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Nutrient digestibility and blood profile of rabbit fed aflatoxin contaminated diets containing sweet orange (*Citrus sinensis*) peel meal

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Target Audience: *Rabbit Farmers, Animal Nutritionists, Feed Millers*

Abstract

The nutrient digestibility and blood profile of mixed breed grower rabbits fed aflatoxin contaminated diets containing sweet orange (Citrus sinensis) peel meal were evaluated in an 8-week feeding trial. Aspergillus flavus (NRRL 1999) was cultured and used for aflatoxin production in inoculated groundnut cake using the solid state fermentation method. Treated groundnut cake was incubated for seven days with incremental incubation temperature from 20-25^{o}C. The groundnut cake was autoclaved, milled and, aflatoxin extracted from 10 g sample of the milled cake with 50 ml chloroform, and its concentration quantified by TLC. Treated groundnut cake was included at 0, 50, 100 and 150 gram in grower rabbit basal diets containing 5% sweet orange peel to produce diets T1, T2, T3 and T4 having 0 ppb, 50 ppb, 100 ppb, 150 ppb levels of aflatoxin, respectively. Twenty four healthy mixed breed grower rabbits of between six to eight (6-8) weeks old used were randomly allocated to these dietary treatments. Significant ($p<0.05$) decreases occured in the digestibility of nitrogen free extract, ether extract and dry matter for rabbits fed 100ppb aflatoxin contaminated diet whereas, digestibilities of crude protein, and crude fibre were not significantly ($p>0.05$) affected among the treatments. Packed cell volume and mean corpuscular volume, serum albumin, urea, glucose and cholesterol differed significantly ($p<0.05$) but, red blood cell and white blood cell counts, haemoglobin, mean corpuscular haemoglobin, total protein and globulin did not ($p>0.05$) among the treatments. The declining nutrient digestibility, coupled with the deviations of PCV and MCV and, serum albumin, urea, glucose and cholesterol from normal physiological values showed the health risk associated with the feeding aflatoxin contaminated diet. The incorporation of sweet orange peel meal at 5% level in grower rabbit diet cannot mitigate the adverse effect of aflatoxin on nutrient digestibility and health.

Key words: Rabbit, aflatoxin, orange peel, digestibility, blood profile

Description of Problem

The exorbitant prices of livestock feeds in many developing countries including Nigeria and, the shortage of conventional proteins and energy concentrates for the formulation of feeds have geared up a double-pronged approach to overcoming this problem. First, is the search for alternative animal feed resources that are cheap and readily available as protein and energy sources. Second, is the focus on nutritional strategies that can translate these alternative feeds into beneficial use by poultry, pigs and rabbits, which are the animal types of choice in fast-tracking the production of animal protein for human consumption. It has been reported, that one possible solution to the increasing shortage of meat production problem is by using pseudo-ruminant species such as rabbits (1). Rabbit meat is considered an important protein source to human due to

the higher quality and lower fat content.

Aside, the on-going search for cheaper feed resources, improving the nutritional strategies to enhance greater productivity of rabbits fed non-conventional feedstuffs is also tied to efficient nutrient utilization of available feed ingredients by rabbits. The digestibility and more effective utilization of nutrients by rabbits are being threatened by aflatoxin contamination in feeds. Poor growth and feed conversion among other loses attributed to aflatoxin contamination are brought about by poor digestibility and utilization (2). Aflatoxin is known to cause a wide range of metabolic changes in poultry and rabbits associated with reduced digestive enzyme activities, immuno-suppression and liver damage (3). Aflatoxin contaminated feeds decreased the activities of several enzymes important for digestion of proteins, lipid and nucleic acid in broiler chicken. Citrus peel contains essential oils (90% D-Limonene) which are well known antimicrobial agents (4). D-limonene in orange and lemon oil is inhibitory to mold growth and aflatoxin production (5,6). It has been reported that biodegraded sweet orange peel can be used to substitute maize in the diets of rabbits (7,8) The consequences of which will be cost reduction in rabbit feeding and making rabbit meat available at cheaper rate per unit weight and, taking advantage of the presence of D-limonene in the sweet orange peel to possibly handle the presence of aflatoxin in rabbit diets. The objective of this study was to evaluate the potential of sweet orange peels to mitigate the effect of aflatoxin contaminated diets on the nutrient digestibility and blood profile of grower rabbits.

