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Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Effect of season on chemical composition and *in situ* degradability in cows and in adapted and unadapted goats of three Mexican browse species

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ARTICLE INFO

Article history:

Received 10 September 2008

Received in revised form 16 October 2009

Accepted 4 December 2009

Keywords:

Browse

Cows

Degradability

Goats

Dry season

Rainy season

ABSTRACT

Browse foliages from *Lysiloma acapulcensis*, *Quercus laeta* and *Pithecellobium dulce*, native to the subtropical region of southern México, were harvested during the dry season (DS) and rainy season (RS) to determine *in situ* degradability using ruminal inoculum from fistulated cows as well as goats previously adapted (AG) or not adapted (UG) to browse species fed in their daily diet. Browse leaf samples were incubated in the rumen of each group for 48 h. The crude protein (CP) content of browse was considerably higher in RS ($P < 0.001$). *P. dulce* had the lowest neutral detergent fiber (NDFom) and acid detergent fiber (ADFom) in the two seasons; *L. acapulcensis* had the highest values and *Q. laeta* values were intermediate, with an overall increase in fiber fractions in DS browse foliage ($P < 0.001$). The lowest *in situ* degradability values were in *L. acapulcensis* and *Q. laeta* had intermediate values during both seasons. Season of harvest (RS or DS), and ruminal inoculum (cows, UG, and AG) affected ($P < 0.001$) dry matter degradability (DMD), crude protein degradability (CPD) and fiber fractions of browse. Nutrient degradabilities in all species were higher ($P < 0.001$) in DS than RS. Goats previously exposed to these browse species (AG) had higher ($P < 0.001$) *in situ* degradability of the browse species than cows or goats in UG fed diets without browse. Overall, goats had higher ($P < 0.001$) nutrient *in situ* degradability than cows. Our results suggest higher potential of these browse species as forages for ruminants during the dry period in semi-arid regions, but goats previously exposed to diets supplemented with the browse species had a better ability to degrade them than cows or goats in UG. *P. dulce* has the highest potential as a feed protein source in small ruminants during the dry period.

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Abbreviations: ADFom, acid detergent fiber; AG, adapted goats; AOAC, Association of Official Analytical Chemists; CP, crude protein; DM, dry matter; DS, dry season; GLM, general linear model; NDFom, neutral detergent fiber; OM, organic matter; SAS, Statistical Analyses System; RS, rainy season; CT, condensed tannins; UG, unadapted goats.

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doi:10.1016/j.anifeedsci.2009.12.001

1. Introduction

The use of browse species as green forage for ruminants is becoming increasingly important in many parts of the tropics, including México. In free-ranging livestock-production systems, browse species are an important supplement to dry season forage shortages, improving both the quantity and quality of pasture. Trees and shrubs are perennials that allow provision of permanent fodder *versus* herbaceous species which decrease rapidly in quantity and quality after rains. Regular availability of forage from trees and shrubs depends on the diversity of species and their phenological variation in time and space (Grouzis and Sicot, 1980), but they have potential to alleviate feed shortages and nutritional deficiencies during the dry season on smallholder farms. The high quality of some browse in crude protein (CP) content, as well as minerals such as calcium and phosphorus (Paterson et al., 1998), is well appreciated. Tree and shrub leaves can be an important component of small ruminant diets (Papachristou and Nastis, 1996; Salem et al., 2006), and they are important in the nutrition of grazing animals in areas where few, or no, alternative feeds are available (Meuret et al., 1990). However many browse species have secondary compounds that can deter invasion and consumption of their leaves by microbes, insects and herbivorous animals. Tannins are an important class of compounds that can decrease degradability of browse fodders (Salem et al., 2006), but use of tree and shrub leaves by herbivores may be restricted by negative effects on digestion and intake of their generally high levels of several other secondary compounds (Salem, 2005; Salem et al., 2006, 2007).

