

Determination of Potential Nutritive Value of Pepper (*Capsicum annuum* L.) Leaves Using *in vitro* Gas Production Technique

Mahmut Kaplan

Department of Field Crops, Faculty of Seyrani Agriculture, University of Erciyes, Kayseri, Turkey

Abstract: The aim of this study was to determine the potential nutritive value of pepper (*Capsicum annuum* L.) leaves from five pepper species using chemical composition and *in vitro* gas production technique. Chemical composition including Dry Matter (DM) Crude Protein (CP), Neutral Detergent Fiber (NDF) Acid Detergent Fiber (ADF) and ash were determined. Gas productions were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times and their kinetics were estimated using the exponential equation $Y = A(1 - \exp^{-ct})$. The Organic Matter Digestibility (OMD) and Metabolisable Energy (ME) of pepper (*Capsicum annuum* L.) leaves from five pepper species were estimated. The DM, CP, NDF, ADF contents of leaves from five pepper species ranged from 17.44-19.69, 13.88-18.76, 26.57-32.72 and 15.64-20.98%, respectively. The pepper leaves obtained from Maras species had significantly ($p < 0.001$) higher CP contents than those of Hatay, 46, Balo and Sena species. Although, the NDF content of pepper leaves obtained from Hatay species was significantly ($p < 0.001$) higher than those of Maras, 46, Balo and Sena species the ADF content of pepper leaves obtained from Sena was significantly ($p < 0.001$) higher than those of pepper leaves obtained from Hatay, Maras, 46, Balo and Sena. The gas production rate, potential gas production, ME and OMD of pepper leaves from Sena was significantly ($p < 0.001$) higher than those of pepper leaves from Hatay, Maras, 46 and Balo. The species had a significant effect on the chemical composition *in vitro* gas production, ME and OMD of pepper leaves obtained after harvest. Despite there are considerable variations in chemical composition of pepper leaves obtained after harvest among pepper species, pepper leaves had high CP and was quite digestible. It appears that pepper leaves obtained after harvest will provide biomass of an acceptable quality for ruminant animals during critical periods when forage shortage occurs.

Key words: Pepper leaves, *Capsicum annuum*, potential nutritive value, forage, *in vitro* gas production, Turkey

INTRODUCTION

Forage has important role for ruminant animals due to providing the energy, protein and minerals. In the South of Turkey, after the harvest pepper (*Capsicum annuum* L.) the considerable amount of biomass becomes available for grazing ruminant animals. However, there is limited information on the nutritive value of this biomass consumed by ruminant animal. Recently the *in vitro* gas production technique and chemical composition of the uninvestigated forage have been widely used to evaluate the potential nutritive value of ruminant feedstuffs (Mesgaran and Mohammadabadi, 2010; Mesgaran *et al.*, 2010; Chaji *et al.*, 2010; Kamalak, 2010). Therefore, the aim of this study was to determine the potential nutritive value of leaves from five pepper species using chemical composition and *in vitro* gas production technique.

MATERIALS AND METHODS

Hay samples: In the present study, leaves from five pepper (*Capsicum annuum* L.) species were hand harvested after harvest. The collected pepper leave samples were shade-dried and milled to pass through a 1 mm sieve for subsequent analysis.

Chemical analysis: Dry matter content was determined by drying the samples at 105°C overnight and the ash content was determined by igniting the samples in a muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). The CP was calculated as $N \times 6.25$. The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) of pepper leave sample were analyzed with the ANKOM fiber analyzer using reagents described by Van Soest (1963) and Van Soest and Robertson (1985), respectively. All chemical analyses were carried out in triplicate.

In vitro gas production: Pepper leave samples milled through a 1 mm sieve were incubated *in vitro* rumen fluid in glass bottles following the procedures of Theodorou *et al.* (1994). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Approximately 0.200 g dry weight of pepper leave sample was incubated in a glass bottle of 100 mL containing 50 mL of McDougall's buffer/rumen mixture in triplicate. Gas production was determined at 3, 6, 12, 24, 48, 72 and 96 h after incubation using pressure transducer. Total gas production was corrected for blank gas production. The *in vitro* gas production kinetics was estimated using the exponential model:

$$y = A(1 - \exp^{-ct})$$

Where:

y = Gas produced at time t
A = The potential gas production
c = The gas production rate (%)
t = Incubation time (h)

ME (MJ kg⁻¹ DM) contents of pepper leave sample were estimated using equation suggested by Menke *et al.* (1979) as follows:

ME (MJ kg⁻¹ DM) = 2.20 + 0.136 GP + 0.057 CP where GP = 24 h net gas production (mL/200 mg), CP = Crude Protein.

