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Effect of Dietary Probiotic (*Saccharomyses cerevisiae*) Supplementation on the Severity of *Trypanosoma brucei* Infection in Rats

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Abstract: The beneficial effect of *Sacharomyces cerevisiae* supplementation in the diets before and after *Trypanosoma brucei* infection in rats was studied. Thirty adult male albino rats used for this study were divided into 5 groups of 6 rats each. Groups A, B and C were fed with diet containing 0.08, 0.12 and 0.16 mg of S. cerevisiae respectively, while groups D and E were fed with unsupplemented diet. On day 28 post supplementation (PS), groups A, B, C and D rats were each infected with 1.00 x 10^6 trypanosomes intraperitonealy, group E was not infected. The pre-infection supplementation significantly ($p<0.05$) increased the Haemoglobin (Hb) concentration in groups B and C and red blood cell (RBC) count in groups A, B and C respectively when compared with group D. Also, the mean weight of group C increased significantly ($p<0.05$) on day 14 PS. However, following infection, the PCV, Hb and RBC count of group D was significantly ($p<0.05$) lower than other groups from day 35 to 49 PS. The weight of the supplemented groups did not vary with the uninfected control while the mean temperature of group D remained higher than other groups. The parasitaemia level of groups B and C had significantly ($p<0.05$) lower than group D but not with group A on day 42PS. The mean survival interval of group C was significantly ($p<0.05$) higher than groups A and D. The supplementation was able to ameliorate anaemia, reduced weight loss, suppresses parasitaemia and increased survival interval in *T brucei* infected rats.

Key words: *Saccharomyces cerevisiae* · *Trypanosoma brucei* · Anaemia · Parasitaemia · Survival interval

INTRODUCTION

African animal trypanosomosis (AAT) is a very important disease of domestic livestock in sub-Saharan Africa and is probably the only disease which has profoundly affected the settlement and economic development of a major part of the African continent [1]. Direct losses due to trypanosomosis are estimated to amount to between US$ 1-1.2 billion each year whereas the indirect impact of AAT on agriculture in sub-Saharan Africa exceeds this amount [2].

The pathogenesis is dominated by three features: anaemia, tissue lesions and immunosuppression. Anaemia remains a cardinal feature of the disease, the severity of which usually reflects the intensity and duration of parasitaemia which also correlates with the severity of infection [3,4]. The cause of anaemia is complex and involves a variety of mechanisms. Despite the importance of anaemia, the exact mechanisms underlying its induction remained unsolved [5]. The pathogenesis of anaemia in African trypanosomosis has been shown to be due to complex and intricate factors acting together [6-11].

Probiotics had been used as growth promoters due to their ability to suppress the proliferation and activities of growth depressing microflora and their ability to enhance absorption of nutrients through the production of digestive enzymes [12].

In recent years, there were increasing researches which have demonstrated the potential benefits of probiotics in enhancing the blood profiles of animals as a precursor for the formation haemoglobin [13]. Since these probiotics produced increase in the erythrocyte indices of anaemia (PCV. Hb and red blood cell counts), its supplementation in trypanosomosis infection may enhance the animal's ability to control the severity of the disease.

This study was therefore aimed at assessing the effect of dietary probiotics (*Saccharomyces cerevisiae*) supplementation on the severity of *Trypanosoma brucei brucei* infection in rats.

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MATERIALS AND METHODS

Experimental Animals: Thirty adult male albino rats were used for this study. They were acquired from the Laboratory Animal Unit, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. The rats were housed in fly-proof house and provided feed and water ad libitum. Animal studies where in compliance to the ethical procedure of the Animal Use and Care Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Probiotics: The probiotic, *Saccharomyces cerevisiae* was used. It was obtained from B.F.P., Dock Road, Felix Stowe, United Kingdom.

Trypanosomes: The strain of *Trypanosoma brucei brucei* (Federe strain) used was obtained from Nigerian Institute for Trypanosomiasis Research, Vom, Plateau State, Nigeria. The trypanosome was maintained in the Department by serial passages in mice. Each rat in groups A, B, C and D was infected with 1 $x10^6$ trypanosomes in Phosphate buffer saline (PBS) diluted trypanosome infected rat blood.

Experimental Design: The thirty rats were randomly divided into five groups (A, B, C, D and E) of six rats each. Each group was kept in a separate cage and treated as follows;

Group A: Fed with feed supplemented with 0.08 g of *S cerevisiae* per kg of the feed,

Group B: Fed with feed supplemented with 0.12 g of *S cerevisiae* per kg of the feed,

Group C: Fed with feed supplemented with 0.16g of *S cerevisiae* per kg of the feed,

Group D: Fed with feed not supplemented with *S cerevisiae* as positive control.

