BLOOD PROFILE OF RABBITS FED WITH VARYING LEVELS OF *Phyllanthus amarus* LEAF AQUEOUS EXTRACT

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Abstract

Blood parameters of rabbits were evaluated in a 10-week experimental trial. A total of thirty-six (36) weaned rabbits were randomly allotted to four groups in a completely randomised design. Each group were fed varying levels of *Phyllanthus amarus* aqueous leaf extract at 0, 2, 4, and 6 Mls. Significant differences ($p<0.05$) were observed in some haematological parameters (Packed cell volume, haemoglobin, red blood cell, mean corpuscular haemoglobin, mean corpuscular volume, neutrophils, monocytes, and eosinophils) among the treatments. No significant difference was observed in white blood cell count. Albumin, globulin, creatinine, urea, aspartate aminotransferase, alanine transaminase, and alkaline phosphate were not affected by the administration of *P. amarus* extract, while glucose concentrations differed ($p<0.05$) significantly. For serum electrolytes, Calcium, phosphorus, bicarbonate, and sodium were significantly influenced ($p<0.05$) by the treatment however, chloride concentration was not significantly ($p>0.05$) affected by leaf extract of *P. amarus*. However, these values fall within reference ranges for healthy rabbits. The results indicate that *P. amarus* (aqueous leaf extract) up to 6 mls does not have any negative effect on the haematological and serum indices of weaned rabbits and could be used to maintain their health status and general performance.

Keywords: Rabbit, blood profile, *Phyllanthus amarus*, extract

INTRODUCTION

Blood is a complex fluid containing a large variety of dissolved suspended inorganic and organic substances or specialized circulating tissues and cells suspended in the intercellular fluid substance (Polat *et al.,* 2011). The various functions of blood are made possible by the individual and collective actions of its constituents – the biochemical and haematological components (Akinmutimi, 2004). Generally, blood components are greatly influenced by a variety of factors such as nutrition, age, sex, health status and environmental conditions.

In animal production and physiology, blood serum is used to monitor the health status as well as the effect a diet is having on the animal. In the case of any malfunctioning in the animal system, serum enzymes and other components provide a pointer to the affected part of the

body if there is any cellular damage. This becomes glaring due to an increased permeability that is likely to occur within the damaged cell membrane which may subsequently lead to the release of one enzyme more than another, and other factors such as differently altered rates of excretion may affect the serum components. Changes in these biochemical and haematological values can also be used as indicators of welfare in rabbit production (Yahaya *et al.*, 2023). The level of the serum enzyme in an animal is important for disease diagnosis as these enzymes at a high level in the serum are an indication of tissue or cellular damage. The activities of both Aspartate amino Transferase (AST) and Alanine amino Transferase (ALT) are high in tissues especially the liver, heart, and muscles. Any damage or injury to tissues may cause the release of these enzymes along with other intracellular proteins/enzymes into the circulation leading to their increase in the blood (Garba and Mohammed, 2015). Alanine aminotransferase (ALT) exists in particularly large amounts in the liver where it plays an important metabolic role. Normally, ALT is found inside liver cells, if the liver is inflamed or injured, it is leaked into the blood as a pointer to the liver. Measuring blood levels of serum biochemical indices provides good information on the overall health status of the animal. Varieties of plants with medicinal values have been used in animal production to prevent illnesses, treat diseases and also improving their overall health status. *Phyllanthus amarus* is a medicinal plant used in folkloric medicine for the treatment of several diseases.

Phyllanthus amarus has broad-spectrum antibacterial activity on variety of bacteria (Komuraiah *et al*., 2009). It is known for its antiviral potential thus often administered in the curing process of Hepatitis in humans (Blumberg *et al*., 1990). Research has also shown antioxidant activities showing significant improvement of body antioxidant activities in both insulin and non-insulin-dependent diabetes mellitus animals (Danladi *et al*., 2018). Studies have also shown that the extract of *P. amarus* has an anti-inflammatory effect; preventing persistent neuropathic pain (Kassuya *et al*., 2003). In view of all these benefits, it is vital to establish the safe level of usage of *P. amarus* aqueous leaf extract on the blood profile of grower rabbits.

