Haematological indices of experimental rats fed with rat chow fortified with processed breadfruits.

ABSTRACT

Background: The abundance of nutrients, vitamins, and fiber in breadfruit has been documented in many literature studies that can hardly be exhausted and are consumed majorly by the Igbos and other southern parts of Nigeria to ameliorate hunger.
Aim: This study however aims at determining the effect of feeds fortified with grilled, parboiled, and cooked breadfruit on hematological indices of Wistar rats.
Methods: Hematological parameters were determined using an automated hematology analyzer (Mindray-BC-5300). The parameters analysed include Hemoglobin (HGB), Packed Cell Volume (PCV), Red Blood Cells (RBC), Platelets (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells (WBC), Neutrophils (NEUT), Lymphocytes (LYMPH), Monocytes (MON), Eosinophils (EOS) and Basophils (BAS).
Results: The result from the experiment indicated that all fortified groups showed no significant increase or decrease ($p>0.05$) in their HGB, PCV, RBC, PLT, MCV, MCH and MCHC when compared with their respective controls. The white blood cell, neutrophil and eosinophil concentration of the experimental rats significantly increased ($p<0.05$) compared with the normal control group. A significant decrease ($p<0.05$) was observed in the lymphocyte and basophil concentration. The monocyte levels of the experimental rats showed a non-significant increase or decrease ($p>0.05$) in all the groups with respect to the normal control.
Conclusion: It may however be safe to infer that fortification of feed with breadfruit has no negative effect on the physiological status of the experimental animals as all the hematological parameters remained within range.

Keyword: Breadfruit, Hemoglobin, Cooked, Parboiled, Grilled, Platelets, Lymphocyte.

1.0 INTRODUCTION

The popular food crop known as breadfruit (*Treculia Africana*) has been known to possess enormous potential for domestic and industrial uses and is widely distributed globally. The commonly known breadfruit does not require much exposition to the science community as a lot of literature research has been carried out on it [1,2] and these literature reviews can hardly exhaust the available information on the nutritional and health benefits of *Treculia Africana*.
Breadfruit is an important source of protein and amino acid that is needed for metabolic well-being of humans. Roasted breadfruit shows significant high levels of sodium, copper, magnesium and phosphorus [3]. It has been reported by Umezuruike *et.al.* [4] that *Treculia Africana* contains Alanine, Isoleucine, lysine, histidine, phenylalanine, glutamic acid, glycine,

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proline, threonine, tyrosine, valine, and methionine. These amino acids are found in concentrations that satisfy the RDA for essential amino acids for infants and adults [5].
This fruit consists of several varieties such as the Africana, inverse, and mollies [6] which basically differ in the size of the tree fruit head, size and color of seeds, rate of fermentation, and ease of seed extraction [1]. in developing countries, breadfruit plays a major role in amelioration of hunger and malnutrition [7,8] as they can be consumed either by Roasting, parboiling, dehulling, steaming or grilling of the extracted breadfruit seeds [4]. Despite the vast nutritional and health benefit of breadfruit, this study tries to investigate the effect of cooked, parboiled, and grilled breadfruit on the hematological parameters of Wistar rats.

2.0 Methods

2.1 Sample Collection and Identification

The breadfruit used for this study was purchased from Orie market, Abagana in Njikoka Local Government Area, Anambra State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of Nnamdi Azikiwe University, Awka is NAUH-77B.

2.2 Processing of Sample

The breadfruit was properly washed and mashed with water to remove its slippery nature and was then dried under room temperature for seven days. After the drying, the breadfruit was shared into three portions for processing.

2.2.1 Cooked Breadfruit

The first portion of the breadfruit was parboiled for 45 minutes in a pot containing only water. The pods were then separated from the chaffs with the help of a corona manual grinding machine. The breadfruit was then cooked using cooking stove for a period of 2 hours until it was soft and edible for consumption. Next, the cooked breadfruit was dried under room temperature and pulverized using corona manual grinding machine, and the now powdered cooked breadfruit was stored inside a well labelled airtight plastic container until use.

2.2.2 Parboiled Breadfruit

The second portion of the breadfruit was parboiled by boiling it inside a pot containing water, on a stove for 45 minutes, till it was partly cooked. The pods were then separated from the chaffs with the help of a corona manual grinding machine, after which it was dried for one week under room temperature. Next, the pods were pulverized using corona manual grinding machine, and the now powdered parboiled breadfruit was stored inside a well labelled airtight plastic container until use.

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2.2.3 Grilled Breadfruit

The third portion of breadfruit was grilled on a frying pan using a stove. The seeds were then separated from the chaffs of the pods and were pulverized using corona manual grinding machine. The now powdered grilled breadfruit was stored inside a well labelled airtight plastic container until use.

2.3 Composition of the Rat Feed

The standard feed used was a product of Novum Agric Industries. It was purchased from a Feed dealer in Awka. The ingredients used in the compounding of the standard feed include grains, cereals, vegetable, protein meals, vitamins, minerals, essential amino acids, anti-toxins, enzymes. The composition of the ingredients is as follows: Oil (6%), Protein (16%), Fibre (7%), Ash (10%), Calcium (0.95%) and Phosphorus (0.65%). The feed was fortified with the processed grilled, parboiled, and cooked breadfruit in the following percentages: Using an analytical weighing balance, the feed and respective breadfruits were each measured. To 70g of feed, 30g of grilled breadfruit was added; to 70g of feed, 30g of parboiled breadfruit was added; to 70g of feed, 30g of cooked breadfruit was added; also, to 50g of feed, 50g of grilled breadfruit was added; to 50g of feed, 50g of parboiled breadfruit was added; and to 50g of feed, 50g of cooked breadfruit was added. These formulations were repeated until enough feed was prepared which lasted for a period of one month.

