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Research Article

Effects of the Probiotic *Saccharomyces cerevisiae* on Some Haematological Indices of Female Rats (*Rattus norvegicus*)

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Abstract: This study aimed to investigate the safety of *Saccharomyces cerevisiae* (SC) supplementation as probiotic on haematological indices (HI) of female Albino rats. Twenty female rats of 12-14 weeks old were used for the study. The rats were randomly assigned to four groups designated A, B, C, and D (N=5). Distilled water was administered to group A (control), while groups B, C and D received 0.5, 1.0 and 1.5 g/kg body weight of SC respectively by oral gavage for twenty weeks. One milliliter of blood was collected from each rat through the ophthalmic venous plexus using a micro-capillary pipette into a labelled EDTA sample bottle at 4th, 8th and 20th weeks of treatment. Haematological indices were determined using automated haemo-analyzer. The HI investigated included: packed cell volume, haemoglobin concentration, red blood cells, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total leucocytes count (TLC), lymphocyte, neutrophil, monocyte, basophil, eosinophil and platelets. The results showed that the erythrocytic indices were not altered among the groups ($p>0.05$) throughout the study. There was a significant decrease ($p<0.05$) in monocytes of the treatment groups at 4th week. There was a significant decrease in TLC, monocytes, basophil and eosinophil of the treatment groups at 8th week. There was no significant difference ($p>0.05$) between the treatment groups and the control at 20th week in all the HI investigated. It was concluded that SC supplementation up to 1.5 g/kgbw has no adverse effects on HI of female rats.
Keywords: Haematological indices, *Saccharomyces cerevisiae,* female rats, safety.

Abbreviations: **SC**=*Saccharomyces cerevisiae,* **HI=**Haematological indices, **PCV=** Packed cell volume, **HbC=**Haemoglobin concentration, **RBC=**Red blood cells, **MCV=**Mean corpuscular volume, **MCH=**Mean Corpuscular haemoglobin, **MCHC=**Mean Corpuscular haemoglobin concentration, **TLC=**Total leucocytes count, **WBC=**White blood cells, **Lymph=**Lymphocyte, **Neut=**Neutrophil, **Mono=**Monocyte, **Baso=**Basophil, **Eosin=**Eosinophil, **PLT=**Platelets.

Introduction

Probiotics are live microorganisms that when administered in adequate amounts confer health benefits on the host (Reid *et al*., 2003; Shane, 2008). *Saccharomyces cerevisiae* (SC) is one of the probitics commonly used as feed additives in avian and bovine species to boost eggs production,

growth rate, weight gain, reduction of methane production among other applications (Ezema, 2012; Agbonu *et al*., 2016). They have also been reported to have antiseptic, antioxidant, anti-aging, anti-inflammatory and antidiabetic activities (Kusmiati *et al*., 2007). Some investigators have incriminated SC as a source of opportunistic infections such as pneumonia, peritonitis, vaginitis and fungemia (Munoz *et al*., 2005). Metabolic processes in the body of a living organism result in the production of reactive oxygen species (ROS) that induce oxidative stress which is responsible for many degenerative and and non-degenerative diseases like pulmonary diseases, cardiovascular diseases and Alzheimer's disease (Lodovici and Bigagli, 2011). These disease' conditions alter the haematological profiles of an organism.

Investigation of haematological indices (HI) is a very useful tool of assessing the safety of a biologically active substance in the body of an organism. Haematological studies are important in animals and humans because the blood is the major transport system of the body and both the input and output substances of almost all the body's metabolic processes and any deviations from normal are detectable in the blood profile (Ihedioha *et al*., 2004). An evaluation of the haematological profile usually furnishes vital information on the response of the body to injury, deprivation and/or stress. Such an evaluation is indispensably important in arriving at a diagnosis, making a prognosis, assessment of the efficacy of therapy and toxicity of drugs and chemical substances (Ihedioha *et al*., 2004). Most reports on the effects of SC are on avian, bovine, caprine and ovine species. There is paucity of information on its effects in monogastric animals especially, rats which have the closest physiology to that of human beings. This study therefore, reports the investigation of the effects of SC on haematological indices of rats with a view to ascertaining its safety as food supplement in monogastric animals.

