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Processed Cocoa (*Theobroma cacao*) Pod Husks in Rabbits Diet: Effect on Haematological and Serum Biochemical Indices

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SAA, JOA and VAA designed the study. Authors SAA, JOA and ODO carried out the feeding trial and performed the statistical analysis. Author SAA wrote the protocol and wrote the first draft of the manuscript. Authors SAA, JOA and ODO managed the analyses of the study. Authors SAA and JOA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to determine the effect of processed Cocoa pod husk on haematological and biochemical indices of rabbits.
Study Design: Completely Randomized Design.
Place and Duration of Study: The experiment was carried out between February and April 2016 at the Teaching and Research Farm, Federal College of Agriculture, Akure, Nigeria. The experimental site lies about 7°25' north and 5°19' east. The average annual temperature and rainfall is 25.3°C and 1455 mm, respectively.
Methodology: Four diets were formulated in which processed cocoa pod husk (PCPH) was included in rabbits' diets at 0, 10, 20 and 30% and designated as diets 1, 2, 3 and 4 respectively.

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One hundred and twenty, 35-day old rabbits of cross-breeds (New-Zealand white X Chinchilla) of equal sexes and average body weight 523.9±43 g were randomly allotted to the 4 dietary treatments (30 rabbits/treatment; 3 rabbits/replicate). On day 56, blood samples were collected from selected rabbits (20 rabbits/treatment) into plain bottles and potassium EDTA bottles for serum biochemical and haematological studies respectively.

Results: Haemoglobin concentration improved (P<0.05) with increased inclusion of PCPH up to 20% level but declined (P<0.05) at 30% dietary level. The red blood cells count and mean cell volume of rabbits fed diets including PCPH at 10, 20 and 30% were higher than the control diet. The albumin, globulin, creatinine, bilirubin and aspertate amino transferase were stable (P>0.05) across the diets. The total protein of rabbits fed the control diet was lower (P<0.05) than those fed the rest diets containing varying levels of PCPH (diets 2, 3 and 4). Cholesterol level of the rabbits reduced (P<0.05) with increase in dietary PCPH. High density lipoprotein increased (P<0.05) at 20 and 30 % dietary PCPH; while the low density lipoprotein decreased (P<0.05) at both 20 and 30% PCPH levels.

Conclusion: Inclusion of PCPH in rabbits' diet up-to 30% level did not affect their haematological and serum biochemical indices.

Keywords: Cocoa pod husks; agro-wastes; blood and serum indices; rabbits; processing methods.

1. INTRODUCTION

The problem of low animal protein intake is a great challenge in developing countries. The average animal protein intake of an average individuals in most African and Pacific countries falls between 8-15 g per day [1], which is far below 65 g per recommended by FAO [2]. This is because of high cost of feed production due to increase in price conventional livestock feed ingredients and consequent rise in cost of animal protein beyond the purchasing power of most people in these regions [3]. Since feed cost ranged between 60 to 70% of total cost of production [4], it is believed that replacing some of the expensive and scarce conventional feed ingredients in part or full will go a long way in reducing the production cost and improving the animal protein consumption.

Processed by-products of cocoa harvesting industry had been reported as a suitable replacement for some conventional feed stuff in monogastric production [5,1]. Cocoa pod husk forms about 80% of cocoa fruit and between 0.8 and 1.0 million tons of cocoa pod husk is generated annually in Nigeria, most of which are considered a waste except for the very negligible quantity being used in local soap making [6]. Nutritional value of cocoa pod is relatively low because of its low crude protein (9.14%) and high crude fibre (35.78%) [5]. It also contains anti-nutrients such as theobromine (2.64%), caffeine (1.14%) and tannin 0.917%) [6]. However ash extract treatment and/or fermentation are being used in processing of cocoa pod husk and other crop residues with remarkable improvement in their nutritional value being recorded [7,8,6]. Therefore, the inclusion of ash extract treated and /or fermented cocoa pod husk in monogastric nutrition could be a way of reducing cost of production and improve animal protein consumption.

Like several other processing methods, ash extract treatment do not completely eliminate anti-nutrients in feeds stuffs but only reduce them to tolerable levels [6]; and these anti-nutrients and other constituents of diets affect blood haematological and biochemical properties [9,3,10]. In addition, blood biochemical components are feed toxicity elements' sensitive; while haematological components are useful in feed toxicity monitoring [11,9].

