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Ruminal Fermentation and Nutrient Digestion in West African Dwarf (WAD) Sheep Fed *Dialium guineense* Supplemental Diets

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Abstract

Ruminant livestock production in sub-Saharan Africa is based on forage as the major feed resources, which of course is highly seasonal with low nutritive quality during dry seasons. Multipurpose trees (MPTs) which are part of the natural vegetation and are accessible to farmers have always been a useful protein supplement (Osakwe et al., 2000). Studies by a number of researchers (Osakwe, 1999; Osakwe and Steingass 2006) have indicated that some MPTs are less suitable as protein supplement because their soluble phenolic and condensed tannin compound limit protein digestion. It was against this background that an experiment was designed to study the fermentation profiles of dried *Dialium guinense* leaves as supplement to grass hay fed West African Dwarf (WAD) sheep. Eight 24 months old WAD sheep (28.8 kg ±4.2) BW), fed a basal hay diet at 2.5% BW dry matter was used to evaluate the fermentation profiles and nutrient digestion of *Dialium guineense* leaves. Four of the sheep were fistulated ruminally and used for rumen pH, ammonia and volatile fatty acid (VFA) in the rumen fluid. Dried leaves of *D. guineense* were offered at two levels (25% and 50% of DMI, diets 2 and 3, respectively) as supplement to a basal hay diet. Rumen liquor was sampled one hour before and one, three and five hours after the morning feeding. Rumen pH of diet 3 was higher ($p<0.05$) compared to the controls. Diet 3 also had a lower ($p<0.05$) (14.6 mg dl $^{-1}$) rumen ammonia concentration compared to the controls (30.6 mg dl $^{-1}$). The total VFA of diet 3 was lower ($p<0.05$) (67.9 mmol^{-1}) when compared to the controls (94.1 mmol $^{-1}$). Diet 3 showed a negative N- retention (-3.5%) compared to the control diet (16.8%). These results demonstrate that dried *Dialium guineense* leaves have a potential as forage during dry season feeding. Even though it showed a lower total VFA and rumen ammonia concentration, the value from this study was within the range of 5 to 23 mg dl $^{-1}$ recommended for optimum microbial protein synthesis.

Key words: *Dialium guineense*, chemical composition, fermentation profiles, WAD sheep.

Introduction

Ruminant livestock production in sub-Saharan Africa is based on forage as the major feed resources, which of course is highly seasonal with low nutritive quality during dry seasons. Multipurpose trees (MPTs) which are part of the natural vegetation and are accessible to farmers have always been a useful protein supplement (Osakwe et al., 2000). Studies by a number of researchers (Osakwe, 1999; Osakwe and Steingass 2006) have indicated that some MPTs are less

suitable as protein supplement because their soluble phenolic and condensed tannin compound limit protein digestion.
Supplementation with tree fodder has been recommended (Mtenga and Shoo, 1990) as a possible solution to the low dry season feed quality problem but quantitative information on nutritive value and animal performance on diets containing forage from several multipurpose tree and shrub species in existing animal Agroforestry Systems in Africa is scanty (Reed et al., 1990). There is paucity of information on the fermentation profile and nutrient digestibility of WAD sheep on supplementation with multipurpose trees. It was against this background that an experiment was designed to study the fermentation profiles of dried *Dialium guinense* leaves as supplement to grass hay fed West African Dwarf (WAD)

Materials and methods

Leaf samples from matured *Dialium guineense* trees were collected from Cotonou (Benin), sun dried on a raised wooden platform, then packed in plastic containers and transported to the University of Hohenheim, Germany.

Animals and Diets

Eight West African Dwarf sheep (28.8 ± 4.2 kg body weight), all castrates and between 18-24 months, were used in a completely randomized design. Four of the sheep were fistulated at the rumen and used to measure rumen ammonia and volatile fatty acid (VFA).
Dried leaves of *Dialium guineense* were offered at two levels (25% and 50% of DM intake) replacing hay as supplement to the basal hay diet. The hay diet without supplement was the control diet. The experimental diets were as follows:
100% hay (Control diet); 25% *Dialium guineense* leaves + 75% hay (diet D25%);
50% *Dialium guineense* leaves + 50% hay (diet D50%). In addition to the experimental diets animals received a mineral premix supplement (10 g/d). Feed was offered twice a day at 0800 and 1600 hours and water was provided *ad libitum.*

Experimental protocol

Four animals each (two fistulated and two not fistulated) were randomly assigned to D25% and D50% diets, respectively. The animals were adapted for 10 days on the experimental diets. This was followed by a digestibility trial that lasted for 8 days during which animals were kept in individual metabolism cages to measure daily feed intake, and to collect daily faeces and urine outputs. After the digestibility trial, two of the fistulated animals from each group were kept in pens for rumen liquor collection for three consecutive days.
The control diet was tested immediately using two animals selected at random from the D25% and another two animals from the D50% giving a total of four animals (two fistulated and two not fistulated). During the digestibility trial, faeces, urine and feed refusals were collected daily, weighed and stored at –18 ^{0}C until analyses. Samples of faeces were dried at 65 ^{0}C for 48 h, ground through a 1 mm diameter screen and together with urine were analysed for N (AOAC, 1990) and NDF, ADF and ADL (Goering and Van Soest, 1970) Gross energy of feed and faeces were measured by bomb calorimetry using benzoic acid as a standard (26437 J/g).

