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EFFECT OF *ALCHORNEA CORDIFOLIA* LEAF MEAL INCLUSION AND ENZYME SUPPLEMENTATION ON PERFORMANCE AND DIGESTIBILITY OF RABBITS

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Abstract: A feeding trial was conducted to study the performance, digestibility and health status of weaner rabbits fed diets including *Alchornea cordifolia* leaf meal (ALM: 18% crude protein [CP] and 12.9% crude fibre) and supplemented with a multi-enzyme additive (cellulase, xylanase, β-glucanase, α-amylase, protease, lipase). Six experimental diets were arranged factorially: 3 levels of ALM (0, 5 and 10% substituting palm kernel cake: 16.3% CP and 39.1% neutral detergent fibre) combined with 2 levels of enzyme supplementation (0 and 0.35 g/kg). One hundred and eighty healthy, 5-wk-old weaner rabbits of cross-breeds were randomly allotted to 6 dietary treatments (30 rabbits/treatment, 3 rabbits/replicate). Growth rate was not affected (P>0.05) by the main factors (exogenous enzyme and ALM inclusion) and their interactions (13.5 g/d on av.). Daily feed intake and feed conversion ratio decreased (P=0.01) with the ALM inclusion by 8%, but did not affect faecal digestibility. However, enzyme supplementation improved crude protein and crude fibre digestibility (P<0.001) by 6%. In conclusion, ALM inclusion and enzyme supplementation had no adverse effect on the performance and digestibility of rabbits.

Key Words: *Alchornea cordifolia*, enzyme supplementation, health status, performance, slaughter traits, rabbits.

INTRODUCTION

The use of non-conventional feed ingredients (e.g. leaf meals) in place of the relatively expensive conventional feeds for feeding monogastric animals is becoming popular in the tropics. This is primarily due to the erratic supply and high cost of conventional feedstuffs and secondarily because of rapid plant growth when water is available and the relatively low cost of these leaf meals, which are mainly from tropical legumes and browse plants (Agbede and Aletor, 2003). Therefore, leaf meals are included in livestock feed to offset the high cost of conventional protein sources and improve profit margin (Nodu *et al.,* 2014).

The *Alchornea cordifolia* shrub belongs to the family of Euphoribiaceace. It is found along the coastal areas of West Africa, in swampy and/or dry land (Kemeseiyefa *et al.,* 2014). *Alchornea cordifolia* has been reported to have antibacterial (Ajao *et al.,* 1985), antispasmodic (Ogungbamila and Samuelsson, 1990), anti-inflammatory (Osadebe and Okoye, 2003) and anti-diarrhoea (Agbor *et al.,* 2004) properties. However, the use of leaf meals as a protein source has constraints due to the presence of anti-nutritional factors (Tewe, 1991). The role of exogenous enzyme in decreasing mortality and improving the feed conversion ratio in rabbits has been reported (García-Ruiz *et al.,* 2006). Similarly, Gutiérrez *et al.* (2002) observed that fibre digestibility improved in rabbits supplemented with an enzyme pool (xylanase, β-glucanase, pectinase and amylase) supplementation. Exogenous enzymes also remove the effect of anti-nutritional factors and improve nutrient availability (Kiarie *et al.,* 2013). A multi-enzyme approach was reported to be a viable way to fully capture the nutritive value of feed by degrading the anti-nutritional factors.

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Therefore, this work was designed to investigate the effect of inclusion levels of *Alchornea cordifolia* leaf meal and enzyme supplementation and their interactions on performance and health status of growing rabbits.

MATERIALS AND METHODS

Experimental site

This feeding trial was carried out at the Rabbit Unit of the Teaching and Research Farm of the Agricultural Technology Department, The Federal Polytechnic, Ado-Ekiti. Nigeria. The site had a mean temperature of 29.2°C during the experiment.

