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Effect of *Alchorneal cordifolia* leaf meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits

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Abstract

The chemical compositions of *Alchornea cordifolia* leaf meal (ACLM) were determined. Thereafter, the effect of ACLM inclusion with enzyme supplementation on rabbits' growth and health status was carried out in an eight weeks feeding trial. Six experimental diets were arranged in a 3x2 factorial arrangement i.e. 3 levels of ACLM inclusion (0, 50, and 100g/kg in place of palm kernel cake) combined with 2 levels of enzyme supplementation (0 and 0.35 g/kg). One hundred and eighty, five-week old rabbits were randomly allotted to six dietary treatments (10 replicate/treatment, 3 rabbits/replicate). The ACLM contained crude protein (180g/kg), crude fibre (129 g/kg), ash (12.8 g/kg), ether extract (41.2g/kg), nitrogen free extract (433g/kg), cardiac glycosides (0.14 mg/g), saponin (1.98 mg/g), phenols (1.21mg/g), phytate (1.08 mg/g), oxalate (1.30 mg/g), hydrogen cyanide (0.18 mg/g) and tannin (0.57mg/g). Potassium (76.00 mg/100g) was the most abundant mineral, while calcium, magnesium, iron, copper, zinc and manganese were also detected. Relatively, the weekly growth rate depression was recorded in diet 3 (10-E) throughout the feeding trial period. White blood cells, lymphocytes, monocytes and granulocytes decreased significantly ($P<0.05$) with increased ACLM levels. The urea, creatinine, cholesterol, bilirubin and serum glutamic oxalo acetic transaminase reduced ($P<0.05$) with increased ACLM level.

Key words : Alchornea, health status, enzyme, rabbits, serum, blood, leaf meal

INTRODUCTION

High cost of livestock production often precipitated mainly by high cost of finished feed is one of the major causes of animal protein shortage in developing countries [1]. This could be attributed to scarcity of plant protein and energy concentrates. Therefore, reduction of the total production cost through the replacement of the expensive conventional feed stuffs by the unconventional ones that are not of high or any demand by human had been recently advocated [2]. Leaf meals are one of the suitable replacements for some conventional feedstuffs, and in particular, *Alchornea cordifolia* leaf meal (ACLM) is among the leaf meals that could be used as feed alternatives for commercial livestock production in the tropics. *Alchornea cordifolia* leaf meal has protein content, which range from 12.66 to 17.94 g/100g, fibre content of 12.86 g/100g and minerals such as calcium, magnesium, potassium, cobalt, manganese, iron, and copper [3]. In addition, *Alchornea cordifolia* leaf has antibacterial property [4]; this may promote its use as phytobiotic growth promoter or alternative to antibiotic growth promoter from plant source when incorporated in animals' diets. In addition, the capability of some leaf meals to stimulate the immune system; in particular the innate immunity which could be beneficial in immunotherapy had been reported [5]. Generally, the major constraints to the utilization of leaf meals as protein source in monogastrics are high fibre contents and presence of anti-nutritional factors. However, enzyme supplementation had been reported to improve fibre digestibility, remove the effect of anti-nutritional factors and improve nutrient availability [6] and feed conversion ratio [7] in rabbits, although the use of exogenous enzyme has not been extensively studies in rabbits when compared to poultry [8]. Previous studies had shown that the use of some legumes as protein source could cause deviations from the normal physiological conditions and reflect in the blood and serum indices of the animals [9]. There is paucity of information on the effect of *Alchornea cordifolia* leaf meal on growth pattern, haematological paramters, immunodulatory response and serum biochemicals of rabbits. Thus, this study was conducted to analyse *Alchornea cordifolia* leaf meal and to assess the effect of its inclusion with exogenous enzyme supplementation on growth and health status of growing rabbits.