Fermentation parameters

Rumen liquor was taken 1 h prior to feeding, and 1, 3 and 5 h after feeding directly by means of a vacuum pump with plastic tube thrust into the rumen compartment. Immediately after collection, pH was measured with a Schott CG 840 pH-meter. The samples were then immediately freed of coarse particles by filtration through cheese cloth and were centrifuged at 2500*g* for 20 min under refrigeration. Ruminal ammonia was determined as described by Cammann (1979) and determination of VFA in ruminal fluid was as described by Zijlstra et al. (1977).

Statistical analyses

The data was analysed using the General Linear Model Procedure (SAS, 1990). The model for ruminal data included variation due to diet, animal within treatment, time of sampling and the time x diet interactions. Diet, animal within treatment effects, diet x time effect interaction were all tested against residual error. Treatment means were differentiated using Duncan's Multiple Range Test (Duncan, 1955).

Results

The chemical composition and gross energy content of *Dialium guineense* and the experimental diets are presented in Table 1.

Table 1: Chemical composition of experimental diet and *Dialium guineense* (% DM)

	*Control diet	Diet D25%	Diet D50%	*D. guineense*
CP	11.5	12.2	12.9	14.3
Ash	9.9	9.2	8.5	7.1
Ether extract	1.5	1.4	1.2	0.92
NDF	59.7	60.2	60.7	61.7
ADF	36.5	38.7	40.8	45.1
ADL	4.3	8.4	12.4	20.5
Cellulose	32.1	30.3	28.4	24.7
Hemicellulose	23.2	21.5	19.9	16.5
Total phenols [1]	-	1.5	3.1	6.1
Tannin Phenol [1]	-	1.3	2.6	5.1
Condensed tannins [2]	n.a.	1.5	3.0	5.9
GE [kJ/g DM]	17.8	18.2	18.6	19.4
Mineral premix [3] g/d	10	10	10	n.a.

[1]As tannic acid equivalent; [2]As leucocyanidin equivalent; n.a.: Not applicable; [3]Composition/kg: vit A 600,000 IU, vit. D3 75,000 IU, vit. E 300 mg; Zn 3,000 mg; Mn 480 mg; Co 12 mg; Se 10 mg. * Control diet=hay+ mineral

Ruminal pH, ammonia and total VFA

Mean ruminal pH, ammonia and total VFA concentration data are presented in Table 2.

Diet D50% had a higher ($P < 0.05$) pH than both the control and D25% diets, respectively. There was no difference in the pH value between the control and D25% diets. The ruminal ammonia concentration of sheep fed diets D25% and D50% were significantly ($P < 0.05$) lower than those fed the control diet. The ammonia concentration of D25% was however, not different from that of D50% diet. Diet D50% had an inferior ($P < 0.05$) total VFA concentration compared to both the control and D25% diets, respectively.

Table 2: Diet effects on pH, ruminal ammonia and total VFA concentration of sheep supplemented with *Dialium guineense*

	Time after feeding (h)	Control	D25%	D50%	SEM	Sig. Level Time
Rumen pH	-1	6.64^{aB}	6.65^{aB}	6.96^{aA}	0.06	*
	1	6.23^{dB}	6.31^{dB}	6.52^{dA}	0.06	*
	3	6.41^{cB}	6.40^{cB}	6.60^{cA}	0.09	*
	5	6.44^{bB}	6.45^{bB}	6.63^{bA}	0.08	*
Sig. Level	(Diet)	*	*	*		
Rumen NH_3	-1	45.3^{bB}	19.5^{bA}	12.5^{bAB}	3.03	*
(mg/dl)	1	57.3^{aB}	26.1^{aA}	21.4^{aAB}	5.85	*
	3	50.6^{bB}	16.3^{bA}	14.2^{bAB}	5.56	*
	5	19.3^{cB}	11.0^{cA}	10.1^{cAB}	1.44	*
Sig. Level	(Diet)	*	*	*		
Total VFA (mmol/l)	-1	84.98^{cC}	75.5^{cB}	54.7^{cA}	1.95	*
	1	102.93^{aC}	82.2^{aB}	77.6^{aA}	1.62	*
	3	97.24^{aC}	86.6^{aB}	71.6^{aA}	2.16	*
	5	91.13^{bC}	83.0^{bB}	67.7^{bA}	2.24	*
Sig. Level	(Diet)	*	*	*		

a,b,c, d Means in a column with common letter(s) superscript do not differ ($P > 0.05$)
A,B,C Means in a row with common letter(s) superscript do not differ ($P > 0.05$) * $P < 0.05$; n.s.= Not significant

