Effect of Dietary Probiotic Supplement on the Haematological Status of Experimental Wistar Rats Infected with *Trypanosoma brucei brucei*

ABSTRACT

Animal African Trypanosomiasis (AAT) is a debilitating protozoan disease of domestic animals caused by *Trypanosoma*spp. which remains a major setback to animal health and the livestock industryin sub-Saharan Africa. The effect of treatment with probiotic on the haematological changes in Wistar rat infected with *Trypanosoma brucei brucei*was investigated. The probiotic strain used was *Lactobacillus acidophilus*manufactured by PURITAN'S PRIDE, INC, Ronkonkoma, NY 11779 USA, with each tablet of 0.5mg containing 100 million live culture colony forming unit (CFU). Twenty-four male wistar rats randomly assigned to six groups were used in the experiment. Groups C to F received 2×10^8 CFU, 5×10^8 CFU, and 8×10^8 CFU of the probiotic daily from day 1 post supplementation (PS) to termination on the 18th day. Group A and B were the negative and positive controls, respectively. On day seven post supplementation (PS), group B to E were challenged intra-peritoneally with approximately 1.0×10^6 trypanosomes. Haematological parameters were monitored post-infection.The study found significant difference in lymphocyte concentration, platelet concentration, white blood cell (WBC), red blood cell (RBC), haemoglobin, and packed cell volume (PCV) among different treatment groups. Group C had the highest lymphocyte concentration(74.00±1.00), while group B had the highest platelet concentration(171.00 ±1.00). Furthermore, Red blood Cell, White blood Cell, haemoglobin, and Packed Cell volume showed higher levels in group A and lowest in group B.The result of this study suggested that oral administration of the probiotics (*Lactobacillus acidophilus)* supplement ameliorated the negative effect of *trypanosoma brucei brucei* infection on the haematology of wistar rat, although there was a high mortality rate and no curable effects.Oral administration of the probiotics (*Lactobacillus acidophilus)* supplement could therefore be incorporated in the management protocol of Animal African Trypanosomiasis (AAT).

Keywords: Dietaryprobiotics,haematologicalstatus, *Trypanosoma bruceibrucei*, wistar rats.

1.0 INTRODUCTION

Among domestic animals in the tsetse belt of Africa, African Animal Trypanosomiasis (AAT) is a prevalent vector-borne disease (1). Parasites of the genus Trypanosoma, which are members of the phylum Sarcomastigophora and the order Kinetoplastida, are the culprits. In AAT, the Trypanosoma species are the causative agents, and the Glossina spp. tsetse fly is the vector for their cyclical transmission. Animals such as goats, sheep, donkeys, and cattle are susceptible to AAT (2). Some of the most noticeable clinical signs of this severe protozoan infection include abortion, neurological problems, edoema in dependent body parts, high intermittent fever, anaemia, and weight loss. Significant productivity losses are known to be caused by this illness (3). Trypanosoma brucei brucei is the salivarian trypanosome that causes African trypanosomiasis, which is also known as sleeping sickness in humans and nagana in animals. Their antigenic diversity, which helps them elude immune clearance, is another well-established trait of these organisms. Parasites rely on the host immune response system to control their early stages of reproduction, which in turn affects the host's defences against the parasites and how sick they become (4).

Reigning as a major livestock disease in Nigeria, trypanosomiasis is mostly impacting small ruminants and has spread into regions that were formerly free of tsetse flies (5). There is evidence that the disease outbreak has spread to many additional communities in Nigeria, in addition to the initial Gboko endemic site (6). Over the last several years, researchers in Nigeria have looked at the prevalence rate of different animal breeds; the results ranged from 8.4 to 15.53% (7).

