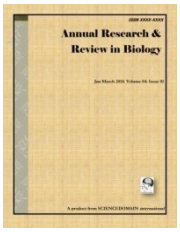

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Effect of Sowing and Cutting Age of *Kochia scoparia* in the Chemical Composition, *In vitro* Gas Production and Rumen Degradation

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Authors' contributions

The present study was carried out in collaboration between all authors. Authors JRB and CCY develop the in vitro gas production, the chemical composition of the samples, and the statistical analyses. Author AZMS help us with the redaction of the manuscript, author JRF develop the crop study and the recollection of the samples, author MGR supervise the study and write the manuscript.

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ABSTRACT

Aims: Determine the chemical composition and ruminal degradation and fermentation of *Kochia scoparia* at three sowing dates (D1, D2, D3) and two cutting ages, 120 (C1) and 165 (C2) days post seeding.
Place and Duration of Study: Department of Animal Nutrition, between August 2011 and June 2013.
Methodology: Samples were analyzed using a randomized statistical design 3x2. Chemical composition, *in vitro* gas production (GP) 96 h and *in sacco* ruminal degradation at 24h was determined.
Results: Crude protein content was higher ($P< .05$) for dates D2 > D3 > D1, and for cutting interval, C1 > C2 (P<0.05). Interaction, D2C2 and D3C1 were higher in comparison with the rest of interactions. *In vitro* GP (ml gas/ g DM) was not different (P>0.05) for *A*, *b*, *c* and lag time, for the main effects of sowing date and cutting interval. *In sacco* rumen degradable

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protein [RDP], D2 was higher (*P*< *.05*) in comparison with the rest of the treatments. There were not differences (*P*>*.05*) among cuts.
Conclusion: *Kochia scoparia* could be used as a protein source for ruminant feeding with a higher rumen degradability, which will be influenced by the sowing establishment date and in lower magnitude for the cutting age.

Keywords: Degradability; Forages; in vitro gas production; in sacco; Kochia scoparia.

1. INTRODUCTION

There are several species of plants used in feed for ruminants in arid and semiarid areas. *Kochia scoparia* represents an alternative forage for their adaptive characteristics [1], forage yield [2,3] and protein quality [4,5], being one of the main factors affecting the nutritional characteristics at the time of year that was cultivated and harvested in their chemical composition and nutritional value [4.6]. The gas production technique to predict *in vitro* fermentation and digestibility of feed [7,8], requires less time, easy to use with high correlation with *in vivo* methods [9] and *in sacco* [10]**.** The *in sacco* technique [11] with nylon bags, is a procedure used to determine the ruminal degradation of feed in ruminants, is also used to estimate the rumen degradable protein (RDP) witch is utilized by the microorganisms in the rumen. The aim of this study was to determine the chemical composition, *in vitro* gas production and in*sacco* technique for determine rumen degradable protein of *Kochia scoparia* at three sowing dates and two cutting ages.

2. MATERIALS AND METHODS

2.1 Study Site

The cultivation of Kochia (*Kochia scoparia* L. Schrad var. Emerald) was held in the town of Telpintla, Temascaltepec municipality, State of Mexico (19° 03' and 100° 02', at an average altitude of 1740 m above sea level) [12]. The cropping was established in the lower rainfall season, of December 7, 2011 (D1), January 6 (D2) and February 5, 2012 (D3). The stocking density was 5.5 kg of seed per ha, using a plot of 1000 m^2 for each sowing date, using three replicates per treatment. They conducted irrigation during germination and every 15 days. Fertilization was applied 100-80-40 (N-K-P) in a ratio of 1 kg m^2, divided in 50% nitrogen at sowing, with all the phosphorus and potassium. The rest of nitrogen was added when the plant reached 35 cm in height. The weeds were removed manually during the initial stage of crop development (30 and 45 days). Forage plots were sampled at two cutting dates (C1, C2 at 120 and 165 days after planting), for each of the dates of establishment.

