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Flemingia macrophylla in goat feeding

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ABSTRACT - The objective of this work was to evaluate the inclusion of *Fabaceae Flemingia macrophylla* (Willd.) Kuntze ex Merr. in the diet of lactating dairy goats arranged in a 5 × 5 Latin square. The diets were composed of 40% of concentrate and 60% of roughage, and the dietary treatments were defined by the level of *Flemingia* hay inclusion (0%, 8%, 16%, 24%, and 32% in the diet dry matter) replacing *Cynodon dactylon* cv. Tifton 85 hay. The diets were isonitrogenous, with 14% crude protein. Feed intake, nutrient digestibility, feeding behavior, and ruminal pH and ammonia nitrogen were evaluated. There was no difference in dry matter intake with the inclusion of *Flemingia* hay in the diet. The digestibility of dry matter, organic matter, crude protein, neutral detergent fiber, and total carbohydrates decreased with the inclusion of *Flemingia* in the diet. The diet did not change rumen ammonia nitrogen concentration or ruminal pH. There were no differences in the feeding behavior or feed and rumination efficiencies. *Flemingia macrophylla* can be used up to the level of 32% in the dry matter in diets for lactating goats.

Key Words: digestibility, feeding behavior, leguminosae, ruminal pH, tropical forage

Introduction

The seasonality of forage production leads to the use of alternative foods as an option in animal feeding, especially in the dry season, when the pastures, mostly formed by grasses, are scarce and have low nutritional value. Thus, forage legumes have advantages in terms of nutrition, because they have a high protein content, good digestibility, and low decline in nutritional value with advancing phenological stages. Legumes are an important reserve of green food for the dry season and transfer atmospheric nitrogen into the soil through biological fixation (Ben Salem et al., 2005).

Flemingia macrophylla (Willd.) Kuntze ex Merr. is a legume that is adapted to acid soils of low fertility, sandy or clayey, and is drought-tolerant (Salmi et al., 2013). Legumes, such as *Flemingia*, produce secondary metabolites such as tannins, which can be hydrolyzed or condensed.

Condensed tannins are the most common secondary compound in legumes (Min et al., 2003). They may negatively influence feed intake by the animals by two factors: one is the astringency, which reduces the acceptability of fodder by animals (Reed, 1995), reducing feed intake or the number of visits to the trough (McLeod, 1974; Jansman, 1993; Reed, 1995). The other factor is the effect of tannin on the nutrient digestibility by forming complexes with proteins and carbohydrates, reducing the ruminal degradation of these nutrients, or complexation with microbial enzymes, decreasing its activity and consequently the digestibility of the feed (Makkar et al., 1988). Additionally, tannins can reduce enteric methane production, so they are important for mitigating greenhouse gas emission by ruminants (Makkar, 2003).

Lignin, another plant compound, contributes to the structural integrity, resistance to degradation, and water impermeability of plants (Hatfield et al., 1999). According to Van Soest (1994), lignin is present in greater amounts in legumes than in grasses and its main effect on animal nutrition is the reduction of digestibility.

This study was conducted to evaluate the feeding behavior, feed intake, ruminal pH and ammonia nitrogen, and digestibility of the nutrients in lactating goats fed increasing levels of *Flemingia macrophylla* (Willd.) Kuntze ex Merr.

Material and Methods

This work was conducted in accordance with the ethical standards of the institution.

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The experiment was carried out in 2011 from August to December in Seropédica - RJ, Brazil. The *Flemingia macrophylla* (Willd.) Kuntze ex Merr. plants were cut to 1 m height and the thick stems were separated from the leaves and thin stems. This fraction and the grass *Cynodon dactylon* cv. Tifton 85 were sun-dried.

Five crossbred dairy goats (Saanen × Boer) with an initial weight of 46.5 kg and 1.5 kg milk/day, in mid-lactation, were arranged in a 5 × 5 Latin square. The animals were kept in individual pens with apparatus for total collection of feces. Each experimental period lasted 11 days: seven for adaptation to the diet and four for the collection of samples and data.