MATERIALS AND METHODS

Alchornea cordifolia leaf collection, processing and chemical analysis

Fresh *Alchornea cordifolia* leaves were plucked, chopped, air-dried under a shed and thereafter milled with hammer mill to produce *Alchornea cordifolia* leaf meal (ACLM). The ACLM was analysed for proximate composition [10]. Cardiac glycosides, saponin, phenols and tannin were determined [11]. Oxalate, phytate and cyanide contents were also determined [12]. The mineral compositions (K, Ca, Mg, Fe, Cu, Zn, Mn and Co) of ACLM were determined using atomic absorption spectro-photometer (model: 210 VGP. Buck Scientific. USA). The gross energy was determined against thermo-chemical grade benzoic acid using combustion calorimeter (Model:e2k combustion calorimeter, www.cal2k.com). Each sample was analysed thrice.

Exogenous enzyme and experimental diet

The exogenous enzyme used in this study contains a minimum of α-amylase (400μ/g), β-glucanase (700μ/g), phytase (130μ/g), cellulase (6,000μ/g), zylanase (10,000 μ/g), and protease (700μ/g). Six experimental diets were formulated and designated

Table 1: Chemical composition of *Alchornea cordifolia* leaf meal

Proximate Composition and Energy		Phytochemicals and anti-nutrients		Minerals	
Parameters	g/kg	Parameters	mg/g	Parameters	mg/g
Crude protein	180	Cardiac glycosides	0.14	Potassium	76.00
Crude fibre	129	Saponins	1.98	Calcium	25.30
Ash	128	Phenols	1.21	Magnesium	21.70
Ether extract	41.2	Phytate	1.08	Iron	18.96
Nitrogen free extract	433	Oxalate	1.30	Copper	35.30
Organic matter	872	Hydrogen cyanide	0.18	Zinc	12.00
Energy (MJ/100g)	1194.54	Tannin	0.57	Manganese	6.12

as diet 1 (0-E), diet 2 (5-E) and diet 3 (10-E) for the diets without enzyme but with 0, 50 and 100 g/kg ACLM levels, in place of palm kernel cake respectively, while diet 4 (0+E), diet 5 (5+E) and diet 6 (10+E) were enzyme supplemented and had 0, 50 and 100 g/kg ACLM inclusion respectively. The diets were thereafter pelletised (4mm diameter and 8 mm long) and fed for 8 weeks to their respective treatments. The experimental diets' compositions are reported in table 3. The experiment was carried out in a 2x3 factorial arrangement in a completely randomized design comprising 2 enzyme levels (0 and 0.35 g/kg) and 3 ACLM inclusion levels (0, 50 and 100g/kg).

Animals

Permission for the use of animal and animal protocol was obtained from the Research and Ethics Committee of the Federal Polytechnic, Ado-Ekiti, Nigeria. The recommendations of Fernandez-Carmona [13], for applied nutrition experiments in rabbits were followed in the management of the rabbits. One hundred and eighty healthy, five-week old weaner rabbits of cross-breeds (New-Zealand white and Chinchilla) of equal sexes and weighing 707±56 g were randomly allotted to six dietary treatments (10 replicates/treatment, 3 rabbits/replicate). The rabbits were fed *ad libitum* in a wire-mesh cage arranged in a well ventilated pen.

Collection of data/samples and analytical methods

The weights of the rabbits were determined on weekly basis to determine the weekly growth pattern. At the end of 8 weeks, blood samples were collected from the marginal vein of the dorsal surface of pinna of fifteen randomly selected rabbits from each treatment with syringe and needle. On the day of collection, the blood indices were accessed by Shenzhen Mind ray Auto Haematology Analyzer, Model Bc-3200 (Shenzhen Mind ray Biomedical Electronics Co. Hamburg 20537, Germany) while the serum was analyzed for serum biochemical parameters with a Reflectron® Plus 8C79 (Roche Diagnostic, GonbH Mahnheim, Germany), using commercial kits.

Statistical analysis

Data were analyzed using General Linear Model procedure of the SPSS version 20 for complete randomized design with 2x3 factorial arrangements of treatment. The data were tested for the main effects and their interactions while statistical significance was assessed at ($P<0.05$).

