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Chemical composition and *in situ* digestion kinetics of urea-molasses treated fallen leaves of *Quercus rugosa*

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Abstract: The aim of the study was to determine the effect of the use of urea (0, 2, 4 and 6%) and or molasses (0, 15, 30 and 45%) on the nutritive value of leaves of *Quercus rugosa* Née The *in situ* digestibility of dry matter (ISDDM) was also estimated. The metabolizable energy (ME), net energy of lactancy (NEl), the organic matter digestion coefficient (OMD) and short chain volatile fatty acids (SCFA) were determined from data of the *in vitro* gas production. Leaves were collected from three sites: site 1 was located in Tequila, county of the state of Jalisco; site 2 was situated in Teul de González Ortega county of the state of Zacatecas, México, and site 3 in San Cristobal de La Barranca county of the state of Jalisco, México. In general, the organic matter, neutral detergent fiber and acid detergent fiber contents diminished as the levels of molasses increased in diets. Conversely, crude protein, nonfiber carbohydrates, ash, ME, NEl and SCFA contents and ISDMD augmented as molasses increased. In general, when urea and molasses were higher, the fractions **a**, **b**, **a**+**b** of the *in situ* digestibility were higher. In all the molasses mixtures, with addition of urea at 4 or 6% had higher chemical composition and *in situ* digestibility.

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Keywords: *Quercus rugosa;* chemical composition; *in situ* digestibility; urea; molasses.

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1. Introduction

Recently, the use of forest residues as foods for ruminants has been focused mainly in find alternatives for the utilization of feed sources of low cost. The great amounts of fallen leaves may be considered as biomass that is ignored as animal feed, and may be considered as an alternative source of forage for ruminants (De la Paz *et al*., 1998). In this region, the fallen leaves of *Q. rugosa* may be used for food source for ruminants at the end of winter when defoliation occurs (Valencia, 2004). However, fallen leaves from Quercus have high concentrations of structural carbohydrates and low crude protein content and low of dry matter digestibility. Thus, to improve the rumen digestion of straw fed animals in terms of the availability of nonfiber carbohydrates and ammonia, supplementation of urea and molasses in the form of block or as liquid feed is often suggested (Reis *et al*., 1995; Sahoo *et al.,* 2004). Other studies have reported beneficial effects of urea and molasses treatment on rumen degradability of wheat, oat and barley straws (Van Thu and Uden, 2000; Souza y Dos Santos, 2002; Montiel *et al*., 2012).

In situ digestion parameters: the soluble fraction **a**, the degradable fraction **b**, the potential degradable fraction **a**+**b** and rate constant **c** are important characteristics of forage digestion in ruminants. Such parameters can be used to predict the nutritive value more accurately and compare the utility of forages in the diets for ruminants (Ørskov, 2000). Therefore, this study was carried out with the aim to determine the effect of the inclusion of urea (0, 2, 4 and 6%) and molasses (0, 15, 30 and 45%) to the fallen leaves of *Q. rugosa* Née on the chemical composition and *in situ* digestion parameters. It was hypothesized that addition of the mixture of a nitrogen source (urea) and a soluble carbohydrate (molasses) on fallen leaves of *Q. rugosa* will improve its solubility of structural carbohydrates and crude protein.

2. Material and Methods

2.1. Study sites

The collection sites were characterized by a similar edaphic pattern, ground elevations, climate conditions and vegetation (CONAFOR, 2009). Predominant soil type was extrusive igneous rock, and phaeozem leptosol. The climate of the region is sub humid warm with summer rains. The annual mean rainfall is about 600 mm. The collection site 1 was located at 21°15'07.30" latitude N and 103°39'48.12" longitude W, in the municipality of Tequila, Jalisco, Mexico; at an altitude of 1,617 m. The collection site 2 was located at 21°17'46.02" latitude N and 103° 34'52.03" longitude W, in the municipality of Teul de Gonzalez Ortega, Zacatecas, Mexico; at an altitude of 1,997 m. The collection site 3 was located at 21°11'30.14" latitude N and 103°35'59" longitude W, in the municipality of San Cristobal de la Barranca, Jalisco, Mexico; at an altitude of 1,738 m (INEGI, 2009abc).

