Journal of Animal and Veterinary Advances 10 (15): 2028-2031, 2011
ISSN: 1680-5593

Determination of Potential Nutritive Value of Sainfoin (*Onobrychis sativa*) Hays Harvested at Flowering Stage

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 sainfoin (*Onobrychis viciaefolia*) hays harvested at flowering stage from Afsin, Tekir, Pazarcik, Baskonus and Kapicam using chemical composition and *in vitro* gas production technique. Chemical composition including Crude Protein (CP), Neutral Detergent Fiber (NDF) Acid Detergent Fiber (ADF), ash Condensed Tannin (CT), Organic Matter Digestibility (OMD) and Metabolisable Energy (ME) were calculated. Gas productions were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times and their kinetics were described using the equation $Y = a+b(1-exp^{-ct})$. The CP, NDF, ADF and CT content of sainfoin hay ranged from 11.39-17.70, 43.31-47.64, 35.61-43.30 and 4.19-9.95%, respectively. The sainfoin obtained from Afsin and Baskonus had significantly ($p<0.001$) higher crude protein contents than those of sainfoin hays from Tekir, Pazarcik and Kapicam. The NDF content of sainfoin hay obtained from Pazarcik and Kapicam was significantly ($p<0.001$) higher than those of sainfoin hays from Afsin and Tekir the ADF content of sainfoin hay from Kapicam was significantly ($p<0.001$) higher than those of sainfoin hays from Afsin, Tekir, Pazarcik and Baskonus. The CT content of sainfoin hay from Pazarcik was significantly ($p<0.001$) higher than those of sainfoin hays from Afsin, Tekir, Baskonus and Kapicam. The gas production from slowly soluble fraction of sainfoin hay from Afsin was significantly ($p<0.001$) higher than those of sainfoin hays from Tekir and Kapicam. The ME and OMD ranged from 6.86-7.79 MJ kg^{-1} DM, 47.82-53.17%, respectively. The ME and OMD content of sainfoin hay from Afsin was significantly ($p<0.001$) higher than those of sainfoin hays from Tekir and Kapicam. As a conclusion, the growing site had a significant effect on the chemical composition *in vitro* gas production, ME and OMD of sainfoin hay harvested at flowering stage. There are considerable variations in chemical composition of sainfoin hay harvested at flowering stages among growing sites. The ME and OMD values of sainfoin hay are negatively correlated with cell wall and CT contents but positively correlated with CP content.

Key words: Potential nutritive value, forage, *in vitro* gas production, tannin, sainfoin hay, Turkey

INTRODUCTION

Native pasture is a very important in providing forages for ruminant animals in Mediterranean areas. The legume plants in native pasture are one of the very important components of pasture since they are rich in protein. Sainfoin is one of the perennial legume plants in native pasture in Turkey. Sainfoin is preferable to alfalfa due to due to its forage quality (Carleton *et al*., 1968). Sainfoin is also preferable to alfalfa due to low incidence of bloat (Majak *et al*., 1995). It was also suggested that tannin containing legume hays such as sainfoin (*Onobrychis viciaefolia*) offer an alternative to anthelmintic chemicals to control gastrointestinal nematodes (Brunet *et al*., 2008). Although, sainfoin (*Onobrychis viciaefolia*) is one of the important perennial legume plants in Mediterranean wild pastures due to excellent nutritional and palatability properties there is limited research on the wild sainfoin hays obtained from different growing sites.

Recently some researches have used the *in vitro* gas production technique to evaluate the fermentation kinetics 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 Sainfoin (*Onobrychis viciaefolia*) hays harvested at flowering stage from five different growing sites using chemical composition and *in vitro* gas production technique.

MATERIALS AND METHODS

Hay samples: In the present study, sainfoin (*Onobrychis viciaefolia*) hays from five different growing sites (Afsin, Tekir, Pazarcik, Baskonus and Kapicam) were hand harvested from native pasture at flowering stage which is common practice in Turkey for hay production. The hay 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 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×6.25. The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) of sainfoin hay sample were analyzed with the ANKOM fiber analyzer using reagents described by Van Soest (1963) and Van Soest and Robertson (1985), respectively. Condensed tannin contents were determined by butanol-HCl method as described by Makkar *et al.* (1995). All chemical analyses were carried out in duplicate.

