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


Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

**Highlights**

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

**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/\(\mathrm{kg}\) 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 \(1 \times 10^6\) 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.

© 2012 Elsevier Inc. All rights reserved.

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...
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 trypanosomoses. 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 $1 \times 10^6$ 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 $1 \times 10^6$ blood stream forms of T. brucei brucei intraperitoneally. 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
prepared at weekly intervals and stained by the Leishman technique, and the different cells of the leucocytic series (including neutrophils lymphocytes, monocytes, eosinophils and basophils) enumerated by the longitudinal counting method.

2.9. Statistical analysis

The data obtained from the study were summarized as means ± standard error of means. Statistical comparisons between the treatment groups were made by One Way Analysis of Variance (ANOVA). Means were considered significant at p < 0.05 and the means separated using Duncan’s multiple range test.

3. Results

Supplementation resulted in increase in total leucocyte count and at day 28 PS there were significant differences (p < 0.05) between the supplemented and non supplemented groups (Table 1). Following infection, the leucocyte count decreased, with the infected non supplemented group being significantly (p < 0.05) lower than other groups on days 35 and 42 PS. The differential counts showed a similar pattern with the total leucocytes count. On day 28 PS the neutrophil counts (Table 2) of the supplemented groups were significantly (p < 0.05) higher than the control groups. Rats given feed supplemented at 0.16 g/kg had higher neutrophil counts that were significantly (p < 0.05) higher than the infected untreated group on days 35 and 42 PS. Similarly, the lymphocyte count (Table 3) increased following supplementation at 0.16 g/kg with counts significantly (p < 0.05) higher than the infected unsupplemented group on days 28, 35 and 42 PS. The monocyte basophil and eosinophil counts did not follow a consistent pattern, however they increased following supplementation.

There was an increase in mean antibody titres following dietary supplementation with S. cerevisiae (Table 4). The antibody titres in groups supplemented at 0.12 g and 0.16 g/kg of feed were significantly higher (p < 0.05) than in other groups. The antibody titres at day 28 PS led to increase in the mean antibody titres though they remained higher than the pre supplementation values. The mean antibody titre of the infected unsupplemented group decreased steadily unlike the supplemented groups that showed gradual decline and was significantly (p < 0.05) lower than other groups on days 35 and 42. The pre-infection dietary supplementation with S. cerevisiae prolonged the onset of parasitaemia in T. brucei infected rats. The pre patent period of the group receiving 0.16 g of S. cerevisiae/kg of feed was significantly (p < 0.05) longer than that of the infected untreated group but was not significantly different from other groups (Fig. 1). The mean parasitaemia of the supplemented groups were significantly lower (p < 0.05) than the infected unsupplemented group at days 35, 42 and 49 PS (Table 5). On day 49 PS there was no significant difference in parasitaemia between the supplemented groups.

4. Discussion

The pre-infection supplementation with S. cerevisiae was necessary to allow for colonization of the gastrointestinal tract of the rats where they will exert their influence and possible effects. Such influences may include regulation of immune function, enhancement of intestinal barrier to unwanted microbes and increase in the bioavailability of dietary minerals (Choudhari et al., 2008). These will most often translate to enhanced feed conversion efficacy and growth rate (Friend and Shalani, 1984; Choudhari et al., 2008). The rats were given feed supplemented with S. cerevisiae throughout the experimental period, since the ingestion of probiotic strains does not lead to measurable long-term colonization and survival in the host. The use of probiotics likely confers more transient than long-term effects, and so continued intake appears to be required (Tannock et al., 2000).