The devastating disease known as African animal trypanosomiasis (AAT) strikes cattle in sub-Saharan Africa at an alarming rate. According to Ali and Bitew in 2011 (8), it is thought to be the only disease that significantly impacted the formation and economic growth of a large portion of Africa. An annual loss of $1 billion to $1.2 billion is thought to be the primary result of African armyworm (AAT). This sum, however, is dwarfed by the indirect effects of AAT on sub-Saharan African agriculture (9).

Chemotherapy and vector control remain the mainstays of disease management in the absence of safe and effective immunisations. As a result of their harmful side effects, resistance development, and unsuitable delivery modalities in comparison to field settings, current chemotherapeutic regimens are obviously inadequate (10).

To a large extent, the genes that determine the host organism's general health and function are impacted by probiotics, which are good microbes in the digestive tract (11). Because of their well-documented beneficial effects on host health, several strains of bifidobacteria and Lactic Acid Bacteria (LAB) are often used as probiotics (12,13,14). Probiotics can boost immunity, lower blood cholesterol, alleviate lactose intolerance, fight infections, act as antibiotics, inhibit tumour growth, and protect against colon and bladder cancer, according to research (15,16.17.18). Because of their ability to suppress the growth and actions of microbes that hamper development and their potential to promote nutrient absorption by creating digestive enzymes, probiotics have also been utilised as agents that stimulate development in further studies (19).

According to research conducted by Lutgendorff*et al.* (2008)[20] and Majlesi*et al.* (2017) [21], there are certain strains of probiotics that may normalise serum biochemistry and exhibit antioxidant capabilities. Probiotics may be able to supplement many types of traditional chemotherapy, according to several studies [22,23,24,25,26].

A number of conditions unrelated to the digestive system have found relief with the use of probiotics. A significant reduction in Plasmodium levels was seen when probiotics Bifidobacterium species and Lactobacillus species were administered to mice infected with malaria [27]. Eze *et al.* (2012) [28] showed that in rats infected with Trypanosoma brucei, the probiotic Saccharomyces cerevisiae enhanced immunosuppression and reduced parasitaemia. Animal blood profiles may be improved by probiotics, according to many studies, since they increase haemoglobin production [29]. New evidence suggests that macrophages in the peritoneum and spleen may destroy probiotics. These beneficial bacteria stimulate the production of immunoglobulin G by way of cytokines, which in turn activate helper T-lymphocyte cells. In order for the immune system and the mucosa's systemic response to work properly, bacterial cell walls are essential. According to Liebl *et al.* (2009) [30], probiotics may thus affect many immune system pathways.

2.0 MATERIALS AND MATERIALS

2.1 Experimental Location

This study was carried out in animal house of the Department of Animal and Environmental Biology, Rivers State University NkpoluOroworukwo Port Harcourt.

2.2 Experimental Animals and Management Protocol

Two rats infected with *Trypanosoma brucei*brucei were obtained from the Nigerian Institute for *Trypanosomiasis* Research (NITR), Kaduna State, Nigeria. Twenty-four male wistar rats weighing 232g to 293g were obtained from the Department of Animal and Environmental Biology, Rivers State University. The rats were housed in 6 plastic cages under standard conditions (12 hours of light and 12 hours of dark) and was allowed to acclimatise for two weeks prior to the commencement of the experiment. All animals were fed with standard rodent pellet and clean water *ad libitum*, the cages and drinkers were cleaned daily to prevent infection of the animals. All experiments were conducted according to the institutional animal care protocols at the Rivers State University and followed approved guidelines for the ethical treatment of experimental animals.