2.4 Study Design

A total of 35 Wistar rats weighing between 120g to 150g were purchased from Chris Experimental Animal Farm and Research Laboratory, Awka, Anambra State, and randomized into seven groups of five rats each and used for the study. They were maintained and housed in cages under standard environmental conditions (27°C ± 3°C, 12-hour light/dark cycle) in Chris Experimental Animal Farm and Research Laboratory, Awka. The rats were weighed, marked, and put into labeled cages. The rats were grouped as follows:

Group A – Normal Control
Group B – 70% Standard Feed fortified with 30% cooked breadfruit
Group C – 70% Standard Feed fortified with 30% parboiled breadfruit
Group D – 70% Standard Feed fortified with 30% grilled breadfruit
Group E – 50% Standard Feed fortified with 50% cooked breadfruit
Group F – 50% Standard Feed fortified with 50% parboiled breadfruit
Group G – 50% Standard Feed fortified with 50% grilled breadfruit

2.4.1 Feeding of the Experimental Animals

The experimental rats were fed accordingly using the feed prepared for each of the groups. The feeding was done for a period of four weeks after which the rats were fasted and anesthetized with chloroform before blood collection. Blood was collected by cardiac puncture and put in the

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EDTA bottles for hematological analysis. The carcasses were properly disposed of by burying them.

2.5 Hematological Analysis

Hematological parameters were determined using automated hematology analyzer (Mindray BC-5300). The hematological parameters that were analyzed include Hemoglobin (HGB), Packed Cell Volume (PCV), Red Blood Cells (RBC), Platelets (PLT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cells (WBC), Neutrophils (NEUT), Lymphocytes (LYMPH), Monocytes (MON), Eosinophils (EOS), Basophils (BAS).

2.6 Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained were performed by using ANOVA Tests to determine if a significant difference exists between the mean of the test and control groups. The limit of significance was set at $p<0.05$.

3.0 RESULTS

Table 1: Effect of feed fortified with cooked, parboiled, and grilled Breadfruit seeds on some of the hematological parameters (HGB, PCV, RBC, PLT, MCV, MCH and MCHC) on Wistar rats expressed as mean ± SEM.

Groups	**HGB (g/dl)**	**PCV (%)**	**RBC (g/dl)**	**PLT (x10d/L)**	**MCV (fl)**	**MCH (Pg)**	**MCHC (g/dl)**
Normal Control	11.95±0.20	35.78±0.63	6.47±0.12	833.25±46.57	55.28±0.56	18.48±0.85	25.93±7.64
70% Std Feed + 30% cooked Breadfruit	12.40±0.65	38.23±1.44	6.78±0.19	717.25±74.45	56.38±0.97	18.25±0.45	32.40±0.71
70% Std Feed + 30% Parboiled Breadfruit	12.28±0.36	38.20±1.65	6.68±0.28	886.25±143.34	57.28±1.67	18.43±0.39	32.20±0.46

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70% + Std Feed + 30% grilled Breadfruit	11.63±0.24	35.12±0.79	6.22±0.35	727.25±135.70	56.88±1.98	18.83±0.68	33.05±0.06
50% Std Feed + 50% cooked Breadfruit	12.15±0.44	37.53±1.51	6.53±0.11	701.25±36.11	57.50±1.45	18.63±0.43	104.83±72.39
50% Std Feed + 50% parboiled Breadfruit	12.50±0.33	38.30±0.83	6.70±0.30	778.25±74.94	57.30±1.46	18.73±0.38	32.63±0.19
50% Std Feed + 50% grilled Breadfruit	11.65±1.10	35.45±3.90	6.35±0.72	681.75±158.65	55.90±0.58	18.50±0.85	33.10±0.70

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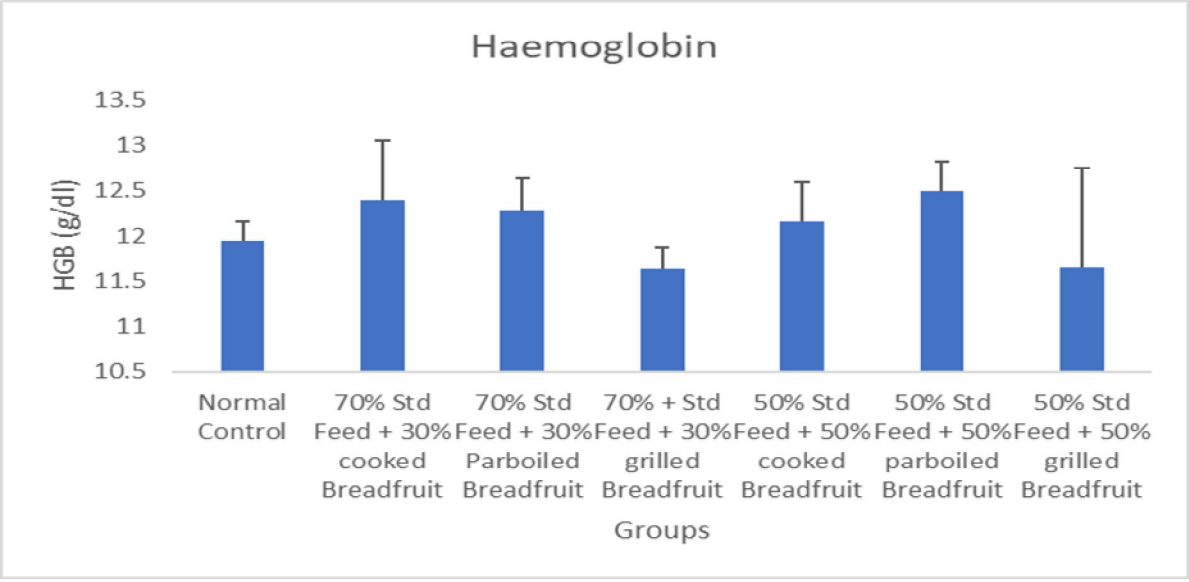

Figure 1: Hemoglobin concentration of normal rats fed with feed fortified with processed breadfruit.

Figure 1 indicates that all fortified groups showed no significant increase or decrease (p>0.05) although these fortified groups showed a slight increase in their haemoglobin concentration when compared to the normal control with the exception to groups fortified with 30% and 50% of grilled breadfruit. The group of rats fortified with 30% and 50% of grilled breadfruit showed a slight decrease (p>0.05) in their haemoglobin concentration with respect to the normal control.

