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Pawpaw Leaf Meal and Exo-enzyme in Rabbit Diet: Effect on Hematological and Serum Biochemical Indices

O. D. Oloruntola[1*] and S. O. Ayodele[1]

[1]Animal Production Department, The Federal Polytechnic, Ado Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ODO managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the effect of dietary enzyme (E) and pawpaw leaf meal (PLM) on hematological and biochemical indices of rabbits.
Study Design: Complete randomized design with 2x2 factorial arrangements.
Place and Duration of Study: The experiment was carried out between November and December, 2016 at Agricultural Technology Department Teaching and Research Farm, The Federal Polytechnic, Ado Ekiti, Nigeria.
Methodology: Four experimental diets were formulated (diet 1 (0%-E), diet 2 (0%+E), diet 3 (10%-E) and diet 4 (10%+E)). Diets 1 and 3 were not supplemented with enzyme but had PLM inclusion at 0 and 10% level respectively, while diets 2 and 4 were enzyme supplemented at the rate of 0.5g/kg but had PLM inclusion at 0 and 10% level respectively. One hundred and twenty (120) healthy, 35 day old weaner rabbits of equal sex and cross breed (Chinchilla x New-Zealand white) were randomly allotted to four dietary treatments (30 rabbits/treatment; 3 rabbits/replicate). The rabbits were fed their respective experimental diets *ad libitum* for 56 days.

**Corresponding author: Email: oloruntoladavid@gmail.com*;

Results: Dietary enzyme significantly (P<0.05) increased the lymphocyte value while pawpaw leaf meal (PLM) inclusion significantly (P<0.05) increased the white blood cells (WBC) and lymphocytes but reduced the platelet values. Significant (P<0.05) interaction exists between dietary enzyme and PLM for white blood cells, monocytes, red blood cells, haemoglobin concentration, mean cell volume and mean cell haemoglobin concentration. Urea, alanine aminotransferase (ALT) and amylase values reduced (P<0.05) with the dietary enzyme supplementation. Cholesterol, low density lipoprotein and amylase reduced due to dietary PLM.
Conclusion: The pawpaw leaf meal (10%) and exo-enzyme (0.5 g/kg) did not pose any threat to the normal health of the rabbits.

Keywords: Herbs; enzyme; rabbits; blood indices; health status.

1. INTRODUCTION

Rabbit have various characteristics that support its production at both subsistence and large scale levels as one of the suitable solutions to the problem of low protein intake in the developing countries [1]. Among these characteristics are short generation time, small body size, high reproduction potential, rapid growth, production of high quality meat, good potential for genetic improvement, ability to utilize non-competitive feeds among others [2,3,1]. Rabbit, being non-ruminant herbivores can be raised on forages and cereal by-products based diet. In rabbits' nutrition, forages plays roles in increasing the fibre content of the diet to the required level and supply some vitamins [4] and protein [5]. Several tropical forages had been used successfully in rabbit nutrition [6,1,7]; most of these forages are green throughout the year [8]. At present, more attention is being given to tropical plants of medicinal values and economic importance in monogastric animal production [9] among which is pawpaw. Pawpaw (*Carica papaya* L), belongs to family of Caricaceae. It is a self pollinated herbaceous succulent plant which produces a pear shaped fruit [10]. Pawpaw contains a broad spectrum of phytochemicals (enzymes in latex and alkaloids and phenols in leaves). Furthermore, papain, the cysteine protease in pawpaw possesses protein digestion and utilization enhancing property [11]. Anti-nutritional factors play vital role in determining the use of herbs or plants in animal nutrition [12]. Therefore, the presence of anti-nutritional factors (secondary metabolites) in pawpaw leaves may pose a problem to the effective utilization of nutrients and undesirable physiological and biological alterations of hematological and serum metabolite indices when pawpaw leaves is used in reasonable large quantity in rabbit diets. The use of exogenous enzymes to remove the effect of anti-nutritional factors and improve the availability of nutrients has been earlier reported [13,7]. In addition, there could be positive interactive effects of the dietary exo-enzyme and pawpaw leaf meal on the physiological status of the consuming animals. Since the heamatological and serum metabolites indices values depict the physiological responses of animals to nutrition and the study of effect of dietary leaf meals as protein source and exo-enzyme supplementation on blood indices are relatively low; this study therefore aimed at ascertaining the effect of pawpaw leaf meal and enzyme supplementation on hematological and serological indices of rabbits.