Utilization of cocoa by products by rabbits had been recognized by Ogunsipe et al. [1]. However, information on effect of processed cocoa pod husk on haematological and biochemical indices in rabbits is relatively rare. Therefore, this study examined the effect of dietary ash extract treated cocoa pod husk meal on haematological and serum biochemical properties of rabbits.

2. MATERIALS AND METHODS

2.1 Experimental Site and Cocoa Pod Husk Collection

The experiment was carried out at the Rabbitary of Teaching and Research Farm of The Federal College of Agriculture, Akure, Nigeria. The experimental site lies about 7°25' north and 5°19'

east. The average annual temperature is 25.3°C while the average rainfall is 1455 mm. Cocoa pods were collected from a Cocoa farm in Oda, Akure, Nigeria, cut into two parts to remove the pulpy seeds, thoroughly washed to remove the residual mucilage of the pods and thereafter chopped into smaller pieces with sharp stainless steel knife. The chopped cocoa pods were spread lightly on polythene and sun dried for 7-14 days, hammer milled, bagged and kept in cool dry place until used.

2.2 Preparation of Corn Stalk Ash Extract

Dried corn (*Zea mays*) stalks were collected after harvesting from the Teaching and Research farm of Federal College of Agriculture, Akure (FCAA), burnt and ashed at 600°C. The corn stalk ash solution (CSAS) was prepared as described by Adamafio et al. [7]. 39.2 g of corn stalk ash was suspended in 100 mls of deionized water for 48 hours at room temperature and filtered first through cheese cloth, then through Wathman No 1 filter paper [7]. The corn stalk ash extract (CSAE) was kept until use.

2.3 Collection of Layers' Wastes and Rumen Liquor

Droppings of commercial layers' wastes (LW) devoid of feathers and broken egg shells were collected from Teaching and Research Farm, FCAA, sundried for 14 days to moisture level of less than 6%, milled with hammer mill, bagged and kept in cool dried place until used. The rumen liquor was manually squeezed out of rumen content of freshly slaughtered cattle (White Fulani) through a clean mushlin cloth at the Central Abattoir, Akure, Nigeria and used almost immediately.

2.4 Bio-degradation of Cocoa Pod Husks

Milled cocoa pod husk was mixed with CSAE at rate of 188 g/L, stirred properly in black plastic container and covered tightly for one hundred and sixty-eight (168) hours. The CSAE soaked cocoa pod husk was transferred into bags, pressed with hydraulic press to drain out the moisture, spread lightly on polythene and sundried for about 14 days to form dried Ash Treated Cocoa pod Husk (ATCH).

Thereafter, ATCH was mixed successively with dried layer wastes and molasses at rate of 100 g/kg and 50 ml/kg; respectively in air tight black plastic container, sprayed with rumen liquor and allowed to ferment for the duration of 168 hours anaerobically as described by Oloruntola et al. [8]. The fermented ATCH was thereafter spread lightly on polythene and allowed to sundried for 7 days, analyzed (Table 1) for proximate analysis [12], theobromine [13], caffeine [14], tannin [15] and thereafter labeled as processed cocoa pod husk (PCPH).

2.5 Experimental Diets and Animals

Four (4) experimental diets were formulated in which the PCPH was included in rabbits' diets at 0, 10, 20 and 30%, designated as diets 1, 2, 3 and 4 respectively and pelletized (4 mm diameter and 8mm long). The composition of the diets is as presented in Table 2. The recommendations and guidelines for applied nutrition experiments in rabbits as described by Fernandez-Carmona et al. [16] were followed in this study. One hundred and twenty (120) healthy, five-week old weaned rabbits of cross-breeds (New-Zealand white and Chinchilla) of equal sexes and average initial weight of 523.9±43 g were randomly allotted to the four (4) dietary treatments (30 rabbits/treatment; 3 rabbits/replicate). The rabbits were housed in a 2 storey, wooden framed wire-meshed cages being placed in a well ventilated pen. Prior to the commencement of the experiment, the rabbits were treated against coccidiosis, mange and bacterial infections by administering prophylactic coccidiostat, ivomectin and tetracycline respectively. Thereafter, they were made to undergo a week adaptation period in their individuals cage and fed their respective diets *ad-libitum* throughout the period of the experiment (56 days).