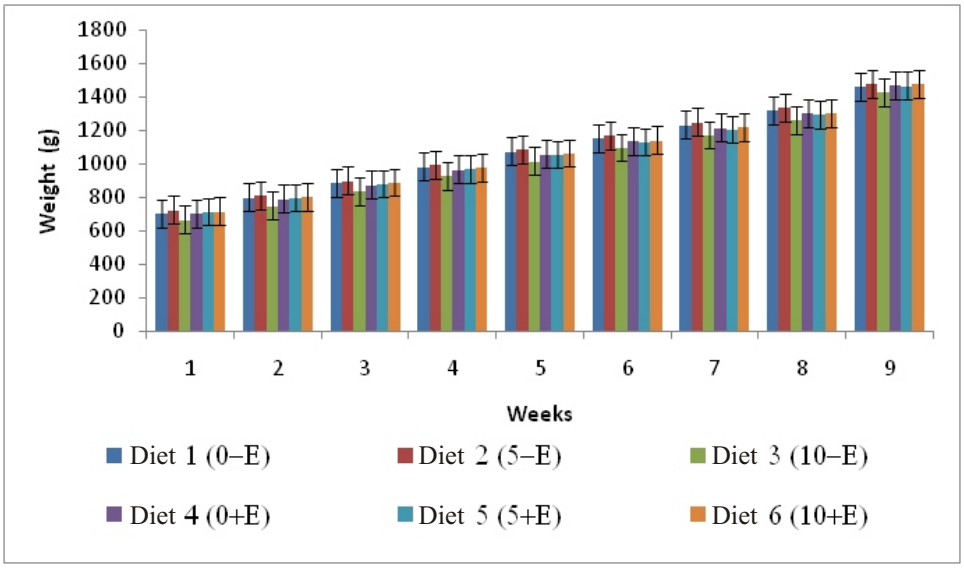

Figure 1: Growth pattern of rabbits on different levels of *Alcornea cordifolia* leaf meal with enzyme supplementation

Table 2: Composition of experimental diets

Ingredients (g/kg)	Level of *A. cordifolia* leaf meal (g/kg) and enzyme (g/kg)					
	Diet 1 (0-E)	Diet 2 (50-E)	Diet 3 (100-E)	Diet 4 (0+E)	Diet 5 (50+E)	Diet 6 (100+E)
Maize	60	60	60	60	60	60
Cassava peel	290	290	290	290	290	290
ACLM	0	50	100	0	50	100
Soybean meal	108.5	108.5	108.5	108.5	108.5	108.5
GNC	75	75	75	75	75	75
PKC	120	70	20	120	70	20
Wheat offals	70	70	70	70	70	70
Molasses	30	30	30	30	30	30
Veg. oil	15	15	15	15	15	15
Rice bran	154	154	154	154	154	154
Maize husk	60	60	60	60	60	60
Bone meal	10	10	10	10	10	10
Premix	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	1.5	1.5	1.5	1.5	1.5	1.5
Lysine	1	1	1	1	1	1
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Determined analysis						
Crude protein (g/kg)	162.6	162.7	162.9	162.8	162.9	163.1
Crude fat (g/kg)	22.3	22.2	22.1	22.0	21.9	22.0
Crude fibre (g/kg)	115.1	115.9	117.1	115.0	116.1	117.3
Energy (MJ/kg)	11.29	11.54	11.88	11.29	11.54	11.29
Neutral detergent fibre (%)	38.6	39.3	39.4	39.6	39.3	39.4
Acid detergent fibre (%)	19.7	49.8	49.9	19.7	19.8	19.9

ACLM: *Alchornea cordifolia* leaf meal; GNC: Ground nut cake; PKC: Palm kernel cake