2. MATERIALS AND METHODS

2.1 Experimental Site

The pawpaw leaf collection and feeding trial was carried out at the Teaching and Research Farm of Agricultural Technology Department, The Federal Polytechnic, Ado Ekiti, Nigeria. The site is situated at about 437 mm above sea level with latitudes of 7°37´N and 7°12´N and longitudes 5°11´E and 5°31´E; mean rainfall of 1247mm and mean temperature of 26.2°C.

2.2 Experimental Diets

Fresh pawpaw leaves were chopped into smaller pieces, spread lightly to air-dry under a shed for 14 days, and sun-dried for 2 hour just before being hammer milled with 2mm screen hammer mill to form pawpaw leaf meal (PLM) and analyzed for proximate composition [14], phytate [15], oxalate [16], saponin and tannin [17]. Chemical composition of PLM is shown in Table 1. The exogenous multi-enzyme used in this study was produced by Bioproton PTY Limited. Brisbane Qld. Australia. Is composed of alpha amylase/*Bacillus subtilis* (400 µ/g), beta-Glucanase/*Trichoderma longibrachiatum* (700

Bioproton bu/g), phytase/*Aspergillus niger* (130 µ/g), cellulase/*Trichoderma longibrachiatum* (6,000 Bioproton µ/g), zylanase/*Trichoderma longibrachiatum* (10,000 Bioproton X µ/g), protease/*Aspergillus niger* (700 µ/g). Four experimental diets were formulated and designated as diet 1 (0%-E), diet 2 (0%+E); diet 3 (10%-E) and diet 4 (10%+E). Diets 1 and 3 were not supplemented with enzyme but had PLM inclusion at 0 and 10% level respectively, while diets 2 and 4 were enzyme supplemented at the rate of 0.5 g/kg but had PLM inclusion at 0 and 10% level respectively. The diets were thereafter pelletized (4 mm diameter and 8 mm long) and analyzed for chemical composition (Table 2).

Table 1. Chemical composition of pawpaw leaf meal

Parameters	Quantity (%)
Crude protein	26.14
Ash	16.44
Crude fibre	16.05
Ether extract	5.40
Energy (MJ/100g)	1135.98
Phytate	2.03
Flavonoid	6.21
Saponin	1.98
Tannin	0.001

2.3 Animals

The recommendations and guidelines for applied nutrition experiments in rabbits [18] were followed in the management of these experimental rabbits. One hundred and twenty (120) healthy, 35 day old weaner rabbits of equal sex and cross breed (Chinchilla x New-Zealand white) were randomly allotted to four dietary treatments (30 rabbits/treatment; 3 rabbits/replicate). The rabbits were raised in galvanized wire meshed cages placed in a well ventilated pen and fed *ad libitum* with their respective experimental diets for 56 days.

2.4 Blood Collection, Hematological and Biochemical Analyses

On day 56, twenty (20) rabbits were randomly selected from each treatment and bled as described by Burnett et al. [19]. About 7 milliliters of blood were collected from the marginal ear vein of each rabbit and thereafter divided equally into plain bottle (without anticoagulant) and potassium EDTA bottle for biochemical and hematological studies respectively. On the collection day, hematologicalindices (white blood cells, lymphocytes, monocytes, granulocytes, red blood cells, haemoglobin concentration, packed cell volume, mean cell volume, mean cell haemoglobin, mean cell hemoglobin concentration and platelets) were determined by