Organic matter digestibility (%) of samples was estimated using equation suggested by Menke *et al.* (1979) as follows:

$$\text{OMD (\%)} = 14.88 + 0.889\text{GP} + 0.45\text{CP} + 0.0651 \text{XA}$$

where, XA is ash content (%).

Statistical analysis: One-way analysis of variance was carried out to determine the effects of species on the chemical composition and *in vitro* gas production kinetics of pepper leaves using the General Linear Model. Significant differences between individual means at $p < 0.05$ were identified using the Duncan multiple range tests. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS AND DISCUSSION

The chemical composition of pepper leaves from five species obtained after harvest is shown in Table 1. The species had a significant effect on the chemical composition of pepper leaves. The CP content of pepper leaves ranged from 13.88-18.76%. The pepper leaves

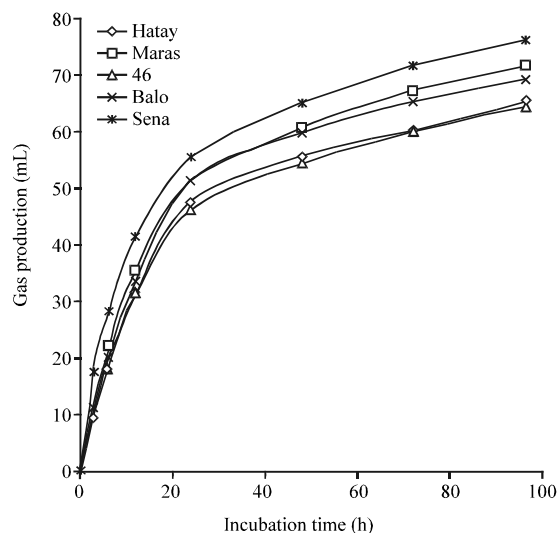


Fig. 1: *In vitro* gas production of pepper leaves from different pepper leaves obtained after harvest

Table 1: The chemical composition of pepper leaves from five species

Composition	Species					SEM	Sig.
	Hatay	Maras	46	Balo	Sena		
DM	17.44 ^b	19.69 ^a	17.67 ^b	18.43 ^{ab}	17.57 ^b	0.869	***
CP	15.14 ^d	18.76 ^a	13.88 ^e	17.06 ^c	17.87 ^b	0.047	***
NDF	32.72 ^a	31.12 ^c	32.46 ^b	28.46 ^d	26.57 ^e	0.057	***
ADF	19.68 ^e	18.50 ^d	19.93 ^b	15.64 ^f	20.98 ^a	0.108	***
Ash	19.95 ^a	15.90 ^c	15.24 ^d	17.02 ^b	14.70 ^e	0.217	***
Yield	2900.09 ^a	920.15 ^c	1460.73 ^b	1390.54 ^b	910.65 ^c	90.074	***

^{a-e}Row means with common superscripts do not differ ($p > 0.05$); SEM-Standard Error Mean; Sig.: Significance Level; DM: Dry Matter, CP: Crude Protein, NDF: Neutral Detergent Fiber, ADF: Acid Detergent Fiber, CT: Condensed Tannin

obtained from Maras species had significantly ($p < 0.001$) higher CP contents than those of Hatay, 46, Balo and Sena species. Although, the NDF content of pepper leaves obtained from Hatay species was significantly ($p < 0.001$) higher than those of Maras, 46, Balo and Sena species the ADF content of pepper leaves obtained from Sena was significantly ($p < 0.001$) higher than those of pepper leaves obtained from Hatay, Maras, 46, Balo and Sena. The yield per hectare was 910.65-2900.09 kg ha⁻¹.

The *in vitro* gas production of pepper leaves from different species obtained after harvest is shown in Fig. 1. At 3, 6, 12 and 24 h incubation times the *in vitro* gas production of pepper leaves from Sena was significantly ($p < 0.001$) higher than those of pepper leaves from Hatay, Maras, Balo species whereas the *in vitro* gas production of pepper leaves from Maras and Sena at 48, 72 and 92 h was significantly higher than those of pepper leaves from Hatay, 46 and Balo.

