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Full Length Research Paper

Use *IN VITRO* gas production technique for assessment of nutritional quality of diets by range steers

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This study evaluated the seasonal changes in the *IN VITRO* gas production of diets consumed for grazing steers. The gas produced by the soluble fraction "a" and insoluble but slowly fermenting fraction "b", were highest in summer and fall (p<0.01). The constant gas production rate "c" was affected by season of the year (p<0.01). The organic matter digestibility (OMD) and short chain fatty acids (SCFA) was affected by season of the year (p<0.05). The *IN VITRO* gas production is a good indicator of the nutritive quality diet consumed by grazing cattle.

Key words: Steers, grazing, *in vitro* gas production, rangelands.

INTRODUCCION

The determination of the *in vitro* gas production is of value to nutritionist because it provides information on fermentation kinetics of forage consumed by ruminants, which is dependent on the rate of passage and the degradation rate (Mould et al., 2005). The rate and extent of DM fermentation in the rumen are crucial determinants of the nutrients utilized by ruminants (Jancík et al., 2010).The relevance of evaluating the nutritional value of forage makes an important contribution to the protein and energy intake of grazing cattle (Cline et al., 2010). This is particularly important in arid and semi arid regions where availability forage and quality may be severely limited during the dry season. Traditionally, the energetic value of forage consumed by grazing cattle is estimated from *in vitro* organic matter digestibility or *in situ* organic matter degradability obtained after 48 h incubation in the rumen (Waterman et al., 2007). Nonetheless, these methods are laborious, expensive and time consuming (France et al., 2005). Menke and Steingass (1988) developed the *in vitro* gas production technique to evaluate the nutritive value of forages and to estimate the rate and extent of DM degradation indirectly using the gas production during the fermentation. *In vitro* gas production method has numerous advantages over *in vitro* methods. This method is less animal dependent, more appropriate for characterizing soluble or small particulate feeds and it can be automated thus reducing the labour input (Adesogan, 2005). This technique simulate the digestive processes generated by microbial activity it help us to understand feed fermentation and degradability as a function of nutritional quality and nutrient availability for the bacteria. The *in vitro* gas production has been widely used to estimate the nutritive quality of cereal straws (Valizadeh et al., 2010) as well as in different classes of forages (Njidda, 2010). However, there is little information about *in vitro* ruminal gas production of the diet consumed by grazing cattle in native rangelands. Consequently, the aim of this study was to evaluate the seasonal variations in the *in vitro* gas production in diets by range steers.

MATERIALS AND METHODS

Study area

The study was carried during two consecutive years (2004 and 2005) in a medium-sized shrub-grassland east of the city of Durango, Mexico (24° 22'N, 104° 32'W, at an altitude of about 1938 m above sea level), with a dry temperate (BS_1k) climate and average annual temperature and rainfall of 17.5°C and 450 mm,

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respectively. Rainfall in 2004 was above average at 547.5 mm and 2005 was drier than normal at 238.0 mm (INEGI, 2007).The study area covers 2,000 ha (6 ha/AU) with an average of forage biomass of 1,796 kg of DM/ha. During the two years of the study, we estimated vegetation cover using minimum area sampling with nested points. Dominant grass species included *Melinis repens* Willd (rose natal grass), *Chloris virgata* (feather fingergrass), *Bouteloua gracilis* (blue grama), *Aristida adscensionis* (sixweeks threeawn) and *Andropogon barbinodis* (cane bluestem); bushes: *Acacia tortuosa* (poponax)*, Prosopis juliflora* (mezquite)*, Opuntia spp* (prickly pears and chollas), *Mimosa biuncifera* (mimosa); plus a wide variety of annual herbs.

Animals and collection of diet samples

We used four steers fistulated in the esophagus with a live weight of 350 ± 3 kg. Surgery was performed on the steers according to procedures approved by the University of Durango Laboratory Care Advisory Committee. We collected diet samples with the steers fistulated of esophagus on four consecutive days at 07:00 during a 45 min period (Karn, 2000), eight times annually: (1) Jan. 2-5, (2) Feb. 4 to 7, (3) Apr. 13 to 16, (4) May 15 to 18, (5) Jul. 20 to 23, (6) Aug. 11 to 14, (7) Oct. 12 to 15 and (8) Nov. 20 to 23. The first two collection periods were considered to be in winter; 3 and 4, spring; 5 and 6, summer; and 7 and 8, fall.

