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Chemical composition, *in vitro* and *in situ* dry matter digestibility and preference of *Quercus resinosa* foliage

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ABSTRACT

The objectives of this study were to determine the chemical composition and dry matter digestibility of *Quercus resinosa* leaves harvested at different seasons of the year and to assess the intake and palatability of lamb diets containing foliage of *Q. resinosa*. There were three sites of collection, two located in the state of Jalisco and one in the state of Zacatecas, México. The young leaves harvested in spring, had greatest (P <0.05) nutritional value than leaves harvested in other seasons. The *in vitro* digestibility of organic matter (IVOMD), rapidly degradable fraction (a) and the degradation rate (c) were greater (P <0.05) in leaves harvested in spring and winter. The slowly degradable fraction (b) was greater (P <0.05) in spring, summer and autumn, and lesser in winter. The potential degradation fraction (a + b) and the effective degradability of dry matter were maximized (P <0.05) in spring than in the other seasons. The consumption was similar (P >0.05) among diets with different inclusion levels of leaves, but sheep consumed greater (P <0.05) amounts of diet containing leaves collected in spring. In conclusion, the *Q. resinosa* foliage contains the nutritional value and the digestibility and palatability that makes it suitable to replace completely the forage in the fattening diets of sheep, being preferable to use leaves harvested in spring and winter.

Key words: Chemical profile; Consumption and palatability; Degradability of dry matter; *Quercus resinosa* Liebm, Sheep.

INTRODUCTION

The foliage is an important source of food for ruminants in areas with scarcity of other alternative fodder plants (Singh and Todaria, 2012). Their importance lies in that it does not compete with human food and can provide nutrients throughout the year (Makkar, 2003). Verma and Mishra (1999) reported that *Quercus* foliage is available in seasons of the year when other fodder sources are scarce, so it could be useful as ruminant feed at these times. In these production systems, feeding of ruminants depend on more than 70% of the foliage (Singh and Todaria, 2012). In India and Nepal, the foliage of *Quercus* is harvested for its use as forage, and is administered either fresh or dried during food shortages (Makkar *et al*., 1986). In addition, the *Quercus* foliage contains anti-nutritional substances in moderate quantities that could potentially have beneficial effects if used to feed ruminants. Thus the animal nutritionists are more focused towards utilization of non-conventional feed resources at the maximum level for livestock feeding (Swain *et al.,* 2016). However, there are limited reports on the use of *Q. resinosa* as fodder for ruminants, and scarce information on its nutritional value, degradability, and inclusion levels in diets for ruminants.

The objectives of this study were: 1) to determine the chemical composition and the *in situ* degradability of leaves of *Q. resinosa* through seasons of the year and 2) to evaluate the consumption and palatability of *Q. resinosa* at different inclusion levels in diets for fattening lambs.

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MATERIALS AND METHODS

Area description

The climate of the region is sub-humid warm with summer rains. There were three collection sites with a distance of 35 km among them. Two sites (Tequila and San Cristobal) were located in the state of Jalisco and one site (Teul de Gonzalez Ortega) was located in the state of Zacatecas, México. In these sites, the annual mean rainfall is 600 mm and the average altitude was 1,750 MASL.

Harvest procedure and sample handling

Sampling was conducted the first day of each month, from April, 2014 until March, 2015. The foliage samples were collected manually from the same 15 trees located in a plot of about 50 m × 50 m at each collection site. Leaves were stored in identified paper bags and kept at 4°C on ice to preserve them until processing in laboratory. The leaves were placed in a circulating air oven at 60°C to constant weight, ground in a Wiley mill with a 2 mm mesh, and stored at ambient temperature in identified paper bags for subsequent determinations.

Chemical analysis

Samples were analyzed in triplicate to determine dry matter (DM) and ash (AOAC, 1995) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al.,* 1991). Hemicellulose (NDF - ADF) and OM (DM - ash) were determined by difference. Non fiber carbohydrate (NFC) were calculated by the equation of Sniffen *et al.* (1992). The Dumas method (AOAC, 1995) was used for to determinate crude protein (CP) with a FP-528 LECO nitrogen analyzer. The ether extract (EE) was determined using the extractor of Ankom^{xt15} (AOCS AM 5-04). The concentration of condensed tannins (CT) was estimated using the butanol-HCl method reported by Porter *et al*. (1986).

***In vitro* digestibility**

The *in vitro* procedure adopted in the present study was as proposed by Theodorou *et al.* (1994). Since the *in vitro* production of gas is proportional to the DM degraded, the net yield of gas at 24 h (ml/700 mg) incubation of the substrate was used to calculate the metabolizable energy (ME) and IVOMD using the equations proposed by Menke and Steingas (1998). The short chain fatty acids (SCFA) were calculated using the equation of Makkar (2005).

