Tropical and Subtropical Agroecosystems, 14 (2011): 683 - 689

CHEMICAL COMPOSITION, *in situ* AND *in vitro* DEGRADATION OF *Kochia scoparia* IN RELATION TO DATE OF SOWING AND AGE OF CUT

[COMPOSICIÓN QUÍMICA, DEGRADACIÓN *in situ* E *in vitro* DE *Kochia scoparia* CON RELACIÓN A LA FECHA DE SIEMBRA Y EDAD DE CORTE]

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SUMMARY

The *Kochia scoparia* is an alternative source of medium-quality protein for food in ruminants, for this, it is important to know its chemical composition and its degradation and fermentation in the rumen, as this can vary depending on the date of establishment and cutting age. In the present study evaluated the chemical composition. The degradation assessment was performed using the technique of *in vitro* gas production and ruminal *in sacco* degradation. The *Kochia scoparia* cultured on three dates (D) of sowing(D1, 07/12/2001; D2, 06/01/2002; and D3, 05/02/2002) and the cut was made at two different ages (78 to 119 days post sowing, C1 and C2, respectively). OM content (g 100g DM) was higher ($p \leq 0.05$) for D1 (90.8) Than for D2 and D3 (89.1) and CP content exchange between due dates, ($P \leq 0.05$) D3 > D2 > D1 (16.4, 15.3, 13.3 %, respectively) the *in vitro* production of gas(ml gas g^{-1} DM)was not different($P \geq 0.05$) between planting dates (185) but for cuts($P \leq 0.05$), being C1 > C2 (199.6 *vs* 171.7); *in vitro* degradation of dry matter, there were no differences observed ($P \geq 0.05$) between dates (53.11 mg 100 mg of DM) but C1 was superior ($P \leq 0.05$) to C2 (59.3 *vs* 46.9 mg 100 mg of DM).The Protein degradation was estimated by *in situ* ruminal incubation (RDP, g 100g of DM),being superior ($p \leq 0.05$) D2 (71.8) with regards to D1 and D3 (62.9 y 59.3) and C1 > C2 ($P \leq 0.05$) (67.6 *vs* 61.8). Due to the CP content of the *Kochia scoparia* it is suggested as an alternative source of medium-quality protein to ruminants, having degradation in the rumen of more than 60 %, which is influenced by the maturity of the plant and its date of establishment.

Key Words: degradation; *Kochia scoparia*; gas production; *in situ*; *in vitro*.

RESUMEN

La *Kochia* scoparia es una fuente alternativa de proteína de calidad media para la alimentación en rumiantes; por lo anterior, es importante conocer su composición química, así como su degradación y fermentación en el rumen, ya que esta puede variar en función de la fecha de establecimiento y la edad al corte. Para ello se evaluó la composición química. La evaluación de la degradación se realizo mediante el uso de la técnica de producción de gas *in vitro* y la degradación ruminal *in sacco*. La *Kochia scoparia* se cultivo en tres fechas (F) de siembra (F1, 07/12/2001; F2, 06/01/2002; y F3, 05/02/2002) y se realizó el corte a dos edades distintas (78 y 119 días postsiembra, C1 y C2, respectivamente). El contenido de MO (g 100g de MS) fue superior ($P \leq 0.05$) para F1 (90.8) con respecto a F2 y F3 (89.1), y el contenido de PC cambio entre fechas de corte, ($P \leq 0.05$) F3 > F2 > F1 (16.4, 15.3, 13.3 %, respectivamente). La producción de gas *in vitro* (mL gas g^{-1} de MS) no fue diferente ($P \geq 0.05$) entre fechas de siembra (185) pero sí para cortes ($P \leq 0.05$), siendo C1 > C2 (199.6 *vs* 171.7); en la degradación *in vitro* de la materia seca, no se observó diferencias ($P \geq 0.05$) entre fechas (53.11 mg 100 mg de MS) pero C1 fue superior ($P \leq 0.05$) a C2 (59.3 *vs* 46.9 mg 100 mg de MS). La degradación de la proteína fue estimada mediante la incubación ruminal *in situ* (PDR, g 100g de MS), siendo superior ($p \leq 0.05$) F2 (71.8) con respecto a F1 y F3 (62.9 y 59.3) y C1 > C2 ($P \leq 0.05$) (67.6 *vs* 61.8). Por su contenido de PC, la *Kochia scoparia* se sugiere como una fuente alternativa de proteína de calidad media para los rumiantes, por tener una degradación en rumen superior al 60 %, la cual está influenciada por el estado de madurez de la planta y su fecha de establecimiento.