Diets were isonitrogenous, with 14% crude protein (CP), and were composed of 40% concentrate and 60% hay (dry matter basis) (Table 1). The treatments were the levels of inclusion of legume hay (*Flemingia macrophylla*) replacing grass hay (*Cynodon dactylon* cv. Tifton 85) in the forage part of the diet.

The treatments were the following: Control - 60% *Cynodon dactylon* hay + 40% concentrate; 8% *Flemingia macrophylla* hay + 52% *C. dactylon* hay + 40% concentrate; 16% *F. macrophylla* hay + 44% *C. dactylon* hay + 40% concentrate; 24% *F. macrophylla* hay + 36% *C. dactylon* hay + 40% concentrate; and 32% *F. macrophylla* hay + 28% *C. dactylon* hay + 40% concentrate.

The diet was formulated according to the nutritional requirements of goats (NRC, 2007) (Table 2). The goats received feed twice daily to allow for 15% of leftovers in relation to the total offered, thus ensuring animal selectivity and the voluntary feed intake. The animals received water and mineral mixture for goat *ad libitum*.

The samples of feed, orts, and feces were oven-dried at 55 ºC and finely ground (1 mm). These samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), and total ash using AOAC methods 934.01, 976.05, 954.02, and 942.05, respectively (AOAC, 1990).

The concentrations of neutral detergent fiber (NDFom) and acid detergent fiber (ADF) were determined using the method proposed by VanSoest et al. (1991). NDF analysis was not performed in the presence of α-amylase and sodium sulfite (Na_2SO_3). In addition, the residue from the NDF analyses was filtered in Goochs crucibles and the concentration of ash in the residual material was determined by combustion at 600 ºC for 3 h (AOAC, 1990; method 942.05).

The carbohydrate fractions were obtained using the methodology described by Sniffen et al. (1992) (Table 3).

Saponins were identified by dissolving the extract in water with constant stirring. The formation and persistence (for 15 min) of foam indicates the presence of saponins in plant extracts (Dewick, 2002).

Samples of rumen contents were collected by an esophageal tube and a vacuum pump four hours after meal on the 11th day of each experimental period for determination of pH and $N\text{-}NH_3$ concentration in the rumen fluid. The pH was measured immediately after collection. For the quantification of the ammonia concentration, 50 mL of rumen fluid were filtered and 1 mL of sulfuric acid - H_2SO_4 (1:1) was added to the filtrate and subsequently distilled over potassium hydroxide - KOH 2N (Vieira, 1980).

The observation of the feeding behavior began on the 5th day of each experimental period and lasted 24 h. The interval between observations was 20 min, as validated

Table 1 - Chemical composition of the diet ingredients

Component	*Flemingia*	Tifton 85	Corn meal	Soybean meal
Dry matter (%)	86.76	83.76	87.16	87.23
Crude protein (%DM)	16.32	14.53	9.15	52.37
Ether extract (%DM)	3.40	2.06	3.97	1.62
Neutral detergent fiber (%DM)	61.30	68.93	15.28	14.06
Acid detergent fiber (%DM)	47.59	34.45	3.78	9.88
Mineral matter (%DM)	6.08	6.44	1.73	6.76

Table 2 - Ingredients and chemical composition of the diets according to the level of the *Flemingia* hay

