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Nutritive Value of Sainfoin (*Onobrychis viciaefolia*) Harvested at Different Maturity Stages

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Abstract: The nutritive value of sainfoin (*Onobrychis viciaefolia*) hay harvested at vegetative, flowering, late maturity stages was evaluated by chemical composition and *in vitro* gas production techniques. *In vitro* gas production was determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times and their kinetics were described using the equation $p = a+b(1-e^{-ct})$. Maturity had a significant effect on the chemical composition. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) contents increased with increasing maturity whereas Crude Protein (CP), ash and Condensed Tannin (CT) contents decreased. Maturity stage had also a significant effect on *in vitro* gas production and estimated parameters except for gas production rate (c).

Key words: Sainfoin, nutritive value, gas production, metabolizable energy, digestibility

INTRODUCTION

Forages are the major part of diet for ruminant animals and provide energy, proteins and minerals. Several researchers (Ammar *et al*., 1999, Kamalak *et al*., 2005a, b) showed that the maturity stage had a significant effect on the nutritive value of forages. Recently Buxton (1996) indicated that herbage maturity influences forage nutritive value than any other single factor. In addition, Min and Hart (2003) indicated that CT had also significant effect on the nutritive value of forages. Although sainfoin was one of the legume forages used as high quality hay in Turkey, the information on the nutritive value of sainfoin harvested at different growth stages was considerably limited when compared with alfalfa. Accurate prediction of forage quality during the growth cycle would allow targeting of harvest or grazing to desired levels of nutritive composition to meet specific animal requirements (Valente *et al*., 2000).

In addition to chemical composition, *in vitro* gas production technique has been developed to evaluate the nutritive value of forages and to estimate the rate and extent of DM degradation indirectly using gas production (CO_2) during fermentation (Menke *et al*., 1979, 1988).

The aim of this study was to determine the effect of maturity stage on the nutritive value of sainfoin hays in terms of chemical composition and *in vitro* gas production.

MATERIALS AND METHODS

Forage samples: Sainfoin (Varyete, Anatolian) was sown in three replicate plots in 2004 at the seed rate of 30 kg ha^{-1}. Forty kg ha^{-1} N and 80 kg ha^{-1} P_2O_5 fertilizers were used. Each plot was irrigated as required for optimal growth.

Sainfoin hays were obtained at vegetative, flowering and late maturity stages in 2005. Sainfoin plants were hand harvested from at three replicate plots of 10x2 m established in the experimental field. The experimental design was a randomized complete block design with three replications. Samples were shade-dried and representative dry samples (approximately 2.5 kg) from each plot was taken to laboratory and milled in a hammer mill through a 1 mm sieve for subsequent analysis.

Chemical analysis: Dry matter was determined by drying the samples at 105°C overnight and ash by igniting the samples in muffle furnace at 525°C for 8 h. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein was calculated as Nx6.25. The NDF, ADF and ADL contents were also determined (AOAC, 1990). Condensed tannin was determined by butanol-HCl method as described by Makkar *et al*. (1995). All chemical analyses were carried out in triplicate.

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***In vitro* gas production:** Sainfoin hay samples milled through a 1 mm sieve were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke *et al.* (1979). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Digestion medium was prepared mixing 500 mL of distilled water, 0.1 mL micro-mineral solution, 200 mL buffer solution, 200 mL macro-mineral solution and 1 mL Resazurin solution. CO_2 gas was bubbled through the solution until the colour turned pink/purple or for 3 h. Approximately 0.2 g of dry sample was weighed into calibrated glass syringes of 100 mL. The syringes were prewarmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture consisting of 10 mL rumen liquor and 20 mL digestion medium into each syringe followed by incubation in a water bath at 39°C. Triplicates of each sample were used in two separate runs. Readings of gas production were recorded before incubation 0 and after 3, 6, 12, 24, 48, 72 and 96 h of incubation. Total gas values were corrected for blank incubation. Cumulative gas production data were fitted to the model suggested by Orskov and McDonald (1979),

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

Where;

a = The gas production from the immediately soluble fraction (mL)
b = The gas production from the insoluble fraction (mL)
c = The gas production rate constant
a+b = The potential gas production (mL)
t = Incubation time (h)
y = Gas produced at the time t

The fermentation kinetics was estimated using a computer package programme called Fig P (Biosoft, Cambridge, UK).