Goats are effective browsers, that have the ability to utilize woody species and low-quality forages better than cattle and sheep, and can adapt to harsh environments (Silanikove, 2000; Salem et al., 2004). Extensive shrublands of evergreens and small trees, known as garrigue or maquis, that are often high in tannins and other secondary compounds are the basic components of diets of goats in the Mediterranean area (Salem et al., 2006, 2007). Previous studies suggest that progressive adaptation to tannins can be induced in any herbivore species in response to regular feeding of tanniniferous feeds (Pell et al., 2000). Animals adapted to tannins in their diet exhibit changes in rumen microbial populations, and tannin-resistant, or tolerant, bacteria can be isolated particularly from the rumen of animals previously exposed to tanniniferous feeds (Brooker et al., 2000; Odenyo et al., 2003). In México, there is limited information on the nutritive value of tree shrubs fed to livestock (Ramirez et al., 2000; Cerrillo and Juárez, 2004). Our aim was thus to investigate impacts of growing season on ruminal *in situ* degradability of some tree foliages using rumen fluid from goats (adapted or unadapted to eating tree foliage) and cows under the southern subtropical conditions in México.

2. Materials and methods

2.1. Area of sample collection

Tree fodder species were collected from the South of Estado de México (Tejupilco) and the related metabolic work and laboratory analyses were completed at the Centro Universitario UAEM Temascaltepec located in the municipality of Temascaltepec de González Estado de México. Geographically, this is located at 19°02'04" north latitude and 100°02'14" west longitude at an elevation of 1720 masl. The climate is moderately humid with an average temperature of 15–18 °C and annual rainfall of 950–1000 mm (García, 1987).

2.2. Browse species

We sampled a mixture of young and mature leaves from three browse species – Tepehuaje (*Lysiloma acapulcensis*), Encino prieto (*Quercus laeta*) and Pinzan (*Pithecellobium dulce*) – that contain different levels of CT (high, medium, and low) from several locations in the South of Estado de México. Plant samples (~5 kg) were randomly collected up to a height of 1.5 m from at least 7 trees for each browse species during the rainy (i.e., August/September, 2006) and dry (April/May, 2007) seasons. Samples of each species were pooled to three samples and air dried in the shade to minimize changes in tannins content and activity (Makkar and Singh, 1991; Yousef and Rouzbehan, 2008; Robinson et al., 2006).

2.3. Analytical methods

Fresh samples were dried at 45 °C for 48 h for moisture determination and ground in a Willey-mill to pass a 1 mm screen. Ground samples were analyzed for dry matter (DM) by drying at 105 °C for 24 h in a forced air oven. Ash content was measured after igniting samples in a muffle furnace at 550 °C for 4 h. The CP was determined by the Kjeldahl method (AOAC, 1990; ID 954.01). Neutral detergent fiber (NDFom) and acid detergent fiber (ADFom) were determined by the methods of Van Soest et al. (1991) with NDFom assayed without use of an alpha amylase, but with sodium sulfite. Both NDFom and ADFom are expressed without residual ash. Total condensed tannins (TCT) were assayed using the butanol-HCL method (Terrill et al., 1992), modified by López et al. (2004), as internal standards using *L. acapulcensis*. Analyses of the free, protein-bound and fiber-bound CT were conducted, according to the method of Porter et al. (1986). The purification was performed with Sephadex LH-20 as described by Asquith and Butler (1985) with modifications by Hagerman (1991). Tannin results were the starting point for categorizing the species in three types: high (*L. acapulcensis*), medium (*Q. laeta*) and low (*P. dulce*) tannin concentrations (Table 1).

Table 1
Chemical composition and condensed tannins (g/kg DM) of the leaves of three species of browse tree during the rainy and dry seasons.