2.3. Experimental Design

A total of 24 adult male wistar rats were randomly selected into cages and grouped A-F with 4 rats per group (cage). Group A served as the negative control, group B were infected and untreated, group C was infected and treated with probiotic (2×10^8) cfu/ml, group D was infected and treated with probiotic (5×10^8) cfu/ml, group E was infected and treated with probiotic (8×10^8) cfu/ml, group F was uninfected and treated with probiotic (8×10^8) cfu/ml, as indicated below;

Group A: Healthy + untreated (control 1)

Group B: Infected + untreated (control 2)

Group C: Infected + treated with 2×10^8 CFU probiotic (*Lactobacillus acidophilus*)

Group D: Infected + treated with 5×10^8 CFU probiotic (*Lactobacillus acidophilus*)

Group E: Infected + treated with 8×10^8 CFU probiotic (*Lactobacillus acidophilus*)

Group F: Healthy + treated with 8×10^8 CFU probiotic (*Lactobacillus acidophilus*)

2.4 Source and type of Probiotics used

The probiotic, *Lactobaccillus acidophilus* manufactured by PURITAN'S PRIDE, INC, Ronkonkoma, NY 11779 USA was used in the experiment. It was purchased at Care Forte Pharmacy Health Shop, Lagos, Nigeria. Each probiotic tablet (*Lactobacillus acidophilus*) of 0.5mg contained 100 million live culture colony forming unit (CFU).

2.5 Parasite Strain

The parasite *Trypanosoma brucei brucei* was obtained from the Nigeria Institute for Trypanosomiasis Research (NITR), Kaduna State, Nigeria. The Parasite was maintained in the laboratory by serial blood passage into normal albino Rats until required. The parasitized wistar rat were kept in a plastic cage and transported in an air conditioned bus to Rivers State University, Port Harcourt.

2.6 Inoculation

Male wistar albino rat already infected with *trypanosome brucei brucei* was euthanized with chloroform, stunned by cervical dislocation and the thoracic region was opened. Two millilitre of blood sample was acquired via cardiac puncture using syringes and then diluted with 2ml of saline water (ratio 1:1), after which those in groups (B, C, D, E) was inoculated with 0.1mililiters of infected blood containing 1million (10^6*trypanosome brucei brucei* intra-peritoneally). The inoculation of the parasite into the rat was done at the Microbiology Laboratory of Rivers State University Port Harcourt.

2.7 Blood Collection

At the end of exposure period, blood was withdrawn from the experimental animals two (2) hours before euthanization with chloroform, the blood samples were collected via cardiac puncture into a well labelled Ethylenediaminetetraacetic acid (EDTA) bottles which were used for haematological and physiological analysis respectively.

2.8 Supplementation and Treatment

Supplementation with the probiotic strain (*Lactobacillus Acidophilus*) in the groups indicated below started from day 1 and continued for 7 days before the rats were challenged with trypanosomes and till the end of the experiment. The probiotic was given to wister rats in groups B, C, D, E, F as a suspension in 1ml of distilled water administered orally using a 2ml syringe.

2.9 Preparation of Thin and Thick Blood Film

Thin and thick blood films were prepared with the collected blood for viewing. For the thin film preparation, a drop of freshly collected blood from the tail of the wistar rat was placed on a clean slide and with the edge of another clean slide held at an angle of 45˚, the blood was gently smeared and then stained with Giemsa stain. In the thick film, fresh blood was collected with the use of a micropipette on a clean microscope slide and with the aid of edge of he pointed edge of another clean microscope slide, the blood was spread to make a dot of even spread and allowed to dry with necessary protection from damage.

2.10 Determination of Baseline Parasitaemia

Baseline parasitaemia was determined after three days of inoculation of the wistar rat with the parasite. Blood was collected from the tail of the mice from where thin and thick films were prepared on microscope slides for viewing to establish the presence of the parasites in the experimental rat. The identification of parasites was done using morphological description [31]

2.11 Microscopy

The prepared slides were immersed with oil and were viewed under the microscope, model Olympus CX 21 using 40 and 1100 objectives lens. Several fields were examined in each slide and recorded to determine the level of parasitaemia.