Materials and Methods

Experimental site: The study was conducted at the Rabbitary unit on the Livestock Farm of the Teaching and Research Farm, Federal University of Agriculture, Makurdi, Nigeria. Makurdi is located between latitude $7^{o}44'$ N and longitude $8^{o}21'$ E in the Guinea savanna zone of West Africa (9). The area has an annual rainfall lasting between 6-8 months (March-October) and ranging from 508 to 1016 mm, a minimum temperature range of 24.20 $\pm$1.4 ^{o}C, maximum temperature range of 36.33 $\pm$3.70 ^{o}C and relative humidity between 39.50 $\pm$ 2.20% and 64.00 $\pm$ 4.80% (9).

Production and quantification of Aflatoxin: The fungal culture used was *Aspergillus flavus* (NRRL 1999) for aflatoxin production. Aflatoxin was produced in groundnut cake by employing solid substrate fermentation method. Groundnut cake was inoculated with a spore suspension of *Aspergillus flavus* (NRRL 1999) and the treated groundnut cake was then incubated for 7 days with incremental increases in the incubation temperature from 20 - 25 ^{o}C. The groundnut cake was then autoclaved at 121 ^{o}C for 30 minutes to kill the mold. It was then washed, dried, and ground to fine particles. Aflatoxin was extracted from 10 g sample of the ground cake powder with 50 ml chloroform, and its concentration quantified by Thin Layer Chromatography (TLC) at the Pathology unit, International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria.

Experimental diets

The experimental diets were prepared from the mixture of maize, groundnut cake, full-fat soyabean, sweet orange peel meal, rice offal, brewers' dried grain, bone ash, mineral-vitamin premix and common salt. Treated groundnut cake was included at 0, 50, 100 and 150 gram in grower rabbit basal diets containing 5% sweet orange peel to produce diets T1, T2, T3 and T4 having 0 ppb, 50 ppb, 100 ppb, 150 ppb levels of aflatoxin, respectively (Table 1).

Table 1: Composition of experimental diets

	Diets			
Ingredients	T1	T2	T3	T4
Maize	30.00	30.00	30.00	30.00
Groundnut cake	10.00	10.00	10.00	10.00
Full fat soyabean	15.00	15.00	15.00	15.00
Sweet orange peel	5.00	5.00	5.00	5.00
Rice offal	20.00	20.00	20.00	20.00
Brewers' dried grain	16.00	16.00	16.00	16.00
Bone ash	3.50	3.50	3.50	3.50
Mineral-vitamin premix	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25
Aflatoxin	-	+	++	+++
Total	**100**	**100**	**100**	**100**
Calculated Nutrients				
Crude protein (%)	18.71	18.71	18.71	18.71
Crude fibre (%)	12.26	12.26	12.26	12.26
Ash (%)	6.78	6.78	6.78	6.78
ME (kcal/kg)	2582	2582	2582	2582

Each 1kg of Mineral-vitamin premix manufactured by BEAUTS Co. Inc. Man, U.S.A., contains Vitamin A 220,000, Vitamin D 66,000, Vitamin K 88 mg; Vitamin B12, 0.76 mg, Niacin 1122 mg, Calcium 27%, Phosp-orus 10%, Iron 0.6%, Zinc 0.35%, Manganese 0.25%, Copper 0.06%; Iodine 0.002%, Cobalt 26 ppm, Selenium 4 ppm

- = 0 ppb aflatoxin, + = 50 ppb aflatoxin, ++ = 100 ppb aflatoxin, +++ = 150 ppb aflatoxin