2.6 Blood Collection, Haematological and Serum Biochemical Analyses

On day 56, blood samples were collected from the randomly selected rabbits (20 rabbits/treatment) using method described by Burnett et al. [17]. About 7 milliliters of blood was collected from the marginal ear vein of each rabbits using 18 gauge 3.8 cm needle. Thereafter, each sample was divided equally into two and placed into plain bottle (without anticoagulant) and potassium EDTA bottle for biochemical and haematological studies. On the collection day, haematological indices were determined by Shenzhen Mind ray Auto Haematology Analyzer, Model Bc-3200 (Shenzhen Mind Ray Biomedical Electronic

Company. Hamburg 20537, Germany). Refloctron® Plus 8C79 (Roche Diagnostic, GonbH Mahmheim, Germany), using commercial kits was used to determine the serum biochemical parameters.

2.7 Statistical Analysis

The experiment data collected were subjected to analysis of variance and regression using General Linear Model procedure of SPSS version 20. Means were separated using Duncan's multiple range test [18].

3. RESULTS

3.1 Haematological and Erythrocytic Indices

The values recorded for packed cell volume (PCV), white blood cells (WBC), lymphocytes, monocytes, granulocytes, MCH, MCHC and platelets were similar (P>0.05) across the dietary treatments. However, the haemoglobin concentration improved significantly (P=.05) with 10 and 20% PCPH inclusion level while haemoglobin concentration at 30% PCPH level was similar to the control. The red blood cells (RBC) count and mean cell volume (MCV) of rabbits fed diets including PCPH at 10, 20 and 30% were higher when compared to the control diet (Table 3).

Table 1. Chemical composition of processed cocoa pod husk (PCPH)

Composition	Quantity (%)
Crude protein	13.66
Crude fibre	14.83
Ether extract	6.44
Ash	15.05
Nitrogen free extract	39.31
Tannin	0.012
Caffeine	0.003
Threobomine	0.036

Table 2. Gross composition (%) of the experimental diets

Ingredients	Levels of PCPH (%)			
	0	10	20	30
	Diet 1	Diet 2	Diet 3	Diet 4
Maize	16.00	17.50	15.50	14.50
PCPH	0.00	10.00	20.00	30.00
Wheat offals	2.50	1.50	1.50	1.50
Soya bean meal	8.65	7.65	7.65	7.65
Breweries Dried Grain	25.90	24.40	19.40	13.40
Rice Bran	26.90	18.90	15.90	12.90
Soya bean Hay	5.00	5.00	5.00	5.00
Maize husk	13.00	13.00	13.00	13.00
Bone meal	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25
Methionine	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
Vegetable oil	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated analysis (%)				
Energy (Kcal/kg)	2512.80	2511.60	2500.32	2534.88
Calcium	0.50	0.51	0.50	0.52
Phosphorus	0.31	0.30	0.31	0.33
Lysine	0.74	0.73	0.72	0.73
Methionine	0.45	0.44	0.43	0.46
Analyzed composition (%)				
Crude protein	17.26	17.39	17.35	17.36
Crude Fibre	16.77	16.91	17.62	16.82

PCPH: Processed cocoa pod husk

3.2 Serum Biochemical Indices

Table 4 shows the serum metabolites of rabbits fed the experimental diets. The albumin, globulin, creatinine, bilirubin and aspertate amino transferase (AST) were stable (P>0.05) across the dietary treatments. The total protein of rabbits fed PCPH inclusive diets was significantly (P=.05) higher than those fed control diet. Serum cholesterol level of the rabbits reduces significantly (P<0.05) with increase in dietary PCPH. The high density lipoprotein (HDL) increased (P=.05) at 20 and 30% dietary PCPH; while the low density lipoprotein (LDL) decreased significantly (P=.05) at both 20 and 30% PCPH inclusion levels. The PCPH level (x) in the diets showed negative correlation with cholesterol (y_1=-0.0183x+1.3183; 0.6183), low density lipoprotein (y_2=-0.1818x+56.277; 0.8714) and positive correlation with high density lipoprotein (y_3=0.0711x+35.971; 0.6017) (Table 5).