Palabras clave: Degradación; *Kochia scoparia*; producción de gas; *in sacco*; *in vitro*.

INTRODUCTION

The *Kochia scoparia* represents an alternative forage for their adaptive characteristics, as it can germinate in a range of 3.5 to 50 °C (Al-Ahmadi and Kafi, 2007), allowing it to have a high potential for establishment., It can be planted in arid and semiarid regions (Garduño, 1993), as it is to be considered emergency crop due to its developmental growth as were other forages could not (Zahran, 1993). It is an alternative source of feed for ruminants in areas where forages are deficient in protein; although it is important to determine the potential degradation of this protein in the rumen.

The technique of *in vitro* gas production (Menke and Steingass, 1988, Theodorou *et al*., 1994) permits the prediction of food fermentation and digestibility. The use of the technique *in situ* in ruminants (Van Keuren and Heinemann, 1968), it allows us to determine the degradation of food at the rumen, and to estimate the protein that is degraded to this level, which is potentially used by microorganisms of the rumen. The study aimed to determine the chemical composition, *in vitro* gas production and rumen degradation of dry matter and crude protein content of *K. scoparia* grown in three planting dates and two harvest ages.

MATERIALS AND METHODS

The study was conducted in 2000 and 2001 when it established a field of *K. scoparia* (*K. scoparia* L. Schrader var. Esmeralda), in the town of San Miguel Ixtapan, Tejupilco municipality, State of Mexico (18 ° 45 '30 'and 19 ° 04'32 ", INEGI, 2004), at an altitude of 1340 m above sea level, dry tropical climate with summer rains. Planting was done in the lower rainfall season in the months of December 2001, January and February 2002. The seeding rate was 5.5 kg seed ha, with a total area of 9000 m^2, utilizing three plots of 1000 m^2 per sowing date. The risks of planting were conducted at two dates during the germination of plants and emerged after plants were irrigated with a frequency of 10 to 12 days. Fertilization was applied 100-80-40 (NPK) at a rate of 217 kg of urea, 174 kg P and 66 kg K ha^{-1}, dividing the N by 50 % for planting along with all the P and K, and the remaining N was added when the plant showed a growth of 35 cm in height. The weeds were controlled manually at the stage of crop development. Two cuts were performed on samples (C1, 78 and C2, 119 days after sowing), recollecting samples of 3 linear m with three replicates per plot for each of the dates of establishment.

Chemical composition

The samples were grounded to 1 mm in diameter with a fixed hammer mill (Wiley Mill ® Mini.) Content was determined in dry matter (DM) by drying at 60 °C for 48 h, organic matter (OM) it was calculated as the weight loss after incineration of the sample at 600 °C, the nitrogen content was estimated by the Kjeldahl method (AOAC, 1999, ID 984.13); The neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined using the Ankom fiber analyzer according to the methodology described by Van Soest *et al*. (1991), without correction for ash and without α-amylase. Analyses were performed in duplicate and average values were used for comparison between samples.

In vitro gas production

It were used three lambs, Rambouillet (20 ± 0.5 kg BW) cannulated in the rumen. *Ad libitum* was fed at 09:00 and 16:00 h with a diet based on alfalfa hay and oat straw (50:50), to which was added 2 % vitamin-mineral supplement (Gold line Hitec -nutrition Multitec Malta Cleyton ®). It was taken from each animal an equal amount (500 ml) of rumen fluid from the middle of the rumen before feeding (08:30 h); a pool was completed and was used as primary inoculums solution. To evaluate the *in vitro* gas production it was used the proposal technique done by Theodorou *et al*. (1994), a day before the incubation, the samples were prepared for it, were used 125 ml flasks, to which they were introduced 800 mg of DM in *K. scoparia* (in triplicate). Later, prepared the incubation solution (Menke and Steingass, 1988) of this mixture was added 90 ml to each vial, which were stored at 4°C. The rumen fluid extracted and filtered in a triple layer of cheese cloth gauze and glass wool, homogenized in an atmosphere of CO_2 for 5 minutes, then was added 10 ml of rumen fluid to each bottle to be incubated in a water bath at 39 °C.