Nutrient Intake, digestibility and nitrogen balance

The results obtained for nutrient intake, digestibility and nitrogen balance are summarized in Table 3. Dry matter digestibility (DMD), Organic matter digestibility (OMD) as well as cellulose digestibility decreased ($P < 0.05$) with level of supplementation. N-intake increased ($P > 0.05$) with level of supplementation. Diet D50% showed a negative N-retention compared to the control

Table 3: Effect of *Dialium guineense* supplementation on feed intake, digestion and nitrogen balance in sheep

	Control	D25 %	D50 %	SEM	Sig. Level (Diet)
Dry matter intake (g/d)	545.20	494.9	545.63	19.22	n.s.
Dry matter digestion (%)	67.2^{a}	57.1^{b}	39.4^{c}	0.98	*
OM intake (g/d)	491.0	449.3	499.2	17.49	n.s.
OM digestion (%)	67.7^{a}	55.5^{b}	38.3^{c}	0.99	*
NDF intake (g/d)	325.1	297.6	330.9	11.59	n.s.
NDF digestion (%)	64.3^{a}	46.2^{b}	19.2^{c}	1.26	*
Cellulose intake (g/d)	174.9	150.1	155.1	5.73	n.s.
Cellulose digestion (%)	65.1a	48.3b	24.6c	1.05	*
N- intake (g/d)	10.1	9.6	11.3	0.39	n.s.
Feacal N (%)	39.2^{c}	49.2^{b}	64.0^{a}	1.37	*
Urinary N (%)	44.1	46.6	39.5	4.32	n.s.
Retained N (%)	16.8^{a}	4.4^{ab}	-3.5^{b}	4.2	*
Digestible N (%)	60.80^{a}	51.5^{b}	36.0^{c}	1.4	*

a,b,c Means in a row with common letter(s) superscript do not differ ($P > 0.05$): * $P < 0.05$ n.s.= Not significant

Discussion

Condensed tannin- protein complex is stable and insoluble in the pH range 3.5-7.0, but is unstable and releases protein at pH < 3.0 and pH > 8.0 (D'Mello, 1992). The higher pH of D50% when compared with the control and D25% diets could indicate an inhibition effect that led to a possible reduction of fermentation activity. The decrease in ruminal ammonia concentration at the higher level of supplementation could indicate an inhibitory effect of tannins on degradability of proteins by rumen microbes (Kumar, 1992). The lower VFA of Diet D50% seemed to confirm the inhibition effect of tannins on VFA. The nitrogen balance trial showed a negative N-retention at the higher level of supplementation compared to the control diet. This would suggest a reduction in fermentative activity. A significantly higher ($P<0.05$) feacal –N loss in D50% compared to the control and D25% diets was observed. There was however, an attempt to compensate for this loss of feacal-N with a lower loss of urinary-N. This compensatory ability was observed by Rittner, (1987) and ILCA, (1988).

It has been reported that tannins may bind bacterial enzymes and/or form indigestible complexes with cell-wall carbohydrates leading to a change in the amount of cell-wall carbohydrates (Kumar, 1992). The decrease in OMD digestibility with level of supplementation could indicate an inhibition of digestive enzyme by dietary tannins. This observation is consistent with the findings of Kumar (1992). Kumar and D'Mello (1995) reported that inhibition of cellulose digestion should lead to reduction in the yield of metabolisable energy in the rumen. This study clearly showed inhibition of cellulose digestion with supplementation level.

Conclusion

This study clearly showed the fermentation profiles and nutrient digestibility of *Dialium guineense* as supplement to hay in livestock diets, especially in sub-Saharan Africa where the major limiting factor to livestock production is the inadequate feed supply. But it also under-scores the need for research into the anti-nutritive factors acting singly or in combination with one another to reduce the feeding value of leguminous forage species in the existing fallow lands. Since the total phenol, tannin phenol as well as the condensed tannin content of *Dialium guineense* (61 g/kg, 51 g/kg and 59 g/kg) DM respectively, are pretty high, the inhibitory effects observed by its supplementation could be attributed to its condensed tannin content.

It was concluded that dried leaves of *Dialium guineense* should be offered only as survival supplementation forage during acute dry season at 25% level of inclusion due to its poor digestibility and negative nitrogen retention.

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