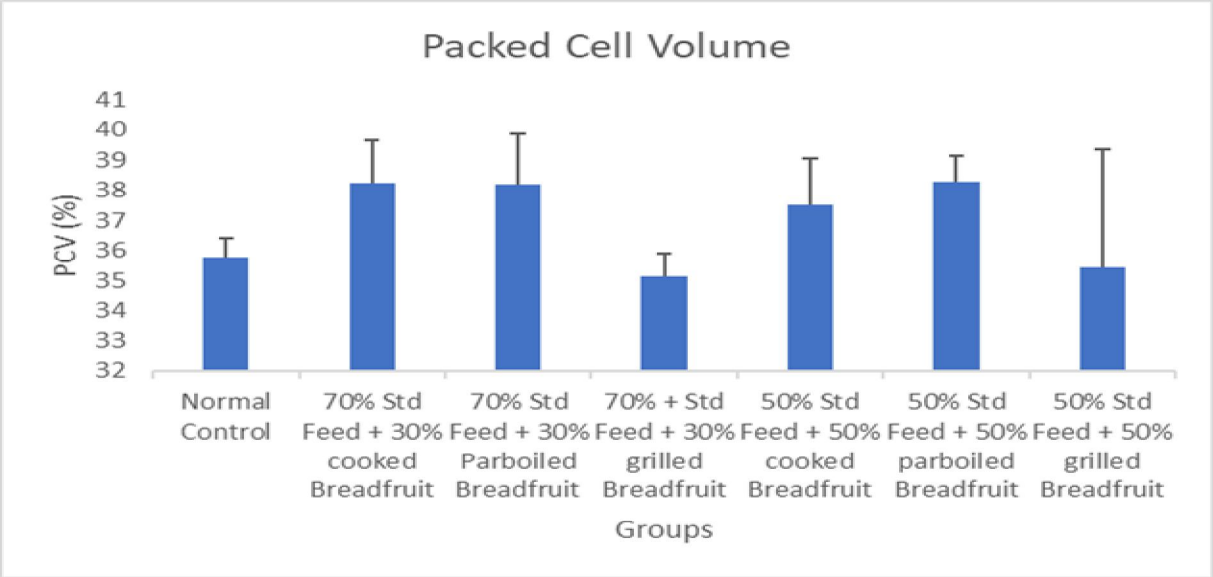

Figure 2: Packed Cell Volume concentration of normal rats fed with feed fortified with processed breadfruit.

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Figure 2 shows that all fortified groups indicated no significant increase or decrease (p>0.05) in their packed cell volume concentration when compared to the normal control with the exception of groups fortified with 30% and 50% of grilled breadfruit. The group of rats fortified with 30% and 50% of grilled breadfruit however showed insignificant decrease (p>0.05) in their packed cell volume concentration with respect to the normal control.

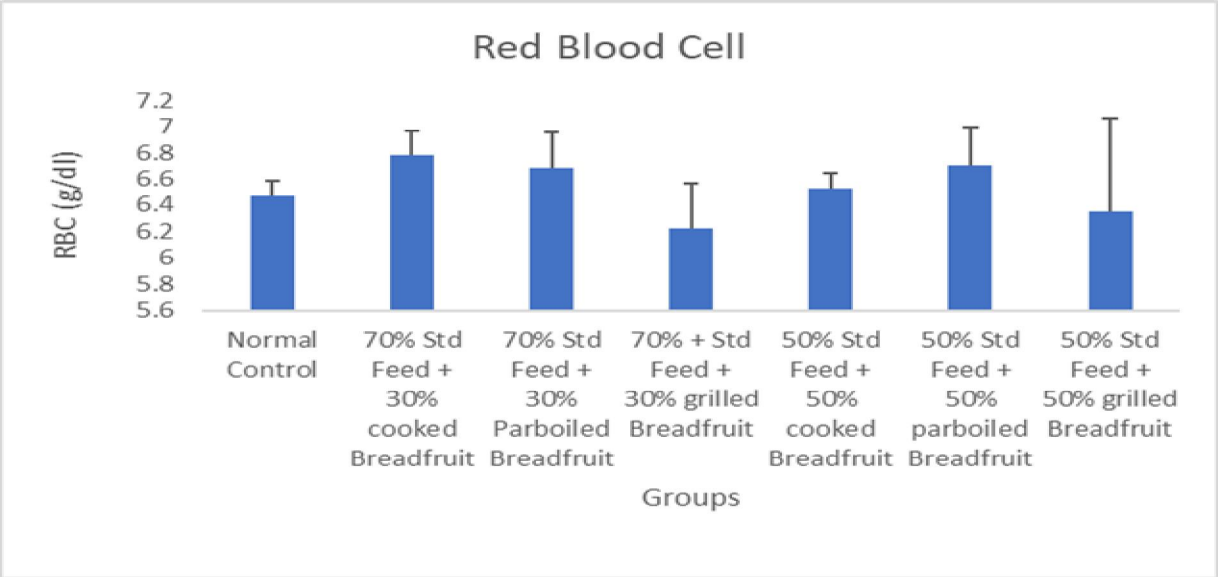

Figure 3: Red Blood Cell concentration of normal rats fed with feed fortified with processed breadfruit.

The red blood cell concentration of all fortified groups as seen in figure 3 showed a slight but insignificant (p>0.05) increase in the red blood cell concentration when compared to the normal control with the exception to groups fortified with 30% and 50% of grilled breadfruit. The groups of rats fortified with 30% and 50% of grilled breadfruit showed a non-statistical decrease (p>0.05) in their red blood cell concentration with respect to the normal control.