Experimental diets

Alchornea cordifolia leaves harvested fresh in Ikere-Ekiti (Ekiti state, Nigeria) were chopped, spread lightly to air-dry under a shed for 1 wk and thereafter sun-dried for 2 h prior to milling with 2 mm screen hammer mill. The chemical composition of *Alchornea cordifolia* leaf meal (ALM) is shown in Table 1. The commercial exogenous enzyme (Natuzyme®), used in this study, according to manufacturer (Biproton, Australia), has a minimum of α-amylase/*Bacillus subtilis* (400 μ/g), β-glucanase/*Trichoderma longibrachiatum* (700 μ/g), phytase/*Aspergillus niger* (130 μ/g), cellulase/*Trichoderma longibrachiatum* (6000 μ/g), xylanase/*Trichoderma longibrachiatum* (10000 μ/g), protease/*Aspergillus niger* (700 μ/g). Six experimental diets were formulated to meet the requirements recommended by NRC (1977) and de Blas and Mateos (2010), and designated as 0-E (diet 1), 5-E (diet 2) and 10-E (diet 3) for the diets without enzyme and 0+E (diet 4), 5+E (diet 5) and 10+E (diet 6) for diets with enzyme. Diets 1 and 4 serve as positive and negative control respectively. The composition of experimental diets is shown in Table 2. The diets were thereafter pelleted (4 mm diameter and 8 mm long).

Animals

The rabbits were managed following the recommendations and guidelines for applied nutrition experiments in rabbits (Fernández-Carmona *et al.,* 2003). One hundred and eighty (180) healthy, 5 wk old weaner rabbit of cross-breeds (New-Zealand white and Chinchilla) of equal sexes and weighing 707±56 g were randomly allotted to 6 dietary treatments (30 replicates/treatment, 3 rabbits/replicate). The rabbits were housed in a 2-storey, wooden framed wire-mesh cage and housed in a well-ventilated (non-forced ventilation) pen. Experimental diets and water were offered *ad libitum* for a period of 8 wk.

Experimental procedure

Daily weight gain and feed intake were determined from 35 to 91 d of age. Nutrient digestibility was carried out from 49 to 53 d of age according to recommendations and guidelines for applied nutrition experiments in rabbits (Fernández-Carmona *et al.,* 2003). Ten 49 d old rabbits were randomly selected from each treatment and caged individually. The faeces were collected using a nylon net placed under the cages of each of the selected rabbits, over a period of 4 d at approximately 08:00 h each morning before the ration for the day is served. Each pooled faecal sample was packed into a 2-layer polythene bag to prevent moisture loss and thereafter kept in a freezer. The frozen samples were individually mixed, pooled and ground. Thereafter, the representative samples were weighed on a aluminium foil pan, oven dried at 80°C for 24 h to a constant weight and kept in a desiccator for chemical analysis.

Table 1: Chemical composition (%) of *Alchornea cordifolia* leaf meal.

Parameters	Literature[1]	Current experiment
Dry matter	89.9	91.3
Crude protein	17.9	18.0
Crude fibre	12.8	12.9
Ash	11.4	12.8
Ether extract	4.34	4.12
Nitrogen free extract	43.5	43.3
Phytate	1.21	10.8
Oxalate	0.86	13.0

[1]Timibitel *et al.,* 2014.

At the end of the feeding trial (8 wk), one rabbit from each replicate (10 rabbits/treatment) was starved overnight,

Table 2: Ingredients and chemical composition of experimental diets.