2.2 Chemical Analyses

Samples of Kochia were analyzed for DM (#934.01), ash (#942.05), N (#954.01) according to Association of Official Analytical Chemists [13]. The neutral detergent fibre [14] and acid detergent fibre (ADFom) (#973.18) [13] analyses used an ANKOM200 Fibre Analyzer Unit (ANKOM Technology Corporation, Macedon, NY, USA). NDFom was assayed without use of an alpha amylase but with sodium sulfite in the NDFom. Both NDFom and ADFom are expressed without residual ash. The residue resulting from the in vitro gas production and *in sacco*was used to determine the DM disappeared (DMd) and rumen degradable protein,

respectively. All analysis was performed in duplicate and the average was used for comparison between samples.

2.3 *In sacco* Procedure

Three rumen-cannulated Rambouillet lambs (LW 20.0 ± 0.5 kg; ≤ 1 year old) were fed twice a day (at 08.00 and 16.00 hours), using a mixed diet (50:50) of forage (oat straw and alfalfa hay) and supplemented with vitamins and minerals (2%) as DM basis. Five grams of the samples under study were incubated in three series, using three repetitions each one. Samples were deposited in nylon bags (16 x 9 cm; 45 μm pore size) and then rumen was incubated during 24h, according to Bagolun et al. [15]. At the end of the incubation, bags were washed with water, and drying (60°C, 48 h), the disappearance of DM and CP (g/100g), were performed using the mathematical adjustment of McDonald et al. [16] to estimate the degradation of DM (DMd) and CP (RDP) by the equation: [1]

2.4 *In vitro* Gas Production

The technique by Theodorou et al. [8] was used to determine *in vitro* gas production; Forage samples were incubated in three series using three repetitions each. Additionally, three blanks per series were used (as well with three repetitions). Before incubation, ingredients were ground, introducing the sample (800 mg) in 120 mL-flasks. The incubation solution was prepared following the Menkeand Steingass [7] method. It was used 500 mL H_2O, 250 mL macromineral (5.7 g Na_2HPO_4, 6.2 g KH_2PO_4, 0.6 g $MgSO_4.7H_2O$ per L), 0.2 mL micromineral (13.2 g $CaCl_2.2H_2O$, 10 g $MnCl_2.4H_2O$, 1 g $CoCl_2.6H_2O$, 0.8 g $FeCl_2.6H_2O$ in 100 mL), 250 mL of buffer solution (35 g $NaHCO_3$, 4 g NH_4HCO_3 per L), 0.5 g cystein and resazurine 0.1%, warmed at 38°C under CO_2. Once homogenized, 90 mL were added from above to each flask together with 10 mL of ruminal liquor; the flasks were then incubated at 38°C. The ruminal liquor was collected (approximately 07:00 hours) from two rumen-cannulated lambs given the rations described above. The gas production (mL gas/g DM) was registered at 3,6,9,12,24,36,48,72 and 96 h and was estimated by the difference of the gas production of the corresponding standard [7]. At the end of the incubation, the residue was filtered and washed with distilled water and dried in an oven (65°C, 48 h) to determine the DMd and relative gas production (RGP, mL of gas 96 h/g DMd) [17].

The *in vitro* gas production was adjusted to the model proposed by France et al. [18].

$$Y=A\ [1 - exp\ (-b^{(t-T)} - c^{(\sqrt{t}-\sqrt{T})})], \quad [1]$$

where Y represents the cumulative gas production (mL), t is the incubation time (h), A is the asymptote of the curve (total gas production, mL), b (h^{-1}) and b ($h^{-1/2}$) are the initial and later gas production rate constants, T represents the lag time (h), which is the time when the food begins to be degraded by microorganisms in the rumen.

2.5 Statistical Analyses

Data were analyzed using a completely randomized design with factorial arrangement (3x2) treatments, which analyzed the effect of three sowing dates, two cutting ages and their interaction. The comparison using statistical differences (*P=.05*) was performed using Tukey test, using the SAS program [19].

3. RESULTS

3.1 Chemical Composition

OM content was higher (*P*< .001) for D3 compared with the rest of the treatments, and when compared between cuts, C1>C2 (*P*< .001, Table 1). CP content was higher (P< .001) at D2 than D3 > D1, CP was higher C2 than C1. NDF and ADF contents were not different (P> .05) between sowing dates and cuts. There were differences (*P*< *.001*) between OM and CP interactions.

