Determination of Nutritive Value of Wild Mustard, *Sinapsis arvensis* Harvested at Different Maturity Stages Using *In situ* and *In vitro* Measurements

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ABSTRACT : The aim of this study was to determine the effect of maturity stage on the nutritive value of wild mustard straw in terms of chemical composition, *in situ*, *in vitro* dry matter degradability and calculated ME. The nutritive values of wild mustard, *Sinapsis arvensis* hays harvested at three stages were evaluated by chemical composition, *in vitro* gas production and *in situ* dry matter degradation methods. Gas production or dry matter (DM) degradation were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h and their kinetics were described using the equation $p = a+b(1-e^{-ct})$. Maturity had a significant effect on both the chemical composition and degradability of wild mustard. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) ($p<0.001$) increased with increasing maturity whereas the crude protein (CP) ($p<0.001$) decreased. The gas produced after 96 h incubation ranged between 64.7 and 81.5 ml per 0.200 g of dry matter. The gas production (ml) at all incubation times and estimated parameters decreased with increasing maturity of wild mustard. The gas production at all incubation times and estimated parameters (a, b (a+b), metabolizable energy (ME) and organic matter digestibility (OMD)) were negatively correlated with NDF and ADF. The DM disappearance after 96 h incubation ranged between 50.8 and 76.1%. The *in situ* DM disappearance at all incubation times and estimated parameters decreased with increasing maturity of wild mustard. The *in situ* dry matter disappearance at all incubation times and some estimated parameters (c, a, b and effective dry matter degradability (EDMD)) were negatively correlated with NDF and ADF but positively correlated with CP. The nutritive value of wild mustard continually changed as it matured. Wild mustard, harvested at the proper stage of maturity offers considerable potential as a high quality forage for ruminants during the winter feeding period. The present study showed that if higher quality forage is an objective, wild mustard should be harvested at the early flowering stage. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1249-1254)***

Key Words : Wild Mustard, Nutritive Value, Degradability, Gas Production, Metabolizable Energy

INTRODUCTION

Forages are the major part of diet for ruminant animals and provide energy, proteins and minerals. Wild mustard, *Sinapsis arvensis*, is a weed which is grazed by the ruminant animals or collected and dried for winter forage for ruminant animals in most parts of Turkey.

The nutritive value of mustard (*Brassica campestris*) straw has been evaluated by Mishra et al. (2000), Misra et al. (2000) and Vaithiyanathan et al. (2003). However there is no previous report on the nutritive value of wild mustard (*Sinapsis arvensis*) straw. 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).

The *in vitro* gas production and *in situ* nylon bag techniques were widely used to evaluate the nutritive value of forages used in ruminant nutrition (Tolera et al., 1997; Larbi et al., 1998; Evitiyani et al., 2004).

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The aim of this study was to determine the effect of maturity stage on the nutritive value of wild mustard straw in terms of chemical composition, *in situ*, *in vitro* dry matter degradability and calculated metabolisable energy.

MATERIALS AND METHOD

Straw samples

Wild mustard straw samples were obtained at early flowering, mid-flowering, and late maturity stages in 2004. The experimental design was a randomized complete block design with three replications. Wild mustard plants were hand harvested from at least three replicate plots of 5×2 m established in the experimental field. Samples were shade-dried and representative dry samples (approximately 2.5 kg) from each plot were taken to the laboratory and milled in a hammer mill through a 1 mm sieve for subsequent analysis. The sampling area is located at an altitude of 630 m above sea level. The mean annual rainfall and temperature are 857.5 mm and 16.2°C.

Chemical analysis

Dry matter (DM) was determined by drying the samples at 105°C overnight and ash was determined by igniting the dry samples in muffle furnace at 525°C for 8 h. Nitrogen

(N) content was measured by the Kjeldhal method (AOAC, 1990). Crude protein was calculated as N×6.25. Neutral detergent fibre content was determined by the method of Van Soest et al. (1991). Acid detergent fibre content was determined using the method described by Van Soest (1963). All chemical analyses were carried out in triplicate.