***In vitro* gas production:** Sainfoin hay 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 samples 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:

$$y = a+b\ (1-\exp^{-ct})$$

Where:

y = Gas produced at time t
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)
t = Incubation time (h)

ME (MJ kg^{-1} DM) content of samples was calculated using equation of 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 calculated using equation of Menke *et al.* (1979) as follows:

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

Where, XA is ash content (%).

RESULTS AND DISCUSSION

The chemical composition of sainfoin hay from different growing sites harvested at flowering stage is shown in Table 1. The growing site had a significant effect on the chemical composition of sainfoin hay harvested at flowering stage. The CP content of sainfoin hay ranged from 11.39-17.70%. The sainfoin obtained from Afsin and Baskonus had significantly ($p<0.001$) higher crude protein contents than those of sainfoin hays from Tekir, Pazarcik and Kapicam. Although, the NDF content of sainfoin hay obtained from Pazarcik and Kapicam was significantly ($p<0.001$) higher than those of sainfoin hays from Afsin and Tekir the ADF content of sainfoin hay from Kapicam was significantly ($p<0.001$) higher than those of sainfoin hays from Afsin, Tekir, Pazarcik and Baskonus. The CT content of sainfoin hay from Pazarcik was significantly ($p<0.001$) higher than those of sainfoin hays from Afsin, Tekir, Baskonus and Kapicam. The chemical composition of sainfoin hay obtained in the current study was consisted with findings of Bal *et al.* (2006). It was reported that the growing site had an effect on the condensed tannin contents of shrub and tree leaves (Ozturk *et al.*, 2006). Tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein such as digestive enzymes (Kumar and Singh, 1984) and microbial enzyme (Singleton, 1981; Lohan *et al.*, 1983; Barry *et al.*, 1984; Makkar *et al.*, 1989). CT content of forages in the range of 60-100 g kg^{-1} DM depresses intake and growth of animals (Barry *et al.*, 1984). CT contents of sainfoin hay obtained from Afsin and Tekir were lower than this range whereas CT contents of sainfoin hays obtained from Pazarcik, Baskonus and Kapicam were within this range. Therefore, supplementation of Polyethylene Glycol (PEG) or other alkali can be recommended to reduce the possible detrimental effect of condensed tannin on feed intake.

Table 1: The chemical composition of sainfoin hay from different growing sites harvested at flowering stage

	Sites						
Composition	Afsin	Tekir	Pazarcik	Baskonus	Kapicam	SEM	Sig.
DM	94.67^{c}	95.10^{c}	95.73^{a}	95.58^{a}	95.40^{ab}	0.155	***
CP	17.39^{a}	15.23^{b}	15.16^{b}	17.70^{a}	11.39^{c}	0.169	***
NDF	43.31^{b}	43.56^{b}	47.64^{a}	44.61^{ab}	47.59^{a}	0.945	***
ADF	35.62^{c}	35.61^{c}	38.28^{b}	34.34^{c}	43.30^{a}	0.521	***
Ash	5.97^{bc}	7.30^{a}	5.21^{d}	6.40^{b}	5.61^{c}	0.137	***
CT	4.19^{c}	5.76^{bc}	9.95^{a}	6.59^{b}	6.82^{b}	0.500	***

$^{a-c}$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

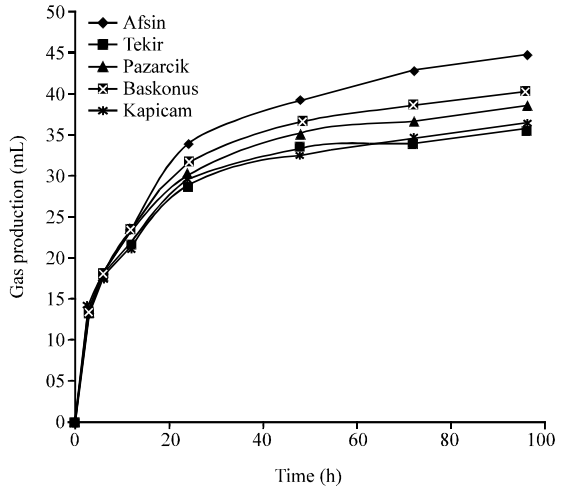

Fig. 1: *In vitro* gas production of sainfoin hay from different growing sites harvested at flowering stage

The *in vitro* gas production of sainfoin hay from different growing sites harvested at flowering stage is shown in Fig. 1. After 24 h incubation times the *in vitro* gas production of sainfoin hay from Afsin was significantly ($p<0.001$) higher than those of sainfoin hays from Tekir, Pazarcik, Baskonus and Kapicam.

