinaemia in trypanosome-infected rats.

MATERIALS AND METHODS

Twenty 90-day old male albino rats (*Rattus norvegicus*) weighing approximately 145g, were used for this experiment. The rats were marked for identification and held in stainless wire-rats-cages in clean experimental animal house. The cages were labeled A to D corresponding to four groups and each group had five rats. Diet 1 was given to rats in Cage A which contained 1kg of chick mash without vitamin A. Diet 2 was given to rats in Cage B which contained 1kg of chick mash mixed with 50mls of vitamin A. Diet 3 was used to feed rats in Cage C which contained 1kg of chick mash mixed with 100mls of vitamin A and Diet 4 was used to feed rats in Cage D which contained 1kg of chick mash without any vitamin A. Rats in Cage A were not infected while rats in Cages B, C and D were infected with *Trypanosoma brucei*. One rat was first inoculated with trypanosome of NITR type from Veterinary Medicine Faculty, University of Nigeria, Nsukka. It was isolated from other animals and after 14 days of inoculation, the

blood of that rat was used to inoculate others. Each experimental rat that was inoculated was given 0.1ml of infected blood in normal saline, which contained about eight thousand trypanosomes, using a matching chart (Herbert and Lumsden, 1976) to determine the level of parasitemia. Rats in Cages A and D served as control groups. Each experimental set up was replicated three times. The rats had unlimited supply of clean water.

Five (5) ml of the blood of the rats were collected in each experimental day which was four days intervals for sixteen days of the experimental period to determine the total serum protein. The collected blood was allowed to clot for about 30 minutes at room temperature. Then each sample was centrifuged at 3,000 rpm for 10 minutes and the serum was removed. The sera were used immediately for serum protein determination using Bradford method. The absorbance of the solutions was read at 520nm-wavelengths using spectrophotometer.

Statistical Analysis: The data on change in total serum protein level were statistically analysis using analysis of variance (ANOVA) at 95 percent probability level. Results obtained were reported as mean concentrations.

RESULTS

The administration of vitamin A positively influenced the serum protein of trypanosome-infected rats. There was increased total serum protein, raised albumin and beta-globulin in the infected and treated rats. The lowest level of mean total serum protein of $49.27 \pm 5.32\text{g/l}$, albumin of $17.85 \pm 3.35\text{g/l}$ and beta-globulin of $3.46 \pm 0.82\text{g/l}$ were observed in infected and untreated rats (Cage D). Furthermore, $50.79 \pm 5.05\text{g/l}$ of total serum protein, $20.77 \pm 3.53\text{g/l}$ of albumin and $5.15 \pm 0.48\text{g/l}$ of beta-globulin were observed in infected rats treated with 50ml of vitamin A per kg of chick mash (Cage B). Then infected rats which were treated with 100ml of vitamin A per kg of chick mash had $52.2 \pm 4.24\text{g/l}$ of total serum protein, $22.99 \pm 2.97\text{g/l}$ of albumin and $5.36 \pm 0.64\text{g/l}$ of beta-globulin (Cage C).

Table 1: Total serum protein, albumin and beta-globulin of rats infected with *Trypanosoma brucei* fed dietary vitamin A supplement

Length of post-infection	Group A (uninfected and untreated)	Group B (infected and treated with 50ml vitamin A/kg of feed)	Group C (infected and treated with 100ml vitamin A/kg of feed)	Group D (infected and untreated)
Total serum protein (g/l)				
4	60.63	59.80	59.82	59.53
8	59.84	55.88	56.24	54.04
12	61.65	50.83	52.46	48.79
16	61.86	36.67	40.31	34.71
Total	243.98	203.18	208.83	197.07
Mean	60.99 ± 0.47	50.79 ± 5.05	52.21 ± 4.24	49.27 ± 5.32
Albumin (g/l)				
4	32.68	30.02	30.21	27.40
8	30.93	20.43	24.62	16.51
12	32.69	19.75	21.06	15.73
14	32.64	12.87	16.09	11.74
Total	128.94	83.07	91.98	71.38
Mean	32.24 ± 0.44	20.77 ± 3.53	22.99 ± 2.97	17.85 ± 3.35
Beta-globulin (g/l)				
4	7.88	6.45	6.92	5.84
8	8.66	5.18	5.77	3.19
12	8.16	4.21	4.86	2.68
14	8.35	4.76	3.91	2.12
Total	33.05	20.60	21.46	13.83
Mean	8.26 ± 0.16	5.15 ± 0.48	5.36 ± 0.64	3.46 ± 0.82

The uninfected and untreated rats (Group A) had the highest mean levels of total serum protein (60.99 ± 0.47 g/l), albumin (32.24 ± 0.44g/l) and beta-globulin (8.26 ± 0.16g/l) (Table 1).