Supplementation with S. cerevisiae resulted in a significant increase in total and differential leucocyte count during the study. Leucocytes counts during stress and infectious disease is a measure of immune response (Hardie et al., 1991; Dufva and Allander, 1995; Ufere et al., 2007) as they are important in protecting our body against infection (Schalm et al., 1975). The increase in leucocytes recorded in this work is consistent with the findings of other workers (Perdigon and Alvarez, 1992; Marteau and Rambaud, 1993; Donnet-Hughes et al., 1999; McCracken and Gaskins, 1999, Perdigon et al., 1999, Nyamaganda et al., 2009). However, some workers did not find a significant improvement of total and differential leucocytes count (Anukam et al., 2004, Cetin et al., 2005; Strompfova et al., 2006). The decrease in leucocytes count following trypanosomiasis infection is consistent with reports that trypanosomosis causes leucopenia (Anosa et al., 1997; Nfon et al., 2000; Biryumumaisho and Katunguka-Rwakishaya, 2007). The ability of the total leucocyte count to remain higher than pre-supplementation values following T. brucei infection could be attributed to S. cerevisiae.

The neutrophils are principally responsible for phagocytosis of pathogenic microorganisms while lymphocytes are involved in humoral and cellular immunity (Schalm et al., 1975: Baker and Silverton, 1985). In essence, the increase in the neutrophil and lymphocyte count observed in our study favored enhanced immune protection of the rats. Probiotics have been found to modulate the functions of immune cells (Gill and Guarner, 2004) such as the major cellular effectors of innate immunity including epithelial cells, phagocytic cells (monocytes, macrophages, and neutrophils), and natural-killer cells (NK-cells). The decrease in differential leucocytes count following trypanosomiasis infection is consistent with reports that leucopenia characterized by neutropenia, eosinopenia and lymphopenia is seen in cats experimentally infected with T. brucei (Nfon et al., 2000), as well as goats infected with T. congolense (Biryumumaisho and Katunguka-Rwakishaya, 2007). The antibody titre to sheep red blood cells (SRBC) increased following S. cerevisiae supplementation. The increase in antibody titre following probiotics supplementation is in agreement with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Mean total leucocyte counts (10³/ml) of T. brucei infected rats given varying levels of S. cerevisiae in the diet and controls.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days PS</td>
<td>A 0.08 (mg/kg)</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td>0</td>
<td>9.17 ± 0.37</td>
</tr>
<tr>
<td>14</td>
<td>12.74 ± 1.11</td>
</tr>
<tr>
<td>28</td>
<td>11.91 ± 0.94</td>
</tr>
<tr>
<td>35</td>
<td>11.47 ± 2.08</td>
</tr>
<tr>
<td>42</td>
<td>7.68 ± 0.99a</td>
</tr>
<tr>
<td>49</td>
<td>3.54 ± 0.41a</td>
</tr>
</tbody>
</table>

*a,b* Different superscripts in a row indicates significant difference between the means at the level of probability: p < 0.05, PS means post supplementation.
Table 2
Mean neutrophil counts (10³/ml) of T. brucei infected rats given varying levels of S. cerevisiae in the diet and controls.

<table>
<thead>
<tr>
<th>Days PS</th>
<th>A 0.08 (mg/kg)</th>
<th>B 0.12 (mg/kg)</th>
<th>C 0.16 (mg/kg)</th>
<th>D Infected not supplemented</th>
<th>E Uninfected not supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.780 ± 119.54</td>
<td>2.827 ± 153.04</td>
<td>2.931 ± 209.90</td>
<td>2.801 ± 216.59</td>
<td>3.377 ± 226.90</td>
</tr>
<tr>
<td>14</td>
<td>3.730 ± 476.87</td>
<td>3.874 ± 603.54</td>
<td>3.650 ± 332.04</td>
<td>2.585 ± 117.01</td>
<td>3.627 ± 443.95</td>
</tr>
<tr>
<td>28</td>
<td>3.728 ± 279.42</td>
<td>4.178 ± 398.52</td>
<td>3.830 ± 223.21</td>
<td>3.202 ± 159.24</td>
<td>3.021 ± 210.21</td>
</tr>
<tr>
<td>35</td>
<td>4.727 ± 570.57</td>
<td>3.100 ± 179.35</td>
<td>3.734 ± 181.11</td>
<td>1.317 ± 62.87</td>
<td>3.164 ± 227.60</td>
</tr>
<tr>
<td>42</td>
<td>2.485 ± 342.29</td>
<td>2.376 ± 235.23</td>
<td>2.741 ± 352.89</td>
<td>0.962 ± 55.24</td>
<td>3.676 ± 405.01</td>
</tr>
<tr>
<td>49</td>
<td>1.071 ± 150.00</td>
<td>1.217 ± 168.32</td>
<td>1.910 ± 218.53</td>
<td>0.754 ± 145.16</td>
<td>2.912 ± 182.96</td>
</tr>
</tbody>
</table>