MATERIALS AND METHODS

Experimental Site

This experiment was carried out at the University of Abuja, Animal Science Research Farm, along Airport Road, Gwagwalada, Abuja, Nigeria. It is along Latitudes 8^0 57^1 and 8^0 551N and Longitudes 7^0 051 and 7^0 061E.

Collection and Processing of *Phyllantus amarus*

The leaves of *Phyllantus amarus* were harvested from within University of Abuja, recognised and validated at the Crop Science Department, University of Abuja. The leaves were cleaned, air-dried and ground into fine powder which was processed for the extraction. 100g of *P. amarus* powder was soaked in 50mls of distilled water for 24 hours kept at 4°C in a refrigerator. The juice was sieved using a filter paper. The resultant extract was stored and preserved until required, Eweka and Enogieru (2011).

Experimental Animal and Management

A total of thirty-six (36) eight to ten weeks old Newzealand rabbits were used for this experiment. The pen, cages, feeders and drinkers were all thoroughly cleaned. The weights of all the rabbits were noted at the point of arrival to obtain their original body weight and thereafter weekly weight gains were taken. The rabbits were randomly allotted to four treatment groups. T_1 (0ml PALE) T_2 (2ml PALE), T_3 (4ml PALE) and T_4 (6ml PALE) in a completely randomized design, each treatment containing 9 rabbits with 3 replicates and 3 rabbits per replicate. Each rabbit was gavaged with its respective treatment throughout the experimental period of 10 weeks (Plate 1). The rabbits were allowed to acclimatize for a period of 1 week before the commencement of the experiment. The rabbits were kept separately in a metabolism cage.



Plate 1: Oral gavaging process



Plate 2: Blood samples collection from the rabbit leg

Haematology trial

Blood samples were collected from the ear vein of 4 rabbits (per treatment) using a 5 ml sterile syringe (Plate 2) into a tube containing ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant. The hemocytometer was used to count the white blood cells (WBC) and red blood cells (RBC). By comparing an acid hematin solution to a standard colored solution contained in the Sahlis hemoglobinometer, the concentration of hemoglobin (Hb) was ascertained. PCV, or packed cell volume, was calculated using the microhaematocrit method. The three metrics mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentrations (MCHC) were calculated.

Serum biochemical trial

Blood was drawn from the same rabbits into plain tubes for serum biochemical analysis. Serum was collected by allowing the blood to clot in the plain bottle and then centrifuge to obtain a clear serum. Using enzyme/buffer and substrate kits in accordance with the Standard Operating Procedure of Alagbe and Omokore, 2019

serum concentrations of triglycerides, cholesterol, glucose, and aspartate aminotransferase (AST), as well as the activity of alkaline phosphatase (ALP), were determined. Total serum protein was determined by refractometer (Doumas and Briggs, 1972). Total serum immunoglobulin concentration was determined by serum zinc sulphate turbidity test using a spectrophotometer. All the blood parameters were analysed at Golden Era Diagnostic Laboratory, Auta-Balefi Nasarawa State.

Statistical analysis

Data collected were subjected to a one-way analysis of variance (ANOVA) based on the Completely Randomized Design model, using Statistical Package for Social Science SPSS (2011) version 20. Duncan's multiple-range test approach (Duncan, 1955) was used to separate significant differences between means.

RESULTS

The blood profile of a weaner rabbit supplemented with *P. amarus* leaf aqueous extract is presented in Table 1. There were significant differences in the parameters among the treatment groups. The packed cell volume (PCV) was significantly higher ($p<0.05$) in rabbits on T4 and the lowest was noted in T1. The results of red blood cell (RBC) and Haemoglobin (Hb) are similar in all treatments except in T3 where they are both higher than the control group. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were significantly higher ($p<0.05$) in T3 and T4. There were no significant differences ($p>0.05$) in MCHC of rabbits in all the treatments. Generally, there were no significant ($p>0.05$) in the white blood cell counts. However, there were differences in its differentials. The percentage of lymphocytes, eosinophils, and monocytes were significantly higher ($p<0.05$) in T3 and T4. The neutrophil percentage was the same in T2, T3, T4 and higher compared with the control.