2.2. Harvest procedure and sample handling

Sampling of fallen leaves of Quercus was carried out in two years (2013 and 2014), during the second week of March. Leaves were collected in a plot of about 50 m × 50 m randomly located in each collection site. Leaf litter was collected by hand and keep in polyethylene bags of 1.4 m × 1.0 m. The bags were transported to the laboratory and stored at 22 °C. Grinding of leaf litter was achieved with a mill (Azteca brand No. 24) equipped with a 2" screen and powered by a tractor at 105 hp engine and 90.4 hp PTO (New Holland 7610S 4WD Double).

2.3 Ensiled the urea and molasses with the fallen leaves of Quercus

There were 16 treatments (mixtures) composed by fallen leaves of Quercus with four levels of urea (0, 2, 4 and 6%) and four levels of molasses of 85 °Brix (0, 15, 30 and 45%). Grinded leaves (1.5 kg) were mixed with the ingredients for each treatment; first diluted urea with 10% of water was added to leaves, then the molasses were included and completely homogenized the three components of the treatment mixtures. Each treatment mixture were ensiled in cylindrical laboratory silos made by polyvinylchloride of 5 cm in diameter and 30 cm long and sealed in each extreme of the silo. There were four experimental silos for each treatment mixture. After a period of 28 days, the silos that were placed at 22 °C, were opened and mixtures were dried at 60 °C for 48 hours in a air circulating oven. Then were grinded in a Wiley mill to pass a 1mm screen. Finally, grinded samples of each treatment mixture were dried again for 24 hours at 60 °C for chemical and digestion analyses.

2.4 Chemical analyses

By triplicate each treatment mixture, was analyses to determine organic matter (OM), ash (AOAC, 1995), 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. Nonfiber carbohydrate (NFC) were calculated by the equation of Sniffen *et al*. (1992): NFC (%) = DM - (CP + NDF + EE + ash). The Dumas method, (AOAC, 1995) was used for the determination of crude protein (CP) with a FP-528 LECO apparatus. The ether extract (EE) was determined using the extractor of Ankom^{xt15}. The concentration of condensed tannins (CT) was estimated using the butanol-HCl method (Makkar, 2005).

2.5 In vitro gas production

Gas production was determined by the *in vitro* procedure proposed by Theodorou *et al.* (1994). Briefly, 700 $\pm$10 mg of DM of each sample were introduced into fermentation units (FU) of 120 ml, mixed with 90 ml of a buffer solution gassed with CO_2 and stored under refrigeration (4°C). After 24 hours, 10 ml of ruminal fluid were also added to the FU. Finally, the FU were kept in a water bath at 39 °C. The gas production was measured using a pressure transducer (SPER SCIENTIFIC, 840065 gauge with a pressure gauge TA87199). The pressure of gas produced was determined in PSI (Pounds-force per Square Inch) at 24 h of incubation. Samples were assayed by quadruplicate.

The ruminal fluid was obtained from three ruminally cannulated sheep fed a diet (12.8% of CP, 2.34 Mcal/kg of ME and 64.60% of TDN NRC, 2007) containing 82% hay (50% alfalfa and 50% wheat straw), 17% concentrate (63% corn cracked, 25% of cottonseed meal, 5.5% of limestone, 5.5% of monocalcium phosphate), and 1 % of vitamins and microminerals premix. Feed was provided *ad libitum*, twice daily at 0800 and 1600 h, with free access to water.

To correct for contamination with rumen contents three FU without substrate were used as whites. In addition, three FU with bean straw, three with bean straw + corn cracked and three with concentrated were used as standards. Pressure readings (PSI) were converted to volume (ml) using a preset linear regression between recorded in such units and inoculated known volumes of air at the same temperature incubation pressures such as: Volume= (Pressure-0.4009) / 0.3853; R = 0.9959; SEM = 0.0522; and n= 72.