Table 3. Haematology of rabbits fed experimental diets

Parameters	Levels of PCPH inclusion (%)				SEM	P value
	0	10	20	30		
	Diet 1	Diet 2	Diet 3	Diet 4		
Packed cell volume (%)	33.48	33.52	33.68	33.21	0.09	0.30
Haemoglobin conc. (g/dl)	11.65^{ab}	11.69^{a}	11.92^{a}	11.22^{b}	0.10	0.03
Red blood cells (× 10^{12}/l)	5.24^{b}	5.35^{ab}	5.55^{a}	5.39^{ab}	0.05	0.04
White blood cells (× 10^{9}/l)	4.04	4.08	4.12	4.08	0.02	0.25
Lymphocytes (× 10^{9}/l)	2.49	2.54	2.57	2.61	0.06	0.94
Monocytes (× 10^{9}/l)	0.89	0.99	1.14	0.98	0.04	0.20
Granulocytes (× 10^{9}/l)	2.27	2.30	2.46	2.39	0.18	0.98
MCV (fl)	61.81^{b}	62.22^{ab}	62.98^{a}	62.08^{ab}	0.18	0.05
MCH (pg)	20.97	21.08	21.45	21.68	0.14	0.20
MCHC (%)	31.13	31.29	31.39	31.30	0.12	0.92
Platelets (10^{9}/l)	185.00	195.00	260.67	212.33	17.67	0.49

Table 4. Serum metabolites of rabbits fed experimental diets

Parameters	Levels of PCPH inclusion (%)				SEM	P value
	0	10	20	30		
	Diet 1	Diet 2	Diet 3	Diet 4		
Total protein (g/l)	67.02^{b}	70.83^{a}	70.91^{a}	69.48^{a}	0.53	0.01
Albumin (gl)	52.65	52.87	53.62	53.18	0.62	0.96
Globulin (gl)	14.79	14.94	15.37	15.24	0.11	0.26
Cholesterol (mmol/L)	1.36^{a}	1.10^{ab}	0.90^{b}	0.81^{b}	0.08	0.03
Creatinine (mg/dl)	59.45	59.92	61.01	59.82	0.28	0.24
HDL (mg/dl)	35.86^{b}	36.42^{b}	38.23^{a}	37.61^{a}	0.31	0.01
LDL (mg/dl)	56.25^{c}	54.95^{b}	51.74^{a}	51.26^{a}	0.66	0.01
Bilirubin(µ/l)	1.05	1.10	1.22	1.15	0.04	0.41
AST (µ/l)	11.90	12.01	13.20	12.10	0.26	0.28

HDL: high density lipoprotein; LDL: low density lipoprotein; AST: Aspartate aminotransaminase.

Table 5. Regression equations derived from parameters of rabbits fed experimental diets

Parameters	Regression equation	R^2	P
Level of PCPH Vs CHO	y_1=-0.0183x+1.3183	0.6183	0.002
Level of PCPH Vs HDL	Y_2=0.0711x+35.971	0.6017	0.003
Level of PCPH Vs LDL	y_3=-0.1818x+56.277	0.8714	0.000

PCPH: Processed cocoa pod husk; CHO: cholesterol; HDL: high density lipoprotein; LDL: low density lipoprotein

4. DISCUSSION

The crude protein (CP), ether extracts (EE) and ash content of PCPH was higher than 7.66% CP, 4.33% EE and 10.1% Ash earlier reported by Donkoh et al. [19] for cocoa pod husks and 8.75% CP, 2.4% EE and 2.19% Ash reported by Ape et al. [20] for maize. Nutritionally, PCPH could be suitable for inclusion in monogastric nutrition.