IN VITRO **gas production**

The *in vitro* gas production was carried out using the method proposed for Menke and Steingass (1988). Approximately 500 mg of the sample's diet, ground to 1 mm, were placed in triplicate in 100 ml calibrated glass syringes. Buffer and mineral solutions were added in a 2:1 ratio to rumen liquid collected from two fistulated heifers which were fed with alfalfa hay. Forty milliliters of this mixture were introduced in each syringe for incubation. Alfalfa hay whose gas production is known was used control. Syringes were shaken gently at each reading and the gas volume was recorded at 0, 3, 6, 9 15, 24, 36, 48, 72 y 96 h of incubation. Data obtained of gas production were adjusted at the model proposed by McDonald, (1981):

$$GP_{(t)} = a + b \times (1-e^{-c \times (t-L)})$$

where: $GP_{(t)}$= gas produced at time t, a= gas produced by the soluble fraction, b = gas produced by the insoluble but slowly fermenting fraction, c = constant gas production rate, t= time of fermentation, L = lag time.

Organic matter digestibility, energy and short fatty acids estimation

The organic matter digestibility (OME), metabolizable energy (ME) value and the short chain fatty acids (SCFA) of diets were estimated with the following equations:

$$OMD\ (\%) = 0.9991\ (G_{24h}) + 0.0595\ (CP) + 0.0181\ (CC) + 9$$ (Menke and Steingass, 1988)

$$ME\ (MJ/kgDM) = 0.157\ (G_{24h}) + 0.0084\ (CP) + 0.022\ (EE) - 0.0081\ (CC) + 1.06$$ (Menke and Steingass, 1988).

$$SCFA\ (mmol) = 0.0222\ (G_{24h}) - 0.00425$$ (Makkar, 2005)

where: G_{24h} is gas production at 24 h of incubation, CP, EE and CC are crude protein, ether extract and crude ash content of diets, respectively.

Statistical analysis

Data of over a month were analyzed as a repeated measure (split-split plot) design using the MIXED procedure of SAS (2003). The repeated effects of the range steers within years × season was used as the error term for the split-split plot. Autoregressive order 1 was used as the covariance structure, because it was better fitting structure, based on comparison of covariance structures with Akaike and Bayesian information criterions (Littell et al., 1998). The comparison of means between years and season was performed using the LSMEANS (least squares means) statement of MIXED procedure of SAS (2003).

RESULTS

IN VITRO gas production

The parameters of *in vitro* gas production are presented in Table 1. There was no year × season interaction for the parameters of *in vitro* gas production ($p>0.05$). Values of "a", "b" and "c" were different between years, with the highest values in 2004 and the lowest in 2005 ($p<0.01$). Nevertheless, the value of the lag time was higher in 2005 than 2004 ($p<0.01$). The gas produced by "a" and "b"; were greater in summer and fall as compared to winter and spring ($p< 0.01$). The constant gas production rate "c" was affected by season of the year ($p<0.01$). The highest values were registered in summer ($4.5\%h^{-1}$) and the lowest in winter ($2.3\%h^{-1}$).

Organic matter digestibility, energy and short fatty acids

There was no year × season interaction for OMD, ME and SCFA ($p>0.05$; Table 2). The values of OMD, ME and SCFA were higher in 2005 that 2004 ($p<0.05$). The OMD and SCFA were affected by season of the year ($p< 0.05$). The highest values were registered during summer and the lowest during the winter. The ME content was higher in summer and fall as compared to winter and spring ($p<0.05$).

DISCUSSION

IN VITRO gas production

To our knowledge, there are peer reviewed articles that detail information about *in vitro* gas production of diets by grazing steers in native range. However, we cite weather conditions as the cause of these differences between years, given that temperature and rainfall have a direct effect on the gas produced by the insoluble "b" and soluble "a" fractions and the constant gas production rate

Table 1. Least squares means for parameters of *in vitro* gas production in the diet of grazing steers.

	a(ml/gDM)	b(ml/gDM)	c(%h^{-1})	LT(h)
Year				
2004	4.4[a]	99.0[a]	3.5[a]	2.2[b]
2005	3.2[b]	88.1[b]	2.6[b]	3.4[a]
SEM	1.6	2.4	2.2	1.8
p<	**	**	**	**
Season				
Spring	3.2[b]	79.9[c]	2.5[c]	3.9[a]
Summer	4.9[a]	99.4[a]	4.5[a]	2.3[b]
Fall	4.8[a]	97.2[a]	4.0[b]	2.6[b]
Winter	2.9[b]	77.0[b]	2.3[d]	3.3[c]
SEM	3.3	1.5	3.8	1.6
p<	**	**	**	**
		Years x **Season**		
p<	0.23	0.15	0.37	0.18

[abcd]Means with different superscripts, within column, are significantly different ($p < 0.05$). SEM: Standard error of mean.**($p < 0.01$).

