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Full Length Research Paper

Effects of mimosine and tannin toxicity on rabbits fed processed *Leucaena leucocephala* (Lam) De Wit. leaves

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A sixteen-week study was conducted to investigate the effects of mimosine and tannin toxicity on rabbits (*Orytolagus cunniculus*) fed processed *Leucaena leucocephala* leaves (LLL). Thirty-six growing rabbits (mixed breed) of average weight of 625 g were used for the experiment. The rabbits were randomly allotted to nine experimental groups of four rabbits each in a 3 x 3 factorial arrangement. LLL were either sun dried (SDLLL), hot water processed (HWLLL) or ensiled (ELLL) for six weeks and included in three diets at 0, 10 and 20% levels respectively. Data were collected on feed intake, weight gain, feed conversion ratio and other performance characteristics of the fed rabbits. The data collected were subjected to analysis of variance (ANOVA) while the significant means were separated using Duncan's multiple range test. The anti-nutritional analysis showed that SDLLL contained highest tannin (1.20%) and mimosine (1.64%) contents but HWLLL had the least tannin (0.77%) and mimosine (1.25%) respectively. The best feed conversion ratio (FCR) of 4.41[b] and the highest (P < 0.05) weight gain were recorded by rabbits fed diets containing ELLL. The interaction effects showed that rabbits fed diets containing 20% SDLLL had alopaecia, necrotic spots, liver congestion, edema and highest percentage mortality. It was concluded that diets containing more than 1% of mimosine and tannin impaired the growth performance and had deleterious effects on liver of the rabbits.

Key words: *Leucaena leucocephala,* processing methods, rabbits and toxicity, anti-nutritional factors.

INTRODUCTION

The interest of animal nutritionist in recent years is to search for cheaper, locally available and nutritionally viable 'alternative feedstuffs' (Onwuka, 1992; Jegede et al., 2006). One of such alternatives is *Leucaena leucocephala* which has become prominent for its immense contribution to the pool of forage resources in the tropics (Onwuka et al., 1992; Arigbede, 1998; Isah et al., 2007). Its year round availability, drought resistance, luxuriant growth, vigorous re-growth and palatability as a source of high quality nutrients for livestock makes it a novel browse plant for rabbits and other ruminant animals (Akinsoyinu and Onwuka, 1988; Fasae et al., 2005; Amata, 2010). The foliage of this arboreal, tropical species contains 21.0 to 28.0% crude protein content, 20 to 22.00 MJ/kg metabolizable energy, 7.10 to 8.32 ether extracts, 6.80 to 8.32% total ash and a range of other nutrients (Agbede and Aletor, 2003; Aduku, 2005; Otsyina and Dzowela, 2008; Ajit et al., 2010). As a result of these nutritional worth, leucaena leaves have been included at varying levels in the rabbit diets for growing or fattening (Adejumo et al., 2006; Onwuka et al., 1992) or as a supplemental diet with natural pasture (Onwudike, 1995; Rubanza et al., 2007; Amata and Bratte, 2008). Despite the rich chemical composition of leucaena foliage, the presence of inherent anti-nutritional factors such as tannin and mimosine has invariably limited its utilization by the livestock (Onwuka et al., 1992; Ajit et al., 2010). Mimosine which is a non-protein amino acid structurally exerts its toxic action by blocking the metabolic pathways of aromatic amino acids and tryptophan (D'Mello, 2002). Because of its structural

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Table 1. Gross composition of the experimental diets (g/100 g DM).

Ingredient	Sun dried LLL			Hot water processed LLL			Ensiled LLL		
	0 % SDLLL	10 % SDLLL	20 % SDLLL	0 % HWLLL	10 % HWLLL	20 % HWLLL	0 % ELLL	10 % ELLL	20 % ELLL
Maize	44.00	42.50	32.00	44.00	42.50	32.00	44.00	42.50	32.00
Soy bean meal	6.00	7.50	8.00	6.00	7.50	8.00	6.00	7.50	8.00
Groundnut cake	11.00	15.00	18.00	11.00	15.00	18.00	11.00	15.00	18.00
Fishmeal (65%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Wheat offals	34.00	20.00	17.00	34.00	20.00	17.00	34.00	20.00	17.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Grower premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Leucaena leaves (LL)	0.00	10.00	20.00	0.00	10.00	20.00	0.00	10.00	20.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

resemblance to L-tyrosine, it probably acts as a tyrosine analogue or antagonist that inhibits protein biosynthesis in the living body and causes toxic symptoms, including growth retardation (Schofield et al., 2001; Ajit et al., 2010).