RESULTS

Table 1 shows the proximate composition, phytochemicals, antinutrients and caloric value of *Alchornea cordifolia* leaf meal (ACLM). The ACLM had crude protein (180g/kg), crude fibre (129g/kg), ash (12.8g/kg), ether extract (41.2g/kg) and nitrogen free extract (433g/kg) ; while cardiac glycosides (0.14 mg/g), saponins (1.98 mg/g), phenols (1.21mg/g), phytate (1.08 mg/g), oxalate (1.30 mg/g), hydrogen cyanide (0.18 mg/g) and tannins (0.57 mg/g) were detected. Analysis of the leaf meal showed that potassium (76.00 mg/100g) was the most abundant mineral, while other minerals such as calcium (25.3 mg/100g), magnesium (21.70 mg/100g), iron (18.96 mg/100g), copper (35.30 mg/100g), zinc (12.00 mg/100g) and manganese (6.12 mg/100g) were also detected. The caloric value of ACLM (1194.54 MJ/100G) was reported for ACLM. The gross compositions of the experimental diets are presented in Table 2. The rabbits' growth in all diets increased across the period of the experiment. However, the weekly growth rate depression was recorded in diet 3 (10-E) throughout the period of the feeding trial (Figure 1). Enzyme supplementation and ACLM inclusion were not significant ($P>0.05$) for packed cell volume, red blood cell, hematological indices and platelets of the experimental rabbits (Table 3). The white blood cells, lymphocytes, monocytes and granulocytes increased significantly ($P<0.05$) with increase in level of ACLM inclusion from 0 to 100g/kg (Table 4). Table 5 shows that the enzyme supplementation have significant ($P<0.05$) effect on serum glutamic oxalo-acetic transaminase (SGOT), and serum glutamate pyruvate transaminase (SGPT) while the ACLM inclusion significantly ($P<0.05$) reduced urea, creatinine, cholesterol, bilirubin, SGOT, and SGPT. However, the interaction of enzyme and ACLM inclusion was not significant ($P>0.05$) except for SGOT.

DISCUSSION

The crude protein and crude fibre quantities of ACLM could

Table 3: Effect of commercial enzyme supplementation and graded levels of *Alcornea cordifolia* leaf meal (ACLM) on packed cell volume, red blood cells, haematological indices and platelets of weaner rabbits

Enzyme (g/kg)	ACLM (g/kg)	PCV	RBC	M CH	MCV	MCHC	HBC	PLA
0.00		39.88	5.44	22.29	66.06	32.82	13.14	359.10
0.35		39.44	5.31	22.18	65.75	32.77	13.42	358.97
SEM		1.05	0.13	0.25	0.72	0.76	0.64	34.83
P value		0.77	0.49	0.78	0.76	0.95	0.76	0.99
	0	39.64	5.52	22.16	66.10	33.33	13.58	366.33
	50	40.08	5.21	22.02	64.27	32.45	13.29	343.12
	100	39.26	5.39	22.53	67.35	32.61	12.96	367.65
	SEM	1.28	0.15	0.31	0.88	0.93	0.78	42.65
	P value	0.90	0.85	0.50	0.08	0.78	0.85	0.90
Enzyme x ACLM								
SEM		1.81	1.11	0.44	1.25	1.33	1.11	60.32
P value		0.81	0.97	0.76	0.85	0.87	0.75	0.97

Means with different superscripts in the same column are significantly ($p<0.05$).
PCV: Packed cell volume (%); RBC: Red blood cells ($x10^{12}$/l); MCH: Mean cell haemoglobin (pg); MCV: Mean cell volume (fl); MCHC: Mean cell haemoglobin concentration (g/dl); HBC: Haemoglobin conc. (g/dl); PLA: Platelets ($x10^{9}$/l).

Table 4: Effect of commercial enzyme supplementation and graded levels of *Alcornea cordifolia* leaf meal (ACLM) on total white blood cell count and the differential count of weaner rabbits

Enzyme (g/kg)	ACLM (g/kg)	White Blood Cells ($x10^{9}$/l)	Lymphocytes ($x10^{9}$/l)	Monocytes ($x10^{9}$/l)	Granulocytes ($x10^{9}$/l)
0.00		5.35	3.18	0.80	2.38
0.35		5.33	3.13	0.83	2.32
SEM		0.39	0.31	0.11	0.30
P value		0.97	0.91	0.87	0.89
	0	5.00^{b}	2.17^{b}	0.55^{c}	2.26^{ab}
	50	4.34^{b}	1.97^{b}	0.73^{b}	1.58^{b}
	100	6.70^{a}	5.32^{a}	1.16^{a}	3.21^{a}
	SEM	0.48	0.38	0.13	0.37
	P value	0.01	0.00	0.02	0.03
Enzyme x ACLM					
SEM		0.67	0.54	0.19	0.52
P value		0.99	0.99	0.94	0.99