Experimental animals and management

A total of twenty four (24) apparently healthy mixed breed grower rabbits of about six to eight (6-8) weeks old were used for an eight week feeding trial. The cages, feeders and drinkers were properly washed and disinfected using izal seven (7) days before the arrival of the rabbits. On arrival, the rabbits were housed individually in 40 x 60 x 40 cm^3 cages having wire mesh floor 1m above the ground. The feeders were made using empty beverage tins with a 1 cm portion of the opening turned in to prevent feed wastage. Each cage had a feeder and a plastic drinker, both of which were firmly fixed to prevent being tipped over. An adaptation period of seven (7) days was given for the experimental animals to acclimatize to the environment. The rabbits will be grouped into four (4) of similar weight.

Digestibility trial

At the seventh week of the feeding trial, three rabbits, with live weight similar to the treatment average live weight, were selected from each treatment and used for the

digestibility trial according to the European reference method for rabbit digestion trial (10). Faecal collection was done for five (5) days and during this period, the rabbits were fed 90% of their daily feed intake per day. Nylon net was tied under individual cages for daily faecal collection. Prior to the commencement of faecal collection, the rabbits were deprived of feed for 18 hours to ensure that faeces collected correspond to the feed offered. Fresh wet faecal droppings from each replicate were weighed, oven- dried at 75 ^{o}C for 24 hours and re-weighed. At the end of the digestibility study, collected faeces from each replicate were bulked, thoroughly mixed together and ground. Samples of the ground faeces were stored in airtight sample bottles for proximate analysis. The experimental diets and faecal samples from each of the four dietary treatments were analysed for their proximate composition using the Standard methods (11) at the Animal Care Feed Analysis and Quality Control Laboratory, Ogere-Remo, Ogun State, Nigeria. The digestibility coefficient was calculated using the following equation:

Apparent digestibility = [(Nutrient in feed-Nutrient in faeces)/Nutrient in feed] × 100

Blood profile evaluation

Haematological indices determined were red blood cell count (RBC), white blood cell count (WBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). Serum total protein, globulin and blood urea were analysed using Sigma kids, serum glucose was analysed according to the procedure of (12) and serum cholesterol according to (13).

Statistical analysis

All the data generated were subjected to the Analysis of Variance (ANOVA) using (14). The means of significantly different data were separated using Duncan's Multiple Range Test.

Results and Discussion

The coefficient of digestibility of the experimental diets is shown in Table 2. The aflatoxin contaminated diets did not have significant effect ($p>0.05$) on the coefficient of digestibilities of crude protein and crude fibre. The coefficient of digestibility of crude protein was high ranging from 72.02% to 84.91% but, that of crude fibre was low ranging from 31.59% to 51.51%. Coefficient of digestibility of crude fibre in this study was low compared to values reported by other researchers (15,16). Rabbits are hindgut fermenters and, they possess inherent capability to digest fibre. The apparent low crude fibre digestibility obtained suggest that the dietary crude fibre may be high in the neutral detergent fibre (NDF) and acid detergent fibre (ADF) which are fibre fractions partially digestible or more difficult to digest (17), by the activities of the intestinal microflora. There was significant difference ($p<0.05$) observed among treatments for the coefficient of digestibilities of dry matter, ether extract and nitrogen free extract. The coefficient of digestibility for each of the dry matter, ether extract and nitrogen free extract by the rabbits was significantly higher ($p<0.05$) in T1, T2 and T4, suggesting that the digestibility of these nutrients were depressed by aflatoxin contaminated diet T_3 while, the rabbits in the other diet groups were not affected negatively. Thus, digestibility result for all the nutrients seems to show better utilization by rabbits with the exception of those in T_3. The high digestibility of NFE showed that it contained a fairly high readily available carbohydrates which the carbohydrate based enzymes in the stomach were able to act upon.

