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Correlation between chemical composition, kinetics of fermentation and methane production of eight pasture grasses

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ABSTRACT. Eight different grasses collected from pastures of the Kermanshah province (Kermanshah, Iran), at mid-vegetative stage were used to study the relationships between their chemical compositions, kinetic parameters of *in vitro* gas production and rumen methane production. There was a positive correlation ($r = 0.62$, $p < 0.05$) between crude protein (CP) content of grasses and total gas production (*A*) at 96h incubation. Negative correlations were also observed between acid detergent fiber (ADF) content and total gas production ($r = -0.60$, $p < 0.05$). Amongst the nutrients, neutral detergent fiber (NDF) and ADF were positively correlated with methane concentration, ($r = 0.75$ and 0.77, $p < 0.01$). The methane reduction potential (MRP) was negative for *Trachyspermum copticum* indicating higher methane production than the control hay for this grass. The MRP of *Chamaemelum nobile* was more than 25%, indicating plants that reduce methane production more than 20 percent methane in comparison with control actually have ingredients to reduce methane.

Keywords: gas production, pasture grasses, methane, *In vitro*.

Correlação entre a composição química, cinética de fermentação e produção de metano de oito tipos de capim de pastagem

RESUMO. Oito tipos diferentes de capim tirados de pastagens na província de Kermanshah (Kermanshah, Iran), em estágio meio vegetativo, foram analisados para ver sua relação entre as composições químicas, parâmetros cinéticos de produção de gás e produção de metano no rúmen *in vivo*. Houve uma correlação positiva ($r = 0,62$; $p < 0,05$) entre a proteína bruta (PB), conteúdo do capim e a produção total de gás (*A*) após 96h de incubação. Foram observadas correlações negativas entre a fibra de detergente ácido (FDA) e a produção total de gás ($r = -0,60$; $p < 0,05$). Entre os nutrientes, a fibra de detergente neutro (FDN) e a FDA foram positivamente correlacionados com concentração de metano ($r = 0,75$ e $0,77$; $p < 0,01$). O potencial reducional de metano (PRM) era negative para *Trachyspermum copticum* e indicava uma produção maior de metano do que o controle feno nesse capim. O PRM de *Chamaemelum nobile* chegou a mais de 25% e indicava plantas que reduzem a produção de metano em mais de 20% quando comparadas ao controle que tem ingredientes para diminuir o metano.

Palavras-chave: produção de gás, capim de pastagem, metano, *In vitro*.

Introduction

Pastures are the main component of ruminants' diet, especially in tropical regions, where, except in areas with high population density, and when properly managed livestock becomes more lucrative. Research on Iranian rangelands and pastures has been undertaken in Iran on range management and ecology. This new area merits investigation because the amount of forage available, as determined in dry matter per hectare, by itself is not a sufficient measure of forage adequacy for animal use. In fact, there are a number of *in vitro* techniques available to evaluate the nutritive value of forage at relatively low cost (Giger-Reverdin et al., 2002). *In vitro* gas production can be used as a rapid evaluation tool to assess nutritional quality of forages (Abdalla et al., 2012; Wanapat et al., 2012). Recently, there has been an increasing interest to the use of natural ingredients, such as plants, and their extracts containing the active ingredient have been attracted (Bodas et al., 2012; Goel & Makkar, 2012; Jayanegara, Leiber & Kreuzer, 2012). Pasture plants may act in many ways against mammals, including as toxins or digestibility reducers, modifying their food quality and their foraging behavior (Bodas et al., 2012; Iason, 2005). They may in some cases be beneficial to mammals (Athanasiadou & Kyriazakis, 2004), including with decreased methane production (Soliva et al., 2008). Methane production from ruminants contributes to total global methane production, which is an important contributor to global warming (Hartung & Monteny, 2000). Enteric methane contributes 30-40 percent of total methane production from agricultural sources. Many attempts, such as concentrate supplementation (Lovett et al., 2005), use of probiotics and prebiotics (Mwenya et al., 2004; Takahashi et al., 2005; Valero et al., 2014), lipid supplementation (Ungerfeld, Rust, Burnett, Yokoyama & Wang, 2005), and addition of plants and their extracts (Goel, Makkar & Becker, 2008; Patra, Kamra & Agarwal, 2006; Śliwiński, Soliva, Machmüller & Kreuzer, 2002) have been made to decrease enteric methane production. The aim of this study was to investigate chemical composition and kinetics of fermentation from eight common grass species found in the pastures of the province of Kermanshah, Iran, and to determine the relationships between chemical composition with *in vitro* rumen methane production due to the identification of plants with anti-methanogenic properties.