Group E: No supplementation and no infection as a negative control.

Groups A, B, C and D rats were each infected with 1 $x10^6$ trypanosomes diluated in PBS on day 28 PS. The erythrocytic profile and parasitaemia were determined on day 0 and every other 7^{th} day while deaths (survival interval) were recorded as it occurred.

Blood Sampling and Analysis: About 0.5 ml of blood was collected from the retro bulbar plexus of the median canthus of rats into bijou bottle containing ethylenediamine tetra-acetic acid (EDTA) as anticoagulant after adequate restraint. The blood samples were analysed for the following parameters: packed cell volume (PCV), red blood cell (RBC) count, haemoglobin (Hb) concentration. Hemoglobin concentration was determined by the cyanomethaemoglobin technique. The PCV was determined using the microhaematocrit method while the RBC counts was determined using the improved Neubauer counting chamber [14].

Determination of Level of Parasitaemia, Mean Survival Interval and Body Weight: The parasitaemia was estimated using the method of Herbert and Lumsden [15] while deaths were recorded as it occurred and was used to calculate the survival interval. The body weight was determined using electronic balance (Citizens Scales Inc., USA). The weight of the mouse was measured in grams (g).

Determination of Rectal Temperature: The rectal temperature was determined using a digital clinical thermometer and the values obtained were recorded in degree celsius (°C).

Statistical Analysis: Data generated were presented as mean ± standard error of mean (SEM) and were subjected to one-way analysis of variance (ANOVA). Variant means were separated using the Least Significant Difference (LSD) method using SPSS version 15 for Windows.

RESULTS

The result showed that from day 0 to day 28 post supplementation (PS), there was no significant difference ($p>0.05$) in the mean packed cell volume (PCV) of all groups (Table 1), though the supplemented groups maintained higher PCV level. However, following infection with trypanosomes on day 28 PS, the PCV of group D was significantly ($p<0.05$) lower than other groups by day 35PS. By day 42 and 49 PS, the PCV of groups A, B and C were significantly ($p<0.05$) higher than group D but lower than group E.

Supplementation with *S. cerevisiae* led to significant ($p<0.05$) increase (Table 2) in Hb in groups B and C when compared with groups D and E but not with group A on day 28 PS. The haemoglobin concentrations of groups B

Table 1: Mean weekly Packed cell volume (%) of infected rat groups fed different levels of S. cerevisae in their diet.

	A	B	C	D	E
Days PS	0.08(mg/kg)	0.12(mg/kg)	0.16(mg/kg)	Positive control	Negative control
0	42.83±1.74	43.33±0.76	42.50±1.41	43.17±1.33	42.83±1.28
14	43.83±2.15	42.83±1.11	43.50±1.38	42.83±2.55	42.67±1.67
28	44.50±1.65	43.83±1.49	44.33±1.23	43.33±0.92	42.47±1.65
35	42.17±1.23a	43.50±0.92a	44.17±1.05a	38.17±2.36b	42.50±1.88a
42	39.33±1.12a	40.83±1.70a	39.67±2.40a	32.50±1.09b	44.00±1.71c
49	37.00±2.27a	35.75±3.45a	34.67±2.52a	28.00±3.33b	45.50±1.78c

[a b c] 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 weekly Haemoglobin concentration of T brucei infected rat groups fed different levels of S cerevise in their diet.

	A	B	C	D	E
Days PS	0.08(mg/kg)	0.12(mg/kg)	0.16(mg/kg)	Positive control	Negative control
0	11.83±0.56	11.20±0.55	11.28±0.62	11.75±0.51	11.30±0.63
14	12.00±0.24	11.95±0.75	12.95±0.35	11.95±0.54	12.20±0.87
28	12.21±0.28a	12.73±0.54a	13.38±0.11a	11.62±0.55b	12.67±0.87b
35	12.33±0.65a	13.12±0.62a	12.87±0.50a	10.45±0.81b	12.07±0.20c
42	10.72±0.46a	11.27±0.56a	10.92±0.46a	9.50±0.68b	11.28±0.14a
49	6.95±0.90a	9.750.68b	9.23±0.15b	7.22±0.98a	11.23±0.73c

[a b c] 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 weekly red blood cell count (10^6 cells per ml) of *T brucei* infected rat groups fed different levels of S cerevise in their diet.