Table 2: Coefficient of digestibility by rabbits fed sweet orange (C*itrus sinensis*) peel meal in Aflatoxin contaminated diets

	Diets				
Parameters	T_1	T_2	T_3	T_4	SEM
Dry matter	74.27^{ab}	76.57^{a}	60.69^{b}	71.17^{ab}	2.40*
Crude protein	79.93	84.91	72.02	77.54	2.14^{ns}
Ether extract	81.24^{a}	81.31^{a}	55.56^{b}	74.53^{a}	2.15*
Crude fibre	51.51	51.14	31.59	46.08	4.20^{ns}
Nitrogen free extract	77.32^{a}	76.72^{ab}	63.33^{b}	74.42^{ab}	2.13*

^{ab}Means on the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard Error of Mean, *Significant difference ($p<0.05$), ^{ns}Not significantly different ($p>0.05$)
T1 = - (0 ppb aflatoxin), T2 = + (50 ppb aflatoxin), T3 = ++ (100 ppb aflatoxin), T4 = +++ (150 ppb aflatoxin)

Table 3: Haematological indices of rabbits fed sweet orange (C*itrus sinensis*) peel meal in Aflatoxin contaminated diets

	Diets				
Parameters	T_1	T_2	T_3	T_4	SEM
Red blood cell x10^6/µl	7.53	8.00	7.74	7.46	0.25^{ns}
White blood cell x10^3/µl	9.53	9.36	12.46	9.40	0.52^{ns}
Haemoglobin (g/dl)	14.03	10.50	10.63	11.53	2.28^{ns}
Packed cell volume (%)	34.66^{ab}	33.00^{b}	33.63^{ab}	37.10^{a}	0.59*
MCV (fl)	65.76^{a}	39.26^{c}	42.43^{bc}	46.23^{b}	0.56*
MCH (g/dl)	19.56	13.33	13.46	14.53	0.21^{ns}

^{ab}Means on the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard Error of Mean, *Significant difference ($p<0.05$), ^{ns}Not significantly different ($p>0.05$)
T1 = - (0 ppb aflatoxin), T2 = + (50 ppb aflatoxin), T3 = ++ (100 ppb aflatoxin), T4 = +++ (150 ppb aflatoxin)

The haematological parameters of rabbits fed diet containing sweet orange (*Citrus sinensis*) peel in aflatoxin contaminated diets is presented in Table 3. Significant ($p<0.05$) values were obtained for packed cell volume (PCV) and mean corpuscular volume (MCV). The Packed cell volume values of 33.00 - 37.10% obtained in this study were within the normal range of 26.7 - 47.2% and 25 - 45% (18) and (19), respectively for normal rabbits. Non-significant ($p>0.05$) values were obtained for haemoglobin concentration (Hb), white blood cell (WBC), red blood cell (RBC) and mean corpuscular haemoglobin (MCH). Haemoglobin values of 10.5 - 11.5 g/dl recorded in this study was lower than the normal range of 11.6 - 14.0 g/dl for normal rabbits (20). It was observed that rabbits on the SOP in the aflatoxin contaminated diets had lower Hb concentration. This could be an indication of impaired availability of nutrient in the diet that could cause anaemic condition due to the aflatoxin toxicity. It has been reported that decrease in haemoglobin concentration results in normocytic normochromic anaemia in aflatoxin application alone (21). Furthermore, broiler chicks given 2.5 mg aflatoxin per kg diet showed decreased haemoglobin (22). It has been found that even small amount of aflatoxin are dangerous for animal health because of its detrimental effect on haematology (23). The red blood cell counts of 7.53 - 8.00 x 10^6 µl obtained in the study was skewed towards the

