NSAP 47th Annual Conference (JOS 2022) CONFERENCE PROCEEDINGS
THEME: SECURING ANIMAL AGRICULTURE AMIDST GLOBAL CHALLENGES

HAEMATOLOGICAL STUDIES OF GROWER RABBITS FED PROCESSED KOLANUT (*Cola nitida*) POD MEAL DIETS SUPPLEMENTED WITH MULTI-ENZYMES

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ABSTRACT
This study aimed at studying the effects of diets containingprocessed kolanut (Cola nitida) pod meal (PKPM) and multi-enzymes (E) on haematological indices of growing rabbits. Five diets (diet 1: control; diet 2: 0.35g/kgE; diet 3: 125g/kg PKPM; diet 4: 125g/kg PKPM+0.35g/kgE; diet 5: 150g/kg PKPM and diet 6: 150g/kg PKPM+0.35g/kgE) were formulated. One hundred and eighty5 week-old rabbits of cross-breeds and mixed sexes were used randomly allocated (30 rabbits/treatment; 10 replicates/treatment; 3 rabbits/replicate)to the 6 dietsin a completely randomised design. The rabbits were fed ad libitum throughout the period (10 weeks) of the feeding trial. The blood indices were not significantly (P>0.05) affected by the dietary treatments, except (P<0.05) for haemoglobin concentration and lymphocytevalues. In conclusion, dietary inclusion of PKPM upto150g/kg level with or without 0.35g/kg multi-enzyme supplementation does not affect the normal blood production in growing rabbits.
Keywords: Haematology, rabbits, kolanut, diets, multi-enzymes

INTRODUCTION
One of the major factors responsible for the slow progress of the rabbit industry in Nigeria is the unavailability of good quality pellet feed. The nutritional potential of forages and industrial by-products or wastes is particularly significant for rabbits. The importance of kolanut*(Cola nitida)* cannot be overemphasized in Nigeria and some West African countries; Harnessing this by-product and utilizing it in the formulation and compounding of livestock feed will not only eradicate its environmental disturbances in the kolanut-producing areas but will remove the rivalry for maize between man and animals, thus bringing about a great reduction in the cost of producing livestock feed and hence reducing the cost of production (Osineye*et al.*,2008). The enzymes are effectively manufactured and added to animal feeds to increase digestibility, remove anti-nutritional factors and improve nutrient availability, as well as for environmental reasons and can be an improvement to intestinal health (Selle*et al.*,2009). Blood is a medium for transporting oxygen and nutrients that fight infection; it brings waste products to the kidney and liver, which filters, cleanses the blood and regulates body temperature. The low level of haemoglobin in the blood is an indication of poor nutrition including dietary deficiency of iron, copper, vitamins or amino acids (Esonu*et al*., 2001). Agbede (2003) reported that high-quality feed results in normal haemoglobin, packed cell volume and red blood cell values in monogastric animals. Therefore, this study was carried out to determine the influence of the kolanut pod husk diet supplemented with commercial enzymeson the haematological indices of rabbits.

MATERIALS AND METHODS
The experiment was carried out at the Rabbitry of Teaching and Research Farm (TRF) of the Federal College of Agriculture (FECA), Akure, Nigeria. The kolanut pod husks (KPH)mealweresubjected to two rounds of 7-day-solid state fermentation successively with corn stalk ash solution (CSAS) (Adamafio*et al*., 2004; Adeyeye*et al*., 2016) and thereafter with bovine rumen contents (Adeyeye*et al*., 2016; Adeyeye*et al*., 2019). The kolanut pod meal that resulted from the two fermentations was sundried and given the name processed kolanut pod meal (PKPM). Multi-enzyme (E) manufactured by Biomix S.A,

Carrera 47C, Sabaneta-Colombia was procured from a commercial feed mill ingredients seller in Akure, Ondo State, Nigeria. The cellulose (700,000U.A), α-amylase (800,000U.A), β-glucanase (300,000U.BG), phytase (1,200F.T.U), protease (800,000 UP), lipase (20, 000 UI) and xylanase (500,000U.X) are all present in the multi-enzyme. Thereafter, six diets were formulated, designated as diets 1 (control), diet 2 (control+0.35g/kgE), diet 3 (125g/kgPKPM), diet 4 (125g/kgPKPM+0.35g/kgE), diet 5 (150g/kgPKPM) and diet 6 (150g/kgPKPM+0.35g/kgE) and pelletized (4mm diameter and 8mm long) (Table 1). One hundred and eighty 5-week-old crossbreed (New Zealand × Chinchilla) rabbits of mixed-sex were randomly allotted to the six dietary treatments (10 replicates/treatment, 3 rabbits/replicate) after balancing for weight. The rabbits were fed their respective diets *ad-libitum* throughout the 10 weeks experimentation period.Thereafter, blood was collected from the marginal ear vein of each rabbit using syringes and a needle. The blood samples were collected into the blue top bottle containing K_3-ethylene diamine tetra-acetate (K_3-EDTA) anticoagulantfor haematological analysis. Following that, the blood samples were examined using Shenzhen Mind ray Auto Haematology Analyzer, Model Bc-3200 (Shenzhen Mind ray Biomedical Electronics Co. Hamburg 20537, Germany).The data obtained were subjected to one way analysis of variance using SPSS version 20. Duncan multiple range test of the same package was used to determine the differences between treatment means.