Materials and Method

Location: The study was carried out in University of Nigeria, Nsukka, Enugu State, Nigeria. Nsukka is situated within latitudes 5º50' and 7º00' north and longitude 6º52' and 7º54' east, at an average elevation of approximately 500 metres above sea level. The yearly minimum and maximum temperature range between 21.17 ^{0}C and 29.67 ^{0}C with a mean of 25.42 ^{0}C (Ihedioha *et al*., 2004). The difference between the longest and shortest days in the year is only 48 minutes (FMANR, 1999). There is rainy season from March to October and dry season from November to February with a yearly average rainfall of 119.5 mm; the relative humidity in Nsukka is about 70% during rainy season and falls to about 20 % during the dry season (FMANR, 1999).

Animals

Twenty female rats (Sprague-Dawley Albino strains) used for the study were maintained at the laboratory animal unit of Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The rats were 12-14 weeks of age and were kept in clean cages in a screened animal house with an average of 12:12 hours light and dark cycle. They were acclimatized for two weeks before the commencement of the study. They were fed on standard rat diet composed of 16% crude protein, which was formulated to meet their nutritional requirements (NAS, 1972). Clean drinking water was also provided *ad libitum.* All experimental protocols were performed in tandem with the National Institute of Health Guidelines for the Care and Use of laboratory animals (NIH, 1999b).

Experimental design

The twenty nulliparous female rats used for the study were randomly assigned to 4 groups of five rats each; designated A, B, C and D. Group A served as untreated control and received only distilled water, while groups B, C and D received graded doses of SC at 0.5 grams per kilogram body weight (g/kgbw), 1.0 g/kgbw and 1.5 g/kgbw respectively for 20 weeks. The probiotic was first weighed using a digital weighing balance and dissolved in distilled water. Oral administration of the probiotic was done daily (24 hourly) with the aid of orogastric cannula, and cage side examination was done to detect any overt signs of toxicity, morbidity or mortality.

Probiotic *Saccharomyces cerevisiae* (SC)

The probiotic SC was sourced from B. F. P. Dock Road, Felix tower, United Kingdom. It was weighed according to the varied dosages of 0.5, 1.0 and 1.5 g/kgbw of rats, dissolved in distilled water and administered daily by oral gavage.

Blood Sample Collection and Haematological Procedure

The blood samples for the haematological study were collected between 8:00AM and 9:00AM at each of the experimental periods (4^{th} week, 8^{th} week and 20^{th} week). About 1ml of blood was collected into a clean labeled sample bottle containing 1mg of sodium ethylene diamine tetra acetic acid (EDTA). Collection was via the ophthalmic venous plexus located in the orbital sinus through the median canthi using a micro-capillary pipette (Ihedioha *et al*., 2004). The relevant haematological determinations were performed on the blood samples immediately upon collection using an automated hematology system analyzer (Orphee mythic 18, JAPAN).

Ethics

The housing, handling and welfare of the rats used for the study were in accordance with the ethics and regulation guiding the use of research animals as approved by the University of Nigeria, Nsukka.

Data Analysis

Data generated were subjected to one-way analysis of variance (ANOVA), and variant means were separated by the least significant difference method using Statistical Package for the Social Sciences (SPSS) version 16. Probability less than 0.05 was considered significant. The results were presented as mean (± standard error of mean).

Results

The probiotic dissolved in distilled water to form a cream coloured homogenous solution. At 4^{th} week of treatment, there was a significant reduction ($p<0.05$) in monocytes of the treatment groups (B-2.00 ± 0.00 %, C-2.20 ± 0.20 %, D-2.24 ± 0.25 %) compared with the control group A-3.40 ± 0.51% (Table 1). There was no significant difference ($p>0.05$) between the treatment groups and the control group in the rest of the haematological indices investigated at 4^{th} week.