Table 1. Chemical composition (g/kg DM) of *Kochia scoparia*at different dates of sowing (D) and cut (C) and *in sacco* degradation (g/100 g DM) of dry matter (DMd) and rumen degradable protein (RDP)

	Date of sowing			Cutting age			*P value*		
Item	**D1**	**D2**	**D3**	**C1**	**C2**	**SEM**	**D**	**C**	**DXC**
OM	883^{B}	885^{B}	889^{A}	893^{A}	878^{B}	0.79	0.001	0.001	0.001
CP	149^{C}	187^{A}	162^{B}	157^{B}	175^{A}	1.79	0.001	0.001	0.001
NDF	603	614	574	611	583	37.32	0.74	0.52	0.48
ADF	405	390	419	400	409	10.01	0.16	0.46	0.37
DMD	51^{B}	62^{A}	57^{AB}	56	57	2.25	0.007	0.07	0.001
RDP	74^{B}	83^{A}	77^{B}	77	78	1.16	0.001	0.68	0.001

^{AB}Different letters within the same row show differences (P< .001). SEM= Standard Error of the Mean.

3.2 In Sacco Ruminal Degradation

The effect on DMd between sowing dates (*P= .007*) (Table 1), was higher D2 than D1. The DMd was higher (*P= .002*) C2vs C1. RDP showed differences (*P< .001*) between sowing dates, being higher D2 than D1 and D3, but there were not differences between cuts (*P= .68*), while for the interactions, D2C2 was higher (*P< .001*) than the rest of the interactions (Table 2).

3.3 *In vitro* Gas Production

Sowing date was not affected on the total gas production (A, 179 ± 12 ml gas/g DM), b (0.033 ± 0.003), c (- 0.022 ± 0.01) and T (0.753 ± 0.27), DMd at D2 was higher (*P= .008*) than the rest of the sowing dates. Age at cut was not affected on any of the variables, showing trend (*P= .06*) for total gas production, DMd was higher for D2 (*P< .008*) in relation with the rest of the dates (Table 2), with out effect between cuts (*P> .59*), but there were differences between interactions (*P< .001*) (Fig. 1). RGP were not different between dates (*P> .05*), but show a trend (*P= 0.06*) between cuts and their interaction.

Table 2. Effect of sowing date (D) and age at cutting (C) on the parameters of the fitted curves from *in vitro* gas production due to the fermentation of *Kochia scoparia*

	DATE OF SOWING			CUTTING AGE			*P VALUE*		
ITEM	**D1**	**D2**	**D3**	**C1**	**C2**	**SEM**	**D**	**C**	**DXC**
A	172	190	174	174	184	5.45	0.08	0.14	0.10
B	0.030	0.036	0.033	0.033	0.033	0.003	0.53	0.96	0.75
C	-0.015	-0.024	-0.028	-0.013	-0.031	0.008	0.58	0.10	0.12
T	0.594	0.713	0.953	0.559	0.948	0.20	0.46	0.12	0.23
DMD	58^{E}	64^{D}	55^{E}	60	59	1.70	0.0085	0.59	0.001
RGP	299	296	316	292	315	10.03	0.36	0.06	0.05

Values are expressed as mean. ^{DE}Different letters indicate significance (d < e, P= .05). †SEM = Standard error of mean. A = total gas production (ml gas/g incubated DM); b = fermentation rate (h^{-1}); c = fermentation rate ($h^{-1/2}$); T = lag time (the time in that the fermentation is initiated); DMd (mg/100 mg DM) = disappeared dry matter proportion; RGP = relative gas production (ml gas/g DM disappeared).

4. DISCUSSION

4.1 Chemical Composition

Madrid et al. [5] show similar values on average of OM (835 g kg DM), NDF (518 g kg DM) and ADF (267 g kg DM) contents, while the CP in the present study was lower when it was expressed for the principal effects between sowing dates and cutting ages compared with values of Madrid et al. [5] (206 g kg DM), but it was lower to D2C2, likewise CP obtained in the present study were similar to Casado et al. [4] and Romero-Bernal et al. [6] at 119 d of age. Other authors obtained values of 91 and 117g CP/ kg DM, respectively [20,21], being lower than the present study. Probably due to the age cutoff, although the plant was in a state of flowering, has a lower CP content compared with a pre-flowering stage [3]. These studies imply that although the *Kochia scoparia*, can be used as a source of protein for feeding ruminant nutrition concentration varies according to the age [4].