Ingredient	*Flemingia* level				
	0%	8%	16%	24%	32%
Cynodon dactylon hay (%)	60	52	44	36	28
Flemingia macrophylla hay (%)	0	8	16	24	32
Corn meal (%)	36.2	36.6	36.8	37.2	37.5
Soybean meal (%)	3.8	3.4	3.2	2.8	2.5
Nutrient					
Dry matter (%)	83.0	83.7	84.0	84.7	85.3
Organic matter (%DM)	95.5	95.5	95.6	95.6	95.7
Crude protein (%DM)	14.0	14.0	14.0	14.0	14.0
Ether extract (%DM)	2.7	2.9	3.0	3.1	3.2
Neutral detergent fiber (%DM)	47.4	46.8	46.2	45.6	45.0
Acid detergent fiber (%DM)	22.4	23.4	24.5	25.5	26.6
Lignin (%DM)	3.5	4.8	6.1	7.6	8.8
Total carbohydrates (%DM)	78.6	78.5	78.4	78.3	78.2
Non-fiber carbohydrates (%DM)	33.0	33.6	34.1	34.7	35.2
Mineral matter (%DM)	4.45	4.45	4.41	4.36	4.30

Table 3 - Fractionation of carbohydrates and lignin content in *Flemingia macrophylla* and Tifton 85 hays

Component	Tifton 85	*Flemingia macrophylla*
Total carbohydrates (%DM)	76.9	74.2
Fraction "C" (%DM)	12.1	49.6
Fraction "B2" (%DM)	56.8	11.7
Non-fiber carbohydrates (%DM)	8.0	12.9
Lignin (%DM)	5.06	20.66

by Carvalho et al. (2007), in which the feeding (including consumption of feed, water, and mineral salts) idling, and rumination times were recorded. During the night time observation the shed was lit minimally to facilitate the observation, but without interfering with the animals' natural behavior. Trained observers took turns of two shifts. Based on the ingestive behavior data, it was possible evaluate feed efficiency (FE), rumination efficiency (RUE), and total time spent chewing (TSC) according to the formulas of Bürger et al. (2000): FE_{DM} = DMI (g/day)/TSF (h/day); FE_{NDF} = NDF (g/day)/TSF (h/day); RUE_{DM} = DMI (g/day)/TSR (h/day); RUE_{NDF} = NDF (g/day)/TSR (h/day); TSC (min/day) = TSF + TSR, in which: FE_{DM} (g DM consumed/h); FE_{NDF} (g NDF consumed/h); RUE_{DM} (g DM ruminated/h); RUE_{NDF} (g NDF ruminated/h), DMI (dry matter intake); TSF (time spent feeding); and TSR (time spent ruminating).

The results were subjected to analysis of variance and regression through the PROC MIXED procedure of SAS (Statistical Analysis System, version 9.0). Effects were considered significant at $\alpha = 0.05$.

The following statistical model was used:

$$Y_{ijk} = \mu + P_i + A_j + \alpha_k + e_{ijk},$$

in which Y_{ijk} = observation of animal *j* subjected to treatment *k* in period *i*; μ = overall mean effect; P_i = effect of period *i*; A_j = effect of animal *j*; α_k = treatment effect *k*; and e_{ijk} = random error *ijk* (*i* = period (1, 2, 3, 4, 5), *j* = animal (1, 2, 3, 4, 5), and *k* = treatment (0%, 8%, 16%, 24%, and 32% of *Flemingia* replacing Tifton hay)).

Lilliefors and Cochran's and Bartlett's tests were performed to check the normal distribution of the evaluated data.

Results

Saponin was detected in samples of *Flemingia*. The test conducted was qualitative, so there was no quantification of saponin content in the legume in question.

There were no differences in feeding behavior, feed and rumination efficiencies, or total chewing time for the animals subjected to different treatments (Table 4).