The serum profile of weaner rabbit-fed graded levels of *P. amarus* leaf extract are presented in Table 2. Albumin was between 2.13 – 2.46 g/dL, glucose (107.40 – 124.86 g/dL), globulin (2.90 – 3.08 mg/dl), creatinine (1.96 – 2.31 mg/dl), urea (3.11 – 3.46 mg/dl), alkaline phosphate (2573 – 26.95 iu/l), aspartate transaminase (62.70 – 68.03 iu/l) and alanine transaminase (44.80 – 48.20 iu/l). Albumin, globulin, urea, creatinine, alanine transaminase, aspartate transaminase and alkaline phosphate were not significantly ($p>0.05$) different among the treatments. However, glucose was significantly ($p<0.05$) higher in rabbits treated with *P. amarus* extract.

Serum electrolytes were significantly ($P<0.05$) influenced by the inclusion of *P. amarus* leaf extract except chlorine which was not significantly ($p>0.05$) affected. Phosphorus varied between 1.65 – 1.90 mmol/l, calcium (2.33 – 2.79 mmol/l), chloride (83.90 - 8679 mmol/l), bicarbonate (22.49 – 24.94 mmol/l) and sodium (119.90 – 130.76 mmol/l) (Table 3).

Table 1: Haematological parameters of weaner rabbit fed *Phyllanthus amarus* leaf aqueous extract.

Parameters	T_1	T_2	T_3	T_4	SEM
PCV (%)	30.02^{c}	31.43^{b}	32.31^{b}	33.68^{a}	0.42
Hb (g/dL)	9.95^{b}	10.58^{ab}	11.29^{a}	10.32^{ab}	0.20
RBC (10^{12}U/L)	11.29^{b}	12.90^{ab}	13.50^{a}	12.58^{ab}	0.34
MCH (pg)	17.51^{b}	20.48^{b}	23.78^{a}	23.82^{a}	0.88
MCHC (g/dL)	33.59	32.23	32.36	32.55	0.49
MCV (fl)	52.75^{b}	54.68^{b}	59.31^{a}	59.83^{a}	1.06
WBC (10^{9}/L)	11.70	11.50	12.01	11.85	0.12
NEU (%)	20.40^{b}	23.21^{a}	23.01^{a}	24.14^{a}	0.51
MONO (%)	2.08^{b}	2.13^{b}	3.00^{a}	3.02^{a}	0.14
EOS (%)	1.11^{b}	1.62^{ab}	1.73^{a}	1.99^{a}	0.12
LYM (%)	62.11^{b}	62.05^{b}	70.47^{a}	71.53^{a}	1.49

^{abc}Means in the same row with different superscripts differ significantly ($P<0.05$) RBC=red blood cell, HB= haemoglobin, PCV= packed cell volume; MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, WBC= white blood cell; NEU= neutrophils; MCHC= mean corpuscular haemoglobin concentration, LYM= lymphocytes, EOS=eosinophils, MONO= monocytes.

Table 2: Serum metabolites of weaner rabbit fed *Phyllanthus amarus* leaf aqueous extract.

Parameters	T_1	T_2	T_3	T_4	SEM
Albumin (g/dl)	2.13	2.35	2.46	2.44	0.04
Globulin (mg/dl)	2.90	3.08	3.01	3.02	0.03
Glucose (mg/dl)	107.40^{b}	121.20^{a}	124.86^{a}	123.66^{a}	2.28
Creatinine (mg/dl)	2.31	1.96	2.04	2.16	0.06
Urea (mg/dl)	3.14	3.46	3.11	3.19	0.08
AST (I.U/L)	62.70	67.29	68.63	67.10	0.70
ALT (I.U/L)	44.82	44.83	46.28	48.20	0.71
ALP (I.U/L)	26.95	26.66	25.73	26.89	0.43

^{abc}Mean values on the same row with different superscripts vary significantly.
AST = aspartate aminotransferase; ALT = alanine transaminase; ALP = alkaline phosphate; SEM = standard error of the mean.

Table 3: Serum electrolytes of weaner rabbit fed graded levels of *Phyllanthus amarus* leaf extract

Parameters	T_1	T_2	T_3	T_4	SEM
Calcium (mmol/l)	2.79[a]	2.63[ab]	2.33[b]	2.51[ab]	0.07
Phosphorus (mmol/l)	1.65[c]	1.75[b]	1.90[a]	1.76[b]	0.02
Bicarbonate(mmol/l)	22.49[b]	24.60[a]	24.94[a]	24.43[a]	0.36
Sodium (mmol/l)	119.90[c]	125.60[b]	130.76[a]	128.70[ab]	1.31
Chloride (mmol/l)	83.90	86.36	86.41	86.79	0.63

[abc]Means on the same raw with different superscripts varies significantly.