Dynamics *in situ* degradability

The DM degradability was determined with the nylon bag technique (Ørskov, 2000). Foliage samples (5 g) were introduced in nylon bags and incubated in the rumen of five rams equipped with ruminal cannula for 0, 6, 12, 24, 48, 72 and 96 h. To determine the parameters of *in situ* degradability and passage rate, the data obtained were processed with the Neway computer program, applying the equation proposed by Ørskov and McDonald (1979).

Intake and palatability

The cafeteria technique was used to estimate the intake and preference of diets by sheep (Ganguli *et al.,* 2010).

Test 1: Inclusion levels of Q. resinosa Liebm

The leaves used in this test were collected in the first week of October. Eight uncastrated Rambouillet lambs (41±1.2 kg) with no history of consumption of *Quercus* leaves were used. The experimental diets consisted of incremental levels of foliage (0, 10, 20, 30 and 40%) that replaced sorghum hay of the basal diet (Table 1). The basal diet was formulated to meet the gain requirements of lambs (NRC, 2007).

Test 2: Harvest dates of Q. resinosa

The leaves of *Q. resinosa* used in this test were the leftovers for the chemical composition procedures and were grouped by season of the year. Eight uncastrated Rambouillet lambs (42.0±0.8 kg) with no history of consumption of *Quercus* leaves were fed four experimental diets containing a 40% monthly mixture of each season of the year of *Q. resinosa* leaves and 60% of the basal diet described in Table 1.

In both tests, the feeder had eight dividers, at each of which were placed 0.1 kg of each one of the experimental diets, the position of the troughs was randomized each day to avoid "habit reflex" (Brown *et al.,* 2016). Observations were made for 30 minutes (0830-0900) each time. Once this time was elapsed, the diet leftovers that were in the different compartments of the feeder were removed, weighed and identified. Animal behavior was recorded with eight security cameras, one per each pen. Samples of approximately 100 g of diet offered and refusals were obtained and dried for 48 h at 60°C. The DMI by each animal was measured by subtracting the difference between the offered and refused, adjusting losses inherent in the spontaneous dehydration of forages in time.

Table 1: Ingredients and chemical composition of the basal diet.

Item	% as fed
Ingredients	
Molasses	3.8
Rolled corn	60.0
Vegetable fat	3.6
Soybean meal	28.3
Limestone	1.6
Monocalcium phosphate	0.8
Sodium bicarbonate	1.5
Vitamin premix*	0.2
Micromineral premix**	0.2
Total	100.0
Chemical composition	
Total digestible nutrients, %	87.0
Metabolizable energy, MJ/kg^{-1} dry matter	13.0
Net energy of maintenance, MJ/kg^{-1} dry matter	9.2
Net energy of gain, MJ kg^{-1} dry matter	6.3
Crude protein, %	20.2
Calcium, %	0.9
Phosphorus, %	0.7
Magnesium, %	0.3

*Vitamins A (5,000,000 IU), D (2,000,000 IU) and E (10,000 IU). Excipient c.b.p. 1,000 g. **Microminerals Co (0.5 g), Fe (50 g), I (2.5 g), Mn (50 g), Zn (50 g), Se (0.2 g) & Cu (15 g). Excipient c.b.p. 1,000 g.

The coefficient of consumption was estimated as the relationship between the consumption of each individual treatment, divided by the average consumption of all treatments. Those treatments with a coefficient of consumption greater than one, were considered greater than other treatments.

The coefficient of effective time consumption was calculated as the ratio between the time consumption of each individual treatment, divided by the average time of consumption of all treatments. Animals spend more time consuming than other animals, when the coefficient is greater than one. Data of DMI were used to determine the relative palatability index (RPI) of different treatments, which was obtained by dividing the data of the treatment with higher DM consumption with the other treatment, and multiplied by 100 (Abdulrazak *et al.,* 2001).

Statistical analysis

Data were analyzed as a randomized block design using the GLM procedure of the SAS statistical software [SAS/STAT® User's Guide (8.1Edition)], SAS Inst. Inc., Cary, NC, USA. The collection site was considered as block effect for the chemical composition, *in vitro* and *in situ* degradability studies. In the study of intake and palatability, the animals were used as block effect. When significant effects were observed, a comparison of means with the Tukey method using the MEANS statement was made. *P* values observed were considered different if P<0.05.