Table 4 - Means and coefficients of variation (CV) for feeding-behavior activities in different treatments

Activity	*Flemingia* level					Mean	CV
	0%	8%	16%	24%	32%		
Rumination (min)	260	288	308	296	296	Ŷ = 289.6	23.09
Idling (min)	792	748	748	728	776	Ŷ = 758.4	11.48
Feeding (min)	388	404	384	416	368	Ŷ = 392.0	19.81
FE (g DM/h)	255	268	284	275	305	Ŷ = 277.4	23.69
FE (g NDF/h)	0.09	0.10	0.11	0.11	0.12	Ŷ = 0.11	27.99
RUE (g DM/h)	404	364	376	387	394	Ŷ = 385.0	27.71
RUE (g NFD/h)	0.15	0.14	0.15	0.15	0.15	Ŷ = 0.15	28.50
Total chewing time (h)	10.8	11.53	11.53	11.87	11.07	Ŷ = 11.4	16.33

FE - feed efficiency; RUE - rumination efficiency; DM - dry matter; NDF - neutral detergent fiber.

Table 5 - Means, coefficients of variation (CV) and determination (r^2), and regression equations for the intake of nutrients in the different treatments

Item	*Flemingia* level					CV	r^2	Regression
	0%	8%	16%	24%	32%			
DM[1]	1.62	1.68	1.79	1.85	1.78	13.00	-	Ŷ = 1.74
DM[2]	3.16	3.24	3.40	3.53	3.40	11.52	-	Ŷ = 3.35
OM[1]	1.56	1.61	1.72	1.77	1.71	12.88	-	Ŷ = 1.67
CP[1]	0.23	0.25	0.27	0.27	0.26	12.50	-	Ŷ = 0.26
EE[1]	0.056	0.063	0.065	0.065	0.093	16.60	0.47	Ŷ = 0.0533 + 0.00094X
NDFom[1,3]	0.60	0.63	0.70	0.73	0.69	15.86	-	Ŷ = 0.67
NDFom[2,3]	1.17	1.22	1.33	1.39	1.32	14.11	-	Ŷ = 1.28
ADF[1]	0.28	0.32	0.38	0.41	0.42	15.35	0.45	Ŷ = 0.2912 + 0.00448X
Lignin[1]	0.048	0.074	0.099	0.133	0.153	16.91	0.83	Ŷ = 0.0474 + 0.00335X
Hemicellulose[1]	0.39	0.29	0.26	0.25	0.23	47.71	-	Ŷ = 0.28
TC[1]	1.27	1.31	1.39	1.44	1.39	13.27	-	Ŷ = 1.36
NFC[1]	0.70	0.71	0.73	0.75	0.73	13.57	-	Ŷ = 0.72
TDN[1]	1.21	1.17	1.23	1.21	1.19	14.36	-	Ŷ = 1.201

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDFom - neutral detergent fiber; ADF - acid detergent fiber; TC - total carbohydrates; NFC - non-fiber carbohydrates; TDN - total digestible nutrients.
[1] kg/day.
[2] %BW.
[3] Not assayed with heat-stable amylase and expressed exclusive of residual ash.

The time spent on each feeding behavior is related to the intake and digestibility of the diet. There were no differences in the intakes of dry matter, organic matter, mineral matter, crude protein, or neutral detergent fiber by goats subjected to different treatments. The intake of ether extract, acid detergent fiber, and lignin increased linearly with the dietary inclusion of *Flemingia* (Table 5).

There were differences (P<0.05) in the digestibility of dry matter, organic matter, crude protein, ether extract, NDFom, ADF, and total carbohydrate (Table 6).

No differences for the values of ruminal ammonia nitrogen and pH were found (Table 7) with the dietary inclusion of *Flemingia*.

Discussion

Flemingia saponins may have had effects on the the feeding and nutrition of animals. Saponins can inhibit the growth and activity of ruminal microorganisms (Taiz and Zeiger, 2004), decreasing the acetate:propionate ratio (Kamra, 2005). Furthermore, the saponins may bring some nutritional benefits related to the reduction of the protozoa population in the rumen (Lu and Jorgensen, 1987).