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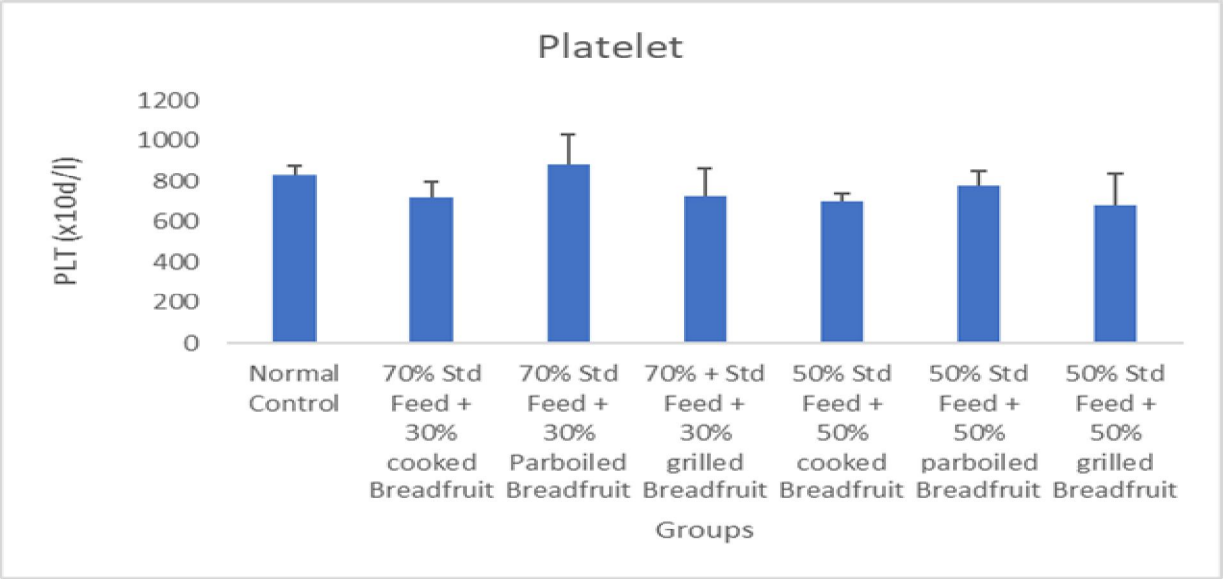

Figure 4: Platelet concentration of normal rats fed with feed fortified with processed breadfruit.

Statistical analysis revealed that only the group of rats fortified with 30% Parboiled Breadfruit indicated a non-significant increase (p>0.05) in its platelet concentration. All other groups were slightly but non statistically decreased (p>0.05) when compared with the normal control (figure 4).

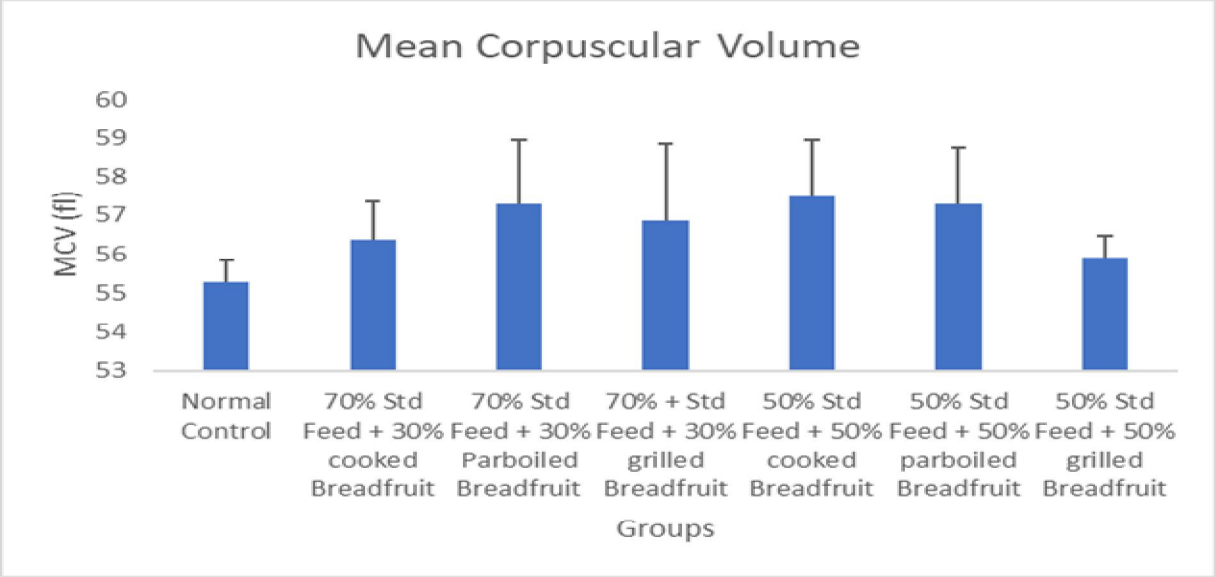

Figure 5: Mean Corpuscular Volume concentration of normal rats fed with feed fortified with processed breadfruit.

The Mean Corpuscular Volume concentration of the rats showed a non-significant increase (p> 0.05) in all groups with respect to the normal control group (figure 5).

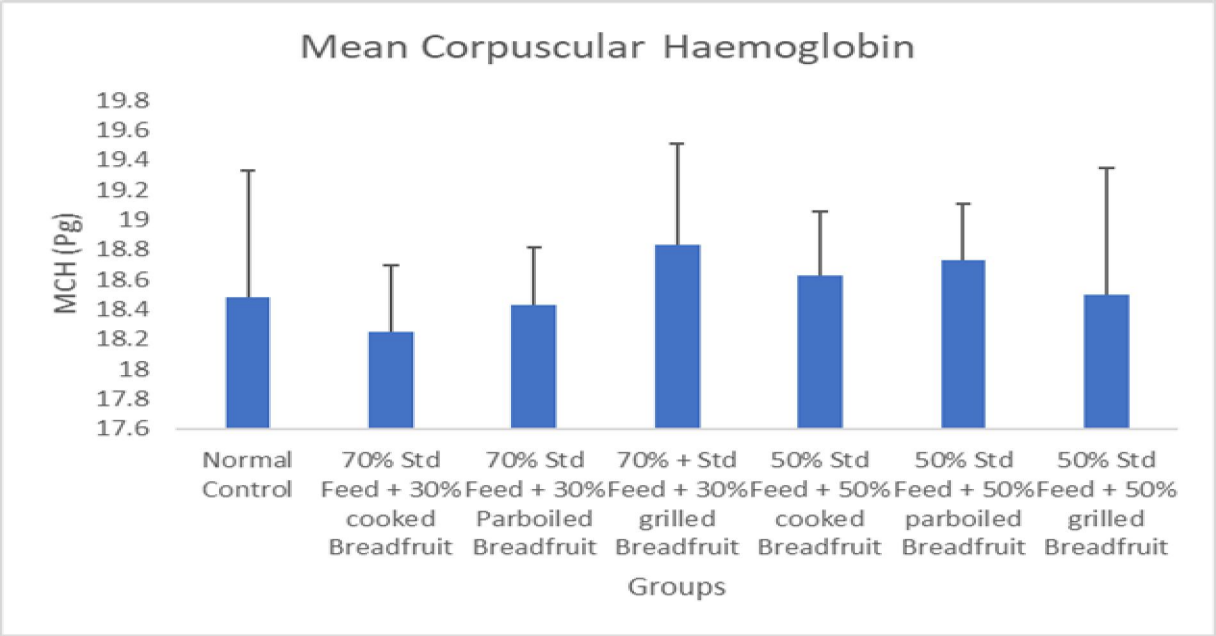

Figure 6: Mean Corpuscular Hemoglobin of normal rats fed with feed fortified with processed breadfruit.