The metal-chelating ability of the 3-hydroxy-4-oxo function of the pyridine ring in mimosine could possibly disturb the action of metal-containing enzymes, especially those containing iron actions and cause inhibition of some biological reactions (Astuti et al., 1989). *L. leucocephala* foliage does not only contain mimosine but also both hydrolysable and condensed tannins (Onwuka et al., 1992; Poonam and Pushpa, 1994; Kariuki and Norton, 2002). Tannins are natural highly polymerized and oligomeric compounds which have the ability to bind proteins and form either soluble or insoluble tannin-protein complexes (Makkar et al., 1997; Bravo, 1998; Norton, 2000). The astringent taste of tannin-rich feeds reduces feed intakes; decreases *in-vivo* protein utilization, affect various enzyme activities and hinder the *in-vitro* digestibility (Makkar, 2003). Although, tannins decrease the availability of nutrients, yet they cause a shift in the partitioning of nutrients so that a higher proportion of available nutrients are channeled to microbial mass synthesis and less to short-chain fatty acid production (Makkar, 1997; Waghorn and Shelton, 1997). In order to determine the effects of mimosine and tannin toxicity on the performance characteristics of growing rabbits.

MATERIALS AND METHODS

Description of the experimental site and animals

This study was conducted at the rabbit unit of the Ministry of Agriculture and Co-operatives, Ikorodu area (Latitude 6° 37'N and Longitude 3° 31' E) of Lagos State, Nigeria. Thirty-six growing rabbits of 6 to 8 weeks of age and of average weight of 625 g were used for this study. The animals were randomly allotted to nine experimental groups of four rabbits each. Each group was further divided into four replications with a rabbit per replicate. This replication was done in accordance to Animal Protection Act (Act 71 of 1962) and Animal Welfare Act 2008 to 2012 (Review of Animal Care Legislation, 2008). A rabbit was housed in a hutch (of 82 cm length, 44 cm height and 150 cm width respectively) for a period of ten weeks-during which they were fed the experimental diets. The test ingredient (the processed leucaena leaves) was either sun dried (SDLLL), hot water processed (HWLLL) or ensiled (ELLL) and each of these was included at 0, 10 and 20% levels in the diet. The conventional feedstuffs were used as the control diet and also, to supplement the test ingredient. The respective gross and chemical compositions of both groups are shown in Tables 1 and 2; the inclusion levels of each feed component are also shown in Tables 3 to 4.

Processing methods of the experimental diet

Fresh leucaena leaves were harvested and were either sun dried, hot water processed or ensiled in accordance to the experimental design. The harvested leucaena leaves were divided into three groups. The first group was ensiled in an airtight container for a period of six weeks; the second group was submerged in 70°C hot water for 10 min while the third group was sun dried for 36 h. The sun dried leaves were included in the basal diet at 0, 10 and 20 as diets 1, 2 and 3. The hot water treated leaves were also included at 0, 10 and 20% as diets 4, 5 and 6. The ensiled leaves were included at 0, 10 and 20% as diets 7, 8 and 9 respectively.

Analysis of the feed samples

The feed samples were analyzed to determine the gross

Table 2. Gross and chemical composition of the experimental diets (g/100 g DM).