DISCUSSION

Haematological parameters of growing rabbit supplemented with *Phyllanthus amarus* leaf aqueous extract.

Packed cell volume (PCV) also known as haematocrit is a measurement of the proportion of Red Blood Cells (RBC) present in the blood and this is expressed in percentage. The percentage of PCV is a pointer to the health status of animals. High or low levels of PCV in animals may indicate several ill-health conditions such as heart, lung or bone marrow disorders. More so, it can also occur as a result of dehydration which can be linked to gastrointestinal stasis. The PCV results obtained from this experiment are within the range of 30% to 50% for growing rabbits (Mathew *et al.,* 2023). Hb and PCV are markers for assessing circulatory RBC and are useful in the diagnosis of anaemia (Alagbe and Adegbite, 2019). Haemoglobin values grew in this experiment with the degree of inclusion of *P. amarus* increased; this suggests that there is adequate circulation of oxygen throughout the system hence better nutrient transport and performance (Isaac *et al*., 2013; Alagbe and Adegbite, 2019). This outcome aligns with the values reported by Mathew *et al.* (2023). The blood indices, MCV, MCH, and MCHC can be used in disease diagnosis since they are good pointer to the conditions of animal's health including the nutritional sufficiency or deficiency in a flock. The MCH and MCHC obtained from this study relate to the report of Amaza *et al.* (2020), who reported similar results for MCH (19.2 - 29.5 pg) and MCHC (27 - 37 g/Dl). The MCV from this study falls within the ranges (50 – 70 fL) reported by Medirabbit, (2023) for rabbits. A low level of MCV is generally associated to low levels of iron, and lead poisoning among others. However, the values recorded in this study (52.75 -59.83 fL) are not at an alarming level and the physical good health condition of the rabbits during the experiment was apparent. Variations in the values of MCV could be ascribed to the differences in diets and the physiological status of the rabbits, Addass *et al.* (2012), Alagbe and Omokore (2019). WBC from this study fell within the standard range of 2.5 to 12.5 as reported by Amaza *et al.* (2020). High levels of WBC indicate that the immune system of animals is been challenged. The proportion of neutrophils and lymphocytes should be at a ratio of 1:1 (Azama *et al.*, 2020), a value higher than

3.0 and lower than 0.7 may be an indication of infection, Zahorec, (2021). Neutrophils and lymphocytes fell within the range of 34 to 70% and 43 to 80% respectively for rabbits (Medirabbit, 2007). Similarly, the monocytes recorded in this study fell within the 2 to 10% reported by Gillet (1994).

Serum metabolites of weaner rabbit supplemented with *Phyllanthus amarus* leaf aqueous extract.

The glucose values obtained in this study were within the range reported Wang *et al* (2020), higher than the values reported by Alagbe and Omokore (2019) but lower than those reported by Esiegwu *et al*. (2013) for rabbits. These differences could be attributed to the subjected environmental conditions. Jenkins (2008) reported that an increase in serum glucose level can be attributed to severe stress conditions. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) values were within the range of reference values reported for rabbits in previous studies (Jurcik *et al*., 2007 and Medirabbit, 2007) this is a clear indication that the integrity of the liver was maintained. AST is an enzyme used in amino acid transamination and its high level can be triggered under serious diseases and morbid conditions. Similarly, ALT is responsible for the synthesis of non-essential amino acids found in the plasma and various bodily tissues (Alagbe and Omokore, 2019). Serum biochemical parameters provide essential information about visceral organ damage, particularly for the liver and the kidneys (Amaza *et al*., 2020).

The concentration of bicarbonate from this study is within the range reported by Medirabbit (2003) indicating that there were adequate but higher than the range of 18.33 to 19.33 mmol/l reported by Amaza *et al.* (2020). Calcium, phosphorus, sodium and chloride from this study are lower than the ranges reported by Yahaya *et al.* (2023). Variations are largely attributed to the composition of the feed and the physiological state of the animal. The sodium levels found in this investigation were in line with those documented by Obikaonu *et al.* (2011). Kidney injury and hypernatremia are avoided when sodium and chloride ions are within normal limits (Alagbe and Adegbite, 2019). The sodium and calcium values reported here are lower than those reported by (Saleh *et al*., 2014), while that of bicarbonates (HCO_3) was lower than the range of values obtained by (Enyenihi *et al*., 2019). A high content of minerals might have caused the elevations of the serum minerals from the experiment conducted by Saleh *et al*. (2014).