The presence of tannin in *Flemingia macrophylla* was confirmed by other authors, who found levels of 2% and 5% (Tiemann et al., 2008), and by Fagundes (2012), who observed 10.5% condensed tannin in *F. macrophylla*. However, Aviz et al. (2009) described a low content of this compound in *Flemingia*, which was set at 1.37%. This divergence of values may occur because of the influence of factors such as climate, plant mineral nutrition, growth, and chemical composition (Waterman and Mole, 1994), which directly affect the formation of tannin.

The total carbohydrates from grass and legume were similar, 76% and 74.2% respectively; the fraction "C" content of *Flemingia* (49.6%) was higher than that of the grass (12.1%). The fraction "C" is the fiber indigestible fraction (Sniffen et al., 1992), and so the observed fraction "C" is consistent with the high lignin content in *Flemingia*, which was greater than 20%, in agreement with reports of a high content of lignin in legumes (Grenet and Besle, 1991; Valente et al., 2011). Furthermore, the methodology used for determining lignin, which was the potassium permanganate, according to Jung (1997) and Goering and Van Soest (1970), overestimates the amount of lignin by the removal of other cell wall components, by interference from other solubilized compounds, and because particles of various sizes are not treated equally. Even with this, the lignin content of *Flemingia* was high and may have had a great influence on the digestibility of polysaccharides.

The fraction "B2", which is slowly digested, was superior in Tifton (56.8%) than in *Flemingia* (11.7%). Fagundes (2012) made similar a report, in which he observed 22.6% fraction "B2" in *Flemingia* and 51.8% for Tifton. The levels of non-fiber carbohydrates (NFC) were

Table 6 - Means, coefficients of variation (CV) and determination (r^2), and regression equations for the digestibility of nutrients in the different treatments

Item	*Flemingia* level					CV	r^2	Regression
	0%	8%	16%	24%	32%			
DM (g/kg)	718	669	661	628	605	5.29	0.54	$\hat{Y}$ = 709 – 3.3X
OM (g/kg)	737	685	676	644	621	5.07	0.56	$\hat{Y}$ = 727 – 3.4X
CP (g/kg)	683	605	588	527	488	7.87	0.68	$\hat{Y}$ = 672.2 – 5.87X
EE (g/kg)	752	737	720	727	818	5.95	0.005	$\hat{Y}$ = 726.3 + 1.53X
NDFom (g/kg)[1]	585	437	465	406	353	14.02	0.37	$\hat{Y}$ = 548.7 – 6.209X
ADF (g/kg)	569	431	436	373	316	20.61	0.45	$\hat{Y}$ = 537.9 – 7.06X
Hemicellulose (g/kg)	626	403	595	580	520	41.9	-	$\hat{Y}$ = 544.8
TC (g/kg)	746	699	693	665	643	5.24	0.47	$\hat{Y}$ = 737.4 – 3.00X
NFC (g/kg)	898	946	923	931	930	4.08	-	$\hat{Y}$ = 925.6

DM - dry matter; OM - organic matter; CP - crude protein; EE - ether extract; NDFom - neutral detergent fiber; ADF - acid detergent fiber; TC - total carbohydrates; NFC - non-fiber carbohydrates.
[1] NDF not assayed with heat-stable amylase and expressed exclusive of residual ash.

Table 7 - Means, coefficients of variation (CV), coefficients of determination (r^2), and regression equations adjusted to the values of ammonia nitrogen (NH_3-N) and ruminal pH in different treatments

Item	*Flemingia* level					Mean	CV
	0%	8%	16%	24%	32%		
N-NH_3 (mg/dL)	19.12	18.10	16.68	14.20	11.17	$\hat{Y}$ = 15.85	29.765
Ruminal pH	6.64	6.72	6.94	6.90	6.92	$\hat{Y}$ = 6.82	0.02

higher in *Flemingia* (12.9%) compared with Tifton (8%), due to the higher content of cellular matter in legumes (Bumbieris Junior et al., 2011). However, Fagundes (2012) found a higher NFC level in *Flemingia* (54.1%) than in this study, possibly because only the leaves of the legume were used and not the leaves and stems, as tested in this study. Carvalho et al. (2004) reported that rumination efficiency is an important tool for analyzing low-digestibility foods. The level of dietary *Flemingia* did not influence the feed and rumination efficiencies expressed in g NDF/h, because the intakes of NDF did not differ between the diets.