Blood examination is helpful in detecting the effect of nutrition on the physiological and pathological status of animals [10]. In this study, the haematological indices values fall within the normal range (PCV: 33-50%; Hb conc.: 9.4 -17.4 g/dl; RBC: 4.7- 7.2 $x10^{12}$/l; WBC: 5-12 $x10^{9}$/l; lymphocyte: 2-20 $x10^{12}$/l; monocytes: 0-1.8 $x10^{12}$/l; MCV: 50-75 fl; MCH: 16-23 pg; MCHC: 280-360 g/l) reported by Flecknell [21] and Oloruntola et al. [10]. In addition, the stability of some of these haematological indices (PCV, WBC, monocytes, granulocytes, MCH, MCHC and platelets) across the experimental diets implies nutritional adequacy and support of the diets' composition for the normal haempoiesis in the rabbits. Similar result was reporded by Ogunsipe et al. [1] in rabbits fed varying levels of cocoa bean shell meal inclusive diets. Heamoglobin is the oxygen carrying pigment in the red blood cells. In this study, the drop in the haemoglobin concentration of rabbits fed 30% PCPH inclusive diet when compared to those fed 10 and 20% PCPH inclusive diets tends to indicate occurrence of anaemia. This may be due to possible increase in quantity of anti-nutritional factors (tannin, theobromine and caffeine) in the diets with increase in inclusive level of PCPH up to 30%. Tannin, when consumed in large quantity was found to adversely affect the bioavailability of the iron [22]; an important mineral necessary in blood formation and relationship exists between dietary iron and haemoglobin [11]. In another way, the observed similarity between haemoglobin concentrations of those rabbits fed 30% PCPH inclusive diet and those on the control diet suggests the levels of the anti-nutritional factors in these experimental diets were tolerable and may not have caused anaemia in the rabbits. The nutritional adequacy of PCPH inclusive diets (diets 2, 3 and 4) were further supported by the similarities in their RBC and MCV values; which were higher than those recorded for the rabbits fed control diet (0% PCPH). By implication, dietary PCPH enhanced red blood cells production and in particular the higher PCV, RBC and MCV indices of rabbits fed 20% PCPH inclusive diet could speculatively be associated with superior nutrition because dietary components have effects on constituents of blood [23].

Serum metabolites levels are determined to provide a view of the metabolic state of an organism. The serum biochemical constituents (total protein, albumin, globulin, cholesterol, creatinine, high density lipoprotein, low density lipoprotein, bilirubin and AST) were generally in consonance with reported reference variables [24,17]. The stability of serum albumin, globulin, creatinine, bilirubin and AST concentration in rabbits across the dietary treatment indicated the safety of the various diets and their support for the normal physiological function of the rabbits. For instance, serum biochemical constituents are released into bloodstream when body tissues or organs are diseased or damaged; therefore the stability of creatinine, bilirubin and AST levels in the rabbits across the diets in this study indicated normal functioning of the kidney, liver and heart of the rabbits fed those diets [25]. Total protein is an indicator for measuring nutritional protein adequacy [26]. The higher total protein levels recorded in PCPH inclusive diets which are similar to the reported normal values [24,17,3] suggests the suitability of nutritional quality of PCPH as feed ingredients in rabbit nutrition. Cholesterol is found in all body cells and travels through blood streams in small packages called lipoprotein. Prolonged high cholesterol levels could result in its deposition on the walls of the blood vessels and consequently leading to atherosclerotic plaque and blockage of important blood vessels which results in a myocardial infarction or heart attack [27]. The progressive decreased in cholesterol level of rabbits with increased PCPH in this study suggests decreased uptake of cholesterol or decreased cholesterol production due to the dietary treatments [28]. This was corroborated by the regression of Cholesterol (x) against PCPH (y), which has the prediction equation y_1=-0.0183x+1.3183 (R^2= 0.61, P<0.002) and implies that PCPH levels accounted for about 61% of the variation in cholesterol level of the rabbits. In addition, for every gram increase in PCPH level in diet, there was a corresponding decrease of 0.0183 mmol/L cholesterol of the rabbits. It is therefore suggested that PCPH may contain components that exert inhibitory effect directly or indirectly on a key enzyme (hydroxymethylglutaryl-coenzyme A reductase) involved in cholesterol biosynthesis. The increase of high density lipoprotein (HDL) levels