Ingredient	Sun dried LLL			Hot water processed LLL			Ensiled LLL			Processed LLL		
	0 %	10 %	20 %	0 %	10 %	20 %	0 %	10 %	20 %	SDLLL	HWLLL	ELLL
Determined analysis (%)												
Dry matter	88.45	88.31	88.26	88.45	88.23	88.23	88.45	88.15	88.56	88.69	92.65	89.56
Crude protein	16.54	16.67	16.89	16.54	16.97	17.23	16.54	16.65	17.15	19.95	22.27	22.13
Ether extract	3.55	3.58	3.74	3.55	3.62	3.64	3.55	3.65	3.69	5.91	7.41	6.87
Ash	7.94	9.09	9.39	7.94	9.18	9.41	7.94	9.28	9.49	6.01	7.16	6.93
Crude fibre	8.53	11.82	11.86	8.53	11.97	12.04	8.53	11.92	12.13	23.69	27.26	26.54
NDF	33.21	35.97	36.09	33.21	37.17	37.45	33.21	36.38	36.49	56.79	59.86	55.76
ADF	16.97	19.67	19.96	16.97	19.98	20.15	16.97	19.78	19.91	43.69	47.31	39.65
ADL	5.83	6.92	7.16	5.83	7.07	17.24	5.83	6.67	6.84	13.21	15.33	12.75
Cellulose	11.14	12.75	12.80	11.14	12.11	17.30	11.14	16.60	16.58	30.48	21.98	26.90
Hemicellulose	16.24	16.30	16.13	16.24	17.19	12.91	16.24	13.11	13.07	13.10	12.53	16.11
Mimosine										1.64	1.20	1.24
Condensed tannins										1.20	0.77	0.84

Table 3. Main effects of processing methods of Leucaena leaves on the performance characteristics of rabbits.

Parameter	SD	HW	E	SEM
Initial weight (g)	635.56	624.44	679.17	
Final weight (g)	1576.00^{ab}	1386.94^{ab}	1874.69^{a}	89.98
Total weight gain (g)	940.44^{b}	762.50^{b}	1195.53^{a}	76.91
Daily weight gain (g)	13.43^{b}	10.89^{b}	17.08^{a}	1.099
Total feed intake (g)	4584.42	4188.58	5009.99	145.16
Daily feed intake (g)	65.49	59.84	71.57	2.073
Feed conversion ratio	4.96^{ab}	5.58^{a}	4.41^{b}	0.204

$^{a, b, c}$means in the same row having different superscript are significantly (P<0.05) different. SDLLL (sun dried *Leucaena leucocephala* leaves); HWLLL (hot water processed *L. leucocephala* leaves) and ELLL (ensiled *L. leucocephala* leaves) respectively.

and composition of the experimental diets as shown in Table 2. All the analyses were done according to the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C., 1995) and Makker (1997).

RESULTS

The gross and chemical compositions of the experimental diets (g/100 g DM) are shown in Tables 1 and 2 respectively. Rabbits fed diets containing ensiled LLL had the highest (P<0.05) weight gain. The trend observed was that rabbits fed diets containing ensiled LLL recorded a

Table 4. Main effects of level of inclusion of processed Leucaena leaves on the performance characteristics of rabbits.

Parameter	0%		10%		20%		SEM	
Initial weight (g)	644.72		616.67	677.78				
Final weight (g)	1575.42	1723.33	1538.89	34.51				
Daily weight gain (g)	13.30		15.81		12.30		0.64	
Daily feed intake (g)		61.98		70.83		64.09		1.63
Feed conversion ratio		4.74		4.92		5.29		0.09
Total weight gain (g)		930.69		1106.67	861.11		44.72	
Total feed intake (g)		4338.78	4958.17	4486.03		114.34		

Table 5. Interaction effects of processing methods and levels on inclusion of LLL on the performance characteristics of rabbits.

Parameter	Sun drying			Hot water			Ensiling			SEM
	0	10	**20**	**0**	**10**	**20**	**0**	**10**	**20**	
Initial weight (g)	656.67	550	700	640	600	633.33	637.50	700	700	
Final weight (g)	1683	1550	1495	1457.50	1270	1433.33	1585.75	2350	1688.33	59.5
Daily weight gain (g)	14.66^{b}	14.29^{b}	11.36^{de}	11.68^{cd}	9.57^{e}	11.43^{de}	13.55^{bc}	23.57^{a}	14.12b	0.70
Daily feed intake (g)	62.52	68.60	65.36	60.57	59.35	62.86	34.54	67.31	1.84	
Feed conversion ratio	4.31^{d}	4.80^{cd}	5.76^{b}	5.21^{c}	6.36^{a}	5.17^{c}	4.71^{cd}	3.59^{e}	4.93^{c}	1.84
Mortality (%)	0.00	50.00	25.00	25.00	50.00	25.00	0.00	50.00	25.00	
Livability (%)	100.00	50.00	75.00	75.00	50.00	75.00	100.00	50.00	75.00	