	Level of *A. cordifolia* leaf meal (0, 5 or 10%) with (+E) or without (-E) enzyme					
Ingredients (%)	0-E	5-E	10-E	0+E	5+E	10+E
Maize	6.00	6.00	6.00	6.00	6.00	6.00
Cassava peel	29.00	29.00	29.00	29.00	29.00	29.00
Alchornea leaf meal	0.00	5.00	10.00	0.00	5.00	10.00
Soybean meal	10.85	10.85	10.85	10.85	10.85	10.85
Ground nut cake	7.50	7.50	7.50	7.50	7.50	7.50
Palm kernel cake	12.00	7.00	2.00	12.00	7.00	2.00
Wheat offal	7.00	7.00	7.00	7.00	7.00	7.00
Molasses	3.00	3.00	3.00	3.00	3.00	3.00
Palm oil	1.50	1.50	1.50	1.50	1.50	1.50
Rice bran	15.4	15.4	15.4	15.4	15.4	15.4
Maize husk	6.00	6.00	6.00	6.00	6.00	6.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00
Premix*	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Determined analysis (%)						
Crude protein	16.3	16.3	16.3	16.3	16.3	16.3
Crude fat	2.23	2.22	2.21	2.20	2.19	2.20
Crude fibre	11.5	11.6	11.7	11.5	11.6	11.7
Neutral detergent fibre	38.6	39.3	39.4	38.6	39.3	39.4
Acid detergent fibre	19.7	19.8	19.9	19.7	19.8	19.9

*Provided per kg diet: Vitamins A (8,500,000 IU); D_3 (1,500,000 IU); E (10000 mg); K_3 (1500 mg); B1 (1600 mg); B_2 (4000 mg); B_6 (1500 mg); B_{12} (10 mg); Niacin (20000 mg); Pantothenic acid (5000 mg); Folic acid (500 mg); Biotin H_2 (750 mg); Choline chloride (175,000 mg); Cobalt (200 mg); Copper (3000 mg); Iodine (1000 mg); Iron (20000 mg); Manganese (40000 mg); Selenium (200 mg); Zinc (30000 mg); and Antioxidant (1250 mg) per 2.5 kg.

weighed, tagged and slaughtered according to World Rabbit Science Association guidelines (Blasco *et al.,* 1993). The skin of the slaughtered rabbits was removed with scalpel and scissors. Then, the removal of head and legs was followed by evisceration of the carcass and dressing out percentage calculated. The skin, head and limb were weighed together while various internal organs (lung, liver, kidney, heart, gall bladder and gastrointestinal tracts) were weighed separately and expressed as a percentage of slaughtered weight.

Chemical analysis

The experimental diets and faeces were analysed for moisture, protein (Nx6.25), fat, ash and crude fibre using AOAC (2000) procedures (934.01, 988.05, 920.39, 942.05, and 962.09, respectively). Neutral detergent fibre and acid detergent fibre were determined by Van Soest method of partitioning fibre in feeds (Goering and Van Soest, 1970). The phytate content was quantified as described by Wheeler and Ferrel (1971), and oxalate as described by Moir (1953).

Statistical analysis

Analysis of variance was performed using General Linear Model procedure for complete randomised design with 2 enzyme doses×3 ALM levels factorial arrangements of treatment. The data were tested for the main effects (enzymes and leaf meal levels) and their interaction, while statistical significance was assessed at ($P<0.05$). When the treatment effect was significant ($P<0.05$), differences between means were determined using Duncan test (SPSS).

Table 3: Effect of a commercial enzyme supplementation and graded levels of *Alcornea cordifolia* leaf meal (ALM) on growth traits of rabbits from 35 to 91 d of age.

	Enzyme (g/kg)				ALM (%)					Enzyme×ALM	
	0	0.35	SEM	*P*-value	0	5	10	SEM	*P*-value	SEM	*P*-value
Initial live weight (g/rabbit)	697	717	21.9	0.54	717	686	718	26.9	0.65	38.2	0.91
Daily weight gain (g/rabbit/d)	13.5	13.6	0.80	0.60	13.5	13.7	13.5	0.90	0.32	0.13	0.88
Daily feed intake (g/rabbit/d)	46.1	45.8	0.52	0.65	47.7[b]	46.0[ab]	44.1[a]	0.64	0.01	0.90	0.89
Feed conversion ratio	3.41	3.37	0.04	0.48	3.54[b]	3.37[a]	3.26[a]	0.05	0.01	0.07	0.93

Means with different superscripts in the same row are significantly different ($P<0.05$).
n=10 replicates/treatment (3 rabbits/replicate) for each combination enzyme×ALM.