Metabolizable Energy (ME) (MJ kg^{-1} DM) contents of sainfoin harvested at different maturity stage were calculated using the equation of Menke *et al.* (1979) as follows:

$$ME\ (MJ\ kg^{-1}\ DM) = 2.20+0.136\ GP+0.057\ CP$$

GP: Gas production at 24 h incubation time
CP: Crude protein

Organic matter digestibility (OMD) of sainfoin hay was calculated using the equation of Menke *et al.* (1979) as follows:

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

Where, XA is % ash content

Statistical analysis: Data of chemical composition and *in vitro* gas production were subjected to standard analysis of variance using General Linear Model (GLM) of statistica for windows (Statistica, 1993). Significance between individual means was identified using the Tukey's multiply range test (Pearse and Hartley, 1966). Mean differences were considered significant at $p<0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS AND DISCUSSION

There was considerable variation among chemical compositions of sainfoin hays harvested at different maturity stages (Table 1).

The NDF, ADF and ADL contents of sainfoin were significantly ($p<0.001$) increased with advancing maturity. The NDF, ADF and ADL contents ranged from 46.14 to 55.71%, 33.40 to 40.15% and 7.10 to 11.10%, respectively. These results are in agreement with findings of Long *et al.* (1999) and Ayed *et al.* (2001), Gulsen *et al.* (2004) and Kamalak *et al.* (2005a) who found that cell wall contents (NDF and ADF) increased with increasing maturity. On the other hand CP, CT and ash contents of sainfoin decreased with increasing maturity and ranged from 13.05 to 19.50%, 42.66 to 105.12% and 7.18 to 8.31%, respectively. The CP, CT and ash contents of sainfoin harvested at vegetative stage was significantly ($p<0.001$) higher than those obtained at flowering and late maturity stages. The decline in CP concentration with advancing maturity occurs both because of decrease in CP in leaves and stems and because stems, with their lower protein concentration, make up a larger portion of the herbage in more mature forage (Buxton, 1996). This is in agreement with findings of Kamalak *et al.* (2005a, b). Minson (1990) found that the average decreases in CP concentration with advance in maturity for several forages averaged 1 g kg^{-1} d^{-1}. The CP, NDF and ADL contents of alfalfa hay were comparable with those obtained by Holechek *et al.* (1986). However the CT content of sainfoin obtained in this experiment is not consistent with those reported by Bermingham *et al.* (2001) and Min and Hart (2003). The differences CT contents may be due to differences in growing condition and cultivars.

The CT content of forages in the range of 60-100 g kg^{-1} DM depresses intake and growth of animals (Barry *et al.*, 1984). The CT contents of the leaves of *sainfoin* at stage 1 and 2 fell into this range. Therefore the CT of sainfoin seems to have a detrimental effect on digestion of nutrients in ruminants. Therefore, supplementation of polyethylene glycol (PEG) can not be

Table 1: The chemical composition of sainfoin hay harvested at different maturity stages

	Maturity stages				
Constituents (%)	Vegetative	Flowering	Late maturity	SEM	Sig.
DM	94.39	94.18	94.29	0.241	NS
NDF	46.14[a]	49.27[b]	55.71[c]	0.448	***
ADF	33.40[a]	37.21[b]	40.15[c]	0.626	***
ADL	7.10[a]	8.20[a]	11.10[b]	0.216	***
CP	19.50[c]	14.5[b]	13.05[a]	0.193	***
Ash	8.31[b]	6.88[a]	7.18[a]	0.067	***
CT	10.51[c]	6.96[b]	4.26[a]	0.138	***

Means within the same row with differing superscript are significantly different, p<0.001, SEM: Standard Error Mean, Sig: Significance level, NS: Non-significant, DM: Dry Matter, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ADL: Acid Detergent Lignin, CP: Crude Protein, CT: Condensed Tannin