in rabbits fed PCPH inclusive diets when compared to the control diet is in consonance with earlier reports of Baba et al. [29] and Khan et al. [30]. The regression of HDL(y) against PCPH(x) which had the prediction equation y_2= 0.0711x+35.971 (R^2= 0.60, P<0.003). This implies that PCPH level was responsible for about 60% increase in HDL and for every gram increase in PCPH level, there was a corresponding increase of 0.071 mg/dl in HDL levels of the rabbits. The reduction of serum HDL-cholesterol was linked to bioactive component of cocoa (theobomine) [31], although the possible physiologic mechanisms of action that explain how theobromine increases HDL cholesterol is yet to be reported. Low level lipoprotein (LDL) cholesterol otherwise called bad cholesterol at high level leads to buildup of cholesterol in arteries. The decrease in low density lipoprotein (LDL) with increased inclusion level of PCPH in the rabbits' diets was corroborated by the regression of LDL(y_3) against PCPH(x), which had the prediction equation y_3= -0.1818x+56.277 (R^2=0.87, P<0.000). This implies that, the PCPH level accounted for about 87% of the variation in LDL of growing rabbits and for every gram increase in PCPH level, there was a corresponding decrease of 0.182 mg/dl in LDL levels of the rabbits. The reduction in LDL level due to dietary PCPH in this study supports the wholesomeness of PCPH and possibility of it reducing cardiovascular disease risk in the rabbits. Cocoa and cococa containing products had been reported to have tendency of reducing cardiovascular disease risk [32].

5. CONCLUSION

Within the limit of this study, inclusion of PCPH up to 30% did not affect the haematological and serum biochemical indices of the rabbits. Therefore, dietary inclusion of 30% PCPH could be adapted in rabbits' production.

ETHICAL APPROVAL

All authors hereby declare that "Guide for the care and use of Laboratory Animals" (National Research Council, Copyright 2011, 8th Edition) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Research committee of the Department of Animal Production and Health, The Federal University of Technology, Akure, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ogunsipe MH, Balogun KB, Oladepo AD, Ayoola MA, Arikewuyo MT. Nutritive value of cocoa bean shell meal and its effect on growth and haematology of weaning rabbits. Nigerian J. Agric. Food Environ. 2017;13(1):23-28.
2. FAO. FAOSTAT Production database. Food and Agriculture Organization of the United Nation; 2008.
3. Oloruntola OD, Agbede JO, Onibi GE, Igbasan FA. Replacement value of rumen liquor fermented cassava peels for maize in growing rabbit diet. Arch Zootec. 2016a; 65(249):89-97.
4. Nworgu FC, Adebowale EA, Oredein OA, Oni A. Prospect and economics of broiler production using two plant protein sources. Trop. J. Anim. Sci. 1999;2:159-166.
5. Eghosa OU, Rasheed AH, Martha O, Luqman AA. Utilization of cocoa pod husk (CPH) as substitute for maize in layers mash and perception of poultry farmers in Nigeria. Int. J. Sci. Nature. 2010;1(2):271-275.
6. Adeyeye SA, Agbede JO, Aleter VA, Oloruntola OD, Ayodele SO, Ahaotu EO. Effects of rumen liquor fermentation on the proximate composition and antinutritional factors of Ash- extract treated Cocoa. (*Theobraoma cocoa*) pod husks. Proceedings of 21st Annual Conference of Animal Science Association of Nigeria. 18-22, 2016. Port Harcourt. 2016; Supplementary Volume 1:52-55.
7. Adamafio NA, Cooper Aggrinage E, Onaye EO, Laary JK, Onaye J. Effectiveness of corn stalk ash in reducing tannin level and improving invitro enzymatic degredation of polysaccharides in crop residues. Ghana J. Sci. 2004;44:87-92.
8. Oloruntola OD, Agbede JO, Onibi GE, Igbasan FA. Composition of cassava (*Manihot* spp.) peels fermented with bovine rumen liquor and different nitrogen sources. J. Global Agric. Ecol. 2015;2(1): 26-35.
9. Ahamefule FO, Obua BE, Ukweni IA, Oguike MA, Amaka RA. Haematological and biochemical profile of weaner rabbits