2.12 Packed Cell Volume

Packed cell volume or haematocrit is the ratio of total blood volume occupied by erythrocytes and it is usually expressed in percentage. When anti-coagulant blood is separated with centrifuge, the heavier RBC settled at the bottom before the WBC. In the procedure, blood sample was put in a capillary tube for capillary action to occur. The haematocrit tube scaled and placed in a centrifuge which was placed on flat surface. The centrifuge was connected to a power source with the sample in the carrier and the sealed side facing out in symmetry. The graduated rotor was tightened up with the cover plate closed. The power switch was turned on and the speed selected at 10,000rpm. This was allowed to run for five (5) minutes. It was observed at the end of this period that the red blood cells were pushed to the bottom followed by the white blood cells while the platelets rose to the surface. Test haematocrit was read using haematocrit reader. The height of the red blood cells column was measured as a ratio of the total column in percentage and was expressed as the haematocrit.

2.13 Determination of Red Blood Cells

This was done with the use of System Automated Analyser KX-21N collected blood sample was aspirated from a sample rotor valve. About 4µl of blood measured by the sample rotor valve was diluted into 1:500 using 1-99 of diluents. This was brought to the chamber for mixing as diluted sample. 40µl was measured by the sample rotor out of the 1:500 dilution sample and was again diluted with 1:25000 using 1:960ml of diluent. This was later transferred to the red blood cells transducer chamber and aspirated through the aperture. The RBC were then counted using the DC detention method.

2.14 White Blood Cell Count

Haemocytometer which is a microscope slide modified and designed for quick estimate of the number of cells in a sample was used to estimate the number of white blood cells. The value was expressed as value per ml or µl. The procedure for white blood cell count involves cleaning the Haemocytometer chamber and the cover slip with water and ethanol and later dried. The tip of a moistened finger was uses to dampen the raised glass rail. About 10µl of the blood sample was delivered into the gap between the cover slip. The slide was then placed under the microscope and the cell suspension were counted and recorded.

2.15 Haemoglobin Concentration of Whole Blood

The haemometer tube was filled to the level of lowest graduation with hydrochloric acid (HCL) diluted 1:10 with water. Blood was then poured out of a pipette into the hydrochloric acid in the haemometer tube which was placed in the stand so that the scale is made visible. Injection water was used to dilute until colours were the same and the result was read three times after blood was added on the calibration. Haemoglobin reacts with hydrochloric acid to form a pigment called haematin.

2.16Statistical Analysis

Data was subjected to one-way analysis of variance (ANOVA) using SPSS version 20 software. Data were expressed as mean and standard deviation, Duncan multiple test was used to separate means.

3.0 RESULT

3.1 Effect of dietary probiotic supplement on lymphocytes on wistar rats infected with *Trypanosoma brucei brucei*.

The highest level of lymphocytes was seen in Group C (74.00±1.00) when compared with the other treatment groups. Group A had the lowest (61.00±00) lymphocytes concentration when compared with the other groups (Table 1). Statistical analysis showed there was a significant difference in lymphocytes from all the treatment groups studied.

3.2 Effects of dietary probiotic supplement on platelets of the wistar rats

The highest level of platelet concentration (171.00±1.00 $\times10^3$/μl) was seen in group B, infected untreated rats, when compared with the other treatment groups that had lower platelet concentration. Data also showed that Group E, Infected treated rat with probiotic (8 x 10^8) cfu/ml, had the lowest platelet concentration (125.00±1.00 $\times10^3$/μl) as shown in Table 1.There was however a significant difference in platelet concentration of the experimental animals studied.

3.3 Effect of dietary probiotic supplement on red blood cells (RBC) count of the wistar rats

The highest level of RBC count was seen in Group A (4.80±1.00 $\times10^6$ /μl), with Group B recording the least count (3.40±1.00$\times10^6$ /μl), as shown in Table 1. There was a significant difference in red blood cell count of the experimental animals studied.