Material and methods

Grass samples and chemical composition

Grass samples, *Chamaemelum nobile*, *Trachyspermum copticum*, *Descurania Sophia*, *Urtica dioica*, *Falcaria vulgaris*, *Tragopogon collinus, Gundelia tournefortii* and *Taraxacum officinale* were collected at mid-vegetative stage from natural pastures in Kermanshah province. Grass samples were ground using a lab mill to pass through a 1-mm sieve. Standard methods as described in AOAC (1998) were used to determine organic matter (OM), crude protein (CP) and ether extract (EE). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest, Robertson & Lewis (1991).

In vitro gas production

The method used for gas production measurements followed Theodorou, Williams, Dhanoa, McAllan & France (1994). About 125 mg of each sample was weighed into tubes kept at approximately 39°C. Each sample was incubated in three replicates. Fifteen mL of buffered rumen fluid (in the proportion of 20% rumen fluid + 80% buffer solution) was anaerobically dispensed in each tube. All tubes were crimped and placed in a shaking incubator. The pressure of gas produced in each tube was recorded by a pressure transducer (Manometer Digital testo 512) at 2, 4, 6, 8, 12, 24, 48, 72 and 96h after the start of the incubation. To estimate the kinetics of gas production, data on cumulative gas volume produced were fitted using the generalized Mitscherlich model proposed by France et al. (1993):

$$G = A\left(1 - e^{-c(t-L)-b\left(\sqrt{t}-\sqrt{L}\right)}\right)$$

where G (mL) denotes cumulative gas production at time t; A (mL) is asymptotic gas production; c (h^{-1}) and b($h^{-1/2}$) are rate constants; L (h) is lag time. The half-life ($t_{1/2}$, h) of the degradable fraction of each substrate was calculated as the time taken for gas accumulation to reach 50% of its asymptotic value.

Measurement of methane production

For measuring methane production, after 24 hours of incubation, 2 mL of NaOH (10 M) were introduced to estimate methane production following the method by Fievez, Babayemi & Demeyer (2005). Data were obtained on volume of gas and methane (CH_4) produced. Net methane and gas productions were calculated by the differences of the methane and gas in the test syringe and the corresponding blank; the methane concentration was determined as Jayanegara, Togtokhbayar, Makkar & Becker (2009):

$$\text{methane concentration} = \frac{\text{Net methane production}}{\text{Net gas production}} \text{x}100$$

Methane production reduction potential (MRP) was calculated by taking net methane rates for control (hay) as 100% Jayanegara et al. (2009):

$$\text{MRP} = \frac{\%\text{Net methane in control} - \%\text{Net methane in control}}{\%\text{Net methane in control}} \text{x}100$$

Statistical analysis

Data on gas production parameters and dry matter (DM) digestibility from different eight grasses were subjected to one way analysis of variance using SAS (2004) and significance between individual means was identified using Duncan multiple-range test (Duncan, 1955).

Results and discussion

Crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of grasses are shown in Table 1. The CP content (g kg^{-1} DM) varied widely, from 52.4 to 269.2, with the highest for *Descurania Sophia* and lowest for *Taraxacum* *officinal*e. Gas production kinetic parameters of different species are presented in Table 2 and graphically illustrated in Figure 1. There were significant differences between potential gas production (*A*), constant rates (*d*) and (*c*) of pasture species ($p < 0.01$).

Table 1. Chemical composition (g kg^{-1} DM) of pasture grasses from western Iran.

species	OM	CP	EE	NDF	ADF
G1	954	131.4	27.1	325.8	251.0
G2	730	167.4	93.1	264.8	183.0
G3	835	269.2	16.0	235.2	221.3
G4	818	119.5	14.5	495.2	390.6
G5	870	151.9	24.6	311.6	283.7
G6	888	145.4	38.6	336.6	280.7
G7	832	203.4	12.9	324.2	237.8
G8	683	52.4	8.60	625.4	480.7

G1: Chamaemelum nobile, G2: Trachyspermum copticum, G3: Descurania Sophia, G4: Urtica dioica, G5: Falcaria vulgaris,G6: Tragopogon collinus, G7: Gundelia tournefortii, G8: Taraxacum officinale. OM: organic matter; CP: crude protein; EE: ether extract; ADF: acid detergent fiber; NDF: neutral detergent fiber.