Conclusion

The results indicate that *P. amarus* (aqueous leaf extract) up to 6 mls does not have any negative effect on the haematological and serum characteristics of weaner rabbits even though some of the parameters were higher than that of the control, they are still within the values reported in the literature for rabbits. Therefore *P. amarus* could be used in rabbit production for general performance without posing any threat to their health.

REFERENCES

Addass, P. A., David, D., Edward, A., Zira, K.E. and Midak, A. (2012). Effect of age, sex and management system and some haematological parameters of intensively and semi intensively kept chicken in Mubi, Adamawa State, Nigeria. *Iranian Journal of Applied Animal Science*, 2(3):277-282.

Akinmutimi, A.H. (2004). Evaluation of Sword Bean (*Canavalia Gladiate*) as an Alternative Feed Resource for Broiler Chickens. *A Ph.D Thesis, Michael Okpara University of Agriculture, Umudike, Nigeria*, Pp, 35-38.

Alagbe, J. O. and Omokore, E. A. (2019). Effect of replacing soya bean meal with Indigofera zollingeriana leaf meal on the performance and carcass characteristics of growing rabbits. *International Journal of Multidisciplinary Research and Development*, *6*(5), 74-77.

Alagbe, J.O and Adegbite M.B. (2019). Heamatological and serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* bark mixture. *International Journal of Biological, Physical and Chemical Studies*, 4:34-42.

Amaza, I. B., Maidala, A. and Isidahomen, C. E. (2020). Haematological and serum biochemical indices of growing rabbits fed graded levels of yam peel meal as a replacement for maize. *Nigerian Journal of Animal Production*, 47(4):167-175.

Balogun, O. (2001). The Federal Capital Territory of Nigeria: A Geography of Its Development. University of Ibadan Press Limited.

Blumberg, B.S., Miilman, I., Venkateswaran, P. S. and Thyagarajan, S. P. (1990). Hepatitis B virus and primary hepatocellular carcinoma: treatment of HBV carriers with *Phyllanthus amarus*. *Vaccine* (8): S86-S92.

Danladi, S., Idris M.A. and Umar I.I. (2018). Review on pharmacological activities and phytochemical constituents of *Phyllanthus niruri* (Amarus). *Journal of Phytopharmacol*ogy, 7(3):341 -348.

Doumas, B.T. and Briggs, H.G. (1972). Serum albumin by bromocresol green binding. Standard Methods. *Clinical Chemistry*, 7: 171-179.

Duncan, D. B. (1955). Multiple Range and Multiple F- tests; *Biometrics*, 11:1-24.

Enyenihi, G. E., Etuk, I. F. and Inyang, U. A. (2019). Haematological and serum biochemical characteristics of weaner rabbits fed plantain leaf and concentrate. *Nigerian Journal Animal Science*, 21 (3): 281-287.

Esiegwu, A. C., Enyenihi, G. E., Emenahom, O. O., Okoli, I. C. and Udedibie, A. B. I. (2013). Effect of graded dictary levels of Garciniakola seed meal on performance, intestinal microbial load, haematological and serum biochemical profile of rabbits. *Journal of Tropical Agriculture, Food, Environment and Extension*, 12(1): 66-72.

Eweka, A. and Enogieru, A. (2011). Effects of oral administration of *Phyllanthus amarus* leaf extract on the kidneys of adult wistar rats: a

histological study. *African journal of traditional, complementary, and alternative medicines : AJTCAM*, *8*(3), 307–311. https://doi.org/10.4314/ajtcam.v8i3.65294.

Garba, Y. and Mohammed, L (2015). Haematology and Serum Biochemical Profile of Weaner Rabbits Fed Yam Peels at Graded levels as a Replacement for Maize. IJSRSET, Volume (1)5:34-45.

Gillet, C. S. (1994). Selected drug dosages and clinical reference data. In: Manning P.J., Ringler D.H. Newcomer C.E. (Eds.). The Biology of the Laboratory Rabbits (2nd ed). Academic Press, New York, USA, 467-472. doi:10.1016/B978-0-12- 469235-0.50028-2.