Season (S)	Rainy			Dry			Sed	P value			
	Browse species (B)	<i>L. acapulcensis</i>	<i>Q. laeta</i>	<i>P. dulce</i>	<i>L. acapulcensis</i>	<i>Q. laeta</i>		<i>P. dulce</i>	S	B	S×B
OM		945.9 ^b	950.4 ^a	909.5 ^d	941.5 ^c	905.6 ^e	862.1 ^f	7.98	<0.001	<0.001	<0.001
CP		177.0 ^c	94.1 ^e	261.5 ^a	110.6 ^d	84.7 ^f	187.8 ^b	9.21	<0.001	<0.001	<0.001
NDFom		607.3 ^a	584.1 ^{ab}	495.8 ^c	545.0 ^b	408.3 ^d	357.3 ^e	2.06	<0.001	<0.001	<0.001
ADFom		500.8 ^a	411.8 ^b	365.7 ^c	478.6 ^a	249.8 ^d	274.7 ^d	2.31	<0.001	<0.001	<0.001
Free-CT		116.3 ^a	64.2 ^{cd}	36.6 ^d	101.6 ^{ab}	80.2 ^{bc}	68.3 ^{cd}	6.71	0.077	<0.001	0.016
PCT		67.8 ^a	23.0 ^c	21.8 ^c	55.8 ^b	26.3 ^c	21.1 ^c	4.57	0.067	<0.001	0.005
FCT		3.7 ^b	2.7 ^c	4.1 ^{ab}	2.8 ^c	2.5 ^c	4.7 ^a	0.21	0.190	<0.001	0.003
TCT		187.8 ^a	89.9 ^{bc}	62.6 ^c	160.2	109.0 ^b	94.1 ^{bc}	10.77	0.229	<0.001	0.004

Means with different superscripts within a row differ ($P < 0.05$) between season of harvest of browse species. OM; organic matter, CP; crude protein, NDFom; neutral detergent fiber, ADFom; acid detergent fiber, Free-CT: free condensed tannins; PCT: protein-bound condensed tannins; FCT: fiber-bound condensed tannins; TCT: total condensed tannins. Sed: standard error of the difference.

2.4. Animals and diets

Two brown Swiss American cows (530 ± 5 kg) and 4 male Criollo×Nubia goats (34 ± 4 kg body weight) were used for sample incubation. Animals were fitted with permanent ruminal cannula about 150 days before the ruminal incubations. The 2 cows and 2 goats (unadapted goats; UG) were fed forage:concentrate (80:20; DM basis) diets. The forage contained a mixture of alfalfa hay (400 g/kg), corn silage (400 g/kg) and corn straw (200 g/kg) with a concentrate mixture (220 g/kg CP), containing ground sorghum (380 g/kg), corn (380 g/kg), soybean (120 g/kg), molasses (80 g/kg), urea (20 g/kg) and minerals (20 g/kg). The other 2 goats were fed the same concentrate mixture offered to the cows and UG, but the forage proportion was alfalfa hay (300 g/kg) and browse species (700 g/kg) composed of 128 g/kg *P. dulce*, 198 g/kg *Quercus* sp., 261 g/kg *Q. laeta*, 413 g/kg *L. acapulcensis*. The AG animals were fed this diet for 100 days before sample incubation. All animal groups were fed twice daily at 07:00 and 16:00 h. All animals had free access to vitamin–mineral mixture and clean water.

2.5. In situ degradability

In situ degradability was measured by the method of Ørskov et al. (1980) with nylon bags (5 cm×10 cm) with a pore size of 35 µm diameter. Samples of the three browse species with different contents (high, medium, and low) of CT, collected during the rainy and dry seasons, were ground to pass a 1 mm screen in a Wiley mill. Samples, 2.5 g DM/bag, were incubated in the rumen of each fistulated animal for 48 h. Three bags, of the same browse species, were introduced into the rumen of each animal species (cows, UG, and AG) before the morning feeding. The incubation was repeated with the same fistulated animal. After removal of all bags simultaneously from the rumen, bags were washed with tap water until the water was clear. They were then dried in a forced air oven at 65 °C and weighed to determine DM loss. The N and fiber fractions (NDFom and ADFom) were determined in residues to calculate the degradability.

2.6. Statistical model and analysis

Data for the chemical assays were analyzed using the general linear model (GLM) procedure in SAS (2002) for a completely randomized design with 2 seasons (rainy and dry)×3 browse species in a factorial arrangement with 3 repetitions (pooled samples) (Steel and Torrie, 1980). Means were tested using a least square means test (LSMEANS) with the statistical model:

$$Y_{ik} = \mu + S_j + B_k + S_j \times B_k + E_{jk}$$

where Y_{ik} represents response variables (chemical composition) for the two seasons (j) for each of the three browse species (k); μ = general mean; S_j = effect of j -season; B_k = effect of k -browse species; $S_j \times B_k$ = interaction of the j season with k browse species; E_{jk} = the error term – NI ($0, \sigma^2$).