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), as follows:

$$\text{ME, Mcal/kg}^{-1}\text{ DM} = (2.20+0.136\times GP_{24h}+0.057\times CP+0.0029\times EE^2)/4.184$$

$$\text{IVOMD, \%} = 14.88+0.889\times GP_{24h} + 0.045\times CP + 0.0651\times XA.$$

The net energy of lactation (NEl) was also calculated by the equation of Abas *et al*. (2005):

$$\text{NEl (Mcal/kg}^{-1}\text{ DM)} = (0.115\times GP_{24h} + 0.0054\times CP + 0.014\times EE - 0.0054\times XA - 0.36)/4.184$$

The short chain fatty acids (SCFA) were calculated using the equation of Makkar (2005):

$$\text{SCFA (mmol)} = 0.0222\times GP_{24h} - 0.00425$$

Where GP= net gas production at 24 h (ml/700 mg); CP= crude protein; EE = ether extract; and XA = ash content.

2.5. Dynamics of in situ degradability

The technique of nylon bag (Ørskov, 2000) was used to assess the degradability of MS. Treatment mixtures (5 g) were incubated in the rumen of five rams equipped with ruminal cannula, for which they were suspended in the ventral part of the rumen for 0, 6, 12, 24, 48, 72 and 96 h of incubation. The samples were introduced in the reverse order of incubation time to be later removed all together. The zero time bags were introduced and removed immediately in order to wet with ruminal fluid (Nocek and Russell, 1988). To determine the parameters of *in situ* degradability and passage rate, the data obtained were processed with the Neway (Cheng, 1997) computer program, applying the equation proposed by Ørskov and McDonald (1979):

$$\mathbf{p} = \mathbf{a} + \mathbf{b}\,(1\text{-e-}^{\mathbf{ct}}),$$

Where, **p**= % degradation of dry matter (DM) at time **t**; **a**= soluble or rapidly degradable fraction; **b**= fraction insoluble or slowly degradable in time **t**; **e**= natural logarithm; **c**= degradation constant **b**; **t**= time of incubation. It follows that **a** + **b**= maximum potential food degradability; and effective degradation, which corresponds to the degradation potential (**a** + **b**) adjusted by the effect of passage rate (**k**), by the relationship **p= a** + (**b** × **c**)/(**c** + **Kp**) was calculated for **Kp** values of 3% h^{-1}, corresponding to moderate production diets.

2.6. Statistical analyses

Data was analyzed as a one way analysis of variance in a 4 x 4 factorial arrangement considering the urea (0, 2, 4 and 6%) and molasses (0, 15, 30 and 45%) levels as the main effects. The GLM procedure of the SAS statistical software (SAS/STAT® User's Guide (8.1Edition), SAS Inst. Inc., Cary, NC, USA (SAS, 2000) was used to compute the data. 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 $P<0.05$.

3. Results and discussion

3.1. Chemical composition

The OM content was higher (P<0.01) in treatments only with urea (Table 1). Araiza-Rosales *et al*. (2013) reported similar response in corn silage with molasses at 0, 5 y 10%. The CP content significantly increased as the addition of urea and molasses increased in fallen leaves of Quercus being the higher treatment with 45% molasses and 6% urea (Table 1). Conversely, Vallejo-Solís (1995) argued that The CP content in fallen leaves of native trees and shrubs was reduced as molasses augmented in diets. In general, the EE content significantly the leaf litter was reduced as urea and molasses was increased.

The NDF and ADF of fallen leaves were significantly reduced as the addition of urea and molasses increased. Araiza-Rosales *et al.* (2013) reported similar tendency in reduction of structural carbohydrates in corn silage treated with molasses at 0, 5 y 10%. With exception of the treatment with 0% molasses, all others (15, 30 and 45%) augmented as urea increased. The NFC content significantly reduced as the addition of urea and molasses increased. In general, as the percentage of urea and molasses increased the ME, NEl and SCFA contents in treated fallen leaves, significantly augmented (Table 1). Similar findings were reported by Shultz *et al.* (1971) who reported increments of SCFA in rumen samples of beef cattle fed with *Panicum maximum* hay treated with 2.4% of molasses.