RESULTS AND DISCUSSION

Chemical composition of leaves

The contents of CP and EE were significantly higher in spring and summer than in other seasons. Moreover, the proportions of NFC and CT were significantly higher in spring and minor in summer, autumn and winter (Table 2). Forwood and Owensby (1985) who evaluated the contents of CP and CT in the leaves of *Q. macrocarpa* and *Q. Alba* reported similar results. They mentioned that the chemical composition tends to decrease with increasing sampling time. However, Infascelli *et al.* (2007) noted that the EE of *Q. ilex* foliage is similar in spring and summer.

The NDF and ADF were significantly lower in spring compared to other seasons. Olson and Kelsey (1997) also observed that the fiber content of *Centaurea maculosa* is lower in the early stages of growth. In contrast, Infascelli

Table 2: Chemical composition of the leaves of *Quercus resinosa* harvested in different seasons of the year.

Item	Seasons				SEM
	Spring	Summer	Autumn	Winter	
Ash, %	3.0^{d}	3.6^{c}	4.2^{b}	5.0^{a}	0.1
Crude protein, %	8.8^{a}	8.7^{ab}	7.7^{b}	5.6^{c}	0.2
Ether extract, %	1.7^{a}	1.8^{a}	1.5^{b}	1.3^{b}	0.1
Neutral detergent fiber, %	52.8^{b}	63.7^{a}	66.1^{a}	67.4^{a}	0.9
Acid detergent fiber, %	44.4^{c}	55.5^{b}	57.1^{ab}	59.4^{a}	0.9
Hemicellulose, %	8.4	8.2	9.0	8.0	0.2
Non fiber carbohydrates, %	33.7^{a}	22.2^{b}	20.5^{b}	20.7^{b}	0.8
Condensed tannins, %	12.4^{a}	3.9^{b}	4.0^{b}	4.1^{b}	0.1
Metabolizable energy, MJ/kg DM	6.3^{a}	5.0^{b}	4.2^{c}	5.9^{a}	0.1
NEl, MJ/kg DM	2.9^{a}	2.1^{b}	1.7^{c}	2.9^{a}	0.1
SCFA, mmol	0.6^{a}	0.4^{b}	0.3^{c}	0.6^{a}	0.03

SEM = Standard error of the mean.

NEl = Net energy for lactancy; SCFA = short chain fatty acids.

$^{a,\ b}$Means with different superscript letters in the same row are different (P<0.05).

Table 3: *In vitro* organic matter digestibility (IVOMD), parameters of the *in situ* degradability and effective degradability of dry matter (EDDM) of *Quercus resinosa* foliage harvested in different seasons of the year.

Concept	Seasons				SEM
	Spring	Summer	Autumn	Winter	
IVOMD, %	41.8^{a}	34.5^{b}	33.9^{b}	41.0^{a}	1.3
Quickly degradable fraction (a), %	27.9^{a}	18.9^{c}	21.9^{bc}	24.7^{ab}	1.0
Slowly degradable fraction (b), %	20.3^{a}	18.1^{a}	18.9^{a}	10.0^{b}	2.0
Degradation rate (c), %/h	0.05^{a}	0.01^{b}	0.01^{b}	0.06^{a}	0.01
Potential degradability (a+b), %	48.3^{a}	37.0^{b}	40.1^{ab}	34.7^{b}	2.0
EDDM, %*	41.6^{a}	27.1^{b}	27.0^{b}	30.5^{b}	1.2

SEM = standard error of the mean. *EDDM calculated degradation rate constant of 3%/h^{-1}

$^{a,\ b}$Means with different superscript letters in the same row are different (P<0.05).

et al. (2007) noted that the NDF and ADF of *Q. ilex* foliage was similar in spring and summer. In this study, the hemicellulose content was not different (P>0.05) among seasons. However, concentrations of ME, NEI and SCFA were maximum during the spring and winter and dropped significantly in summer and autumn. In this study, only the leaves collected in spring had ME values to satisfy the maintenance requirements of mature ewes (6.2 MJ/kg DM; NRC, 2007). Safari *et al.* (2011) reported that herbs and native grasses in East Africa contain greater CP and ME at the beginning of the plant growth, but eventually reduced with maturity.

In vitro and *in situ* degradability

The IVOMD was significantly higher in spring than in other seasons. The quickly degradable fraction (a), the potential degradation fraction (a + b), the rate of degradation (c) and effective degradability of dry matter of *Q. resinosa* leaves were significantly higher in spring than in the other seasons (Table 3). High IVOMD values, *in situ* digestion parameters and EDDM in spring leaf samples might be because of their low fiber NDF and ADF content (Table 2). In addition, the decline in digestion of *Q. resinosa* leaves to advance maturity is consistent with previous studies (Hassen *et al.,* 2007; Safari *et al.,* 2011).