The Mean Corpuscular hemoglobin of the rats showed a non-significant increase ($p > 0.05$) in most groups with respect to the normal control group except for groups fortified with 30% cooked Breadfruit and 30% parboiled Breadfruit which showed a slight but insignificant decrease ($p > 0.05$) when compared with the normal control (figure 6).

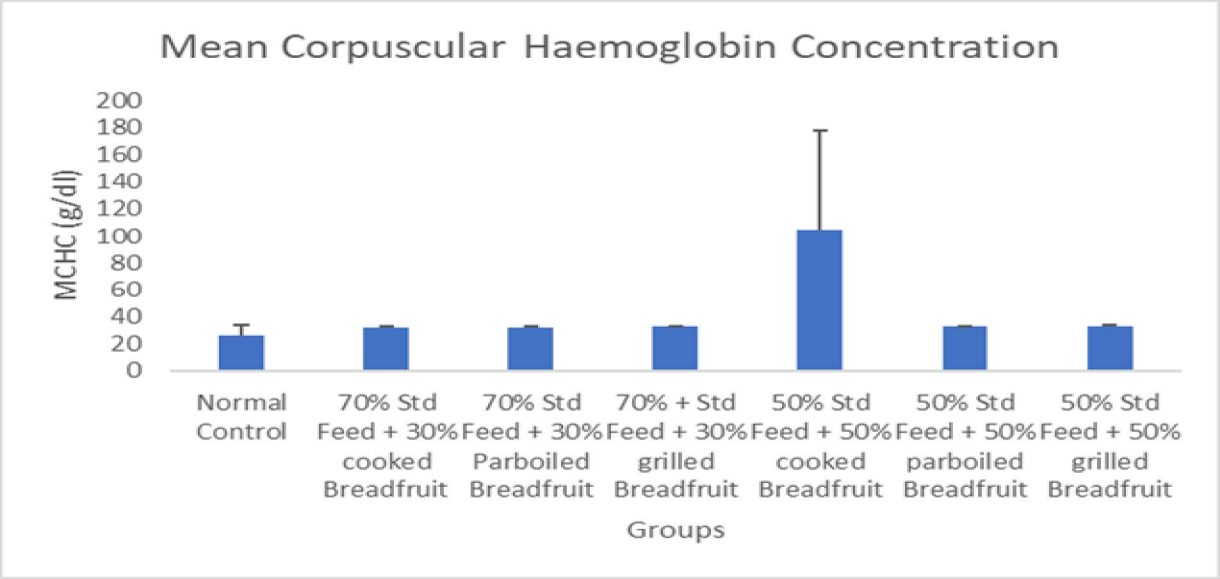

Figure 7: Mean Corpuscular Haemoglobin concentration of normal rats fed with feed fortified with processed breadfruit.

The Mean Corpuscular hemoglobin concentration of the rats showed a non-significant increase ($p > 0.05$) in all groups with respect to the normal control group as seen in figure 7.

Table 2: Effect of feed fortified with cooked, parboiled and grilled Breadfruit seeds on some of the white blood cells and its differentials (Neut, Lymph, Mon, Eos and Bas) on Wistar rats expressed as mean ± SEM.

Groups	**WBC (x10 d/L)**	**NEUT (%)**	**LYMPH (%)**	**MON (%)**	**EOS (%)**	**BAS (%)**
Normal Control	7.40±0.81	22.78±1.97	74.60±2.54	1.78±1.11	1.15±0.43	1.95±1.06
70% Std Feed + 30% cooked Breadfruit	13.48±2.22*a*	38.88±8.12*a*	59.13±8.18*b*	0.88±0.21	3.90±2.17	0.60±0.12
70% Std Feed + 30% Parboiled Breadfruit	14.49±3.14*a*	18.28±1.47	78.30±2.53	2.00±139	0.93±0.36	0.50±0.11*b*
70% + Std Feed + 30% grilled Breadfruit	10.48±1.69	21.50±1.83	76.53±2.12	0.83±0.40	040±0.41	0.75±0.12
50% Std Feed + 50% cooked Breadfruit	15.21±0.97*a*	29.75±4.10	67.88±4.13	0.98±0.10	2.05±1.34	0.70±0.29
50% Std Feed + 50% parboiled Breadfruit	13.18±0.79*a*	28.60±2.38	68.28±2.43	1.35±0.31	1.08±0.21	0.70±0.18
50% Std Feed + 50% grilled	10.04±2.66	26.23±3.06	70.48±2.54	1.58±0.39	0.55±0.24	1.18±0.45

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[a]Significant increase with respect to normal control; [b]Significant decrease with respect to normal control.