Table 1. Haematological Indices (Mean ± SEM) of Rats (*Rattus norvegicus*) Supplemented with Graded Levels of Probiotic *Saccharomyces cerevisiae* (4^{th} Week)

Parameters	Graded Levels of *Saccharomyces cerevisiae*			
	Group A (Control)	Group B (0.5 g/kgbw)	Group C (1.0 g/kgbw)	Group D (1.5 g/kgbw)
PCV (%)	43.60 ± 1.50	44.80 ± 0.66	44.2 ± 1.46	43.6 ± 1.12
HbC (g/dL)	15.08 ± 0.50	15.60 ± 0.24	15.30 ± 0.42	14.82 ± 0.34
RBC ($\times10^{12}$)	7.90 ± 0.25	7.84 ± 0.29	7.80 ± 0.41	7.64 ± 0.30
MCV (fL)	55.18 ± 0.61	56.12 ± 1.06	56.62 ± 0.46	56.76 ± 1.17
MCH (pg)	19.26 ± 0.25	19.60 ± 0.30	19.48 ± 0.16	19.48 ± 0.43
MCHC (g/dL)	35.00 ± 0.20	34.52 ± 0.44	34.34 ± 0.25	34.56 ± 0.18
TLC ($\times10^{9}$)	9.28 ± 0.80	8.58 ± 0.89	10.62 ± 0.20	10.74 ± 0.98
Lymph (%)	75.20 ± 2.33	81.00 ± 1.41	80.80 ± 1.50	80.00 ± 2.07
Neut (%)	15.60 ± 1.86	13.00 ± 1.34	13.00 ± 0.89	14.00 ± 1.64
Mono (%)	3.40 ± 0.51^{a}	2.00 ± 0.00^{b}	2.20 ± 0.20^{b}	2.24 ± 0.25^{b}
Baso (%)	3.60 ± 0.45	3.00 ± 0.37	3.00 ± 0.45	3.00 ± 0.45
Eosin (%)	1.40 ± 0.74	1.20 ± 0.37	1.00 ± 0.31	0.60 ± 0.25
PLT ($\times10^{9}$)	491.80 ± 47.39	506.80 ± 25.86	519.60 ±19.21	539.20 ± 44.91
Different superscripts $^{a, b}$ in a row indicate significant differences between the groups ($p<0.05$)				

Table 2 shows the hematological indices at 8^{th} week of treatment. There was an inverse correlation in total leucocyte count (TLC) or white blood cells count (WBC) with increasing graded levels of SC treatment. Group D (1.5 g/kgbw) of SC had a significantly lowest WBC value of 14.42 ± 0.96 $\times10^{9}$

($P<0.05$) when compared with the control group A ($17.48 \pm 2.02 \times 10^9$). There was a significant reduction ($p<0.05$) in monocytes count of the SC treated groups C (2.57 ± 0.18 %) and D (2.49 ± 0.14 %) compared to the control group A (3.92 ± 0.40). Group B monocyte was lower than group A (control) even though there were not significantly different ($p>0.05$) from each other. The basophil and eosinophil levels of SC treatment groups C and D were significantly lower ($p<0.05$) compare to their control groups while group A and B were not significantly different ($p>0.05$) from each other.

Table 2. Haematological Parameters (Mean ± SEM) of Rats (*Rattus norvegicus*) Supplemented with Graded Levels of Probiotic *Saccharomyces cerevisiae* (8th Week)