upper limit of 3.7 - 7.5 x 10^6 µl (18) for normal rabbit and did not agree with the report of (24) that red blood cell levels were decreased in aflatoxin group compared with the other groups. The effect of experimental diets on white blood cell gave counts which were within the normal range of 5.2 – 16.5 x 10^3 µl (18) but, tended to be high, similar to the trend given by the red blood cell. Some workers (18) reported that increase in white blood cell occurred in aflatoxin contaminated diets as an indication that the toxin elicited an inflammatory response. The MCV for the rabbits on T2, T3 and T4 were significantly lower than the MCV for the rabbits in T1. They varied from 39.26 – 46.23 fl and were lower than the 58.0 – 76.6 fl for normal rabbits (18). The variation in the MCH showed depressed values in the rabbits on sweet orange peel in aflatoxin contaminated diets similar to the pattern observed in MCV. It has been found out that MCV and MCH values were depressed and, increased the sedimentation rate by aflatoxin in broilers (23). Other workers (25) also reported decrease in MCV counts.

Table 4: Serum biochemical indices of rabbits fed sweet orange peel (C*itrus sinensis*) meal in Aflatoxin contaminated diets

			Diets		
Parameters	T1	T2	T3	T4	SEM
Total Protein (g/dl)	5.30	6.10	6.53	5.76	1.89^{ns}
Albumin (mg/dl)	4.63^{ab}	5.06^{a}	4.73^{ab}	4.36^{b}	0.81^{*}
Globulin (mg/dl)	0.67	1.04	1.70	1.40	0.70^{ns}
Urea (mg/dl)	29.93^{c}	40.90^{ab}	40.97^{a}	40.57^{b}	3.50^{*}
Glucose (mg/dl)	65.10^{a}	19.73^{b}	18.43^{b}	12.40^{c}	0.62^{*}
Cholesterol (mg/dl)	73.33^{b}	208.33^{a}	212.67^{a}	201.67^{a}	3.29^{*}

^{ab}Means on the same row with different superscripts are significantly different ($p<0.05$), SEM = Standard Error of Mean, *($p<0.05$), ^{ns}Not significantly different ($p>0.05$)
T1 = - (0 ppb aflatoxin), T2 = + (50 ppb aflatoxin), T3 =++ (100 ppb aflatoxin),
T4 = +++ (150 ppb aflatoxin)

The serum biochemical parameters are presented in Table 4. Feeding rabbits with diets containing sweet orange peel contaminated with aflatoxin had significant effect on ($p<0.05$) on albumin, urea, glucose and cholesterol. The total protein and globulin were not affected significantly ($p>0.05$). The significant effect of the experimental diets on the serum albumin appeared to be more pronounced in the elevated values obtained for rabbits in groups contaminated with aflatoxin which were higher than for the rabbits in the control group. Although the albumin values of 4.36 to 5.06 mg/dl obtained in this study were within the normal reference values of 2.4 to 6.10 mg/dl (20), it has been reported that abnormal high values are indications of liver disease and chronic enteropathy (19). The serum urea nitrogen for the control diet was within the range of 30 - 37.3mg/dl reference value (19) whereas, high levels were observed in other diet groups contaminated by aflatoxin. Blood urea level is influenced by the protein quality and, high urea level in blood is an indication that low protein quality was utilized by the rabbits (26). The result was also in agreement with the findings of (27) and (22). Balanced diets were fed to the experimental rabbits irrespective of their dietary groups. Thus, the high urea nitrogen levels in T2, T3

and T4 showed that the presence of aflatoxin reduced the dietary protein quality. Serum glucose level in rabbits of 65.10 mg/dl was recorded in the control diet ($T_{1)}$ while, the lowest value of 12.04 mg/dl was recorded in T_4. This revealed a significant reduction in serum glucose concentration of experimental animals in diet groups contaminated with aflatoxin. Similar changes have also been reported in other studies (21,22). Aflatoxin contamination in feed has the tendency to induce hypoglycaemia in rabbits (27) a condition caused by low blood glucose level which in extreme cases results in death. The significant increase in the cholesterol level in treatment diets containing aflatoxin gave a variation from 73.33 to 212.67 mg/dl. The rabbits in the control diet had the lowest cholesterol. According to (25), aflatoxin ingestion in diets contributed to inducing the coronary condition that results in ill health when the serum cholesterol is high.