Table 2: The gas production and estimated parameters of sainfoin hay harvested at different maturity stages

	Maturity stages				
IT	Vegetative	Flowering	Late Maturity	SEM	Sig.
3	21.76[b]	19.10[a]	16.86[a]	0.577	***
6	29.50[b]	26.83[a]	25.66[a]	0.399	***
12	44.83[c]	41.00[b]	38.10[a]	0.399	***
24	62.20[c]	57.90[b]	53.26[a]	0.406	***
48	67.80[c]	64.80[b]	59.80[a]	0.465	***
72	77.26[c]	71.23[b]	66.63[a]	0.371	***
96	81.00[c]	74.33[b]	69.26[a]	0.444	***
Estimated parameters					
c	0.068	0.069	0.069	0.001	NS
a	3.65[c]	3.04[b]	2.51[a]	0.113	***
b	72.99[c]	68.40[b]	63.70[a]	0.352	***
a+b	76.64[c]	71.44[b]	66.21[a]	0.381	***
ME	11.77[c]	10.92[b]	10.18[a]	0.054	***
OMD	73.37[c]	68.82[b]	64.47[a]	0.361	***

Means within the same row with differing superscript are significantly different, p<0.001, SEM: Standard Error Mean, Sig: Significance level, Ns: Non-significant, IT: Incubation Times (h), c: Gas production rate, a: Gas production (mL) from quickly soluble fraction, b: The gas production (mL) from the insoluble fraction, a+b: The potential gas production (mL), ME: Metabolizable Energy (MJ kg^{-1} DM), OMD: Organic Matter Digestibility (%)

recommended to reduce the possible detrimental effect of tannin. On the other hand, low level CT in *Lotus corniculatus* and *Hedysarum coronarium* have been shown to offer advantages for ruminants and have resulted in increased in milk production, wool growth, ovulation rate and lambing percentage, as well as reducing bloat risk and reducing parasite burdens (Min and Hart, 2003).

The data related to *in vitro* gas production and estimated parameters of sainfoin hay harvested at different maturity stages are given in Table 2.

Gas production and estimated parameters except for gas production rate (c) significantly (p<0.001) decreased with increasing maturity of sainfoin. The gas production and estimated parameters except for gas production rate (c) obtained at vegetative stage was significantly higher than those obtained at flowering and late maturity stages. These results are in consistent with findings of Long *et al.* (1999), Gülsen *et al.* (2004), Kamalak *et al.* (2005a, b) and Zinash *et al.* (1996). The OMD of sainfoin obtained in the current experiment was consistent with that reported by Holechek *et al.* (1986).

The reduction in gas production and the estimated parameters may be a result of reduction of microbial activity due to increased level of NDF, ADF and ADL with advancing maturity. Recently significant negative correlation between cell wall contents and gas production or estimated parameters has been reported by Kamalak *et al.* (2005a, b), Ndlovu and Nherera (1997), Larbi *et al.* (1998) and Abdulrazak *et al.* (2000). Generally the digestibility decreased with advancing maturity due to increased lignification (Morrison, 1980) and decreased leaf/stem ratio (Hides *et al.*, 1983). It was reported that early stage of growth, all part of plants are highly digestible, but during stem elongation and flowering there is a more rapid decline in the digestibility of stem than of leaf (Terry and Tilley, 1964). The decrease in CP with advancing maturity may also be another reason why there is a decrease in the *in vitro* gas production and estimated parameters. Crude protein is the one of the limiting factors for microbial growth. Tolera *et al.* (1997) and Larbi *et al.* (1998) reported a significant (p<0.001) positive correlation between CP and estimated parameters.

Maturity stage had a significant effect of nutritive value of sainfoin hay. Nutritive value of sainfoin impaired as it matures. Harvesting of sainfoin at early stages offers more digestible dry matter and metabolizable energy. However the dry matter yield of sainfoin

hay per hectare must be determined to find out the best harvest stage.

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