Table 2. Composition of the experimental diets

Ingredients (%)	Level of pawpaw leaf meal (PLM) ± enzyme			
	Diet 1 (0%-E)	Diet 2 (0%+E)	Diet 3 (10%-E)	Diet 4 (10%+E)
Maize	36.5	36.5	31.45	31.45
Maize husk	24.00	24.00	22.00	22.00
Soya bean meal	17.84	17.84	13.84	13.84
Pawpaw Leaf meal	0.00	0.00	10.00	10.00
Enzyme (E)	0.00	0.05	0.00	0.05
Wheat offals	13.91	13.86	14.46	14.41
Rice bran	5.40	5.40	5.40	5.40
Bone	1.00	1.00	1.00	1.00
Premix	0.25	0.25	0.25	0.25
Methionine	0.2	0.2	0.2	0.2
Lysine	0.15	0.15	0.15	0.15
Vegetable oil	0.5	0.5	1.00	1.00
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Determined analysis				
Crude protein (%)	15.01	15.4	15.09	15.04
Crude fibre (%)	11.06	11.08	11.12	11.13
*ME (Kcal/kg)	2589.75	2589.75	2596.7	2596.7

**Calculated analysis*

Shenzhen Mind ray Auto Haematology Analyzer, Model Bc-3200 (Shenzhen Mind Ray Biomedical Electronic Co. Hamburg 20537, Germany) while Refloctron® Plus 8C79 (Roche Diagnostic, GonbH Mahmheim, Germany), using kits was used to determine the serum biochemical parameters (creatinine, urea, cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, aspartate aminotransferase, alkaline aminotransferase, bilirubin and alkaline phosphate).

2.5 Statistical Analysis

All data collected during the experiment were subjected to analysis of variance from General Linear Model procedures using SPSS version 20 while means were separated using Duncan's multiple range test from the same software package.

3. RESULTS AND DISCUSSION

The proximate composition and gross energy recorded for pawpaw leaf meal (Table 1) in this study revealed PLM as a potential source of protein and energy in monogastric nutrition. Hematologicalindices are useful indicators in determining the status of animals nutritionally, pathologically and physiologically [20]. In this study, the values for the hematologicalindices fall within the normal range [21] and most of these indices (monocytes, granulocytes, red blood cells, haemoglobin concentrate, packed cell volume, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration) were not affected ($P>0.05$) by dietary enzyme and PLM (Table 3). This implies that these treatments (dietary enzyme and PLM) support the normal physiological functions of the rabbits. Lymphocytes are types of white blood cells that play important role in immune system. In this study, dietary enzyme significantly ($P<0.05$) increased the lymphocyte value. There exists a strong relationship between nutrition and lymphocytes. In particular, mineral (zinc), some vitamins (vitamin A and B6) and protein helps lymphocytes health and function, while low dietary protein reduces lymphocyte cell count and hinders formation of antibodies. Dietary enzyme might have promoted the increase in lymphocyte cell count in this study by breaking down the anti-nutrients that bind the essential nutrients (Zn, vitamins A, B6) and promote their release for proper lymphocytes development in the rabbits. Increased mineral digestibility as a result of enzyme supplementation was reported by Asmare [22]. Pawpaw leaf meal (PLM) inclusion significantly ($P<0.05$) increased the white blood cells (WBC) and lymphocytes. This agreed with earlier report of Oloruntola et al. [20] and Dashputre et al. [23]. It is therefore suggested that dietary PLM is capable of exerting immunodulatory effect by supporting increased production of WBC. PLM contains papain, an effective natural protein digestive aid [24]. Since dietary protein has effect on WBC and differential cell, the increased WBC and lymphocytes observed in relation to dietary PLM in this study might also be associated to enhanced protein utilization due to action of the proteolytic enzyme present in PLM. However, this fact has to be proven in a digestibility trial to assess the effect of dietary PLM on protein digestibility. The reduced ($P<0.05$) platelet value recorded in the rabbits due to dietary PLM in this study agreed with earlier report of McEwen [25] where reduced platelet aggregation was linked to some fruits and vegetables. Although accelerated platelet loss, decreased platelets production, abnormal platelet distribution and dilution platelet loss was reported by Lording and Friend [26] as major causes of reduced platelet values. The observed reduced platelets recorded in this study in association to dietary PLM may not be due to any physiological disorder but rather due to a constituent of PLM. For instance, feeds high in omega-3-polyunsaturated fatty acids reduce platelets aggregation [25]. However, this hypothesis needs to be proved further, to ascertain the main constituents of PLM responsible for this observed platelets reduction in the rabbits. The significant ($P<0.05$) interactive effect of dietary enzyme and PLM in this study for WBC, monocytes, red blood cells, haemoglobin concentration, mean cell volume and mean cell haemoglobin concentration (Table 3) suggests that the combined activities of these two factors (dietary enzyme and PLM) could be harnessed to promote normal production and function of those hematologicalindices in rabbits. Table 4 shows the effect of exo-enzyme and dietary pawpaw leaf meal on serum biochemical indices of the rabbits. Some enzymes are tissues specific and increase in their concentration in the blood indicates some kind of tissue damage. The values for the serum biochemicals determined in this study are within the normal range [19,21,20]. Urea, alanine aminotransferase (ALT) and amylase reduced ($P<0.05$) with the dietary enzyme supplementation. Elevated blood urea and amylase are associated with renal impairment while increased values of ALT indicate liver problems [26]. Therefore, reduction