	A	B	C	D	E
Days PS	0.08(mg/kg)	0.12(mg/kg)	0.16(mg/kg)	Positive control	Negative control
0	9.46±1.09	10.36±1.25	10.64±0.61	11.04±0.36	10.88±0.71
14	13.93±0.64^{a}	13.26±0.91^{a}	13.91±0.67^{a}	11.20±0.40^{b}	12.36±0.44^{ab}
28	11.63±0.39	12.04±0.43	11.88±0.12	12.55±0.51	12.31±0.40
35	10.87±0.24^{a}	11.61±0.66^{ab}	11.59±0.18^{b}	8.22±0.55^{c}	11.37±0.24^{b}
42	8.64±0.41^{a}	9.27±0.20^{b}	5.85±0.24^{b}	5.38±0.18^{c}	11.39±0.30^{d}
49	6.41±0.52^{a}	6.97±0.39^{a}	7.92±0.25^{b}	4.85±0.74^{c}	11.71±0.36^{d}

[a b c] 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 weekly parasitaemia (10^6 parasites per ml) of *T brucei* infected rat groups fed different levels of *S cerevise* in their diet.

	A	B	C	D
Days PS	0.08(mg/kg)	0.12(mg/kg)	0.16(mg/kg)	Positive control
35	28.98±7.58	42.11±6.64	39.49±7.87	39.98±7.78
42	115.38±29.93^{ab}	36.86±8.78^{a}	62.95±38.43^{a}	198.82±71.22^{b}
49	125.83±44.34^{a}	189.88±31.33^{a}	157.20±31.34^{a}	271.10±35.95^{b}

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

Table 5: Changes in mean body weight (g) of *Trypanosoma brucei* infected rat groups fed different levels of *S cerevise* in their diet.

Days PS	A 0.08 mg/kg	B 0.12 mg/kg	C 0.16 mg/kg	D Positive control	E Negative contrl
0	209.64 ± 9.60	218.72 ± 9.33	217.84 ± 6.14	210.08 ± 9.94	212.48 ± 9.60
14	212.20± 9.16^{a}	221.6 ± 9.57^{a}	226.8 ± 7.89^{a}	212.56 ± 6.89^{b}	213.20 ± 6.94^{b}
28	218.21±8.28	224.34±9.40	228.12±8.96	211.25±10.12	217.99±10.00
35	220.02±9.12	225.64±8.88	231.28±8.24	211.84±9.39	215.80±9.44
42	217.82±7.22^{a}	222.06±10.40^{a}	224.14±9.14^{a}	192.49±6.68^{b}	216.24±10.12^{a}
49	216.24±8.86^{a}	220.34±10.02^{a}	219.98±9.98^{a}	189.27±10.06^{b}	220.12 ± 10.14^{a}

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

Table 6: Mean temperature (^{0}C) of *Trypanosoma brucei* infected rat groups fed different levels of *S cerevise* in their diet.

Days PS	A 0.08 mg/kg	B 0.12 mg/kg	C 0.16 mg/kg	D Positive control	E negative control
0	37.52 ± 0.50	37.46 ± 0.22	37.42 ± 0.32	37.60 ± 0.35	37.32 ± 0.37
14	37.08 ± 0.55	37.70 ± 0.16	38.08 ± 0.53	37.68 ± 0.19	37.36 ± 0.73
28	36.84 ± 0.48	37.24 ± 0.50	37.92 ± 0.40	37.82 ± 0.52	37.66 ± 0.73
35	39.02 ± 0.72^a	38.01 ± 0.38^b	37.62 ± 0.43^b	38.86 ± 0.47^a	37.46 ± 0.63^b
42	37.89± 0.45^a	37.98 ± 0.73^a	38.02 ± 0.34^a	39.22 ± 0.38^b	37.72 ± 0.42^a
49	38.36 ±0.45^a	38.94 ± 0.46^a	38.10 ± 0.35$^{a\,b}$	39.18 ± 0.47^a	37.65 ± 0.38^b

and C were significantly ($p<0.05$) higher than groups A and E and very significantly ($p<0.01$) higher than group D on day 35 PS. On day 42 PS, the Hb of animals in groups A, B, C and E were significantly ($p<0.05$) higher than group D. While on day 49 PS, groups B and C were significantly ($p<0.05$) higher than groups A and D but significantly ($p<0.05$) lower than group E.

The red blood cell (RBC) counts showed that supplementation with probiotics led to significant increase ($p<0.05$) in RBC counts of groups A, B and C when compared with group D but not with group E (Table 3). The infection with trypanosomes on day 28 PS led to significant ($p<0.05$) decrease in RBC of group D when compared with other groups on day 35 PS. Also, on day 42 and 49 PS, the RBC of the supplemented groups were significantly ($p<0.05$) higher than group D but significantly ($p<0.05$) lower than group E.