***In situ* dry matter degradation**

The nylon bag technique (Orskov and McDonald, 1979) was used to measure the kinetics of DM degradation of wild mustard straw harvested at three different maturity stages. Samples were milled in a hammer mill through a 3 mm sieve. Forage samples were subjected to standard rumen degradability procedures using three fistulated male sheep. The sheep were fed a diet containing wild mustard straw (60%) and concentrate (40%). Throughout the experimental period dacron bags with 40-50 µm pore size containing approximately 5 g forage sample were incubated in each sheep for each of the testing time periods: 3, 6, 12, 24, 48, 72 and 96 h. The bags were removed after incubation in the rumen of sheep and washed in cold running water until the washings ran clear and colourless. Time 0 h samples were not incubated in the rumen but were washed in cold water as above to determine solubility at time 0 h. The bags were oven dried at 60°C for 48 h.

The DM degradation data was fitted to the exponential equation $p = a+b\ (1-e^{-ct})$ (Orskov and McDonald, 1979).

Where

p = the disappearance of nutrient during time t

a = The soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable

b = The proportion of insoluble nutrient which is potentially degradable by micro-organisms

c = The degradation rate of fraction b per hour.

t = incubation time (h)

The effective degradability (P) of samples was calculated using the equation shown below, using a rumen out flow rate (r) of 0.02 h^{-1} which is an average value for animals fed at approximately maintenance level (AFRC, 1992):

$$EDMD\ (\%) = a+((bxc)/(c+r)).$$

EDMD = Effective degradability of dry matter

***In vitro* gas production**

Rumen fluid was obtained from two fistulated sheep fed twice daily with a diet containing wild mustard hay (60%) and concentrate (40%). The concentrate consisted of wheat (74%), sunflower meal (24%), calcium carbonate (0.99%), salt (1%) and vitamin and mineral mixture (0.01%). The forage samples (0.200 g dry weight) were incubated in triplicate in rumen fluid (10 ml) in 100 ml calibrated glass syringes following the procedures of Menke and Steingass (1988). The syringes were prewarmed at 39°C before the injection of 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39°C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Readings of gas production were recorded before incubation (0) and 3, 6, 12, 24, 48, 72 and 96 h after incubation. Total gas values were corrected for blank incubation. Cumulative gas production data were fitted to the model of 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 for the insoluble fraction (b)

t = incubation time (h)

y = gas produced at time 't'

Metabolizable energy (ME) (MJ/kg DM) content of mustard straw was calculated using equation of Menke et al. (1979) as follows:

$$ME\ (MJ/kg\ DM) = 2.20+0.136GP+0.057CP+0.0029CP^2$$

Where

GP = 24 h net gas production (ml/200 mg).

CP = Crude protein

Organic matter digestibility (OMD) (%) of mustard straw was calculated using equation of Menke et al. (1979) as follows:

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

OMD: Organic matter digestibility (%)

XA = Ash content (%)

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to compare chemical composition, *in vitro* gas production, *in situ* dry matter degradation and estimated parameters with species as the main factor using General Linear Model (GLM) of Statistica for Windows (1993). Significance between individual means was identified using the Tukey's multiple 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. A simple correlation analysis was used to establish the relationship between

Table 1. The chemical composition of wild mustard hay harvested at three different stages

Constituents (%)	Stages of harvest			SEM	Sig.
	Early flowering	Mid-flowering	Late maturity		
DM	95.9	96.4	96.5	0.469	NS
CP	13.2^{c}	9.8^{b}	7.7^{a}	0.101	***
NDF	66.5^{a}	70.6^{b}	74.1^{c}	0.350	***
ADF	56.4^{a}	60.8^{b}	65.8^{c}	0.372	***
Ash	7.4^{b}	8.6^{c}	5.6^{a}	0.122	***

Means within the same column with differing superscripts are significantly different.
SEM: Standard error mean. NS: Non-significant. DM: Dry matter. *** $p<0.001$.
CP = Crude protein, NDF: Neutral detergent fiber. ADF = Acid detergent fiber.