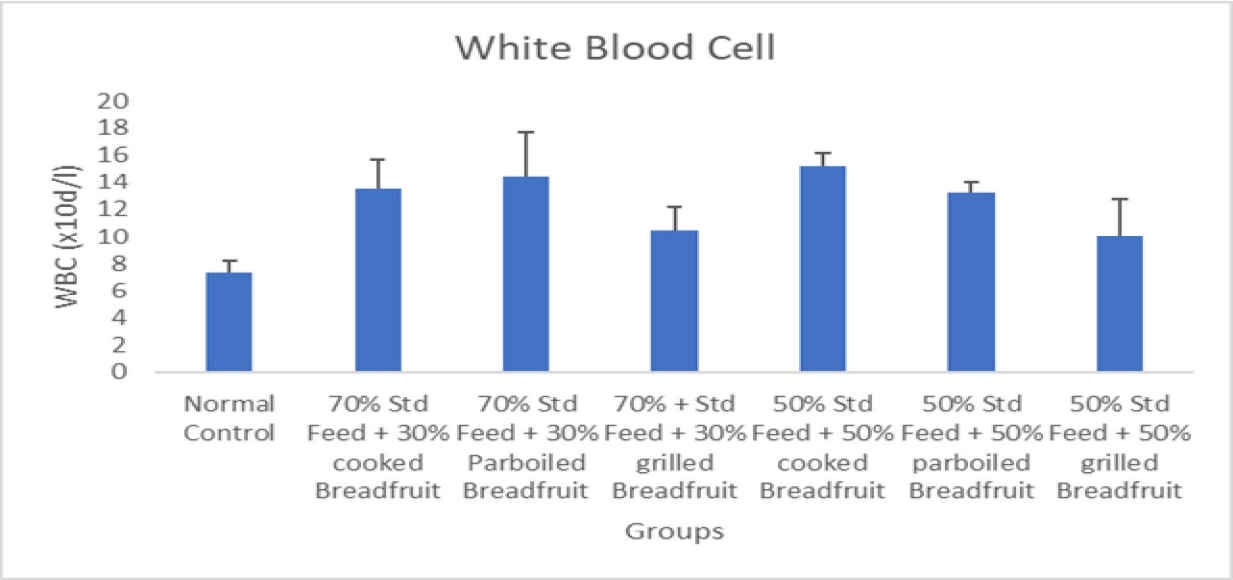

Figure 8: White blood cell concentration of normal rats fed with feed fortified with processed breadfruit.

The white blood cell count of the experimental rats showed significant increase ($p< 0.05$) in all groups except for the groups fortified with 30% and 50% grilled Breadfruit respectively compared with the normal control (figure 8).

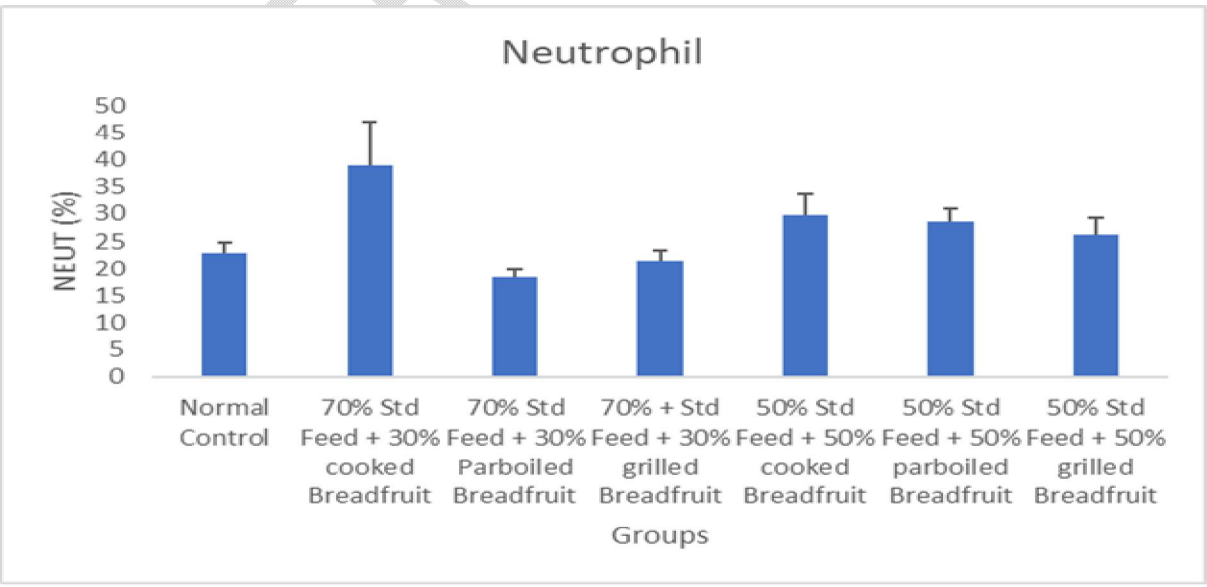

Figure 9: Neutrophil concentration of normal rats fed with feed fortified with processed breadfruit.

Throughout the period of the experiment, only the group fortified with 30% cooked Breadfruit showed a significant increase ($p< 0.05$) with respect to the normal control group (figure 9). The Neutrophil concentration of rats fortified with 30% Parboiled Breadfruit and 30% grilled Breadfruit showed some level of reduction when compared to the normal control, although this reduction was not statistically significant ($p> 0.05$). All the groups that were fortified with 50% of either cooked, parboiled or grilled Breadfruit showed levels of increase in their neutrophil concentration with respect to the normal control group, but this increase was not statistically significant ($p> 0.05$).

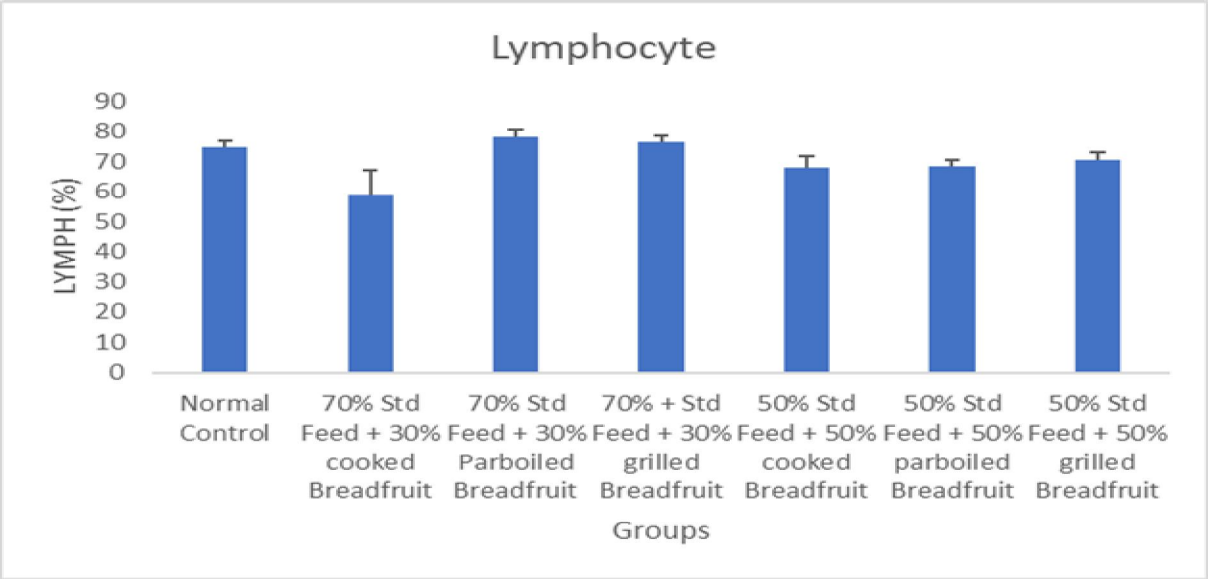

Figure 10: Lymphocyte concentration of normal rats fed with feed fortified with processed breadfruit.