Isaac, L. J., Abah, G., Akpan, B. and Ekaette, I.U. (2013). Haematological parameters of different breeds and sexes of rabbits. Proceedings of the 18th Annual Conference of the Animal Science Association of Nigeria (Pages 24-27).

Jenkins, J.R. (2018). Rabbit diagnostic testing. *Journal of Exotic Pets and Medicine*, 17: 4-15.

Jurcik R, Suvegova K, Hanusova E, Massanyi P, Ryban L, Chrenek P. (2007) Evaluation of haematological, biochemical and histopathological parameters of transgenic rabbits. *Journal of Veterinary Medicine and Anatomy*, 54:527-531.

Kassuya, C.A.L., Sylvestre, A.A., Lucia, V.G.R., Calixto, J. B. (2003). Anti-allodynic and antioedematogenic properties of extract and lignans from *Phyllanthus amarus* in model of persistent imflammatory and neuropathic pain. *European Journal of Pharmacology*, 478:145-153.

Komuraiah, A., Bolla, K., Rao, K. N., Ragan, A., Raju, V. S. and Charya, M. A. S. (2009). Antibacterial studies and phytochemical constituents of South Indian Phyllanthus species. *African Journal of Biotechnology,* 8(19):4991-4995.

Marinou K. A., Georgopoulou K., Agrogiannis G., Karatzas T., Iliopoulos D., Papalois A., Chatziioannou A., Magiatis P., Halabalaki M., Tsantila N., Skaltsounis L., Patsouris E. and Dontas I. A. (2010). Differential effect of *Pistacia vera* extracts on experimental atherosclerosis in the rabbit animal model: an experimental study. *Lipids Health Dis.* Vol 9 p 73.

Mathew, B.K., Awoyomi, O.V., Raji, A.M., Olosunde, A. O., Olaniyi, T.A., Popoola, M.A., MatheAwopetu-Adetoro, B.O. and Akanbi, I.O. (2023). Haematology and Serum Electrolyte indices of Hyla Rabbits fed graded levels of Watermelon waste with wheat offal as absorbent. Forum of Nigerian Toxicologists (Fontox), FTX-2023-77.

Medirabbit (2007). Complete blood count and biochemistry reference values in rabbits. www.medirabbit.com/EN/Haematology/blood_chemistry.htm

Obikaonu, H. O., Okoli, I. C., Opara, M. N., Okoro, V. M. O., Ogbuewu, I. P., Etuk, E. B. and Udedibie, B. I. (2011). Haematological and Serum Biochemical Indices of Starter Broilers Fed Neem (*Azadirachta Indica)* Leaf Meal. *Journal of Agriculture and Environmental Science 1(4), 150-54.*

Polat, U., Yesilbag, D. and Eren, M. (2011). Serum Biochemical Profile of Broiler Chickens Fed Diets Containing Rosemary and Rosemary Volatile Oil. *Journal of Biology and Environmental Science*, 5(13):23-30.

Saleh, J. L., Njidda, A. A. Adeniji, A. A. and Lawan, G. B. (2014). Haematological and biochemical indices of rabbits fed graded levels browse forage (*Balanites aegyptiaca*) in semi and environment. Global Journal of Science Frontier Research: D. Agriculture and Veterinary, 14(2): 42-48.

SPSS (2011). Statistical Package for Social Sciences. SPSS Version 20 IBM Inc. 444 Michigan Avenue, Chicago, IL60611, USA.

Wang R, Liu R, and Li L, (2020). Fasting is not required for measuring plasma lipid levels in Yahaya rabbits. Laboratory Animals.54(3):272-280. doi:10.1177/0023677219855102

Yahaya, I., Ndatsu, Y., Yunusa, A., Musa, A. I., Uthman, A., Amina, M., Abdulkarim, H. and Obewa, I. (2023). Proximate, Electrolyte and Biochemical Profile of Rabbits Fed with Tridax procumbens Residue Meal in Replacement of Vitamins and Minerals in Lapai Niger state. *Nigerian Journal of Applied Science and Environmental Management*, 27 (2) 349-354.

Zahorec R. (2021). Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy.;122(7):474-488. doi: 10.4149/BLL_2021_078. PMID: 34161115.