Table 3. Effect of exo-enzyme and dietary pawpaw leaf meal (PLM) on hematological indices of rabbits

Enzyme (g/kg)	PLM (%)	WBC ($x10^9$/l)	LYM ($x10^9$/l)	MON ($x10^9$/l)	GRA ($x10^9$/l)	RBC ($x10^9$/l)	HBC (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	PLA ($x10^9$/l)
0.00		8.61	3.22^b	1.09	4.28	5.96	12.80	39.22	65.75	21.42	32.55	226.25
0.05		9.61	4.66^a	1.36	3.58	6.09	13.22	40.84	67.00	21.67	32.35	246.75
SEM		0.48	0.42	0.09	0.37	0.11	0.14	0.72	0.66	0.18	0.25	22.22
P value		0.17	0.04	0.06	0.22	0.44	0.07	0.14	0.22	0.35	0.58	0.53
	0	8.10^b	3.18^b	1.27	3.64	6.11	13.07	40.77	66.75	21.40	32.00	272.75^a
	10	10.12^a	4.71^a	1.18	4.22	5.95	12.95	39.31	66.00	21.70	32.90	200.25^b
	SEM	0.48	0.42	0.09	0.37	0.11	0.14	0.72	0.66	0.18	0.25	22.22
	P value	0.02	0.03	0.46	0.31	0.36	0.56	0.19	0.45	0.27	0.32	0.05
Enzyme x PLM												
SEM		0.67	0.59	0.12	0.53	0.16	0.21	1.02	0.95	0.25	0.34	31.43
P value		0.03	0.09	0.01	0.84	0.03	0.05	0.15	0.05	0.02	0.78	0.69

Means with different superscripts in the same column are significantly ($p<0.05$).
WBC: White blood cells; LYM: Lymphocytes; MON: Monocytes;
GRA: Granulocytes, RBC: Red blood cells; HBC: Haemoglobin concentration;
PCV: Packed cell volume; MCV: Mean cell haemoglobin;
MCHC: Mean cell haemoglobin concentration; PLA: Platelets.