$^{a, b, c, d, e}$means on the same row having different superscripts are significantly (P<0.05) different.

slightly higher (P<0.05) feed intake and the best feed conversion ratio (FCR) of 4.41^{b} (Table 3). However, the main effects of inclusion levels of LLL in the basal diet (Table 4) did not have significant impact (P>0.05) on the performance characteristics of the fed rabbits. The only thing observed was that rabbits fed diets containing 10% of processed LLL had the highest (P>0.05) daily feed intake, weight gain and the best FCR. The interaction effects of levels of inclusion and processing methods of LLL (Table 5) showed that the daily weight gain and FCR were significantly influenced by the interaction. Rabbits fed diets containing 10% ELLL had the highest (P<0.05) daily weight gain while the least value was recorded for rabbits fed diet 10% HWLLL. Also from Table 5, rabbits fed diets containing 0% LLL (control diet) had the highest percentage survivability but those on 20% LLL had the highest mortality.

DISCUSSION

The chemical compositions of the diets showed that they were adequate to meet the nutrient requirements of the growing rabbits and also, conformed to the recommended feeding standards (Aduku, 2005). The crude protein ranges of 19.95 to 22.27% (of processed LLL obtained) were within 12 to 30% range reported by Norton (1993), Agbede and Aletor (2003) and Ajit et al. (2010), respectively. The result obtained could be attributed to the similarity of the processing methods imposed on LLL fed to the growing rabbits. The lowest quantities of mimosine and tannin (1.20, 0.77 for HWLLL) and the highest amount of (1.64, 1.20 for SDLLL) were in tandem with the findings of Srinivasulu et al. (2003) that hot water treatment is more effective for the removal of the two anti-nutritional factors. Although the hot water processing method reduced the anti-nutrients greatly, yet a considerable loss of protein (90.92%), water soluble vitamins, minerals and free amino acid losses were reported by Srinivasulu et al. (2003) having boiled LLL in water for few minutes. As a result of this, Fasae et al. (2005) recommended that allowing LLL to rot away would reduce its anti-nutritional factors to innocuous residues. The 'post-mortem' evidence of edema, fragile (and even congested) liver with necrotic spots, confirmed the effects of tannin and mimosine toxicity as earlier reported by Awosanya and Akinyode (2000). The prevalence of alopecia (hair losses) on rabbits fed 20% SDLLL also concurred with the observations of D'Mello (1992), Van-veen (1996) and Awosanya and Akinyode (2000). The result also agreed with the postulation of Makkar (1991) that the inhibitory effects of mimosine on enzymes such as cystathionine synthetase and cystathionase involved in the conversion of cystane to methione causes alopecia. The highest weight gain and the better feed conversion ratio (FRC) observed in rabbits fed diets containing ELLL could be associated to the effects of fermentation on the reduction of mimosine and tannin in

the fed diets (Hongo et al., 1988). While this result agreed with the findings of Schroeder (2004) and Weiss and Underwood (2004), it is contrary to the reports of Awosanya and Akinyode (2000) who reported a poor daily weight gain and feed intake by rabbits fed 20% ensiled LLL meal. This is because they postulated that the presence of 3, 4–di hydroxyl pyridine (DHP) in LLL could act as appetite depressant (when present in the diet) due to its astringent taste.

The disparity in these results could be due to the stage of maturity of the forage at harvest, the type of fermentation that occurs in the silo or bunker, the type of storage structure used and the method of harvesting of the LLL. Hence, the efficiency of feed utilization was better for rabbits fed ELLL. In summary, the results obtained in this study can be adduced to the complex interactions existing between the two major anti-nutrients in leucaena leaves. It can therefore be concluded that rabbits cannot survive on diets containing more than 1% mimosine and tannin, respectively.

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