abc Different superscripts in a row indicates significant difference between the means at the level of probability: p < 0.05; PS means post supplementation.

Table 3
Mean lymphocyte counts (10³/ml) of T. brucei infected rats given varying levels of S. cerevisiae in the diet and controls.

<table>
<thead>
<tr>
<th>Days PS</th>
<th>A 0.08 (mg/kg)</th>
<th>B 0.12 (mg/kg)</th>
<th>C 0.16 (mg/kg)</th>
<th>D Infected not supplemented</th>
<th>E Uninfected not supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.102 ± 190.2</td>
<td>5.115 ± 282.8</td>
<td>5.383 ± 200.1</td>
<td>5.452 ± 268.2</td>
<td>5.247 ± 308.7</td>
</tr>
<tr>
<td>14</td>
<td>6.810 ± 788.3</td>
<td>6.512 ± 513.0</td>
<td>7.412 ± 643.7</td>
<td>5.305 ± 287.6</td>
<td>5.973 ± 892.4</td>
</tr>
<tr>
<td>28</td>
<td>7.265 ± 584.0</td>
<td>8.002 ± 570.7</td>
<td>8.076 ± 462.8</td>
<td>5.069 ± 422.2</td>
<td>5.862 ± 316.9</td>
</tr>
<tr>
<td>35</td>
<td>7.281 ± 767.9</td>
<td>6.353 ± 394.1</td>
<td>7.341 ± 560.7</td>
<td>3.634 ± 118.2</td>
<td>5.980 ± 448.4</td>
</tr>
<tr>
<td>42</td>
<td>5.100 ± 774.9</td>
<td>4.694 ± 461.4</td>
<td>5.790 ± 620.5</td>
<td>2.974 ± 274.8</td>
<td>6.574 ± 436.2</td>
</tr>
<tr>
<td>49</td>
<td>2.259 ± 230.1</td>
<td>3.066 ± 407.7</td>
<td>3.230 ± 298.2</td>
<td>2.399 ± 606.8</td>
<td>6.342 ± 486.3</td>
</tr>
</tbody>
</table>

abc Different superscripts in a row indicates significant difference between the means at the level of probability: p < 0.05; PS means post supplementation.

Table 4
Mean antibody titres (Log 2) of T. brucei infected rats given varying levels of S. cerevisiae in the diet and controls.