Table 1: Gross and analysed composition of the experimental diets

Ingredients	Levels of PKPM inclusion (g/kg)					
	0+E	**0-E**	**125+E**	**125-E**	**150+E**	**150-E**
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**Diet 6**
Maize	160.00	160	135.00	135.00	135.00	135.00
PKPM	0.00	0.00	125.00	125.00	150.00	150.00
Multi-enzyme (E)	0.00	0.05	0.00	0.50	0.00	0.50
Wheat offal	30.00	30.00	12.00	12.00	10.00	10.00
Soybean meal	70.50	70.50	74.50	74.50	74.50	74.50
BDG	229.00	229.00	202.00	202.00	200.00	200.00
Rice bran	304.00	303.50	250.00	249.50	228.00	227.50
Soybean Hay	50.00	50.00	50.00	50.00	50.00	50.00
Maize Husk	135.00	135.00	128.00	128.00	129.00	129.00
DCP	10.00	10.00	10.00	10.00	10.00	10.00
Methionine	2.50	2.50	2.50	2.50	2.50	2.50
Premix	2.50	2.50	2.50	2.50	2.50	2.50
Lysine	1.00	1.00	1.00	1.00	1.00	1.00
Salt	2.50	2.50	2.50	2.50	2.50	2.50
Soy oil	3.00	3.00	5.00	5.00	5.00	5.00
Calculatedanalysis (g/kg)						
Crude protein	161.70	161.70	161.70	161.70	161.50	161.30
Crude fibre	161.30	161.30	161.30	161.30	161.30	161.00
Energy	2522.94	2522.94	2514.70	2514.70	2519.70	2521.90

PKPM = Processed Kolanut Pod Meal, E =Multi-enzyme; BDG = Brewers Dried Grain
DCP =DicalciumPhosphate.

RESULTS AND DISCUSSION

Table 2 shows the haematological values were not affected (P>0.05) by the dietary treatment except (P<0.05) for the haemoglobin concentration (Hbc) and lymphocyte counts. The Hbc recorded in rabbits fed diets 2(control+0.35g/kgE) and 4(125g/kgPKPM+0.35g/kgE) were lower (P<0.05) thanthose fed diets 3 (125g/kgPKPM) and 5(150g/kgPKPM) but similar (P>0.05) to those fed diet 1(control). The lymphocyte counts of rabbits fed diets 2, 4 and 5 were lower (P<0.05) than those fed diet 1.Haematologicalstudies help to understand the relationship of blood characteristics to the environment and haematological indices are good indicators of the physiological status of the animals (Etim*et al.*,

2014a,b). In addition, nutrition was reported as one important factor that affects the haematological indices (Addass*et al*., 2012). The stability of red blood cells, packed cell volume, white blood cells, and platelets among the rabbits given the various experimental diets, as shown in this study, demonstrates that the dietary treatments (PKPM and enzyme) maintained normal blood haematopoiesis in rabbits (Oloruntola*et al*., 2018). The observed variations in the haemoglobin concentration of the rabbits is of no health significance because the values recorded for these indices among the rabbits fed diets 2, 3 4, 5 and 6 were comparable to those fed diet 1. In addition, the haemoglobin concentration recorded in this study falls within the normal range (9.80-15.8 g/dl) reported by Mitruka and Rawnsley(1977). Lymphocytes are a type of immune cell that recognises antigens, produces antibodies and destroys potentially harmful cells. The observed reduction of lymphocytes counts in rabbits fed diets 2, 4 and 5 in this study further unveils the immunomodulatory effects of test diets containing multienzymes and PKPM in the rabbits. This is in agreement with Oloruntola*et al*(2016), who observed thatwith an increase in alchornea leaf meal inclusion from 0 to 100g/kg, the number of white blood cells, lymphocytes, monocytes, and granulocytes increased.Furthermore, the observed lymphocyte values of 6.32-6.78 x 10^9/l were within the normal range of 1.6-10.6x10^9/l and 2.0-20.0 x10^9/l reported by Latimer*et al* (2003) and Flecknell (2000) respectively.

CONCLUSION AND RECOMMENDATION

In conclusion, the dietary inclusion of processed kola nut pod meal up to 150g/kg level with or without 0.35g/kg multi-enzyme supplementation does not affect the normal blood production in growing rabbits.

Table 2: Haematological indices of weaner rabbits fed diets containing graded levels of processedkolanut pod meal (PKPM) supplemented with multi-enzymes (E)

Parameters	Levels of PKPM inclusion (g/kg)						SEM	*P value*
	0+E Diet 1	0-E Diet 2	125+E Diet 3	125-E Diet 4	150+E Diet 5	150-E Diet 6		
Packed cell volume (%)	34.54	34.48	34.62	34.52	34.67	34.54	0.07	0.99
Haemoglobin (g/dl)	14.11^{ab}	13.83^{b}	14.25^{a}	13.85^{b}	14.30^{a}	13.80^{b}	0.07	0.04
Red blood cell(×10^{12}/L)	5.69	5.61	5.96	5.88	6.19	5.29	0.08	0.33
White blood cell(×10^9/L)	7.29	7.23	7.35	7.29	7.43	7.37	0.09	0.99
Lymphocytes (×10^9/L)	6.78^{a}	6.51^{bc}	6.63^{ab}	6.32^{c}	6.40^{c}	6.35^{a}	0.05	0.01
Monocytes (×10^9/L)	1.40	1.36	1.38	1.33	1.34	1.35	0.03	0.99
Granulocytes (×10^9/L)	3.19	3.14	3.22	3.16	3.31	3.17	0.18	1.00
MCV (fl)	64.89	64.71	64.84	63.74	64.89	64.75	0.13	0.05
MCH (g)	19.87	19.73	20.35	19.80	20.64	19.51	0.07	0.46
MCHC (g/dl)	30.50	29.66	30.66	29.72	30.87	30.08	0.15	0.07
Platelets (×10^9/L)	222.08	221.94	222.15	222.31	223.05	221.97	0.20	0.68

^{a,b,c}Means with different superscripts in the same row are significantlydifferent($P<0.05$);
MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentrate.

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