RESULTS AND DISCUSSION

Growth rate was not affected by the main factors (commercial enzyme supplementation and *Alchornea cordifolia* leaf meal inclusion) and their interactions (13.5 g/d on av.; Table 3). Daily feed intake and feed conversion ratio (FCR) decreased ($P<0.01$) with the ALM inclusion levels from 0 to 10% ($P=0.01$). The decreased daily feed intake with ALM inclusion may be due to reduced palatability and a possible increase in the level of antinutritonal factors as the dietary ALM inclusion increased, although it did not affect growth rate. The inclusion of 5% ALM supplied 0.54 g phytate/100 g diet and 1.08 g oxalate/100 g diet, which increased to 0.65 g phytate and 1.3 g oxalate/100g for 10% ALM inclusion. However, the phytobiotic properties of *Alchornea cordifolia* leaf might be linked to the improved FCR with ALM inclusion in this study, as antibiotics when used at low dose-long term administration in feed reduce feed usage per production unit and enhance performance (Barton, 2000). The potential of *Alchornea cordifolia* leaf meal as a good source of nutrients for monogastric was previously reported by Alikwe *et al.* (2014b). According to Falcao-e-Cuncha *et al.* (2007), exogenous enzymes are included in rabbit diets to improve nutrient availability. Reduction of ileal flow and mortality in rabbits (Gutiérrez *et al.,* 2002; García-Ruiz *et al.,* 2006) and improvement of feed conversion ratio (Eiben *et al.,* 2008) had been achieved through addition of proteases in rabbit diets. However, in some studies no improvement of rabbit performance was detected as a result of exogenous enzyme supplementation (Falcao-e-Cunha *et al.,* 2004), which is consistent with the result on performance obtained in this study. This suggests that the ability of enzyme supplementation in rabbit nutrition could vary and might depend on the type of diet. The performance of the rabbits in this study was relatively low, in particular when compared to those of the temperate region. It has been reported that high levels of performance being recorded in developed countries are not always achieved in the developing countries (Ehinobu *et al.,* 1997), as depressed growth rate due to feed quality and reproduction due to selection have been identified as the major reasons for this relatively poorer performance of rabbits in the tropics compared to temperate zones (Ehinobu *et al.,* 1997; Owen, 1976). However, the rabbits' performance in this study was similar to those reported earlier (Ogunsipe, 2014; Oloruntola *et al.,* 2015).

Table 4: Effect of commercial enzyme supplementation and graded levels of *Alcornea cordifolia* leaf meal (ALM) on dry matter (DM) intake and apparent digestibility (%) from 49 to 53 d of age.

	Enzyme (g/kg)				ALM (%)					Enzyme×ALM	
	0	0.35	SEM	*P*-value	0	5	10	SEM	*P*-value	SEM	*P*-value
Feed intake, (g DM/d)	40.1	40.4	1.07	0.87	40.3	40.5	39.99	1.31	0.95	1.86	0.83
Dry matter	64.0	64.3	0.33	0.56	63.9	64.4	64.2	0.40	0.62	0.57	0.96
Organic matter	64.8	65.0	0.21	0.61	64.6	65.0	65.1	0.25	0.26	0.36	0.99
Crude protein	68.3	75.5	0.73	0.001	69.0	70.6	71.6	0.89	0.16	1.25	0.13
Crude fibre	28.7	30.5	0.35	0.001	29.6	29.4	29.8	0.43	0.81	0.60	0.66
Neutral detergent fibre	46.5	47.0	0.54	0.49	46.7	46.7	46.9	0.66	0.98	0.94	1.00
Acid detergent fibre	26.7	26.8	0.33	0.85	26.2	26.6	27.5	0.40	0.14	0.57	0.98

SEM: standard error of the mean.
n=10 rabbits for each combination enzyme×ALM.

Table 5: Effect of commercial enzyme supplementation and graded levels of *Alcornea cordifolia* leaf meal (ALM) on carcass and relative weights (% live weight) of organ of weaner rabbits of 91 d of age.