Data for *in situ* degradability were analyzed using a randomized complete block design with 2 seasons (rainy and dry)×3 sources of inoculum (cows, UG, and AG)×3 browse species in factorial arrangement with 3 repetitions (Steel and Torrie, 1980). The mixed model was:

$$Y_{jkl} = \mu + S_j + TI_k + Sp_l + \beta_n + S_j \times TI_k \times Sp_l + E_{jkl}$$

where Y_{jkl} represents response variables (degradability of DM, CP, NDF and ADF) for the same season (j), source of ruminal inoculum (k), and browse species (l); μ = general mean; S_j = effect of j season; TI_k = effect of k -type of ruminal inoculum; Sp_l = effect of l browse species; β_n = effect of n -block (repeated incubation); $S_j \times TI_k \times Sp_l$ = interaction of the j season with k type of ruminal inoculum and l -browse species; E_{jkl} = the error term – NI ($0, \sigma^2$).

Differences among means with $P < 0.05$, determined using a least square means test (LSMEANS), were accepted as representing statistically significant differences (Steel and Torrie, 1980).

Table 2

In situ degradability (g/kg) of dry matter (DMD), crude protein (CPD), neutral detergent fiber (NDFD) and acid detergent fiber (ADFD) of some browse tree foliages after 48 h of incubation in the rumen of cows as well as unadapted (UG) and adapted (AG) goats during the rainy and dry seasons.

Season	Inoculum	Browse	DMD	CPD	NDFD	ADFD
Rainy	Cows	<i>L. acapulcensis</i>	241.6 ^k	21.7 ^l	61.1 ^{ij}	109.3 ^{hi}
		<i>Q. laeta</i>	331.8 ^{gh}	131.4 ^{hi}	142.0 ^{gf}	230.7 ^{ef}
		<i>P. dulce</i>	544.3 ^c	584.6 ^{bc}	308.6 ^{bc}	303.6 ^{cd}
	UG	<i>L. acapulcensis</i>	273.1 ^j	19.9 ^l	88.0 ^{hi}	121.2 ^{hi}
		<i>Q. laeta</i>	369.5 ^{ef}	176.0 ^{hg}	162.5 ^{ef}	247.6 ^{ef}
		<i>P. dulce</i>	547.8 ^c	581.9 ^{bc}	332.4 ^{ab}	374.7 ^{ab}
	AG	<i>L. acapulcensis</i>	281.9 ^j	108.5 ^{ij}	109.2 ^{hig}	144.3 ^{gh}
		<i>Q. laeta</i>	389.5 ^e	226.4 ^f	163.7 ^{ef}	198.5 ^{fg}
		<i>P. dulce</i>	514.6 ^d	563.1 ^c	273.8 ^c	340.2 ^{bc}
Dry	Cows	<i>L. acapulcensis</i>	296.7 ^{ij}	59.0 ^{kl}	38.1 ^j	61.6 ⁱ
		<i>Q. laeta</i>	499.9 ^d	214.0 ^{if}	121.1 ^{gfh}	112.5 ^{hi}
		<i>P. dulce</i>	596.9 ^b	625.4 ^b	214.7 ^d	291.5 ^{cde}
	UG	<i>L. acapulcensis</i>	310.7 ^{ih}	71.2 ^{jk}	77.6 ^{hij}	101.5 ^{hi}
		<i>Q. laeta</i>	551.7 ^c	304.8 ^e	217.0 ^d	143.9 ^{gh}
		<i>P. dulce</i>	668.9 ^a	730.9 ^a	366.2 ^a	404.1 ^a
	AG	<i>L. acapulcensis</i>	344.7 ^{gf}	152.1 ^{hi}	116.1 ^{gfh}	121.7 ^{hi}
		<i>Q. laeta</i>	558.6 ^c	371.6 ^d	197.7 ^{ed}	105.2 ^{hi}
		<i>P. dulce</i>	652.8 ^a	738.5 ^a	336.2 ^{ab}	381.8 ^{ab}
Sed	5.61	9.73	9.84	10.92		
		<i>P</i> value				
		DMD	CPD	NDFD	ADFD	
Season		<0.001	<0.001	0.232	<0.001	
Inoculum		<0.001	<0.001	<0.001	<0.001	
Browse		<0.001	<0.001	<0.001	<0.001	
Season×inoculum×browse		<0.001	<0.001	<0.001	<0.001	

UG: unadapted goats; AG: adapted goats. Means within a column with different superscripts differ ($P < 0.05$).