Means with different superscripts in the same column are significant ($p<0.05$).

contribute positively to the 13.4-23.2 % crude protein and 12.2-24.44% crude fibre requirement of the rabbits [14]. The presence of some phytochemicals; which are of pharmacological importance in ACLM further shows its suitability in rabbit production. For example, cardiac glycosides functions as stimulants in case of cardiac failure, saponin when consumed at moderate rate have been reported to demonstrate hypocholesterolemic and anticarcinogenic properties [15]; while phenols are known to possess anti inflammatory, anti bacterial, antiviral, antioxidant, anticarcinogenic and anti-allergic activities [16]. However, the presence of some anti-nutritional factors in ACLM could slightly decrease the overall benefits of this plant. However, it had been reported that the anti-nutritional factors level of ACLM is relatively low when compared to most other vegetables [3], meaning that the overall nutritional value of ACLM may not be negatively affected. The detection of some minerals in ACLM in this study indicated that it may complement minerals from other feed sources and ultimately meet the mineral requirements of the rabbits for optimal performance and wellbeing. The energy content of ACLM (1194.54 MJ/100G) revealed its suitability to contribute to the supply of 1319 KJ/day total energy required by growing rabbits [17]. The crude protein, crude fiber and fat content of the experimental diets in this study falls within the range of 16, 10-12 and 2-3 g/100g recommended for growing rabbits by NRC [18].

The relative depression of rabbits' weekly growth rate in diet

Table 5: Effect of commercial enzyme supplementation and graded levels of *Alcornea cordifolia* leaf meal (ACLM) on serum metabolites of weaner rabbits

Enzyme (g/kg)	ALM (g/100g)	TP	AL	GLO	URE	CRE	CHO	BIL	SGOT	SGPT	ALP	GLU
0.00		5.68	3.84	1.83	16.21	0.99	52.20	0.62	77.10[a]	84.85[a]	144.08	122.78
0.35		5.38	3.58	1.79	15.66	0.84	52.73	0.58	70.94[b]	77.77[b]	145.61	111.03
SEM		0.15	0.12	0.07	1.65	0.51	1.81	0.02	1.96	2.08	15.57	8.75
P Value		0.18	0.94	0.68	0.82	0.59	0.83	0.15	0.05	0.03	0.94	0.36
	0	5.36	3.52	1.84	21.05[a]	1.12[a]	58.40[a]	0.66[a]	83.50[a]	95.91[a]	121.22	138.66
	5	5.48	3.69	1.78	14.14[b]	0.81[b]	49.10[b]	0.65[a]	67.75[b]	73.61[b]	150.66	102.69
	10	5.75	3.93	1.82	12.60[b]	0.64[b]	49.90[b]	0.50[b]	70.81[b]	74.40[b]	162.66	109.36
	SEM	0.18	0.15	0.08	2.02	0.06	2.22	0.02	2.40	2.54	19.07	10.72
	P value	0.35	0.19	0.89	0.02	0.00	0.02	0.00	0.00	0.00	0.32	0.07
Enzyme x ALM												
SEM		0.26	0.21	0.12	2.86	0.08	3.13	0.03	3.40	3.60	26.97	15.16
P Value		0.38	0.07	0.40	0.99	0.26	0.94	0.81	0.02	0.77	0.88	0.92

Means with different superscripts in the same column are significantly different ($p<0.05$).
TP: Total protein (g/l); AL: Albumin (g/l); GLO: Globulin (g/l); CHO: Cholesterol (mg/dl); URE: Urea (mg/dl); CRE: Creatinine (mg/dl); (mg/dl); BIL: Bilirubin (µ/l); SGOT: Serum glutamate oxalato acetate transaminase (µ/l); SGPT: Serum glutamate pyruvate transaminase (µ/l); ALP: Alkaline phosphate (µ/l); GLU: Glucose (mg/dl).