From table 4, the parasitaemia level did not show any significant ($p>0.05$) difference on day 35 PS, but on day 42PS, groups B and C had significantly ($p<0.05$) lower parasitaemia than group D but not with group A. The supplemented-infected groups (A, B and C) had significantly ($p<0.05$) lower parasitaemia when compared with the unsupplemented-uninfected group on day 49 PS.

The supplementation led to significant ($p<0.05$) increase in weight (Table 5) with group C being significantly higher than groups D and E on day 14 PS prior to infection. Following infection, the body weight of group D (infected unsupplemented) was significantly lower than other groups from day 42 to 49 PS. The infected supplemented groups did not differ significantly with group E.

The supplementation did not affect mean rectal temperature of the supplemented groups prior to infection (Table 6). The infection on day 28 PS, led to significant increase in mean temperature of groups A and D on day 35 PS when compared with other groups. On day 42 PS, group D was significantly ($p<0.05$) higher than other groups while on day 49 PS, groups A, B and D were significantly ($p<0.05$) lower than group E. Whereas group C did not differ with any of the groups.

The mean survival intervals were 55.8±6.82, 62.00±9.02, 74.8±7.91 and 51.80±7.43 days PS for groups A, B, C and D respectively. The mean survival interval of group C was significantly higher than groups A and D but did not differ significantly with group B.

DISCUSSION

The packed cell volume (PCV), red blood cell (RBC) counts and haemoglobin concentration were used to assess anaemia in this experiment. The significant increase in RBC and Hb of the supplemented groups prior to infection is consistent with the findings of Onifade [16] and Gheisari and Kholeghipour [17] that red blood cell values were higher in chickens fed diets containing granular *S. cerevisiae* than in those fed the control diet. The ability of S cereviviae to enhance the red blood values could be attributed to the fact that it act as growth promoters due to their ability to suppress the proliferation and activities of growth depressing microflora and their ability in enhancing absorption of nutrients through the production of digestive enzymes [18].

Following infection with *Trypanosoma brucei brucei* on day 28 PS, the PCV, Hb and – RBC count decreased progressively. This is consistent with other reports that trypanosome infections cause anaemia [19, 20]. The anaemia in trypanosomosis usually starts early during the first wave of parasitaemia [20]. However, anaemia is not directly dependent on the numbers of parasites, but is controlled by host factors, such as cytokines [21].

The supplemented groups maintained higher red blood values through the experimental period. This may be partly due to their relatively lower parasitaemia, since the degree of anaemia in trypanosomosis has been positively correlated with the onset and level of parasitaemia [22]. However, control of parasitaemia and anaemia in mice are not correlated, as they were in bovine trypanosomosis. Anaemia rather than parasitaemia is best correlated with productivity and is

used as the primary indicator of when to treat the infection [23].

The parastaemia of the probiotic supplementated groups which significantly decreased compared with the infected unsupplemented group, could be attributed to the beneficial effect of S cerevisiae in the rats. This may possible due to the fact that probiotics are known to improve immune response [24], enhance nutrient absorption [18] and also act as antioxidants [25]. Probiotics are important in optimum functioning of the immune system through enhancement of neutrophil production and also through protection against free radical damage, enhance immunity by maintaining the functional and structural integrity of important immune cells [26, 27]. The ability of animals to control anaemia and parasitaemia has been used as indicator of trypanotolerance especially in cattle [29].

The supplementation also resulted in increased survival time when compared with the infected untreated group. The increase supports the findings that probiotic supplemented as water or food additive at a certain concentration could significantly increase survival rate and growth of fish [29]. The increased survival time could also be attributed to the fact that probiotics have the ability to suppress the growth and activities of growth depressing microflora and their ability in enhancing absorption of nutrients through the production of digestive enzymes [18].

Also, probiotic treatment significantly increased body weight and decreased feed intake and the mortality rate on broilers [30], survival improvement and increased resistance of shrimps to pathogenic Vibrio through probiotic administration [25, 31]. The ability of the supplemented groups to maintain weight comparable with the uninfected control could be attributed to the fact that probiotics secrete a number of key nutrients crucial to its host's (our body) immune system and metabolism, including B vitamins pantothenic acid, pyridoxine, niacin, folic acid, cobalamin and biotin and crucial antioxidants such as vitamin K [32]. The supplementation did not affect mean rectal temperature of the supplemented groups prior to infection. However, infection on day 28 PS, led to significant increase in mean temperature of group D. The ability of the infected supplemented groups to maintain temperature comparable to uninfected group is indication that probiotics enhance/stabilize the infected rats.

The ability of *S. cerevisiae* to enhance the Hb, PCV and Rbc count, reduced weight loss and increased survival intervals in *T brucei brucei* infected animals is an indication that probiotics could be useful in the management of trypanosomosis.

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