Conclusion and Applications

1. Rabbits fed sweet orange peel based-diets with aflatoxin contamination showed decreased nutrient digestibility.
2. Blood values of packed cell volume, mean corpuscular volume, and the serum values of albumin, urea, glucose and cholesterol differed from normal, which is an indication that there is health risk in feeding aflatoxin contaminated diet.
3. Sweet orange peel at 5% inclusion level in grower rabbit diet cannot be used to mitigate adverse effect of aflatoxin on nutrient digestibility and health.

Nutrient digestibility by rabbits showed a declining trend in the aflatoxin contaminated diets which will cause a reduction in nutrient availability for growth with such feed. The deviations of the packed cell volume (PCV), mean corpuscular volume (MCV), serum albumin, urea, glucose and cholesterol from the normal physiological values showed the health risk associated with aflatoxin contaminated feed. Thus, the incorporation of sweet orange peel at 5% level in grower rabbit diet cannot mitigate the adverse effect of aflatoxin on nutrient digestibility and health.

References

1. A.O.A.C. (2000). Association of Official Analytical Chemists. Official Methods of Analysis, 16th edition. Washington. D.C., USA.
2. Abd El-Mageed, F.A. (1987). Some biological and nutritional studies on aflatoxins. Unpublished M.Sc. Thesis, Faculty of Agriculture, Zagazig University, Egypt.
3. Abdel-Wahhab, M.A, Nada, S.A. and Khalil, F.A. (2002). Physiological and toxicological responses in rat fed aflatoxin-contaminated diet with or without sorbent materials. *Animal Feed Science and Technology*, 97:209-219.
4. Ball, D.M., Hoveland, C.S. and Lacefield, G.D. (2002). Southern forages, potash and phosphate. Institute and Foundation for Agronomic Research. Norcross, GA.
5. Dhanapac, K.S., Rao, S., Govinderaju, P.K.P., Hukkeri, R. and Nathesh, K. (2014). Ameliorative efficacy of citrus fruit oil in aflatoxicosis in broiler : A growth and biochemical study. *Turkish Journal of Veterinary Animal Science*, 38:207-211.
6. Donmez, N., Donmez, H., Keskin, E., and Kisadere, I. (2011). Effect of aflatoxin on some haematological parameters and protective effectiveness of esterified Glucomannan in Merino rams. *The Scientific World Journal Article ID* 342468, 758-762.
7. Eggum, B.O. (1970). Blood urea measurement as a technique for accessing

protein quality. *British Journal of Nutrition*, 24: 983-988.