There were three series of incubations, in which included three bottles without substrate (as blank), as witnesses for the correction of gas production due to fermentation of rumen fluid itself and oat straw was used, with a known gas production, as standard between sets of incubation.

Once incubation started, there was the reading of gas production by a Delta brand pressure transducer (Model HD 8804), at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation finalizing the period, the samples were filtered, washed with water and dried (65 °C) for 48 h to estimate dry matter digestibility (DMD, mg/100 mg). Gas production at 96 h was related to the DMD for gas production ratio (RGP: gas g ml^{-1} DMD) according to Gonzalez Ronquillo *et al*. (1998).

Gas production was fitted to the model proposed by France *et al*. (1993), $y = A [1 - \exp (-b^{(t - T)} - c^{(\sqrt{t} - \sqrt{T})})]$, where "y" represents the cumulative gas production (ml), "t" is time incubation (h), "A" curve asymptote (total gas production, ml), "b" (h^{-1}) and "c " ($h^{-1/2}$) are

constants of gas production and "T" represents the delay time (h), which is the time it takes for bacteria to initiate fermentation. The model indicates that the fractional degradation rate μ (h-1) is not constant, but varies with the time between the periods of fermentation:

$\mu = b + (c / 2 \sqrt{t}), t \geq T$

Ruminal *in situ* degradation

To determine the dry matter digestibility (DMD, g 100g) and rumen degradable protein (RDP, g 100g) it were used three rumen cannulated sheep's as described in the *in vitro* technique. Nylon bags were introduced (10 x 15 cm, 52 ± 16 microns diameter) in the rumen for a period of 24 h in duplicate by performing two repetitions per animal. It was weighed 5 g of DM in each bag. Before being introduced to the rumen, all bags were incubated for 5 minutes in distilled water at 39°C. Finalized the incubation, the bags were removed, which were hand washed and rinsed with tap water until the water came out clean. The samples were dried for 24 h at 60°C for further analysis. The residue of the incubations *in vitro* and *in situ* were determined the DMD and its protein content (N x 6.25), respectively.

Statistical analysis

The results were analyzed using a variance analyses design, the variables date of planting, cutting age and their interaction. $Y = \mu + Date_i + Courtship_j + (Court \times Date)_{ij} + \varepsilon_{ijk}$. The comparison of means in the variables that had significant differences ($P \leq 0.05$) were made by the method of Tukey (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Chemical composition

Table 1 shows the chemical composition (g/100 g DM) of *K. scoparia*. OM was superior ($P \leq 0.05$) for D1C2, followed by D2C2, and lower ($P<0.05$) for D2C1. Where D1 was higher than the rest and C2 > C1. CP content was superior ($P \leq 0.05$) for D3C1, followed by D2C2 and D3C2, and less for D1C1. The NDF was superior ($P \leq 0.05$) for D1C2 compared to the rest and lower for D2C1 and D3C1. The ADF content was superior ($P \leq 0.05$) for D1C2 > D2C2, D3C2 and lower for D3C1, finally the contribution of NDF and ADF was superior ($P \leq 0.05$) for D1 > D2 and D3, and C2 > C1.

Cohen *et al*. (1989), while evaluating the chemical composition of *K. scoparia* at different stages of plant maturity, you will find values of 9g of CP 100g DM lower than those shown in the present study, possibly due to the age cutoff, as in the present study, this is performed in a state of pre-flowering, the previous suggests the variations in chemical composition as a function of age at cutting and planting date, as presented by Finley and Sherrod (1973) at different stages of flowering, showing higher nutritional content at the stage of pre-flowering. In studies such as that conducted by Zaman *et al*. (2003), *K. scoparia* has been compared with alfalfa, while evaluating different interactions of alfalfa and weeds (*Kochia*), they find ranges from 22 to 18 % of CP superior to the present study, but lower than the wild oats and alfalfa association (11 % CP), allowing to consider Kochia as a source of moderate quality protein.

The forage *K. scoparia* is moderate in protein, similar to certain varieties of alfalfa or clover (Finley and Sherrod, 1973; Rankins and Smith, 1991), by having this feature. However, the difference is that the former can grow in soils with low humidity and low levels of organic matter, unlike the varieties of alfalfa or clover. It has been compared with other halophytes (Riasi *et al*., 2008), having the property to survive in saline and alkaline soils (Zahran, 1993) and resistant to drought, showing similar characteristics (eg. Atriplex) or more beneficial in terms of nutritional content and digestibility compared to other halophytes (eg. Suaeda, Gamanthus).