3.4 Effect of dietary probiotic supplement on white blood cells (WBC) count of the wistar rats.

The highest level of WBC count was seen in group A (7.60±1.00 **(**$\times10^3$ /μl), while group C had the lowest (4.30±1.00**(**$\times10^3$ /μl). The other groups however had concentrations intermediate of the highest and the lowest. Table 1 showed there was a significant difference in WBC in all the experimental animals studied.

Table1 Effect of dietary probiotic supplement on lymphocytes, platelets, red blood cells (RBC), white blood cells (WBC), of the wistar rats.

GROUP	LYMPHOCYTES (%)	PLATELETS ($\times10^3/\mu l$)	RED BLOOD CELLS (RBC) ($\times10^6/\mu l$)	WHITE BLOOD CELLS (WBC) ($\times10^3/\mu l$)
A	61.00±1.00^{e}	140.00±1.00^{d}	4.80±1.00^{a}	7.60±1.00^{a}
B	72.00±1.00^{b}	171.00±1.00^{a}	3.40±1.00^{a}	4.53±0.57^{b}
C	74.00±1.00^{a}	134.00±1.00^{e}	4.30±1.00^{a}	4.30±1.00^{b}
D	69.00±1.00^{c}	161.00±1.00^{b}	4.33±0.57^{a}	5.73±0.57^{b}
E	67.00±1.00^{d}	125.00±1.00^{f}	4.00±1.00^{a}	5.10±1.00^{b}
F	62.00±1.00^{e}	156.00±1.00^{c}	4.40±1.00^{a}	4.40±1.00^{b}
P value	0.000	0.000	0.946	0.005

Significance Level: = p<0.05; numbers with different superscript showed significant difference

<u>Key</u>

A = Healthy untreated rat
B = Infected untreated rat
C = Infected treated rat with probiotic (2×10^8) cfu/ml
D = Infected treated rat with probiotic (5×10^8) cfu/ml
E = Infected treated rat with probiotic (8×10^8) cfu/ml
F = Healthy treated rat with probiotic (8×10^8) cfu/ml

3.5 Effect of dietary probiotic supplement on heamoglobin (Hb) in wistar rats infected with *Trypanosoma brucei brucei*.

The highest level of Hb was seen in group A (13.00±1.00 g/dl), while group B had, 9.20±1.00 representing the lowest concentration of Hb. Group C-F had Hb concentrations lower than the group A and higher than the group B which is the lowest. Table 2 showed there was a significant difference in Hb concentration from all the experimental animals studied.

3.6 Effect of dietary probiotic supplement on packed cell volume (PCV) in wistar rats infected with *Trypanosoma brucei brucei.*

The highest level of PCV was seen in group A (39.00±1.00 %), followed by group F and E, group B, C and D had the lowest PCV concentration of (34.00±1.00%). Table 2 showed there was a significant difference in PCV concentration from the experimental animals studied.

Table 2 Effect of dietary probiotic supplement on heamoglobin and packed cell volume in wistar rats infected with *Trypanosoma brucei brucei*.

GROUP	HEAMOGLOBIN (g/dl)	PACKED CELL VOLUME (PCV) (%)
A	13.00±1.00^{a}	39.00±1.00^{a}
B	9.20±1.00^{c}	34.00±1.00^{a}
C	11.30±1.00^{ab}	34.00±1.00^{a}
D	11.00±1.00^{b}	34.00±1.00^{a}
E	12.00±1.00$^{ab\backslash}$	36.00±1.00^{b}
F	12.30±1.00^{ab}	37.00±1.00^{b}
Pvalue	0.009	0.000

Significance Level: =p<0.05; numbers with different superscript showed significant difference.