4.2 In Sacco Ruminal Degradation

Rankins and Smith, [22] founded that Kochia presented an apparent ruminal degradation of 59 and 72% of DMd and RDP, respectively, compared with the present study had a lower DMd, but higher RDP. Romero-Bernal [23] present similar values for DMd at 78 d (59%), but were lower when the plant reaches an age of 119 d (52%), and showed a difference of 12% between cuts. In the present study there were not differences between cuts (P>0.05), which suggested that Kochia after 120 d of age reaches its maximum growing maturation, and therefore there was no difference in the contents of NDF, ADF, DMd and RDP between cuts. Sowing date (D2 vs D1 and D3) was affected (*P= .05*) on DMd and RDP, but this effect was not shown when the plant was younger [23]. Danesh Mesgaranand Stern [21] presents an average value of 46% of RDP being lower than the present study, but were similar in NDF contents. However, Romero-Bernal et al. [6] found a similar degradation of RDP (69%) to the present study, when the plant reached 78 d of age at the first cut, and this still lower at 119 d (59%) of age compared to the present study. So it is important to consider that the age at cutoff was affected at a younger age of the plant [4] and this effect was lost with the plant maturation, similarly to the present study. *In situ* DMd were lower than *in vitro*, due to several factors such as differences between techniques and incubation times, but shows the same

pattern of degradation, being higher D2C2 and D3C1 versus D2C1 and D3C2. Rankinsand Smith [22] found that Kochia presented an apparent degradation *in vivo* of59 and 72% of DM and CP respectively, being similar to the present study when compared with the *in sacco* degradation. Riasi et al. [24] determined *in sacco* degradation of DM in Kochia at 16 h of rumen incubation and found similar values (51.7%) than those in this study for D1C1, D1C2 and D3C2, but lower than D2C2 and D3C1, which 23.8% lower digestibility. CP and NDF content were higher than Riasi et al. [24]. This confirms that there are variations in the nutritional content and ruminal degradation, depending on the area or region where the crop has been sown, and the stage of maturity.

4.3 *In vitro* Gas Production

Finley and Sherrod [25] compare the different stages of maturity of Kochia, which were higher than this study (53.2-73.0% digestibility of DM). Ahmed and El-Hag [26], present values of total gas production (ml gas/g DM) for different legumes 117.7 *Trebulus terrestres*, 135.5 *Solanum doblium*, 153.6 *Inigofera spp.* 172.7 *Sesamum alatum*, 133.2 *Stylosanthes frauticosa* and 136.8 *Zornia glochidiata*, which were lower than those observed in this study. When compared with results obtained by Casado et al. [4], these showed a higher gas production (186 ml gas / g DM) when the plant reaches the age of 78 d. DMd was similar to that found by Romero-Bernal et al. [6] at 78 and 119 d with respect to the present study.

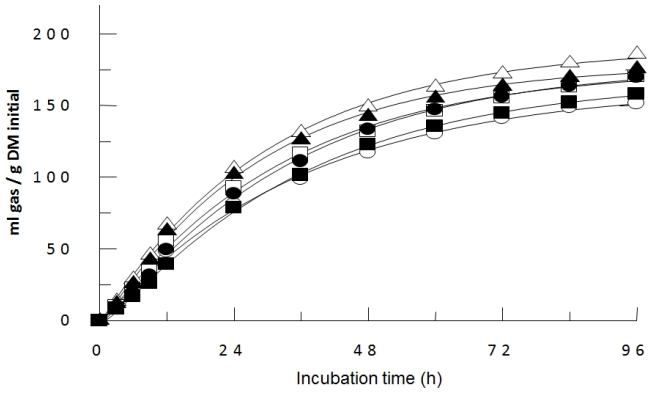

Fig. 1. *In vitro* gas production (ml gas/ g DM) obtained by the adjustment of the incubation of *Kochia scoparia* in relation with the date of sowing and cutting age (●, D1C1; ○,D1C2; ▲, D2C1; △, D2C2; ■,D3C1; □,D3C2).

5. CONCLUSION

Based on the CP contents of *Kochia scoparia* and the rumen degradable protein (> 70%), we conclude that *Kochia scoparia*is a medium-quality protein source for ruminants, whose values were influenced by the date of establishment and in lower magnitude for the cutting age.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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