Table 1. Chemical Composition (g/100g^{-1} MS) of *Kochia scoparia* to different dates (D) of establishment and cuts (C).

	D1		D2		D3					
Variable	C1	C2	C1	C2	C1	C2	SEM	D	C	DxC
OM	88.40^{cd}	93.14^{a}	88.06^{d}	89.90^{b}	89.00^{bcd}	89.67^{bc}	0.312	*	*	*
CP	12.88^{e}	13.74^{de}	14.35^{cd}	16.26^{b}	17.72^{a}	15.11^{c}	0.057	*	NS	*
NDF	66.34^{cd}	72.61^{a}	64.67^{d}	70.03^{ab}	64.82^{d}	67.94^{bc}	0.574	*	*	*
ADF	41.58^{c}	49.54^{a}	40.02^{cd}	45.66^{b}	37.92^{d}	46.68^{b}	0.515	*	*	*

^{a,b} Means with different letters in the same row are statistically different (*$P \leq 0.05$).
SEM, Standard Error of the Mean.

***In vitro* gas production**

Table 2 presents the gas production values that were observed *in vitro* effect of planting date interaction with cutting age for fractions A, b, c and the coefficient of digestibility of dry matter after 96 hours, the maximum gas production (fraction A) (P≤0.05) was for D2C1 and D3C1 and lower for D2C2, as shown in Figure 1.

The constant "b" D3C1 was higher (P≤0.05) with respect to D3C2, no significant differences (P≥0.05) in terms of planting dates, however, was higher C1 than C2 (P≤0.05) (0.031 *vs* 0.018, respectively). As for the parameter "c", D1C2 was higher (P≤0.05) D3C2, and the effect due to the date of establishment, D1 was greater (P≤0.05) at D3, no significant differences between cuts; for the fractional degradation rate (0.584 ± 0.66) and RGP (358 ± 17), no significant differences (P≥0.05) for the dates of establishment and cutting age.

For the DMD, D3C1 was higher (P≤0.05) by 34 % over D3C2, no significant differences (P≥0.05) for sowing dates (53.11 ± 1.7 g 100 g^{-1} DM), but effect was observed (P≤0.05) in the cutting intervals, was higher C1 (59.3 g 100 g^{-1} DM) compared to C2 (46.9 g 100g^{-1} DM).

The fractional rate of degradation at different incubation times; For all D3C2 hours of incubation was lower (P≤0.05) compared to the rest, only showed an effect due to the date for 6 h, and C1 > C2 (P≤0.05). When we compare the fractional fermentation rate (μ h^{-1}) with the DMD_{96h}, F3C2 has the lowest DMS to be less than the rate of fermentation, with the rest.

The disappearance of DM and CP varies due to NDF, ADF (Van Soest, 1994) and even in terms of ash content (Benjamin *et al*., 1995). Mir *et al*. (1991) evaluated the apparent digestibility of *K. scoparia in vivo*, showing a 65.5 g 100g apparent digestibility of DM, which is superior to that obtained in the present study *in vitro*, it is important to note that there were differences in the degradation *in vitro* by 20 % when comparing C1 *vs* C2, Finley and Sherrod (1973) reported similar differences when comparing the state of maturity of the Kochia.

Ruminal *in situ* degradation

Table 3 shows the DMD *in sacco* and RDP (g/100 g DM). There were no differences (P≤0.05) for DMD in terms of interaction and cuts, but effect was observed between dates (P≤0.05), D2 and D3 are superior with respect to D1. For the PDR it presented the lowest D3C2 degradation (P≤0.05), followed by D1C1, no differences were found for other interactions, F2 (71.8) was higher compared to D1 and D3 (63.0 and 59.3, respectively) as for cuts, C1 (67.6) was higher (P≤0.05) C2 (61.8).

Table 2.Parameters of fitted curves from *in vitro* gas production due to fermentation of *Kochia scoparia* on different dates of establishment and cutting age.