The fermentation kinetics, metabolisable energy and organic matter digestibility of pepper leaves from different species obtained after harvest is shown in Table 2. The species had a significant ($p < 0.001$) effect on the

Table 2: The fermentation kinetics, metabolisable energy and organic matter digestibility of pepper leaves from different species obtained after harvest

Parameters	Species					SEM	Sig.
	Hatay	Maras	46	Balo	Sena		
c	0.057 ^b	0.059 ^b	0.058 ^b	0.062 ^b	0.072 ^a	0.004	***
A	62.550 ^c	68.510 ^{ab}	61.440 ^a	64.70 ^{bc}	71.40 ^a	2.148	***
ME	65.210 ^c	69.840 ^b	63.160 ^c	69.15 ^b	73.06 ^a	1.339	***
OMD	9.520 ^a	10.220 ^b	9.260 ^a	10.13 ^b	10.74 ^c	0.205	***

^{a-c}Row means with common superscripts do not differ ($p>0.05$), SEM: Standard Error Mean; Sig.: Significance level; a = The gas production from the quickly soluble fraction (mL), b = The gas production from the slowly degradable fraction (mL), c = The gas production rate constant for the slowly degradable fraction (b) ME: Metabolisable Energy, MJ kg⁻¹ DM, OMD: Organic Matter Digestibility (%) *** $p<0.001$

Table 3: Correlation coefficient (r) relationship of chemical composition with gas production kinetics and some estimated parameters of pepper leaves

Parameters	ADF	NDF	Ash	CP	Yield
c	0.2170 ^{NS}	-0.752 ^{***}	-0.458 ^{NS}	0.384 ^{NS}	-0.451 ^{NS}
A	0.1760 ^{NS}	-0.661 ^{***}	-0.455 ^{NS}	0.753 ^{***}	-0.587 [*]
ME	-0.030 ^{NS}	-0.821 ^{***}	-0.382 ^{NS}	0.835 ^{***}	-0.581 [*]
OMD	-0.015 ^{NS}	-0.828 [*]	0.4110 ^{NS}	0.822 ^{***}	-0.599 [*]

ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber, CP: Crude Protein, A = The potential gas production (mL), c = The gas production rate constant, ME: Metabolisable Energy, MJ kg⁻¹ DM, OMD: Organic Matter Digestibility (%), NS: Non Significant, * $p<0.05$, *** $p<0.001$

fermentation kinetics, metabolisable energy and organic matter digestibility of pepper leaves. The gas production rate of pepper leaves from Sena was significantly ($p<0.001$) higher than those of pepper leaves from Hatay, Maras, 46 and Balo. The potential gas production (A) of pepper leaves from Sena was significantly ($p<0.001$) higher than those of pepper leaves from Hatay, Maras, 46 and Balo. The ME and OMD values of pepper leaves from Sena was significantly ($p<0.001$) higher than those of pepper leaves from Hatay, Maras, 46 and Balo.

Correlation coefficient (r) relationship of chemical composition with gas production kinetics and some estimated parameters of pepper leaves are shown in Table 3. The c, A, ME and OMD were negatively correlated with NDF content of pepper leaves whereas A, ME and OMD were positively correlated with CP content. This result is in agreement with findings of Kamalak (2006).

The reason why the gas production, ME and OMD values of pepper leaves from Sena was significantly higher than those of Hatay, Maras, 46 and Balo species is possible due to high CP and low NDF contents of Sena. Blummel and Orskov (1993) indicated that the gas production is associated with volatile fatty acid production as a result of fermentation of carbohydrate in feedstuffs. The increase in cell wall content of pepper leaves might have resulted in a decrease in fermentable carbohydrate. As a result of decrease in fermentable

fraction of pepper leaves of Hatay, Maras, 46 and Balo species, the production of VFA acid decreased which means that less gas production occurred from carbohydrate fermentation. The estimated ME and OMD values of pepper leaves were decreased with decreasing gas production due to increase in less fermentable cell wall contents of pepper leaves since the ME and OMD were estimated from chemical compositions and gas production at 24 h incubation time.

CONCLUSION

The species had a significant effect on the chemical composition *in vitro* gas production, ME and OMD of pepper leaves obtained after harvest. Despite there are considerable variations in chemical composition of pepper leaves obtained after harvest among pepper species, pepper leaves had high CP and was quite digestible. It appears that pepper leaves obtained after harvest will provide biomass of an acceptable quality for ruminant animals during critical periods when forage shortage occurs.

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