Table 2. Coefficients of curves fitted to the average gas production pattern of each species together with the volume of total gas produced during 96h of incubation (mL 125 mg^{-1} dry matter).

species	*A*(mL gas 125 mg^{-1} DM)	b($h^{-1/2}$)	*c*(h^{-1})	Half life(h)
G1	51.86^{a}	0.087^{a}	0.040^{a}	9.43^{g}
G2	43.12^{d}	0.045^{f}	0.025^{cd}	10.48^{d}
G3	36.48^{e}	0.031^{d}	0.023^{d}	12.50^{c}
G4	27.86^{g}	0.028^{e}	0.020^{d}	14.01^{b}
G5	46.05^{c}	0.041^{c}	0.040^{a}	9.43^{g}
G6	52.25^{a}	0.065^{b}	0.036^{b}	9.70^{f}
G7	48.09^{b}	0.045^{c}	0.28^{c}	10.27^{e}
G8	32.23^{f}	0.033^{d}	0.023^{d}	14.81^{a}
P-value	**	**	**	**
SEM	1.78	0.197	0.014	0.004

G1: Chamaemelum nobile, G2: Trachyspermum copticum, G3: Descurania Sophia, G4: Urtica dioica,G5: Falcaria vulgaris,G6: Tragopogon collinus, G7: Gundelia tournefortii, G8: Taraxacum officinale. A: asymptotic gas production (mL 125 mg^{-1} DM); c: constant rates (per h); Half-life: time (h) required to reach 0.50 total gas production (per h). SEM: standard error of mean. ** $p < 0.01$.

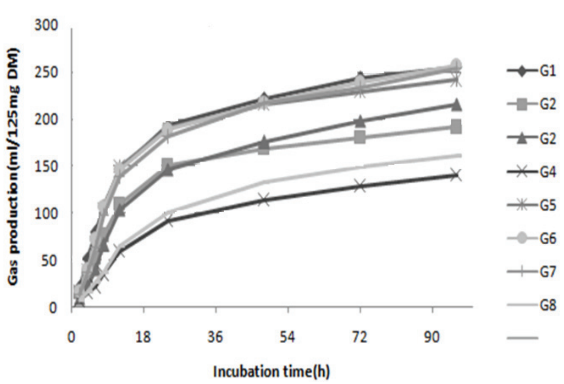

Figure 1. Pattern of in vitro gas production of grasses (G1: Chamaemelum nobile, G2: Trachyspermum copticum, G3: Descurania Sophia, G4: Urtica dioica, G5: Falcaria vulgaris, G6:Tragopogon Collinus, G7: Gundelia tournefortii, G8: Taraxacum officinale) in buffered rumen fluid.

There was a positive correlation ($r = 0.62$; $p < 0.05$) between CP content of grasses and total gas production (*A*) at 96h incubation (Table3). Crude protein concentrations below the threshold of 70 g CP kg^{-1} DM restrict microbial activity due to a lack of nitrogen (Hariadi & Santoso, 2010). However, protein levels above this threshold, as seen in this study, are considered to enhance microbial multiplication in the rumen, thus improving fermentation (Njidda & Nasiru, 2010). Negative correlation was observed between ADF content and potential gas production (*A*) ($r = -0.60$; $p < 0.05$). With increasing NDF and ADF, potential gas production (*A*) and the rate constant (*b, c*) decreased (Table 3). This result is in agreement with the report by Heidary & Kafilzadeh (2012) who found that NDF and ADF content had negative correlation with fermentation parameters (A and b). It may be concluded that although increase in cell wall content showed a decrease in total gas production, the eight grasses behaved differently in the rate at which they produced gas.

Table 3. Correlation (r) between chemical composition (g kg^{-1} DM) and *in vitro* gas production characteristics.