3. Results

The CP content of the browse species ranged from 85 g/kg (DS: dry season) and 94 g/kg (RS: rainy season) for *Q. Laeta* to 262 g/kg DM for *P. dulce* in the RS (Table 1). Overall, CP content of browse species was considerably higher in the rainy season ($P < 0.001$). *P. dulce* had the lowest NDFom and ADFom in the two seasons, *L. acapulcensis* had the highest values, and *Q. laeta* values were intermediate with an overall increase in fiber fractions in the rainy season ($P < 0.001$). The browse species differed in CT contents ($P < 0.01$ —Table 1). Overall, free-CT values were lower in *Q. Laeta* and *P. dulce* in the RS than in the DS, except *L. acapulcensis* had the opposite though non-significant effect. Soluble or free-CT fraction varied from 36.6 in *P. dulce* to 116.3 g/kg DM in *L. acapulcensis* during the RS. The protein-bound CT (PCT) fraction had the same trend of free-CT content in the tree species and varied ($P < 0.05$) from 21 g/kg in *P. dulce* (DS) to 67.8 g/kg DM in *L. acapulcensis* (RS). PCT was not different between *Q. Laeta* and *P. dulce* in the two seasons.

Season of harvest (RS or DS), and ruminal inoculum (cows, goats (UG or AG)) both affected ($P < 0.001$) DMD, CPD and fiber fractions of the browse (Table 2). Nutrient *in situ* degradability of all browse species was higher ($P < 0.001$) during the DS than the RS. *In situ* degradability of browse species in goats previously exposed to the browse species (AG) was higher ($P < 0.001$) than in cows or UG fed diets without browse. Overall, goats had higher ($P < 0.001$) nutrient degradability of browse foliage than cows. However, *P. dulce* had the highest ($P < 0.001$) *in situ* degradability values, while the lowest values were for *L. acapulcensis* with *Q. laeta* intermediate during the two growing seasons.

4. Discussion

4.1. Chemical composition and condensed tannins

Lower levels of CP in browse during the DS versus the RS (Table 1) are consistent with other studies, which indicate that the minimum CP content of fodder tree leaves in the DS is more than twice that of grasses in the RS (Evitayani et al., 2004). Importantly, the CP content of browse species remained relatively high in *P. dulce* and *L. acapulcensis* during the DS, suggesting the possibility that leaves may be used as a DS fodder and feed supplement to low-quality diets. However, high secondary compound levels in *L. acapulcensis* may prevent its use if the nutrients cannot be digested and utilized. The higher

CP content in *P. dulce*, with its low CT during both seasons, suggests that it may be better quality forage for ruminants than the other browse species.

The CT content of browse species increased during the DS versus the RS, likely due to hydric stress in DS, activating enzymatic system phenylalanine ammonia-lyase (PAL) (Hidalgo, 2002) higher DS temperatures a finding consistent with those of Cabiddu et al. (2000) and Salem et al. (2006) for various browse species. Decreased concentrations of CT in browse leaves during the RS season may be due to the plant allocating more soluble carbohydrates to growth and reproduction than to producing tannins (Skogsmyr and Fagerstrom, 1992). This dilution effect during the RS may also explain the lower concentrations of fiber during the DS. Such seasonal variation in response to climatic and physiological changes in browse plants induces changes in chemical composition and, in particular, in concentrations of secondary compounds such as tannins (Salem, 2005; Salem et al., 2006). These differences determine the value of browse plant foliage as forages for ruminants.

4.2. Ruminal digestibility of browse species

Lower *in situ* degradability of browse species during the DS versus the humid RS in cows and goats could be due to the effects of CT on available N in leaves, which could reduce rumen ammonia concentrations and microbial growth (Salem et al., 2007). Cross-linkages of lignin to hemicellulose, polysaccharides and proteins may also depress digestibility (Van Soest, 1994). In the current study, the lignin content of RS browse was likely high, as suggested by the positive relationship between NDFom and ADFom in the studies of Frutos et al. (2002), and this could explain the lower of DM and NDF degradability of browse during the DS.