fed raw or processed pigeon pea seed meal based diets. African J. Agric. Res. 2008;3(4):315-319.
10. Oloruntola OD, Ayodele SO, Agbede JO, Oloruntola DA, Ogunsipe MH, Omoniyi IS. Effect of *Alchornea cordifolia* leaf meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits. Asian J. Bio. Life Sci. 2016b;5(2):190-195.
11. Onifade AA, Tewe OO. Alternative tropical energy feed resources in rabbits diets: Growth performance, diet's digestibility and blood composition. World Rabbit Sci. 1993;1(1):17-24.
12. AOAC. Official Methods of Analysis. 16th edition. Association of Official Analytical Chemists, Inc., Arlington, VA. USA; 1995.
13. Bisto MS, Veloso MCC, Pinheiro HLC, De Oliveira RFS, Reis JON, De Andrade JB. Simultaneous determination of caffeine, theobromine and theophylline by high-performance liquid chromatography. J. Chromatog. Sci. 2002;40:45.
14. Rade I, Branislava S, Matevz P, Marija B, Katarina K, Borut S. Determination of Caffeine and associated compounds in food,beverages, natural Products, pharmaceuticals, and cosmetics by Micellar Electrokinetic Capillary Chromatography. J. Chromatog. Sci. 2008;46:137-143.
15. Shad MA, Nawaz H, Rehma T, Ikram M. Determination of biochemicals, phytochemicals and antioxidative properties of different part of *Cichorium intybus* L.: A comparative study. The J. Anim. Plant Sci. 2013;23(4):1060-1066.
16. Fernandez-Carmona J, Blass E, Pascual JJ, Maertens L, Gidenne T, Xiccato G, Garcia J. Recommendations and Guidelines for Applied Nutrition Experiments in Rabbits. World Rabbit Sci. 2003;13:209-228. Available:http://dx.doi.org/10.4995/wrs.2005.516
17. Burnett N, Mathura K, Metivier KS, Holder RB, Brown G, Campbell M. An investigation into haematological and serum chemistry parameters of rabbits in Trinidad. World Rabbit Sci. 2006;14:175-189.
18. Steele RGO, Torrie JH. Principles and procedures of statistics, McGraw-Hill Comp. Inc. New York, Toronto, London. 2nd Ed. 1960;633.
19. Donkoh A, Atuahene CC, Wilson BN, Adomako D. Chemical composition of cocoa pod husk and its effect on growth and food efficiency in broiler chicks. Ani. Feed Sci. Tech. 1991;35(1-2):161-169.
20. Ape DI, Nwogu NA, Uwakwe EI, Ikedinobi CS. Comparative proximate analysis of maize and sorghum bought from ogbete main market of Enugu State, Nigeria. Greener J. Agric. Sci. 2016;6(9):272-275. Available:http://doi.org/10.15580/GJAS.2016.9.101516167
21. Flecknell P. Manual of rabbit medicine and surgery. Gloucester: British Small Animal Veterinary Association; 2000.
22. Adeyeye EI. Proximate, mineral and antinutrient composition of dika nut (*Irvingia gabonensis*) kernel. Elixir Int. J. 2013;58:14902-14906.
23. Church JP, Judd JT, Young CW, Kebay JL, Kim WW. Relationships among dietary constituents and specific serum clinical components of subjects eating self selected diets. American Journal of Clinical Nutrition. 1984;40:1338- 1344.
24. Mitruka BM, Rawnsley HM. Clinical biochemical and hematological reference values in normal experimental animals. Masson Publishing USA, Inc. 1977;134-139.
25. Kerr M. Veterinary laboratory medicine. clinical biochemistry and haematology. Blackwell Scientific Publications; 1989.
26. Eggum BO. Protein quality of cassava leaf. British J. Nutr. 1987;24:761-768.
27. Mcdonald P, Edwards RA, Greenhalgh JFD, Morgan CA. Voluntary Intake of food. In: Animal Nutrition. 5th edition. Longman, Malaysia. 1995;418-422.
28. Peter ML, Susan EEF. Interpretation of laboratory results. Australian Veterinary Practitioner. 1991;21(4):188-192.
29. Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, Fukuda K, Muto Y, Kondo K. Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol concentrations in humans. American Journal of Clinical Nutrition. 2007;85:709–17.
30. Khan N, Monagas M, Andres-Lacueva C, Casas R, Urpy -Sarda M, Lamuela-Raventos RM, Estruch R. Regular consumption of cocoa powder with milk increases HDL cholesterol and reduces

oxidized LDL levels in subjects at high-risk of cardiovascular disease. Nutr., Metabol. Cardiovas. Dis. 2012;22:1046–53.

31. Neufingerl N, Zebregs YE, Schuring EAH, Trautwein EA. Effect of cocoa and theobromine consumption on serum HDL-cholesterol concentrations: A randomized controlled trial. American J. Clin. Nutr. 2013;97:1201-1209.

32. Buitrago-Lopea A, Sanderson J, Johnson L, Warnakula S, Wood A, Franco OH. Chocolate consumption and cariometbolic disorders: Systematic review and meta-analysis. British Med. J. 2011;343:d4488.

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