	D1		D2		D3					
Variable	C1	C2	C1	C2	C1	C2	SEM	D	C	DxC
A	189.5^{de}	176.8^{de}	204.4^{d}	158.5^{e}	204.8^{d}	179.7^{de}	9.5	NS	*	*
b	0.027^{de}	0.017^{de}	0.032^{de}	0.023^{de}	0.034^{d}	0.015^{e}	0.004	NS	*	*
c	0.007^{de}	0.034^{d}	0.005^{de}	0.002^{de}	-0.010^{de}	-0.024^{e}	0.009	*	NS	*
DMD_{96h}	55.9^{de}	47.8^{de}	58.9^{de}	51.3^{de}	63.0^{d}	41.6^{e}	4.0	NS	*	*
T	0.267	0.089	0.307	0.363	0.577	1.903	0.465	NS	NS	NS
RGP	345.1	373.6	351.9	313.5	327.0	439.6	37.0	NS	NS	NS
μ 6 h	0.029^{d}	0.024^{d}	0.033^{d}	0.024^{d}	0.032^{d}	0.010^{e}	0.0028	*	*	*
μ 24 h	0.028^{de}	0.021^{de}	0.032^{d}	0.024^{de}	0.033^{d}	0.012^{e}	0.0033	NS	*	*

^{d,e} Means with different letters in the same row are statistically different (*P≤ 0.05).
A, total gas production (ml gas g-1 initial DM), fermentation rate and c h-1, h-1/2; DMD96h, proportion of DMD (mg 100 mg DM) at 96 h; T, delay time of incubation (h-1); RGP, relative gas production (ml gas g-1 DMS); μ6, μ24h, fractional fermentation rate (h-1); SEM, Standard Error Mean.

The values of dry matter digestibility *in sacco* were 20 % lower than the values *in vitro*, this is due to several factors such as differences in techniques and incubation times, since *in vitro* was performed 96 h *vs* 24 h incubation *in sacco*. This is important, since *in vivo* tests will be different digestibility's obtained. Rankins and Smith (1991), found that Kochia had an apparent degradation *in vivo* 59 and 72 g 100g of DM and CP, respectively, being lower than the present study for the DM, but similar to the protein content in D2; not for the cuts, which are slightly lower in this study. Mesg Danesh and Stern (2005) indicated a mean RDP of 46 % CP, less than this study (65 %). Riasi *et al*. (2008) determined *in sacco* degradation of Kochia at 16 h of incubation in the rumen, and found values of 51.7 % remaining lower than those shown in this study, giving a difference of 28 % digestibility when compared with D2, whose values have a higher content of CP and NDF with respect to those shown by Riasi *et al*. (2008). Rodriguez *et al*. (2009), there is variation in the nutritional content and ruminal degradation, depending on the region where the crop has been sown and the state of maturity.

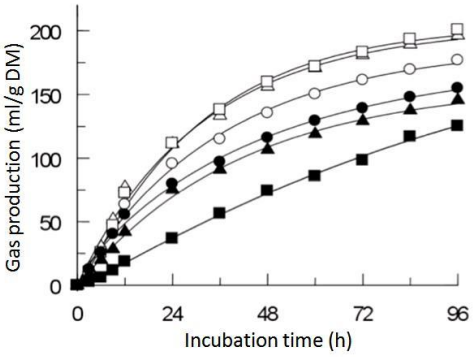

Figure 1. Changes in volume of gas produced (ml gas g-1 DM) in relation to planting date interaction (D) with an age of cut (C): (D1C1, ○; D2C1, □; D3C1, Δ, D1C2, ●; D2C2, ■; D3C2, ▲) of *Kochia scoparia*.

Table 3. Degradation (g/100 g^{-1} DM) *in sacco* dry matter (DMD) and rumen degradable protein (RDP) in *Kochia scoparia*, a different date of establishment and age of cut.

	D1		D2		D3					
Variable	C1	C2	C1	C2	C1	C2	SEM	D	C	DxC
DMD	36.91	33.95	45.07	41.66	47.26	41.77	3.12	*	NS	NS
RDP	57.89[b]	67.90[a]	71.60[a]	71.96[a]	73.26[a]	45.70[c]	2.03	*	*	*

[abc] Different letters within the same row indicate differences (* P≤0.05). SEM, Standard Error of the Mean.

CONCLUSIONS

The results above shows that the *Kochia scoparia*, can be a source of rumen degradable protein level of moderate quality, which is influenced by the date of establishment and the age cutoff, and its higher nutritional value and if harvested ruminal degradation in a state of early maturity.

ACKNOWLEDGEMENTS

This project was funded through the Universidad Autonoma del Estado de México (Project 1771/2003).

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Submitted November 18, 2010 – Accepted January 13, 2011
Revised received March 01, 2011