<table>
<thead>
<tr>
<th>Days PS</th>
<th>A 0.08 (mg/kg)</th>
<th>B 0.12 (mg/kg)</th>
<th>C 0.16 (mg/kg)</th>
<th>D Infected not supplemented</th>
<th>E Uninfected not supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.33 ± 2.21</td>
<td>4.04 ± 1.26</td>
<td>4.19 ± 1.11</td>
<td>4.54 ± 2.11</td>
<td>4.08 ± 1.18</td>
</tr>
<tr>
<td>14</td>
<td>5.25 ± 1.52</td>
<td>5.88 ± 2.80</td>
<td>6.17 ± 2.89</td>
<td>4.05 ± 2.16</td>
<td>5.00 ± 2.15</td>
</tr>
<tr>
<td>28</td>
<td>5.75 ± 3.10</td>
<td>6.50 ± 5.93</td>
<td>6.71 ± 5.09</td>
<td>4.50 ± 1.95</td>
<td>4.33 ± 1.15</td>
</tr>
<tr>
<td>35</td>
<td>5.44 ± 2.85</td>
<td>6.53 ± 5.31</td>
<td>6.58 ± 5.53</td>
<td>4.33 ± 2.23</td>
<td>4.50 ± 0.80</td>
</tr>
<tr>
<td>42</td>
<td>5.100 ± 3.37</td>
<td>6.06 ± 3.38</td>
<td>6.54 ± 5.73</td>
<td>4.00 ± 1.42</td>
<td>4.92 ± 2.24</td>
</tr>
<tr>
<td>49</td>
<td>4.54 ± 1.91</td>
<td>5.10 ± 0.31</td>
<td>5.67 ± 3.06</td>
<td>3.83 ± 1.54</td>
<td>4.42 ± 0.98</td>
</tr>
</tbody>
</table>

abc Different superscripts in a row indicates significant difference between the means at the level of probability: p < 0.05; PS means post supplementation.

Fig. 1. Pre patent period of rats given varying levels of S. cerevisiae in their diet and the infected control.
Table 5
Mean parasitaemia (10^6 tryp/ml of blood) of T. brucei infected rat given varying levels of S. cerevisiae in the diet and controls.

<table>
<thead>
<tr>
<th>Days</th>
<th>PS A 0.08 (mg/kg)</th>
<th>B 0.12 (mg/kg)</th>
<th>C 0.16 (mg/kg)</th>
<th>D Infected not supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>28.98 ± 7.58</td>
<td>42.11 ± 6.64</td>
<td>39.49 ± 7.87</td>
<td>39.49 ± 7.87</td>
</tr>
<tr>
<td>42</td>
<td>115.38 ± 29.93^a</td>
<td>36.86 ± 8.78</td>
<td>62.95 ± 38.43^a</td>
<td>198.82 ± 71.22^a</td>
</tr>
<tr>
<td>49</td>
<td>125.83 ± 44.34^*</td>
<td>219.88 ± 31.33^*</td>
<td>157.20 ± 31.34^*</td>
<td>251.10 ± 55.95^*</td>
</tr>
</tbody>
</table>

^a,b Different superscripts in a row indicates significant difference between the means at the level of probability: p < 0.05; PS means post supplementation.

previous studies that show that the intake of probiotics has a number of effects on the immune system including increased production of IgA antibodies (Dugas et al., 1999). Kaila et al. (1992) reported that consumption of specific probiotics has been shown to enhance antibody responses to natural infections and to immunizations such as in children with rotavirus infections. However, the antibody titre reduced following T. brucei infection, although not to pre supplementation level. Many workers have reported that trypanosome infections led to suppressed antibody response to mitogens (Steverding, 2006).

The significantly reduced level of parasitaemia recorded for the supplemented groups is believed to be due to the possible immunomodulatory effects of S. cerevisiae. This is considered significant as there is a direct correlation between level of parasitaemia and disease severity in trypanosomosis (Anosa, 1988). It is thought that this effect on parasitaemia was mediated by interaction of the enhanced immune system of the host with the parasite (Vanhamme et al., 2001).

The increased total and differential leucocytes count, the significant rise in antibody titre to SRBC and suppression of parasitaemia in this study could be a result of immune stimulatory effect of the probiotic, S. cerevisiae.

5. Conflicts of interests

There are no conflicts of interests related to this work.

References


Mátéová, S., Gaálová, M., Šály, J., Fialková, M., 2009. Investigation of the effect of the increased total and differential leucocytes count, the significant rise in antibody titre to SRBC and suppression of parasitaemia in this study could be a result of immune stimulatory effect of the probiotic, S. cerevisiae.

5. Conflicts of interests

There are no conflicts of interests related to this work.