The fermentation kinetics, metabolisable energy and organic matter digestibility of sainfoin hay from different growing sites harvested at flowering stage is shown in Table 2. The growing site had a significant effect on the fermentation kinetics, metabolisable energy and organic matter digestibility of sainfoin hay harvested at flowering stage. The gas production rate of sainfoin obtained from Tekir and Kapicam was significantly ($p<0.001$) higher than that of sainfoin hay from Afsin. The gas production from quickly soluble fraction of sainfoin from Afsin was significantly ($p<0.001$) higher than those of sainfoin hay from Tekir, Pazarcik and Baskonus. The gas production from slowly soluble fraction of sainfoin hay from Afsin was significantly ($p<0.001$) higher than those of sainfoin hays from Tekir and Kapicam. The ME and OMD content of sainfoin hay from Afsin was significantly ($p<0.001$) higher than those of sainfoin hays from Tekir and Kapicam.

Generally the ME, OMD, gas production and their kinetics except for c and a obtained in the current study were considerably lower than those reported by Bal *et al.* (2006). The differences between these two studies are possibly due to differences in buffered solution used in these two experiments.

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

Table 2: The fermentation kinetics, metabolisable energy and organic matter digestibility of sainfoin hay from different growing sites harvested at flowering stage

	Sites						
Parameters	Afsin	Tekir	Pazarcik	Baskonus	Kapicam	SEM	Sig.
c	0.066^{b}	0.095^{a}	0.086^{ab}	0.088^{ab}	0.093^{a}	0.007	***
a	3.260^{a}	2.090^{b}	2.410^{b}	2.460^{b}	2.650^{ab}	0.196	***
b	39.450^{a}	31.830^{b}	34.130^{ab}	35.850^{ab}	31.570^{b}	1.718	***
ME	7.790^{a}	6.990^{b}	7.160^{ab}	7.510^{ab}	6.860^{b}	0.222	***
OMD	53.170^{a}	47.820^{bc}	48.860^{abc}	51.410^{ab}	46.600^{c}	1.452	***

$^{a-c}$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

Parameters	ADF	NDF	Ash	CP	CT
c	0.327^{NS}	0.354^{NS}	0.282^{NS}	-0.555*	0.574*
a	0.047^{NS}	-0.159^{NS}	-0.422^{NS}	0.210^{NS}	-0.545*
b	-0.547*	-0.444^{NS}	-0.159^{NS}	0.769***	-0.451^{NS}
ME	-0.661***	-0.523*	-0.027^{NS}	0.853***	-0.422^{NS}
OMD	-0.695***	-0.543*	0.007^{NS}	0.877***	-0.412^{NS}

ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber, CP: Crude Protein, CT: Condensed Tannin; 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 (%), NS: Non-Significant, *$p<0.05$, ***$p<0.001$

The gas production is associated with volatile fatty acid production after fermentation of carbohydrate in feedstuffs (Blummel an Orskov, 1993). The increase in cell wall content of sainfoin hay might have resulted in a decrease in fermentable carbohydrate. As a result of decrease in fermentable fraction of sainfoin hay, the production of VFA acid decreased which means that less gas production occurred from slowly fermentable fraction (b). The estimated ME and OMD values were decreased with decreasing gas production due to increase in less fermentable cell wall contents of sainfoin hay since the ME and OMD were estimated from chemical compositions and gas production at 24 h incubation time.

CONCLUSION

The growing site had a significant effect on the chemical composition, *in vitro* gas production, ME and OMD of sainfoin hay harvested at flowering stage. There are considerable variations in chemical composition of sainfoin hay harvested at flowering stages among growing sites. The ME and OMD values of sainfoin hay are negatively correlated with cell wall and CT contents but positively correlated with CP content.

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