Parameters	Graded Levels of *Saccharomyces cerevisiae*			
	Group A (Control)	Group B (0.5 g/kgbw)	Group C (1.0 g/kgbw)	Group D (1.5 g/kgbw)
PCV (%)	46.6 ± 0.93	45.58 ± 1.4	46.54 ± 0.88	44.14 ± 0.72
HbC (g/dL)	17.2 ± 0.39	17.12 ± 0.67	17.3 ± 0.39	16.62 ± 0.42
RBC ($\times 10^{12}$)	8.23 ± 0.12	8.03 ± 0.33	8.42 ± 0.18	7.76 ± 0.15
MCV (fL)	56.65 ± 0.98	56.92 ± 1.78	55.32 ± 1.17	56.89 ± 0.41
MCH (pg)	20.91 ± 0.42	21.35 ± 0.65	20.58 ± 0.63	21.43 ± 0.61
MCHC (g/dL)	36.91 ± 0.29	37.52 ± 0.45	37.17 ± 0.39	37.66 ± 0.83
TLC ($\times 10^9$)	17.48 ± 2.02^a	16.12 ± 0.62^a	15.38 ± 0.58^b	14.42 ± 0.96^b
Lymph (%)	85.28 ± 2.29	79.48 ± 3.18	83.90 ± 2.71	77.30 ± 1.90
Neut (%)	10.80 ± 2.47	14.28 ± 2.26	14.26 ± 1.74	16.00 ± 1.15
Mono (%)	3.92 ± 0.40^a	3.33 ± 0.46^{ab}	2.57 ± 0.18^b	2.49 ± 0.14^b
Baso (%)	1.40 ± 0.24^a	0.80 ± 0.20^{ab}	0.40 ± 0.24^b	0.40 ± 0.24^b
Eosin (%)	1.60 ± 0.24^a	1.20 ± 0.37^{ab}	0.60 ± 0.24^b	0.60 ± 0.24^b
PLT ($\times 10^9$)	433.20 ± 8.11	405.60 ±31.84	414.00 ± 39.75	401.80 ± 63.87
Different superscripts [a, ab, b] in a row indicate significant differences between the groups ($p<0.05$)				

The results of the HI investigation at 20th week are as presented in table 3. In all the HI investigated, there was no significant difference ($p>0.05$) between the treatment groups and the control.

Table 3. Haematological Parameters (Mean ± SEM) of Rats (*Rattus norvegicus*) Supplemented with Graded Levels of Probiotic *Saccharomyces cerevisiae* (20th Week)

Parameters	Graded Levels of *Saccharomyces cerevisiae*			
	Group A (Control)	Group B (0.5 g/kgbw)	Group C (1.0 g/kgbw)	Group D (1.5 g/kgbw)
PCV (%)	41.00 ± 0.45	41.2 ± 0.58	42.2 ± 0.37	41.6 ± 0.24
HbC (g/dL)	13.64 ± 0.36	13.64 ± 0.26	13.76 ± 0.16	13.36 ± 0.05
RBC ($\times 10^{12}$)	7.68 ± 0.13	7.6 ± 0.15	7.62 ± 0.13	7.46 ± 0.15
MCV (fL)	53.41 ± 0.43	54.25 ± 0.57	55.474 ± 1.41	55.85 ± 1.12
MCH (pg)	17.75 ± 0.19	17.96 ± 0.30	18.09 ± 0.43	17.94 ± 0.35
MCHC (g/dL)	33.25 ± 0.59	33.10 ± 0.30	32.61 ± 0.22	32.12 ± 0.17
TLC ($\times 10^9$)	10.86 ± 0.66	10.84 ± 0.59	10.22 ± 1.02	8.94 ± 1.29
Lymph (%)	72.20 ± 2.03	71.40 ± 1.91	73.20 ± 1.39	71.60 ± 4.06
Neut (%)	22.20 ± 1.62	22.80 ± 1.59	20.40 ± 1.25	21.80 ± 3.53
Mono (%)	1.60 ± 0.24	2.00 ± 0.45	2.00 ± 0.00	2.00 ± 0.63
Baso (%)	2.60 ± 0.24	3.00 ± 0.32	2.40 ± 0.51	2.80 ± 0.20
Eosin (%)	1.40 ± 0.40	1.00 ± 0.00	0.60 ± 0.24	0.60 ± 0.24
PLT ($\times 10^9$)	596.20 ± 45.79	558.40 ± 37.75	578.80 ± 28.36	538.60 ± 31.55
Different superscripts [a, ab, b] in a row indicate significant differences between the groups ($p<0.05$)				

