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Effect of probiotic (*Saccharomyces cerevisiae*) supplementation on immune response in *Trypanosoma brucei brucei* infected rats

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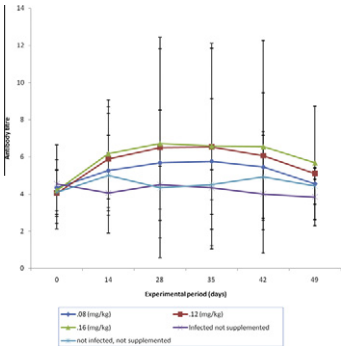
HIGHLIGHTS

- Increased antibody response.
- Increased total and differential leucocyte count.
- Decreased parasitaemia.

GRAPHICAL ABSTRACT

Graph showing the antibody titre of *Trypanosoma brucei* infected rats given varying levels of *Saccharomyces cerevisiae* in their diet and controls.



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ABSTRACT

The immunomodulatory effect of the probiotic (*Saccharomyces cerevisiae*) on *Trypanosoma brucei brucei* infected rats was studied. Thirty (30) rats divided into five groups (A–E) of 6 rats each were used for the study. Groups A, B and C rats received feed supplemented with *S. cerevisiae* (at 0.08, 0.12 and 0.16/kg of feed, respectively) for the duration of the study. Groups D and E diets were not supplemented. All the rats in the 5 groups were immunized with 0.3 ml of 10% sheep red blood cells (SRBC) at day 7 pre-supplementation, and booster doses given every 14 days thereafter. On day 28 post supplementation (PS), rats of groups A–D were infected with 1×10^6 of *T. brucei brucei* intraperitoneally. Supplementation resulted in increases in antibody titres to SRBC which later declined following *T. brucei brucei* infection, but remained higher than the pre supplementation titres. At termination of the study (i.e. day 49 PS) supplemented groups had significantly ($p < 0.05$) higher antibody titres than either the infected or the non infected controls. The total and differential leucocyte counts followed a similar pattern with initial increases in counts following supplementation followed by reductions after *T. brucei brucei* infection. Supplementation also resulted in decline in parasitaemia with significant difference between the supplemented groups and the un-supplemented controls on day 42 post infection. The results are indication that probiotics can be used to ameliorate the immunosuppressive effect of *T. brucei brucei* infections.

1. Introduction

Immunosuppression is a well-documented feature of trypanosomosis in cattle, humans and mice (De Baetselier, 1996; Taylor, 1998; Namangala, 2011). Some of the immunological dysfunctions

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occurring during the disease are a marked elevation of immunoglobulin M (IgM) levels in the serum and other body fluids, the production of free immunoglobulin light chains, and a spontaneous rise in heterophile IgM antibodies specific for antigens such as xenogeneic erythrocytes and bacterial lipopolysaccharide (LPS) (Vincendeau and Bouteille, 1996; Barry and Carrington, 2004). Other dysfunction include the presence of an IgM rheumatoid factor-like antibody, the production of IgM autoantibodies specific for normal tissue antigens, and the suppression of both B and T lymphocyte functions (Vincendeau and Bouteille, 1996; Barry and Carrington, 2004; Namangala, 2011).

There is evidence that infection related immunodepression compromises the animal's capacity to control trypanosomoses (Stenberg et al., 1994), as well as secondary infections (Scott et al., 1997; Onah and Wakelin, 2000; de Sousa et al., 2011). This may result in increased mortality and morbidity from associated diseases exhibited through increased processing plant condemnations, higher feed conversions, and depressed average daily weight gains. Minimizing immunosuppression and its impact is an important strategy for success in increased livestock productivity. Immunomodulators, such as vitamin E, combination of vitamin E and selenium, retinyl palmitate, vitamins A and C are shown to be beneficial in the management of trypanosomosis in animals (Ihedioha et al., 2003; Eze and Ochike, 2007; Ufele et al., 2007; Umar et al., 2007).