Table 2. Least squares means for organic matter digestibility, metabolizable energy and short chain fatty acids of diet of grazing steers.

	OMD(%)	ME(MJ/kg DM)	SCFA(mmol)
Year			
2004	72.6[a]	11.5[a]	1.558[a]
2005	67.8[b]	10.2[b]	1.226[b]
SEM	0.92	0.84	0.13
p<	*	*	*
Season			
Spring	66.3[c]	11.2[b]	1.429[c]
Summer	77.5[a]	12.9[a]	1.667[a]
Fall	71.5[b]	12.0[a]	1.524[b]
Winter	49.4[d]	8.8[c]	1.001[d]
SEM	0.16	0.67	0.91
p<	*	*	*
		Years x **Season**	
p<	0.91	0.61	0.16

[abcd]Means with different superscripts, within column, are significantly different ($p < 0.05$). SEM: Standard error of mean.*($p < 0.05$).

"c" of grasslands. The values of gas production from the soluble fraction "a" observed in summer and fall were similar to those reported in indigenous browses by Ondiek et al. (2010). The differences between seasons in values of "a", "b" and "c" may be attributed to concentrations of soluble carbohydrates in diet selected by grazing cattle (La et al., 2008). The high "b" value found in summer and fall could result in higher energy intake by cattle (Murillo et al., 2006). The value "b" (33 ml/200 mg) reported by Cerrillo et al. (2006) in goats browsing a thorn shrubland in North Mexico were lower than we obtained in the summer (99.4 ml/g DM), fall (97.2 ml/g DM) winter (77.0 ml/g DM) and spring (79.9 ml/g DM). These discrepancies between studies are most likely a result of the different plant communities and climate zones in which the investigations took place. Arigbede et al. (2006) report similar values of "c" in leaves from indigenous multipurpose tree species than

our findings. High “c” degradability rates indicate high nutrient availability for ruminal microorganisms; while lower “c” values may be the result of greater NDF content whose chemical components could slow down substrate fermentation speed (Fievez et al., 2005). The variations observed between years and seasons in the lag time may be explicated by the NDF and lignin content of the diet consumed by grazing cattle, to delay the onset of degradation of nutrients in the rumen (Kamalak et al., 2005).The negative effect of lignin can be attributed to the physical obstruction of the structural carbohydrates like cellulose and hemicelluloses and to a limited attack by microorganism on substrates (Cerrillo et al., 2004).

Organic matter digestibility, energy and short fatty acids

The values of OMD and ME registered in this study between year and seasons were similar to reports in forages during dry season (Babeyemi, 2007). The differences in values of OMD and ME registered in this study may be attributed to soluble carbohydrates content of diet selected by grazing cattle across seasons and years (Getachew et al., 2004). Akinfemi et al. (2009) report low SCFA values for five agricultural wastes (average 0.95 mmol) than we obtained in of diet consumed by grazing steers across seasons (average 1.4 mmol) and years (average 1.3 mmol). In this study, SCFA predicted from gas production in winter was lower (1.0 mmol) as compared to spring (1.4 mmol), summer (1.6 mmol) and fall (1.5 mmol). This may due to a lower absolute gas production which is based mainly on carbohydrate fermentation (Sallam et al., 2007). About 94% of the variation in the *in vitro* gas production of browse leaves was explained by SCFA produced, which mainly comes from carbohydrate fermentation (Njidda and Nasiru, 2010). Akinfemi et al. (2009) suggests that gas production from protein fermentation is relatively small as compared to carbohydrate fermentation; while contribution of fat to gas production is negligible.

The *in vitro* gas production can be used to determine the quality nutritive across of the seasons of year and may indicate deficiencies in the energy content of diet consumed by grazing cattle.

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