Differences between cows and goats in ruminal *in situ* degradability were probably due to differences in rumen microbial populations that affect the kinetics of rumen fermentation. In a comparison of sheep and goats, Salem et al. (2004) concluded that differences between sheep and goats in dentition, chewing/eating behaviour, gut physiology, compartment dimensions and retention time all influence gut microflora. Cone et al. (2002) compared rumen fluid from cows and sheep fed a similar diet, and reported that the volume of gas produced was lower with rumen fluid from sheep, although there was a good relationship between volumes of gas produced by the two sources of inocula. Bueno et al. (1999) compared bovine and ovine rumen fluid inocula adjusted to provide the same microbial mass and concluded that the two sources were comparable under tropical conditions.

Animals that regularly consume tanniniferous feedstuffs adapt to minimize the detrimental effects of tannins. In our study, goats in AG, exposed for 3 months to diets containing 560 g/kg of a mixture of *P. dulce*, *Q. laeta* and *L. acapulcensis*, had higher nutrient *in situ* degradability than unadapted goats (UG) or cows. Wiryawan et al. (2000) observed that *in vitro* digestibility of *Caliandra* foliage was lowest in rumen fluid from goats fed grass, and increased when they were fed *Caliandra* for 3 months before the digestibility determinations. Similar results were reported by Tjakradidjaja et al. (2000) who observed that browse from high-tannin species (*Acacia* or *Caliandra*) were digested better by goats adapted to consuming high-tannin feedstuffs.

In goats, *in situ* degradability of browse species was higher in AG than in UG goats, which is consistent with McSweeney et al. (2001) who reported that within the same animal species responses to tanniniferous diets depend largely on the physiological capacity of the animals to adapt to the high-tannin diets. Mlambo et al. (2007) found that *in vitro* digestibility and fermentation kinetics of tannin-containing substrates improved when rumen fluid was obtained from goats fed a mixture of tannin-containing tree fruits (adapted rumen fluid). This is consistent with our finding that adapted goats had enhanced fermentative activity in the rumen to degrade high-tannin substrates, but this benefit was less evident with feedstuffs with lower concentrations of tannins.

Ruminants have a higher tolerance of tannins than non-ruminants (Smith et al., 2001), due to extra mastication, large amounts of saliva and rumen fermentation (Salem et al., 2001), and our results were likely due in part to acclimatization of microbial populations in the rumen. Odenyo and Osuji (1988) identified some tannin-tolerant ruminal bacterial strains from enriched cultures of rumen microflora of goats to establish a medium containing high concentrations of crude tannin extract or tannic acid. A strain of the anaerobe *Selenomonas ruminantium*, subspecies *ruminantium*, capable of growing on tannic acid or condensed tannin as a sole energy source, has been isolated from ruminal contents of feral goats browsing tannin-rich foliage (Skene and Brooker, 1995). Transferring these micro-organisms from feral goats to domestic goats and sheep fed tannin-rich foliage increased feed intake and N retention in inoculated versus uninoculated goats. Inoculation also improved N digestibility and reduced the rate of live weight loss in sheep and domestic goats (Miller et al., 1995).

5. Conclusions

Higher nutritive value of browse species during the dry season for cows and goats could explain the great importance of those species in the nutrition of grazing ruminants in semi-arid regions of Mexico. *P. dulce* has potential to be used as protein source for sheep and goats during the dry season. Goats previously exposed to diets supplemented with the browse species were better able to degrade the browse species than goats fed diets not supplemented with browse species, which likely enables adapted goats to better use browse scrubland (Salem et al., 2006). Our results are consistent with the hypothesis that microbial populations in the rumen of goats fed a diet supplemented with the same browse species may evolve to become resistant to secondary compounds, in particularly condensed tannins, thereby becoming superior at degrading feedstuffs rich in condensed tannins.

Acknowledgements

This work was undertaken with funds from the Universidad Autónoma del Estado de México (project UAEM 2400/2006U). Our gratitude also to the Mexican National Council for Science and Technology (Consejo Nacional de Ciencia y Tecnología-CONACYT) for the grant received by Luis Miguel Camacho Díaz.

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