8. Esper, R.H., Goncales, E., Marques, M.O.M., Felicio, R.C. and Felicio, J. (2014). Potential of essential oil for protection of grain contaminated by aflatoxin produced by *Aspergillus flavus*. *Frontier of Microbiology*, 5:269.
9. Fayed, A.M.A. (1999): Amononiation of the contaminated crop residues with aflatoxins and its effect on rabbits. M.Sc. Thesis, Faculty of Science, Cairo University, Egypt.
10. Hewitt, C.D., Innes, D.J. Savory, J. and Wills, M.R. (1989). Normal Biochemical and haematological values in New Zealand white rabbits. *Clinical Chemistry*, 35(8):1777-1779.
11. Kececi, T., Oguz, H., Kurtoglu, V. and Demet, O. (1998). "Effects of polyvinylpolyprolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis," *British Poultry Science*, 39:452–458.
12. Mahsoub, H. M. M. (2007). Some factors affecting productive traits in V line rabbits raised under Egyptian conditions. M.Sc. Thesis, Faculty of Agriculture, Alexandria University, Egypt.
13. Makun, H.A., Anjorin S.T., Moronfoye, B., Adejo, F.O., Afolabi, O.A., Fagbayibo, G., Balogun, B.O and Surajudeen, A.A. (2010). Fungal and aflatoxin contamination of some human food commodities in Nigeria. *African Journal of Food Science*, 4(4):127-135.
14. Minitab (1991). Minitab Statistical Software. V.10.2. Minitab Inc., P.A., USA.
15. Mitruka, B.M. and Rawnsley, H. (1977). Clinical Biochemistry and Heamatological Reference values in National Experimental Animals. 1st edition, Masson Publishing Inc., New York, USA. Pp. 106-112.
16. Ochefu, J. (2006). The effect of Kapok (*Ceiba pentandra*) seed meal based diets on growth, carcass yield and blood chemistry of weaner rabbits. M.Sc. Thesis, Department of Animal Breeding and Physiology, University of Agriculture, Makurdi. Nigeria.
17. Oguz, H. T., Kecececi, Y.O., Birdane, F., Onder, V. and Kurtoglu, (2000). Effect of clinopilolite on serum biochemical and haematological characters of broilers during aflatoxicosis. *Research of Veterinary Science*, 69:89-93.
18. Ologhobo, A.D., Ewuola, E.O., Jerome, U.U., Franca, U.O. and Ifarajimi, O. (2015). Growth and nutrient digestibility of Broilers fed Aflatoxin contaminated diets with Aflatoxin binders. Journal of Science and Technology, 5:16 -20.
19. Oluremi, O.I.A., Ejeh, E.A. and Winifred, A. (2018a). Evaluation of rumen filtrate for fermentation of sweet orange (*Citrus sinensis*) peel in rabbit feed. *Animal Veterinary. Science*, 6(1):1-5.
20. Oluremi, O.I.A., Gabriel, O.S., Ipirakwagh, E.N., Ikwue, C.O. and Afolabi, E.T. (2018b). Performance and Blood profile of Rabbits fed Biodegraded sweet orange (*Citrus sinensis*) peel based diets. *Nigerian Journal of Animal Science*, 20(3):287-297.
21. Powell, J.B., and Djuh, Y.Y. (1971). A comparison of automated methods for glucose analysis in patients with uremia before and after dialysis. *American Journal of Clinical Pathology*, 56:8.
22. Perez, J.M., Lebas, F., Gidenne, T., Xiccato, G., Parigi-Bini, R., Dalle Zotte, A., Cossu, M.E., Carazzolo, A., Villamide, M.J., Carabano, R., Fraga, M.J., Ramos, M.A., Cervera, C., Blas, E., Fernandez, J., Falcao, E., Cunha, L. and

Bengala Freire, J. (1995). European reference method for *in vivo* determination of diet digestibility in rabbits. *World Rabbit Science*, 3(1):41-43.

23. Roschlan, P., Bernt, E. and Gruber, I.N. (1974). Enzymatische best immung des gesamtcholesterius in serum. *Journal of Clinical Chemistry and Biochemistry*, 12:402-407.
24. Sung, J. (2007). D-Limonene safety and clinical application. *Alternative Medical Review*, 12(3):259-265.
25. TAGEO (2009). An online Database for Geographical Coordinates Information of Nigeria.
26. Talis De, O.S., Luiz, C.K., Leonardo, J., Koao, B., Auren, B.S. and Cleverson, S. (2005). Reference values for Chinchilla (*Chinchilla laniger*) blood cells and serum biochemical parameters. *Ciencia Rur,* 35(3):1-8.
27. Tuzcu, M., E. Sur., I. Ceuk., and Ciftci, M.K. (2010). Effects of Aflatoxin on the Proportions of Peripheral Blood Leukocytes and Alpha-Naphtyl Acetate Esterase (ANAE) Positive Lymphocytes in the Mouse. *Kafkas University Veterinary Fak Derg*, 16(2):337-341.
28. Yousef, M.I., Salem, M.H., Kamel, K.I., Hassan, G.A. and El-Nouty, F.D. (2003). Influence of ascorbic acid supplementation on the haematological and clinical biochemistry parameters of male rabbits exposed to aflatoxin B1. *Journal of Environmental Science and Health*, 38(2):193-209.