Discussion

This study was designed to investigate the effects of graded levels of the probiotic *Saccharomyces cerevisiae* supplementation on female rats (*Rattus norvegicus*) haematological indices. Our study demonstrated that there was no alteration in the erythrocytic indices between the control and treatment groups in all the treatment periods. The results of erythrocytic indices are in agreement

with the report of Ihedioha *et al.,* (2004) for Sprague-Dawley rats in Nsukka, Nigeria. This suggested that the probiotic did not have an adverse effect on the RBCs, PCV and HbC up to the dose level of 1.5 g/kgbw used in the study. Onifade *et al.,* (1999) reported that SC had no toxic effect on the HI of rabbits when 3.0 g/kgbw of SC was administered for 56 days. The report of Onifade *et al.,* (1999) agrees with the findings of this study where SC was administered for 20 weeks without any adverse effect in the erythrocytic indices of the rats.

There were slight alterations in total and differential leucocytes count at 4^{th} and 8^{th} weeks of the experimental periods. In this study, we found that there was a significant reduction in the total leukocyte count of the treatment groups C and D compared to the control group A at 8^{th} week which showed that the probiotic SC modulated the development of leukocytosis which is a common occurrence during infectious and stressful conditions. This trend was similarly observed at 20^{th} week even though there was no significant difference between the treatment groups and the control. Activation of leukocytes can take place in the presence of certain factors including advanced end glycation products (Pertynska-Marczewska *et al*., 2004), angiotensin II (Shurtz-Swirski *et al.,* 2004), oxidative stress (Lee *et al*., 2004b) and cytokines in a hyperglycaemic state (Scherberich, 2003). The decrease in the TLC or WBC count seen in the rats administered *SC* could be due to the anti-inflammatory and antioxidant properties of the probiotic.

Monocytosis and eosinophilia usually ensue during inflammatory conditions, parasitic and bacterial infections, collagen vascular diseases such as lupus, vasculitis, or rheumatoid arthritis. In this study, the monocytes and eosinophils had a significant negative association with increasing graded level of the probiotic SC particularly at 4^{th} and 8^{th} weeks. The monocytosis and eosinophilia observed in the control group could be triggered by Particulate matters (PM) present in the wood shavings which served as bedding for comfort, urine and fecal absorption in the rats' cages. Also, the environmental air is naturally polluted with fumes from automobiles and local inhabitants burning fossil fuel for cooking and other energy producing processes. These PM are responsible for oxidative stress and trigger activation of antioxidant defense, inflammation, and toxicity in cells (Li *et al*., 2008).

Oxidative stress induced by PM is responsible for causing many degenerative and non-degenerative diseases like pulmonary diseases, cardiovascular diseases and Alzheimer's disease (Li *et al*., 2008) which can elicit monocytosis and eosinophilia. Though, we did not investigate the presence of these diseases condition in this study, but they are not unlikely because of the animals' exposure to the polluted environment. The low level of monocytes and eosinophils in the SC treatment groups may be an affirmation of the antioxidant and anti-inflammatory property of the probiotic SC which perhaps, modulated the immune system of the rats as compared to the untreated control. This assertion is in conformity with the earlier report of Kusmiati *et al.,* (2007) who reported SC to possess anti-oxidant and anti-inflammatory property.

From the findings of this study, it can be concluded that SC supplementation up to 1.5 g/kgbw in female rats had no adverse effects on their haematological indices. Rather, the immune system of the rats was modulated by the probiotic.

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Study Limitation
This study was limited to the effects of SC on some haematological indices of female rats as mentioned above. Further research is necessary to evaluate the effects of SC on male rats haematology, serum biochemistry and other physiologic parameters of both male and female rats.

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Conflict of Interest: The authors declare that they have no competing interest.

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