	Gas production constants		
Predictor	*A*(mL gas 125 mg^{-1} DM)	b($h^{-1/2}$)	*c*(h^{-1})
CP	0.621*	0.246*	-0.031
NDF	-0.207	-0.307	-0.032
ADF	-0.602*	-0.415*	-0.120

CP: crude protein; ADF: acid detergent fiber; NDF: neutral detergent fiber; *A*: asymptotic gas production (mL 125mg^{-1} DM); c, d: fractional rate of gas production. *$p < 0.05$.

Methane production reduction potential of grasses by *in vitro* rumen fermentation

Table 4 shows methane concentration and methane production reduction potential (MRP) which was calculated by assuming net methane concentration for control hay (oat) as 100%. The MRP of *Chamaemelum nobile* was more than 25 percent and showed plants that reduce methane production more than 20 percent methane in comparison with control actually have ingredients to reduce methane (García-González, López, Fernández & González, 2006). The MRP of negative for *Trachyspermum copticum* meant that the methane concentration for this sample was higher than that of the control hay. Amongst the nutrients, NDF and ADF were positively correlated with methane concentration, ($r = 0.75$ and 0.77, respectively; $p < 0.01$), while no relationship existed for CP and EE. Generally, the type of carbohydrate present in the forage is thought to dictate methane production via shifts in the ruminal microbial population (Johnson & Johnson, 1995; Meale, Chaves, Baah & McAllister, 2012). High soluble carbohydrate content is suggested to promote the production of propionate in the rumen, lower ruminal pH and inhibit methanogen growth, thereby reducing methane production per unit of fermented OM

(Kessel & Russell, 1996; Meale et al., 2012), as shown that negative relationships occurred between NDF or ADF and the MRP ($p < 0.05$) (Table 5). As seen in this study, dietary components such as NDF and ADF also contributed to explain total variation in methane production. Higher NDF increases methane production by shifting short chain fatty acid proportion towards acetate which produces more hydrogen (Carulla, Kreuzer, Machmüller & Hess, 2005; Puchala, Min, Goetsch & Sahlu, 2005; Tavendale et al., 2005). EE had no relationship with all methane expressions when it was correlated individually. The role of lipids in decreasing methane is well illustrated, albeit at levels higher than present in most of the samples in this study (Beauchemin, Kreuzer, O'Mara & McAllister, 2008). In addition, most of the samples in current study were forage *in natura* with low levels of EE. The nature of lipid and its form (i.e., bound or free) are also determining factors in their effect on methane production (Beauchemin et al., 2008; Zeitz et al., 2013).

Table 4. Methane concentration and Methane production reduction potential (MRP) from pasture grasses from western Iran.

species	Methane (%)	MRP (%)
G1	18.26^c	26.96^a
G2	30.36^a	-22.50^e
G3	22.24^c	8.040^c
G4	21.40^d	14.42^b
G5	24.53^b	1.89^d
G6	24.13^b	3.47^d
G7	20.93^d	16.30^b
G8	20.96^d	16.17^b
P-value	**	**
SEM	0.671	2.89

G1: Chamaemelum nobile, G2: Trachyspermum copticum, G3: Descurania Sophia, G4: Urtica dioica, G5: Falcaria vulgaris, G6: Tragopogon collinus, G7: Gundelia tournefortii, G8: Taraxacum officinale. MRP: Methane production reduction potential. SEM: standard error of mean. ** $p < 0.01$.

Table 5. Correlation (r) between chemical composition (g kg^{-1} DM) and methane production parameters.

Predictor	Methane (%)	MRP (%)
CP	0.164^{ns}	-0.217^{ns}
EE	0.259^{ns}	-0.347^{ns}
NDF	0.752**	-0.679*
ADF	0.774**	-0.694*

CP: crude protein; EE: ether extract; ADF: acid detergent fiber; NDF: neutral detergent fiber; MRP: Methane production reduction potential. SEM: standard error of mean. ** $p < 0.01$ * $p < 0.05$.

Conclusion

From the results of this study among the nutrients, NDF and ADF were positively correlated with methane concentration. *Chamaemelum nobile* had a better nutritive quality when compared to the others and also reduced methane production. However, any decision with regard to its use as a possible crop in agricultural system or in breeding programs, their yield and some other agronomic factors, requires further study.

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