Table 2. *In situ* dry matter disappearance and estimated parameters of wild mustard harvested at different stages

IT	Stages of harvest			SEM	Sig.
	Early flowering	Mid-flowering	Late maturity		
3	37.9^{c}	31.6^{b}	21.5^{a}	0.391	***
6	49.4^{c}	39.3^{b}	27.8^{a}	0.378	***
12	57.3^{c}	46.0^{b}	35.4^{a}	0.618	***
24	63.9^{c}	58.2^{b}	40.9^{a}	0.610	***
48	72.8^{c}	60.0^{b}	46.5^{a}	0.480	***
72	78.9^{c}	65.4^{b}	50.5^{a}	0.751	***
96	76.1^{c}	66.5^{b}	50.8^{a}	0.751	***
	Estimated parameters				
c	0.063	0.057	0.054	0.002	NS
a	33.1^{c}	27.6^{a}	17.9^{b}	0.194	***
b	42.6^{c}	37.7^{b}	32.5^{a}	0.646	***
EDMD	65.4^{c}	55.6^{b}	41.7^{a}	0.546	***

Means within the same row wit differing superscripts are significantly different.
*** $p<0.001$. NS: Non-significant.
SEM = Standard error mean. Sig. = Significance level.
a = the soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable.
b = the proportion of insoluble nutrient which is potentially degradable by micrograms.
c = the degradation rate of fraction b per h, EDMD = Effective dry matter degradability.

Table 3. Correlation coefficient (r) of relationship of chemical composition with *in situ* dry mater degradation or estimated parameters

IT	DM	Ash	CP	ADF	NDF
3	-0.363^{NS}	0.704^{x}	0.955^{z}	-0.980^{z}	-0.975^{z}
6	-0.357^{NS}	0.640^{NS}	0.978^{z}	-0.980^{z}	-0.980^{z}
12	-0.386^{NS}	0.590^{NS}	0.983^{z}	-0.976^{z}	-0.982^{z}
24	-0.324^{NS}	0.801^{y}	0.900^{z}	-0.951^{z}	-0.933***
48	-0.376^{NS}	0.622^{NS}	0.981^{z}	-0.985^{z}	-0.983^{z}
72	-0.349^{NS}	0.681^{x}	0.955^{z}	-0.973^{z}	-0.965^{z}
96	-0.338^{NS}	0.708^{x}	0.951^{z}	-0.972^{z}	-0.967^{z}
c	-0.291^{NS}	0.391^{NS}	0.798^{z}	-0.736^{z}	-0.776^{z}
a	-0.364^{NS}	0.724^{x}	0.947^{z}	-0.982^{z}	-0.971^{z}
b	-0.325^{NS}	0.600^{NS}	0.952^{z}	-0.947^{z}	-0.943^{z}
EDM	-0.357^{NS}	0.679^{x}	0.962^{z}	-0.978^{z}	-0.973^{z}

^{x,y,z} $p<0.05$, $p<0.01$ and $p<0.001$ respectively.
NS: Non-significant.
a = the soluble nutrient fraction which is rapidly washed out of the bags and is assumed to be completely degradable.
b = the proportion of insoluble nutrient which is potentially degradable by micrograms.
c = the degradation rate of fraction b per h.
EDMD = Effective dry matter degradability. DM: Dry matter.
CP = Crude protein. NDF: Neutral detergent fiber.
ADF = Acid detergent fiber.

chemical composition and gas production or estimated parameters.

RESULT AND DISCUSSION

Chemical composition

Chemical compositions of wild mustard hay harvested at three different maturity stages are given in Table 1. There were significant differences between chemical compositions of wild mustard hays harvested at different maturity stages.

The crude protein content ranged from 7.7% to 13.2% and decreased with increased maturity. Crude protein content of wild mustard harvested at the early flowering stage was significantly higher than those of other maturity stages. The decline in protein concentration with advancing maturity occurs both because of decrease in protein 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). The average decreases in crude protein concentration with advance in maturity for several forages averaged 1 g kg^{-1} d^{-1} in data reported by Minson (1990).