DISCUSSION

Several scientific researches have been done on trying to identify and standardize active food supplement that would be active in treatment of trypanosomiasis. This trypanosomiasis has contributed adversely to the economic and social well being of Sub-Sahara Africans. A lot of scientific investigations have proved that retinol and retinoic acid nutrient produced significant enhancement on the immune system of rats (Nauss *et al.*, 1985).

The observed effect of vitamin A supplement from this study on the serum protein of trypanosome-infected rats is attributed to its effect on the haemopoietic system. The effect of vitamin A on the infected and treated rats when compared with the infected and untreated rats showed that vitamin A had positive influence on the defense capacity of infected and treated animals. Therefore, this study has provided evidence that vitamin A has a potential for influencing the state of hypoproteinaemia in the trypanosome-infected rats. Even if vitamin A cannot destroy the trypanosomes, it can ameliorate the stress of trypanosomiasis and as enhanced the hosts' immune system to fight the invaded pathogens.

REFERENCES

- AGYEMANG, K., DWINGER, R. H., TOURAY, B. N., JEANNIN, P., FOFANA, D. and GRIEVE, A. S. (1990). Effects of nutrition on degree of anaemia and live weight changes in N'Dama cattle infected with trypanosomes. *Livestock Production Science*, 26: 39 – 51.
- BANG, F. B., BANG, B. G. and FOARD, M. (1995). Acute Newcastle viral infection of the upper respiratory tract of the chicken: 2. Effect of diets deficient in vitamin A on the infection. *Journal of Animal Pathology*, 78: 417 – 426.
- BENYENEN, A. C., SIJTSMA, R. S., KIEPURSKI, A. K., WEST, C. E., BAUMANS, V., HERCK, VAN. H., STAFLEU, R. F. and VAN TINTELE, G. (1989). Objective clinical examination of poultry as illustrated by the comparison of chicken with different vitamin A status. *British Journal of Animal Science*, 23: 307 – 312.
- HERBERT, W. J. and LUMBSDEN, W. H. R. (1976). *Trypanosoma brucei*; A rapid matching method for estimating the host's parasitemia. *Experimental Parasitology*, 40: 427 - 431.
- JACKSON, G. J. (1979). *Trypanosoma congolense*: inheritance of susceptibility to infection in inbred strains of mice. *Experimental Parasitology*, 48: 378 - 383.
- JOHANNSEN, A. K. B., JENSEN, S. K. and JAKOBSEN, K. (1998). A note on vitamin A activity of B-carotene in rats. *Acta Agriculturae Scandinavica, Section A - Animal Science*, 48: 260 – 263.
- KATUNGKA-RWAKISHAYA, E. (1996). Interaction between animal nutrition and parasites, studies with experimental trypanosomiasis in sheep. Pages 1 – 9. *In: LEBBIE, S. H. B. and KAGWINI, E., (Eds). Small Ruminant Research and Development in Africa. International Livestock Research Institute (ILRI) Nairobi, Kenya.*
- KRAMPTZ, H. E. (1970). Beobachtungen an experimentell infizierten ostafrikanischen Zebuindern mit wildem Stämmen von *T. congolense*. *Tropenmed Parasitology*, 21: 1 - 30.
- LITTLE, D. A., DWINGER, R. H., CLIFFORD, D. J., GRIEVE, A. S., KORA, S. and BOJANG, M. (1990). Effect of Nutritional level and body condition on susceptibility of N'Dama cattle to *T. congolense* infection in the Gambia. *Proceedings of the Nutrition Society*, 49: 209A.
- MORRISON, W. I., MURRAY, M. and MCINTYRE, W. I. M. (1981). Bovine Trypanosomiasis. Pages: 469 – 497. *In: RISSTIC, M. and MCINTYRE, W. I. M. (Eds), Disease of Cattle in Tropics. Martinus Nijhoff Publishers, The Hague, Netherlands.*
- NAUSS, K. M., PHUA, C. C., AMBRODI, L. and NEWBERNE, P. M. (1985). Immunological changes during progressive stages of vitamin A deficiency in the rats. *Journal of Nutrition*, 115: 909 – 918.
- RAZA, A., KHAN, F. K., SACED, M. A. and BASIR, N. I. (1997). Effect of vitamin A on growth traits, immuno-regulatory organs and immune response in broiler chicken. *Journal of Applied Animal Research*, 12: 81 – 88.
- SIJTSMA, R. S., WEST, F. C., POMBOUT, M. W. J. and VAN DER ZIPP, J. A. (1991). The interaction between vitamin A status and Newcastle disease virus infections in chicken. *Journal of Nutrition*, 119: 932 – 939.