3 (10-E) throughout the period of the feeding trial when compared to other diets in this study may be as a result of possible increase in levels of anti-nutrients being precipitate by increase in ACLM inclusion levels as anti-nutrients such as tannin, when present in high levels has been reported to cause deleterious effects on growth of animals [19]. For instance, in this study, the inclusion of 50g/kg ACLM supplied 2.9 g tannin/kg diet, which increased to 5.7 g tannin/kg diet at 100g/kg ACLM inclusion. Blood indices are usually used as indicator for determining the nutritional, physiological and pathological status of animals. In this study, the haematological parameters (WBC, monocyte, lymphocytes, RBC, PCV, haemoglobin concentration, MCV, MCH and MCHC) determined falls within the normal range (5-12 $x10^9$/l, 0-1.8 $x10^9$/l, 2-20 $x10^9$/l, 4.7-7.2 $x10^{12}$/l, 33-50%, 9.4-17.4 g/dl, 50-75 fl, 16-23 pg and 280-360 g/l respectively) reported by Flecknell [20] for rabbits. The WBC and its differentials are involved in fighting of infections, defending the body against foreign organisms' invasion and production and transportation/distribution of antibodies. Therefore, animals with low WBC are exposed to high risk of disease infection while decreased lymphocytes are linked to stress, debilitating disease, trauma, shock, fever and viral injury [21]. Although there were increase in values of WBC, lymphocytes, monocytes and granulocytes with increase in ACLM inclusion rate, this may not have any negative impact on the health status of the rabbits as all values of these haematological parameters are within the normal range reported by Flecknell [20] for rabbits. These observations suggest that ACLM inclusion enhanced the principal function of phagocytes, which is to defend against the invading microorganisms by ingesting and destroying them [22]. This further supports the earlier report that some herbs are capable of stimulating the immune system [23]. Enzymes are present within the cells and catalyze the reactions for which the cells are responsible. Therefore, these enzymes are leaked into the surrounding fluids and finally into the blood whenever their cells are damaged or destroyed. Urea is a product of protein catabolism whose production site is the liver. Advanced liver disease, decreased urea synthesis, protein malnutrition and prolonged anorexia with glucose therapy are among the cause of decreased urea values [21]. However, reduced urea with increased ACLM inclusion level in this study may not have precipitated any health hazard because the urea range (12.60-21.05 mg/dl) in this study falls within normal range [24]. In addition, the decrease in creatinnine, SGOT and SGPT values with increase in ACLM inclusion level in this study further support the safety of the inclusion of this leaf meal in rabbit ration up 100 g/kg because, not only is decrease in the values of these parameters beneficial or not clinically significant [24], their values falls within the normal range (0.5-2.5 mg/dl, 14-113 µ/l and 48-80 µ/l) reported for creatinnine, SGOT and SGPT respectively by Flecknell [20] and Latimer et al. [24]. Abnormally increased cholesterol value in animals is an indication of increased fat mobilization, decreased fat catabolism, starvation, diabetes mellitus, liver, diseases, and white muscle disease among others [21]. The observed decrease in cholesterol with increase in ACLM inclusion level suggests ACLM has negative effect on uptake of cholesterol by the rabbits. There is the possibility of leaves to contain components that exerted indirect inhibitory effects at the levels of hydroxymethylglutaryl-coenzyme A reductase, a key enzyme in cholesterol biosynthesis. The observed decrease in cholesterol level may also be linked to presence of saponins which has earlier been reported to reduce the uptake of cholesterol in the got

through intra-lumenal physiochemical interaction [19].These observations further support the suitability of ACLM in rabbit nutrition. Decreased bilirubin values have been linked to decreased red blood cell production [21]. In this study, the observed reduction of bilirubin value at 100g/kg ACLM level may not signal any health hazard because the bilirubin level of the rabbits are within the normal range (0.0-0.7 mg/dl) reported by Latimer et al. [24] while the RBC values were not significantly ($p>0.05$) affected by the ACLM inclusion.

CONCLUSION

It was concluded that *Alchornea cordifolia* leaf meal inclusion and exo-enzyme supplementation did not have negative effect on growth and the health status of rabbits.

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