The animals spent more time idle at night and the longest period of rumination occurred at dawn, which is a common behavior according to Gonçalves et al. (2001). The feeding activity predominated in the morning and afternoon, while during the night and early morning the prevailing behavior was idleness.

The lack of difference between the intakes of diets shows good acceptability of *Flemingia* by goats, and the presence of lignin, saponin, and tannin did not influence the acceptability by the animals. The TDN intake was similar and was sufficient to meet the animals' requirements (NRC, 2007).

The higher intake of EE is related to the higher content of this component in the diets with more *Flemingia* and corn, because these ingredients have more EE than Tifton and soybean meal, respectively. The higher intake of ADF in diets with a higher content of *Flemingia* can be justified by the greater percentage in this legume. *Flemingia* is also rich in lignin (20.66% DM), and so the intake of lignin was higher in diets with higher concentrations of *Flemingia* replacing Tifton.

The intakes of DM and CP were higher than the 1.6 kg and 0.14 kg predicted by AFRC (1993) for animals with 46.5 kg and 1.5 kg of milk per day. Diets with *Flemingia* met the animals' nutritional requirements.

Fagundes (2012) fed goats with *Flemingia* and obtained results close to the average values found in this study (DMI of 1.77 kg/day, OMI of 1.71 kg/day, and CPI of 0.27 kg/day) and intakes were similar between animals. Mui et al. (2001) fed goats with *Flemingia*, and when they included the legume, the goats reduced their DMI. Aviz (2007) achieved good values for DMI and CP even with the addition of 100% *Flemingia* in the diet.

The CP intake by goats was equivalent in the five treatments, because the diets were isonitrogenous. Thus, for every increase in the level of the legume in the diet, there was a decrease in soybean meal added to the concentrate.

The apparent digestibility of DM, OM, CP, NDFom, ADF, and TC showed a linear decrease with the increasing level of dietary *Flemingia*. Fagundes (2012) also found decreased digestibility of DM, OM, CP, NDF, and TC with the inclusion of *Flemingia* in the diet of lactating goats. These results may be due to three reasons, two of them related to the presence of condensed tannins in legumes. One explanation is the formation of complexes between tannins and proteins or carbohydrates in the diet, reducing nutrient availability, and the other is the complexation of tannins with microbial enzymes, reducing their activity and thus the digestibility of the diet. The third possibility is related to the lignin content of *Flemingia*, which may have been responsible for the greater decline in digestibility.

The ether extract digestibility showed a linear response with increasing dietary levels of *Flemingia*. This shows that despite a lower digestibility of the other components of the diet, there was an energetic compensation with higher EE digestibility.

The presence of condensed tannins in *Flemingia* could alter the rumen pH, which did not occur in this study. Another factor that could contribute to increased pH is the lignin content of *Flemingia*. Diets with more *Flemingia* had a higher lignin content and could provide lower production of fatty acids (Gomes et al., 2009), raising the pH. The higher concentrations of *Flemingia* also could increase the ruminal pH due to the higher buffering capacity of vegetables compared with grasses (Whittenbury et al., 1967).

According to Satter and Slyter (1974), the minimum value of ammonia nitrogen to maintain normal rumen function is 5 mg/dL. The average ammonia nitrogen found in this study was 15.85 mg/dL of ruminal fluid, a value within the normal range, and did not vary with the different diets. The presence of ammonia nitrogen in the rumen is essential for microbial growth, providing an adequate fermentation rate (Van Soest, 1994).

Conclusions

Flemingia macrophylla hay can be used up to the level of 32% in the dry matter of the diet of lactating goats without impairing nutrient intake.

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