A significant decrease ($p< 0.05$) in the lymphocyte concentration was observed in the group fortified with 30% cooked Breadfruit when compared with the normal control (figure 10). All the groups that were fortified with 50% of either cooked, parboiled or grilled Breadfruit showed levels of decrease in their lymphocyte concentration when compared with the normal control group, but this decrease was not statistically significant ($p> 0.05$). The groups that were fortified with 30% parboiled Breadfruit and 30% grilled Breadfruit both showed an increase in Lymphocyte level, however, this increase was also not statistically significant ($p> 0.05$).

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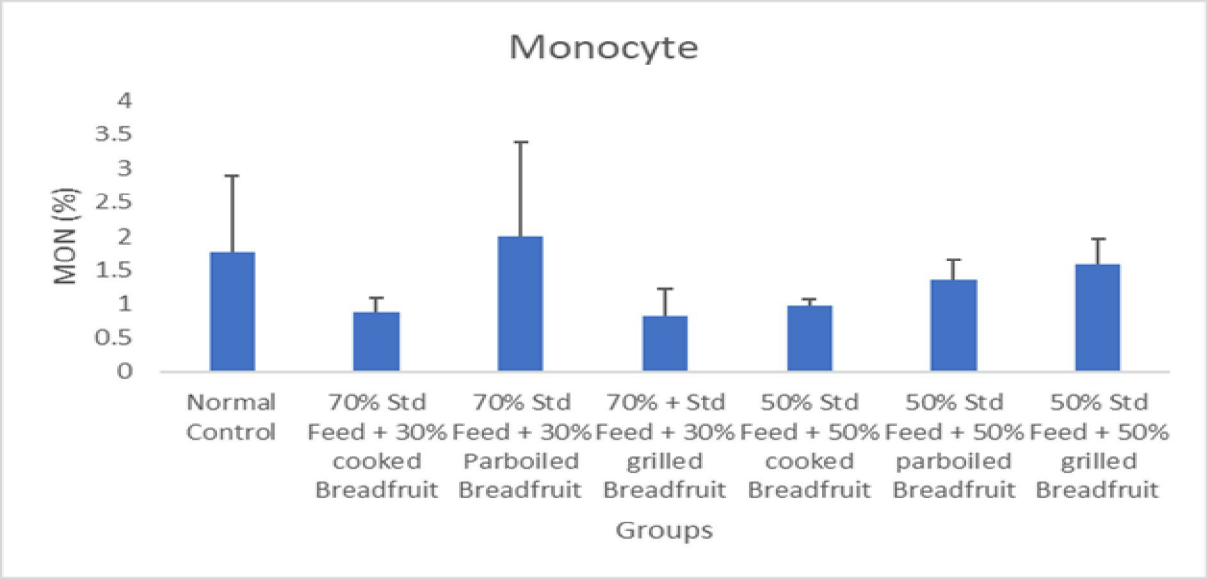

Figure 11: Monocyte concentration of normal rats fed with feed fortified with processed breadfruit.

Figure 11 revealed that the monocyte levels of the experimental rats decreased in all groups with respect to the normal control except for the group of rats fortified with 30% Parboiled Breadfruit which indicated an increase in its monocyte level when compared to the normal control group. Both the increase and decrease observed were not statistically significant ($p > 0.05$).

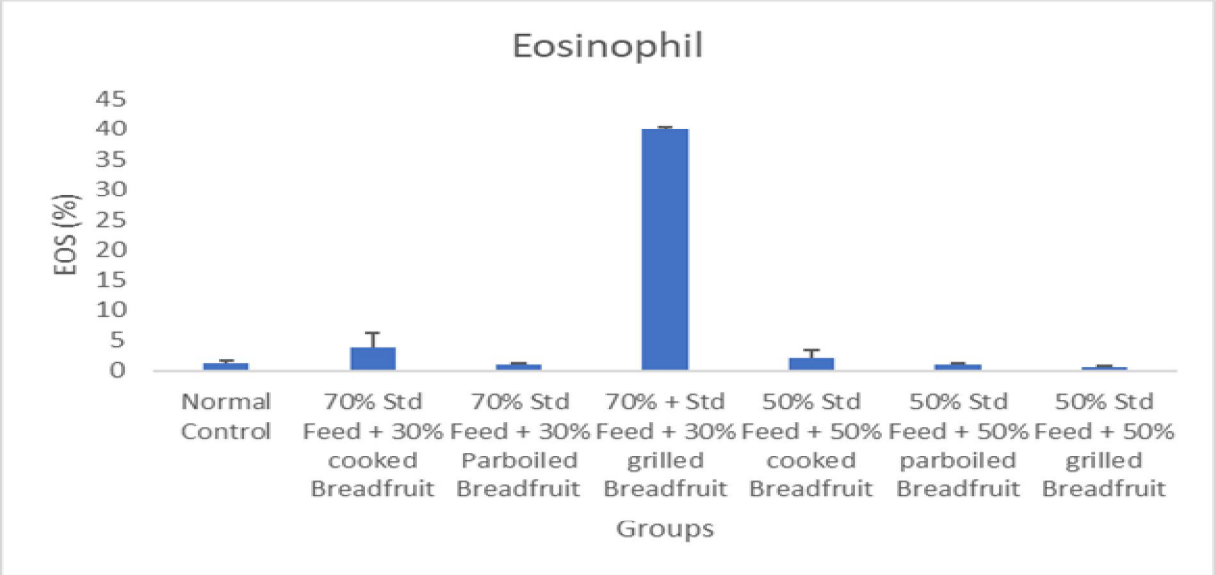

Figure 12: Eosinophil concentration of normal rats fed with feed fortified with processed breadfruit.