<u>Key</u>

A = Healthy untreated rat
B = Infected untreated rat
C = Infected treated rat with probiotic (2x10^{8}) cfu/ml
D = Infected treated rat with probiotic (5 x 10^{8}) cfu/ml
E = Infected treated rat with probiotic (8 x 10^{8}) cfu/ml
F = Healthy treated rat with probiotic (8 x 10^{8}) cfu/ml

4.0 DISCUSSION

4.1 Effect of dietary probiotic supplement on lymphocytes, platelets, red blood cells (RBC), white blood cells (WBC), in wistar rats infected with *Trypanosoma brucei brucei.*

The study showed a reduced blood lymphocyte concentration in the negative control group compared to other groups that were infected with *Trypanosoma brucei brucei*. Group B to E showed elevated lymphocyte concentration when compared to group A and F. The reduction in lymphocytes which is one of the macrophages in infected wister rats is an evidence of immunosuppression of host defence system by the invading parasite. This finding is in accordance with Mabbott *et al.,* 1995 [33] who reported immunosuppression of experimental animal model by *Trypanosoma brucei brucei*.

There was also an increase in platelet in the positive control (group B) when compared with the other treatment groups. Group E had the lowest platelet concentration when compared with the positive control group. *Trypanosoma brucei brucei* infection triggered the increase in platelet in group B, with the other groups that received probiotic showing reduced platelets count. This signified that the probiotic reduced the effect of the parasite as normal range of platelet wasn't exceeded. This conforms to the fact that *Trypanosoma brucei brucei* infection increases platelet concentration and showed that the host organism's response to the evading parasite suppresses the immune system [34].

There was a significant difference in the Red Blood Cell (RBC) of the experimental result. The positive control group had the lowest RBC when compared with the other experimental groups. Despite not having a wide range in the RBC result, it still implies that *Trypanosoma brucei brucei* affected blood circulation and production in the infected groups which is an indication of anaemic condition in the experimental animals. The findings is in agreement with the findings of Thomas *et al.,* (2017) [35] who reported decrease in β-globin chains, which causes excess α globin chains production and results in the destruction of premature red blood cells.

An elevation of white blood cells WBC concentration in group A was noted in the study. The other treatment groups had lower concentration of WBC. The decrease in WBC concentration may be due to the parasite invasion which resulted in an immune response from the host which is a part of host defense system during infection [37]. Administration of probiotic to the experimental animals elicited immune response but not as much as the negative control group which was not infected. The presence of *Trypanosoma brucei brucei* reduced WBC concentration as the host defence system is fighting the invading parasite, the parasite on the other hand is destroying the white blood cells.

4.2 Effect of dietary probiotic supplement on haemoglobin (Hb) and packed cell volume (PCV) in wistar rats infected with *Trypanososma brucei brucei*.

The study noted Group A recording the highest concentration of haemoglobin (Hb) when compared to other experimental groups. This result further validates the effect of *Trypanosoma brucei brucei* in immunosuppression of its host. The administration of probiotic ameliorated the negative effect of *Trypanosoma brucei brucei* in the experimental rats, but is not able to suppress the parasite effect, hence the reduction in immune response that usually leads to anaemia and death.

Group A and F showed elevated pack cell volume (PCV) concentration when compared with the other groups that were infected with *Trypanosoma brucei brucei*. Low PCV concentration experienced in the experimental animals is evident of immunosuppression and the evasive nature of the parasite. Administration of probiotic enhanced immune response to *Trypanosoma brucei brucei* infection and the evasion of host defence system though it was unable to reduce or decrease lysing and destruction of the host antibodies. This have shown that packed cell volume (PCV), red blood cell (RBC), and haemoglobin (Hb) can be used to assess anaemia in trypanosomes infections [38].

5. CONCLUSION

From the results obtained in this study, the supplementation of the probiotics *Lactobacillus acidophilus* ameliorated the negative effect of Animal African Trypanosomiasis in wistar rat since there was an improvement in the haematological indices. This study also showed that the administration of probiotic in higher concentration had a positive effect on the haematological status when compared to the control groups.

Administration of probiotic to infected individuals and domestic animals should be encouraged as this study have shown positive effect of the probiotic supplement in the haematological status. Thus, probiotics may be useful in the management of trypanosomiasis.

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