Intake and palatability

The DMI was significantly higher in sheep fed diets with *Q. resinosa* at 0, 10, 30 and 40%, compared to 20% (Table 4). However, the DM intake did not differ significantly among the sheep fed diets with different levels of *Q. resinosa*. The calculated coefficients of effective consumption and time consumption and the RPI of sheep were also not significantly different between treatments. Telford *et al.* (1983) reported that DM intake of goats did not change when included 25 or 50% of *Quercus* spp in diet, but consumption was reduced by 75%. Similarly, Villena-Rodríguez (1987) reported that DMI in goats was not affected at levels of 0, 25 and 50% of *Q. havardii* in the diet. In contrast, Yildiz *et al.* (2005) recommended that only 50% of the forage could be replaced by *Q. hartwissiana* foliage in diets for lambs. Nastis and Malechek (1981) also pointed out that when using *Q. incana* with 25% in the diet increased the DMI.

In this study, DMI of sheep was significantly higher in spring, followed by winter, autumn and summer (Table 5). The coefficients of calculated and effective time consumption and RPI of animals followed the same patern as DMI. Similarly, Ganguli *et al.* (2010) reported that sheep prefer and consume more foliage from native plants when they

Table 4: Dry matter intake (DMI), coefficients of consumption and palatability of lamb fed diets containing incremental levels of *Quercus resinosa.*

Item	Percentage of inclusion in diet (dry basis)					
	0	10	20	30	40	SEM
Dry matter intake, %*	82.2[a]	78.8[ab]	74.4[b]	74.6[ab]	74.7[ab]	2.2
Coefficient of calculated consumption[¥]	1.2[a]	1.1[a]	0.9[a]	0.9[a]	0.9[a]	0.1
Coefficient of effective time consumption[€]	4.1[a]	3.9[a]	3.4[a]	3.5[a]	3.4[a]	0.2
Relative palatability index[¢]	100[a]	93.1[a]	84.4[a]	86.2[a]	85.7[a]	8.7

SEM = Standard error of the mean. *DMI expressed as % of the consumed of each diet.

[¥]Calculated as the consumption of each individual treatment divided by the average consumption all of the treatments.

[€]Calculated as the ratio between the time consumption of each individual treatment, divided by the average time of consumption of all treatments.

[¢]Obtained by dividing the data of the treatment with higher DM consumption with the others treatment, and multiplied by 100.

[a,b,c]Means with different superscript letters in the same row are different (P<0.05).

Table 5: Dry matter intake (DMI), coefficients of consumption and palatability of lamb diets containing foliage of *Quercus resinosa* harvested at different seasons of the year.

Item	Season				
	Principio del formulario Spring	Final del Summer formulario	Autumn	Winter	SEM
Dry matter intake, %*	76.9[a]	40.6[d]	54.4[c]	66.7[b]	3.0
Coefficient of calculated consumption[¥]	1.5[a]	0.7[b]	0.8[b]	1.0[ab]	0.2
Coefficient of effective time consumption[€]	1.3[a]	0.6[b]	0.8[b]	1.2[ab]	0.2
Relative palatability index[¢]	100.0[a]	44.7[c]	56.1[bc]	69.1[b]	5.1

SEM = Standard error of the mean; *DMI expressed as % of the consumed of each diet.

[¥]Calculated as the consumption of each individual treatment divided by the average consumption all of the treatments.

[€]Calculated as the ratio between the time consumption of each individual treatment, divided by the average time of consumption of all treatments.

[¢]Obtained by dividing the data of the treatment with higher DM consumption with the others treatment and multiplied by 100.

[a,b,c]Means with different superscript letters in the same row are different (P<0.05).

are growing and to a lesser extent during maturity. Moreover, Launchbaugh (1996) reported that sheep could form a strong preference for unpalatable compounds, if their consumption is associated with a high concentration of nutrients or better energy levels. Conversely, Riddle *et al.* (1999) reported that goats had similar intakes of *Q. virginiana* Sarg. var fusiformis in spring and autumn. Furthermore, Papachristou *et al.* (2005) reported that sheep and goats selected more shrubs with high CP content, NDF and of lignin. In addition, Carlson *et al.* (1993) suggested that white-tailed deer preferred browse species with high CP, phosphorus and OMD, regardless of the content of antinutritional compounds.

CONCLUSION

The *Q. resinosa* foliage showed a nutritional value and digestibility that makes it a useful fodder for feeding ruminants at any season of the year. Sheep preferred leaves collected in spring, than leaves collected in winter, autumn or summer. The leaves of *Q. resinosa* can completely replace forage in diets for fattening sheep without having negative effects on palatability and intake.

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