	Enzyme (g/kg)				ALM (%)					Enzyme x ALM	
Parameters	0	0.35	SEM	*P*-value	0	5	10	SEM	*P*-value	SEM	*P*-value
Slaughter weight	1398	1421	21.1	0.46	1414	1395	1418	25.9	0.80	36.6	0.89
Carcass weight	687	710	21.1	0.46	1414	1395	1418	25.9	0.80	36.6	0.89
Carcass weight (%)	49.1	49.8	0.75	0.49	49.7	49.0	49.7	0.92	0.83	1.30	0.84
Liver	3.32	3.25	0.01	0.39	3.28	3.31	3.28	0.07	0.92	0.09	0.94
Heart	0.37	0.37	0.01	0.76	0.37	0.37	0.36	0.01	0.63	0.01	0.94
Lung	0.62	0.61	0.02	0.76	0.62	0.59	0.62	0.03	0.31	0.02	0.99
Kidney	0.58	0.57	0.01	0.62	0.59	0.58	0.56	0.03	0.31	0.02	0.95
Gall bladder	0.04	0.04	0.01	0.51	0.05	0.04	0.04	0.02	0.46	0.01	0.49
Gastro intestinal tract	183	197	12.2	0.45	205	188	178	14.9	0.45	21.1	0.57
Head, leg and skin	17.6	18.1	0.32	0.28	17.3	18.6	17.6	0.40	0.11	0.56	0.17

SEM: standard error of the mean.
Means corresponded to the value recorded from 1 rabbit per replicate. n=10 replicates/treatment (3 rabbits/replicate).

Crude protein and crude fibre digestibility both improved ($P<0.001$) by 6% with enzyme supplementation, although the digestibility of neutral detergent fibre and acid detergent fibre was not ($P>0.05$) affected by the main factors and their interaction (Table 4). However, it did not improve feed conversion ratio and daily weight gain, which might be due to the low growth potential of these experimental rabbits, as observed earlier for tropical breeds of rabbit (Ehinobu *et al.,* 1997; Owen, 1976). Crude fibre digestibility slightly improved with enzyme addition, but this was not the case for NDF and ADF digestibility (Table 4).

Thus, the observed improvement of crude protein and crude fibre digestibility with enzyme supplementation agreed with the results reported previously (Fernández *et al.,* 1996; Bolis *et al.,* 1996; Gutiérrez *et al.* 2002). Nevertheless, while Bolis *et al.* (1996) observed a negative nutrient digestibility following exogenous enzyme (protease) supplementation, others observed positive result when an enzyme cocktail (α-amylase, protease, cellulase, xylanase and β-glucanase) was added to rabbit diets (Bhatt *et al.,* 1996; García-Ruiz *et al.,* 2006). An abnormal increase in relative internal organ weights of animals may indicate the possible response of their internal organs to toxin in their feed. In this study, the fact that there were no significant ($P>0.05$) effects of the enzyme supplementation and ALM inclusion and their interactions on the liver, heart, lung, kidney and gall bladder and that there was no case of mortality during this study (Table 5) suggests that the health status of the rabbits was not compromised. This finding agreed with the report by Alikwe *et al.,* (2014a) that there were no remarkable difference ($P>0.05$) in weight of liver, kidney, heart, lung and spleen of Wistar rats gavaged with aqueous extract of *Alchornea cordifolia* leaf meal. Tannin and flavonoids have been reported to have potent antioxidant and anti-inflammatory activities (Perchellet *et al.,* 1996; Manga *et al.,* 2004). Okuda *et al.,* (1983) also reported the ability of tannin and related compounds to lower the level of lipid peroxide in the liver cells and consequently prevent its negative effect on the liver.

CONCLUSION

Alchornea cordifolia leaf meal inclusion and enzyme supplementation did not have negative effect on the performance, nutrient utilisation and health status of rabbits. Therefore, *Alchornea cordifolia* leaf meal may be recommended as a reliable feed stuff for rabbit feed formulation. Further research is recommended to determine the optimum dose of the exogenous enzyme (Natuzyme®) in rabbit diets.

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