Probiotics are live microorganisms (bacteria and yeast) that when administered in adequate amounts, confers health benefits on the host (Reid et al., 2003; Shane, 2008). It is thought that probiotics may be beneficial in management of trypanosomes. The positive biomedicinal effects of probiotics consist in their ability to inhibit digestive tract pathogens, optimize digestion and stimulate the immune system (Mátéová et al., 2009). Probiotics also exhibit antitumoural, antiallergenic and anticholesterol actions (Soccol et al., 2010). Organisms' that favor the production of lactic acid-producing bacteria in the gut, including *Saccharomyces cerevisiae* are known to stimulate various aspects of the immune system, including phagocytic function of macrophages, natural killer cells, monocytes, and neutrophils (Patterson et al., 2011). Clearly, interaction of commensal gastrointestinal flora with the gut-associated immune system is an important key in maintaining normal immune function (Haghighi et al., 2005).

Applying probiotics to stimulate immune function, especially in individuals with underdeveloped or dysregulated immune function, appears to be sound, considering the positive outcomes of feeding studies targeting viral infections (Ötlesl et al., 2003). The beneficial effects of probiotics on human and animal health and nutrition are becoming increasingly recognized and are believed to play an important role in immunological, digestive and respiratory functions, and could have a significant effect on the alleviation of infectious diseases.

This work was therefore designed to evaluate the possible effect of the probiotic, *S. cerevisiae* on the immune response of *Trypanosoma brucei brucei* infected rats.

2. Materials and methods

2.1. Experimental animals

Thirty adult male albino rats aged 109–118 days and weighing between 230–248 g were used for the study. They were acquired from the Laboratory Animal Unit, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. The rats were housed in a fly-proof house and provided commercial feed (Grand Feeds, Jos-Nigeria) and water ad libitum. Animal studies were in compliance to the ethical procedure of the Animal Use and Care Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka which corresponds with NIH guidelines (NIH, 1996).

2.2. Probiotic

The probiotic, *S. cerevisiae* was used in this study. It was obtained from B.F.P., Dock Road, Felix Stowe, United Kingdom.

2.3. Trypanosomes

The strain of *T. brucei brucei* (Federe strain) used was obtained from Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The trypanosome was maintained in the laboratory by serial passages in mice. Rats were infected with 1×10^6 trypanosomes in PBS diluted rat blood.

2.4. Immunization of rats against sheep red blood cells

Fresh blood was obtained from sheep by jugular venipuncture. Immunization was achieved by an initial injection of 0.3 ml of a 10% sheep red blood cells (SRBC) suspension in normal saline at 7 days pre supplementation, followed by booster doses every 14 days, till termination of the study.

2.5. Assay of antibody response to SRBC

Antibody response to SRBC was assayed in serum samples of the individual rats by the direct haemagglutination technique, with a 2% SRBC suspension in normal saline as described by Ikeme and Adelaja (1990).

2.6. Effect of supplementation on immune response

The rats were randomly divided into five groups (A, B, C, D and E) of six rats each and each group kept in separate cages. From day 0 post supplementation (PS), rats in groups A, B and C were given feed supplemented with *S. cerevisiae* (at 0.08, 0.12 and 0.16 g/kg of feed, respectively) for the duration of the study. Groups D and E mice did not receive supplemented feed. At day 28 PS each rat in groups A, B, C and D was infected with 1×10^6 blood stream forms of *T. brucei brucei* intraperotoneally. Group E rats were not infected and served as negative controls.

The parameters assessed in the study included the antibody response to SRBC using direct haemagglutination test, total and differential leucocyte counts and parasitaemia estimated by the rapid matching method (Herbert and Lumsden, 1976). The antibody response to SRBC was determined on day 0 PS and at 14 day intervals thereafter. The total and differential leucocyte counts were determined on day 0 PS and at 7 day intervals subsequently. Parasitaemia was estimated at weekly intervals from day 35 PS.

2.7. Collection of blood sample from rats

About 0.5 ml of blood was collected from the retro bulbar plexus of the medial canthus of rats. 0.2 ml of the blood was collected into anticoagulant bottles for leucocyte count while the remainder was collected into Ependof tubes, allowed to clot and later centrifuged at 3,000 rpm (rpm) for 10 min to separate the serum for determination of antibody response to SRBC.

2.8. Haematology

The total leucocyte count was done as described by Schalm et al. (1975). Smears for differential leucocyte counts were