From figure 12, an increase in eosinophil concentration was observed in the groups of rats fortified with 50% cooked Breadfruit, 30% cooked Breadfruit and 30% grilled Breadfruit.

However, a significant increase ($p< 0.05$) was only observed in the group fortified with 30% grilled Breadfruit when compared to the normal control.

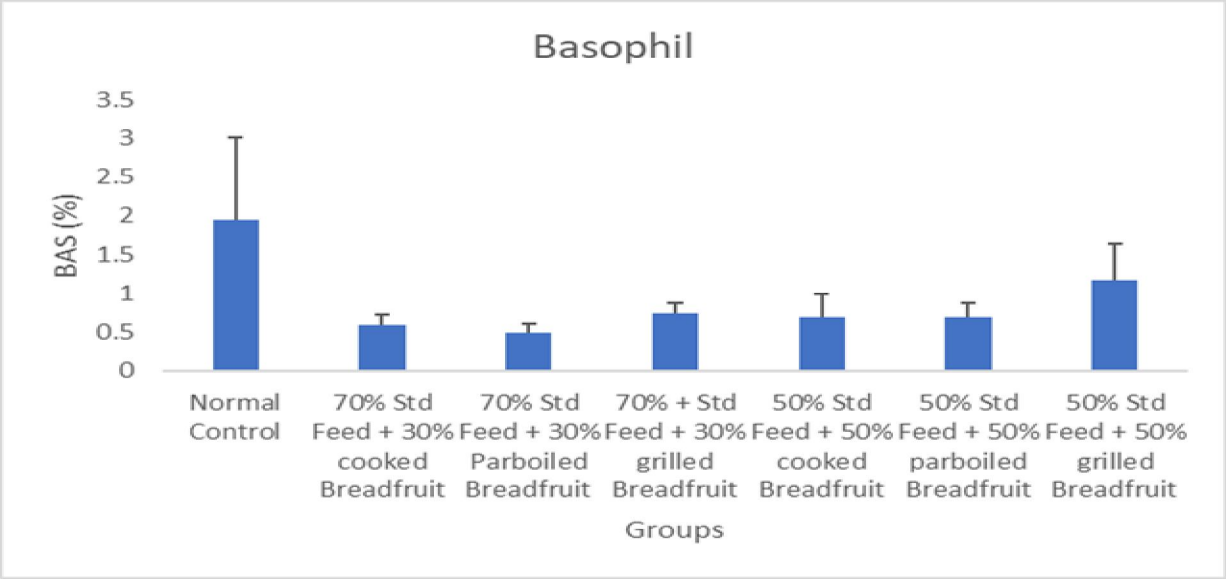

Figure 13: Basophil concentration of normal rats fed with feed fortified with processed breadfruit.

All the groups of experimental rats used showed a decrease in their Basophil concentration, with significant decrease ($p< 0.05$) seen in the group of rats fortified with 30% Parboiled Breadfruit when compared with the normal control group (figure 13).

Discussion

Hematological parameters give an insight into the physiological status of animals [9]. These parameters are of great interest because they help to understand the relation of blood characteristics to the environment and help in the diagnosis and monitoring of diseases [10]. Hemoglobin plays the physiological role of transporting oxygen to tissues of the animal for oxidation of consumed food in order to release the needed energy for the effective functioning of other body parts and also for the transport of carbon dioxide out of the body of animals [11,12,13]. The slight increase in the haemoglobin concentration observed from the result may result in a higher amount of oxygen transported to tissues.

The red blood cell is involved in the transportation of oxygen and carbon dioxide in the body. Thus, a decrease in the red blood cell count indicates a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs [11,14]. The result showed a slight but non-significant decrease in the red blood cell count of feed fortified with 30% and 50% of grilled breadfruit. It could however be safe to infer that the red blood cell count was not affected by the fortification of the feed with processed breadfruit.

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The platelet concentration showed a slight but non statistically decreased (p>0.05) in all groups except in the group of rats fortified with 30% Parboiled Breadfruit when compared with the normal control. This could result in low platelet concentration when consumed over a long period of time as Platelet concentrations are implicated in blood clotting, as research has shown that low platelet concentration indicates that the path to clot-formation (blood clotting) will be prolonged leading to excessive loss of blood in the case of injury [15].

It has been reported that circulatory erythrocytes and diagnosis of anemia are evaluated by some major indices such as Packed Cell Volume, hemoglobin, and mean corpuscular hemoglobin [16]. The packed cell volume concentration of the fortified rats showed a nonsignificant increase (p>0.05) when compared to the normal control with the exception of groups fortified with 30% and 50% of grilled breadfruit. Although the increase was insignificant but may be an indication of either an increase in the number of Red Blood Cells (RBCs) or a reduction in circulating plasma volume as reported by [17].

The Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration indicate blood level conditions and a low level is an indication of anemia [18]. The research result however indicates no negative effect as the slight increase and decrease observed in the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were statistically insignificant. Some medicinal plants are capable of improving the haematological parameters of Wistar rats [19,20,21].

White blood cell and its differentials function majorly as the body's defense against infections through phagocytosis against attacks by foreign organisms and through the production, transportation and distribution of antibodies in immune response [22,14]. The significant increase (p< 0.05) observed in the white blood cell count, neutrophil and eosinophil concentration may indicate higher generation of antibodies and high degree of resistance to diseases which agrees with the findings of Soetan *et al*. [13].
The lymphocyte and basophil concentrations indicated significant decrease (p< 0.05) in groups fed with 70% Standard Feed + 30% cooked Breadfruit and 70% Standard Feed + 30% Parboiled Breadfruit respectively. This reduction may however expose the animal to high risk of disease infection [13].

CONCLUSION

The results of the experiment carried out on the effect of feed fortified with different quantities of processed breadfruit revealed that processing does not have any toxic effect on the haematological parameters of Wistar rats. It may be inferred that the fortification of feed with processed breadfruit has no negative effect on the physiological status of the experimental animals.

ETHICAL APPROVAL

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All experiments were supervised and approved by the Nnamdi Azikiwe University Animal Research Ethics Committee (NAU-AREC) in line with the principles of Animal Care and Use in Research, Education and Testing. The ethical approval number as issued by the NAU-AREC is NAU/AREC/2021/00040.

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