3.2. Digestion parameters

The soluble fraction **a** of fallen leaves significantly augmented as the addition of urea and molasses increased in fallen leaves of Quercus (Table 2). Similar findings were observed by Araiza-Rosales *et al*. (2013) with corn silage added with 0, 5 and 10% molasses. They also reported that with 10% molasses the fraction **a** was significantly higher than other levels of molasses. In this study, the fraction **b** significantly varied among treatments being higher when fallen leaves were added with 6% urea and 45% of molasses. The rate constant of degradation **c** of the dry matter of the fallen leaves of Quercus significantly augmented as the addition of urea and molasses augmented (Table 2). The potential degradability **a**+**b** of the dry matter of fallen leaves treated with urea and molasses varied significantly. This fraction in general augmented as the levels of urea and molasses increased. Conversely, Vallejo-Solís (1995) reported that the potential degradability of silages based on tree and shrub fodders was reduced as molasses as the levels of molasses were increased. In this study, the fraction **c** was significantly different among treatments. Higher digestion rates were achieved in leaf litter samples with molasses and 4 and 6% of urea (Table 2). In concordance with this study, Souza and Dos Santos (2002) reported that the rate of digestion of barley straw increased as the level of urea increased from 0 to 6%. Conversely, Pinto-Hernández *et al.* (1994) mentioned that the digestion parameters do not varied in the forage of *Panicum maximum* treated with or without molasses and urea.

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Table 1. Chemical composition of fallen leaves of *Quercus rugosa* treated with different levels of urea and molasses

Content	0% molasses				15% molasses				30% molasses				45% molasses				SEM
	Urea, %																
	0	2	4	6	0	2	4	6	0	2	4	6	0	2	4	6	
OM, %	92.2^{d}	93.7^{b}	93.9^{ab}	94.2^{a}	92.1^{de}	92.7^{c}	92.7^{c}	92.8^{c}	90.7^{i}	91.3^{g}	91.7^{f}	91.9^{ef}	91.0^{hi}	91.1^{hg}	90.9^{hi}	91.1^{hg}	0.05
Ash, %	7.8^{e}	6.3^{g}	6.0^{gh}	5.8^{h}	8.0^{de}	7.33^{f}	7.3^{f}	7.2^{f}	9.3^{a}	8.7^{c}	8.2^{d}	8.0^{de}	9.0^{ab}	8.9^{bc}	9.1^{ab}	8.9^{bc}	0.05
Crude protein, %	1.9^{k}	7.5^{h}	11.7^{e}	17.2^{c}	2.7^{j}	7.9^{h}	12.0^{e}	17.8^{b}	3.6^{i}	8.5^{g}	13.2^{d}	17.9^{ab}	8.9^{g}	9.4^{f}	13.4^{d}	18.2^{a}	0.08
Ether extract, %	1.6^{a}	1.4^{bc}	1.1^{def}	0.9^{f}	1.5^{ab}	1.3^{bcd}	1.0^{ef}	1.1^{f}	1.4^{bc}	1.3^{bcd}	1.2^{cde}	1.2^{cde}	1.3^{bc}	1.2^{cde}	1.2^{cde}	1.2^{cde}	0.04
NDF, %	67.7^{ab}	70.3^{a}	69.7^{a}	69.5^{a}	63.6^{bcd}	62.7^{bcde}	67.3^{ab}	64.2^{bc}	59.1^{cde}	58.5^{def}	59.8^{cde}	58.0^{ef}	53.8^{fg}	60.1^{cde}	57.6^{efg}	52.6^{g}	0.9
ADF, %	62.1^{ab}	64.1^{a}	64.7^{a}	64.8^{a}	57.9^{bc}	56.6^{bcd}	60.2^{ab}	56.3^{bcde}	53.0^{cdef}	53.5^{cdef}	52.0^{cdef}	50.5^{ef}	48.6^{g}	51.8^{def}	49.8^{g}	44.4^{g}	1.0
Hemicellulose, %	5.6^{bcd}	6.2^{abcd}	4.9^{d}	4.7^{d}	5.6^{bcd}	6.1^{abcd}	7.0^{abcd}	7.9^{ab}	6.1^{abcd}	5.0^{d}	7.7^{ab}	7.4^{abc}	5.2^{cd}	8.3^{a}	7.8^{ab}	8.2^{a}	0.4
NFC, %	20.90^{bcd}	14.3^{fg}	11.4^{fgh}	6.4^{h}	24.1^{ab}	20.6^{bcd}	12.4^{fg}	9.7^{hg}	26.7^{a}	22.8^{abc}	17.6^{ed}	14.8^{ef}	26.8^{a}	20.3^{bcd}	18.9^{cde}	19.0^{cde}	0.90
ME, MJ kg^{-1}	3.3^{h}	3.8^{gh}	3.8^{gh}	4.2^{efgh}	4.2^{efgh}	4.2^{efgh}	4.6^{defg}	5.4^{cde}	5.0^{defg}	5..0^{def}	5.9^{bcd}	6.7^{abc}	6.9^{b}	6.7^{ab}	6.8^{ab}	7.5^{a}	0.06
NEl, MJ kg^{-1}	0.4^{fg}	0.4^{fg}	0.4^{g}	0.8^{efg}	1.3^{def}	0.8^{efg}	0.8^{efg}	2.1^{cde}	1.3^{def}	1.3^{def}	2.1^{bcd}	3.3^{a}	2.9^{b}	2.8^{bc}	2.9^{b}	3.8^{a}	0.05
SCFA, mmol	0.1^{e}	0.1^{e}	0.1^{e}	0.1^{e}	0.3^{cde}	0..2de	0.2^{de}	0.4^{bcd}	0.3^{cde}	0.3^{cde}	0.4^{abc}	0.7^{b}	0.6^{ab}	0.6^{ab}	0.6^{ab}	0.8^{a}	0.05

OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; NFC = non-fiber carbohydrates; ME = metabolizable energy; NEl = net enery for lactancy; SCFA = short chain fatty acid; SEM = standard error of the mean.

[a, b, c, d. f. g. h] Means in a row with different letter superscripts are different ($P<0.05$).

Table 2. Kinetics of *in situ* degradability of fallen leaves of *Quercus rugosa* treated with different levels of molasses and urea

Concept	0% molasses				15% molasses				30% molasses				45% molasses				
	Urea, %																
	0	2	4	6	0	2	4	6	0	2	4	6	0	2	4	6	SEM
a, %	18.2^{g}	18.5^{g}	16.5^{g}	23.8^{f}	22.8^{f}	23.2^{f}	27.4^{e}	29.3^{d}	33.6^{bcd}	33.6^{bcd}	36.0^{ab}	33.4^{bcd}	33.7^{bcd}	35.0^{abcd}	36.8^{a}	35.7^{abc}	0.50
b, %	13.7^{bcd}	13.3^{bcd}	20.8^{a}	14.2^{bcd}	17.9^{ab}	18.2^{ab}	18.0^{ab}	17.2^{ab}	7.9^{d}	8.3^{d}	10.1^{cd}	13.5^{bcd}	12.6^{cd}	13.7^{bc}	11.23^{cd}	22.3^{a}	0.90
a+b, %	31.9^{g}	31.8^{g}	37.3^{f}	38.0^{f}	40.8^{ef}	41.4^{def}	45.4^{bcd}	46.5^{bc}	41.5^{def}	41.9^{def}	46.1^{bc}	46.9^{bc}	46.3^{bc}	48.7^{b}	48.0^{b}	58.0^{a}	0.9
c h^{-1}	0.01^{d}	0.02^{cd}	0.02^{cd}	0.03^{c}	0.03^{c}	0.01^{d}	0.04^{b}	0.05^{b}	0.02^{cd}	0.02^{cd}	0.04^{b}	0.1^{a}	0.02^{cd}	0.03^{c}	0.04^{b}	0.1^{a}	0.02

a = fraction of dry matter lost during washing; b = fraction of dry matter slowly degraded; c = degradation rate of dry matter of fallen leaves treated with urea and molasses.
SEM= standard error of the mean.

$^{a, b, c, d, f, g}$ Mean in a row with different letter superscripts differ (P<0.05).

4. Conclusions

The OM, NDF, ADF and NFC contents in leaf litter samples diminished as the levels of urea and molasses augmented. However, CP, hemicellulose, ME, NEl and SCFA augmented as the levels of urea and molasses increased. In general, the *in situ* digestion parameters **a, b**, **a**+**b** and **c** of the dry matter of fallen leaves were improved as the levels of urea and molasses increased. These results confirm that the nutritive value of fallen leaves can be improved if are ensiled with urea-molasses

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