Table 4. Effect of exo-enzyme and dietary pawpaw leaf meal (PLM) on serum biochemical indices of rabbits

Enzyme (g/kg)	PLM (%)	CRE (µmol/l)	UREA (mmol/l)	CHOL (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	TRI (mmol/l)	AST (u/l)	ALT (u/l)	BIL (µmol/l)	AMY (u/l)	GLU (mmol/l)
.0.00		89.62	7.21[a]	2.92	0.78	1.95	1.40	92.05	109.45[a]	10.47	896.91[a]	7.99
0.05		101.55	6.73[b]	2.63	0.98	1.35	1.49	68.70	79.05[b]	11.56	688.83[b]	8.61
SEM		5.75	0.14	0.26	0.07	0.13	0.15	7.56	8.64	0.86	50.12	0.59
P value		0.18	0.05	0.44	0.08	0.15	0.69	0.06	0.04	0.40	0.02	0.48
	0	94.50	7.15	3.51[a]	0.89	1.91[a]	1.51	89.50	111.50[a]	12.36	960.58[a]	7.93
	10	96.67	6.79	2.04[b]	0.86	1.38[b]	1.37	71.25	77.00[b]	9.67	625.16[b]	8.67
	SEM	5.75	0.14	0.26	0.07	0.13	0.15	7.56	8.64	0.86	50.12	0.59
	P value	0.79	0.11	0.01	0.75	0.03	0.53	0.13	0.02	0.06	0.01	0.41
Enzyme x PLM												
SEM		8.14	0.20	0.36	0.09	0.19	0.21	10.70	12.23	1.22	70.88	0.84
P value		0.13	0.27	0.41	0.64	0.21	0.66	0.49	0.51	0.68	0.09	0.29

Means with different superscripts in the same column are significantly ($p<0.05$).
CRE: Creatinine; CHOl: Cholesterol; HDL: High density lipoprotein,
LDL: High density lipoprotein; TRI: Triglyceride; AST: Aspartate aminotransferase;
ALT: Alanine aminotransferase, BIL: Bilirubin,
ALP: Alkaline phosphate; Amylase: Amylase; GLU: Glucose.

of these biochemical indices suggests the support of dietary enzyme for normal health of the rabbits. Cholesterol is the principal sterol synthesized by all animals and composed of about 30% of all animal cell membranes. Reduction of blood cholesterol due to dietary PLM in this study agreed with Oloruntola et al. [20] who reported decreased cholesterol level in rabbits due to dietary Alchornea leaf meal. Decreased cholesterol values have been associated with decreased uptake of cholesterol, low fat diet and intestinal mal-absorption Lording and Friend (1991). Reduction of cholesterol level recorded in this study may be as a result of activities of some secondary metabolites present in PLM which exert reducing effect on cholesterol uptake in the intestine. Capability of saponin, one of the anti-nutrients present in PLM in reducing absorption of cholesterol through intra-lumenal physiochemical interaction was reported [27]. Low density lipoprotein which is otherwise known as bad cholesterol collects in the blood vessels walls, narrows or blocks the blood vessel lumen and increased risk of atherosclerosis and heart disease. Therefore, reduced low density lipoprotein in rabbits due to dietary PLM in this study is of health benefit and support the wholesomeness of this leaf meal in rabbits' nutrition. Decreased values of alanine amino transferase as observed in this study due to dietary PLM are not clinically significant or important. This is of health benefit because increased alanine aminotransferase values indicate various degrees of hepatic problems [26]. Serum amylase test is used for diagnosing pancreatic or mucosal diseases and decreased serum amylase value as recorded in this study due to dietary PLM is not of clinical importance except in fulminant cases of pancreatic necrosis with thrombosis of pancreatic vessels. However, the observed decreased in serum amylase may not be pathological since its values falls within the normal range (167-315 u/l) reported by Latimer et al. [28] for healthy rabbits.

4. CONCLUSION

The pawpaw leaf meal at 10% inclusion and exo-enzyme inclusion did not pose any threat to the normal health of the rabbits. Furthermore, the blood cholesterol reduction could be achieved in rabbits by dietary 10% PLM.

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 Agricultural Technology, The Federal Polytechnic, Ado Ekiti, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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