The ash content of wild mustard hay was also decreased with increased maturity. On the other hand NDF and ADF content of wild mustard were significantly increased with advancing maturity. NDF and ADF contents ranged from 66.5% to 74.1% and 56.4% to 65.8% respectively.

The NDF and ADF contents of wild mustard harvested at early flowering and mid-flowering stages were significantly lower than that of mustard (*Brassica campestris*) straw reported by Mishra et al. (2000) whereas the NDF and ADF content of mustard straw harvested at late maturity stage was similar to that of mustard (*Brassica campestris*) straw reported by Mishra et al. (2000).

The crude protein contents of wild mustard harvested at three stages were considerably higher than that (4.5% of dry matter) of mustard (*Brassica campestris*) straw reported by Mishra et al. (2000).

Table 4. *In vitro* gas production and estimated parameters of wild mustard harvested at different stages

	Stages of harvest			SEM	Sig.
	Early flowering	Mid-flowering	Late maturity		
3	26.7^{c}	23.5^{bc}	22.5^{ab}	0.335	***
6	42.3^{c}	34.0^{b}	31.2^{ab}	0.518	***
12	53.2^{c}	43.2^{b}	41.5^{ab}	0.844	***
24	62.2^{b}	59.8^{b}	49.2^{a}	0.833	***
48	74.3^{c}	62.3^{b}	56.7^{a}	0.666	***
72	78.2^{c}	69.2^{b}	62.2^{a}	1.027	***
96	81.5^{c}	72.5^{b}	64.7^{a}	0.631	***
	Estimated parameters				
c	0.105	0.092	0.096	0.002	NS
a	3.8	3.6	3.8	0.160	NS
b	71.9^{c}	64.5^{b}	56.4^{a}	0.773	***
a+b	75.7^{c}	68.1^{b}	60.3^{a}	0.730	***
ME	11.9^{b}	11.2^{b}	9.5^{a}	0.112	***
OMD	72.4^{b}	69.9^{b}	60.0^{a}	0.738	***

Means within the same row wit differing superscripts are significantly different.
*** p<0.001, NS: Non-significant.
SEM = Standard error mean. Sig = Significance level.
a = the gas production from the immediately soluble fraction (ml).
b = the gas production from the insoluble fraction (ml).
(a+b) = Potential gas production.
c = the gas production rate constant for the insoluble fraction (b).
ME = Metabolizable energy (MJ/kg DM).
OMD: Organic matter digestibility (%).

In situ dry matter degradation

The DM disappearance (%) and estimated parameters of wild mustard hays harvested at three stages are presented in Table 2. At all incubation times there were significant (p<0.001) differences in DM disappearance of wild mustard hays harvested at different maturity stage with hay harvested at early flowering stage having significantly (p<0.001) higher DM disappearance than hay harvested at mid-flower and late maturity stages. Therefore wild mustard hay harvested at early flowering stage had significantly higher a, b and P values than hay harvested at mid-flower and late maturity stages. The maturity had a significant effect on DM disappearance and estimated parameters. Dry matter degradation and estimated parameters (a, b and EDMD) was significantly reduced with increasing maturity. This result is in agreement with findings of Khazaal et al. (1993).

It can be seen from Table 3 the *in situ* dry matter disappearance at all incubation times and estimated parameters (c, a, b and EDMD) were negatively correlated with NDF and ADF but positively correlated with CP.

This result is in agreement with findings of Abdulrazak et al. (2000) who found that NDF and ADF were negatively correlated with dry matter disappearance or estimated parameters (c, a, a+b). Tolera et al. (1997) found that dry matter disappearance at 24 and 48 h incubation were positively correlated with CP content. This result is consistent with findings observed in this experiment.

Table 5. Correlation coefficient (r) of relationship of chemical composition with *in vitro* gas production and estimated parameters

	DM	Ash	CP	ADF	NDF
3	-0.406^{NS}	0.330^{NS}	0.913^{z}	-0.850^{y}	-0.904^{z}
6	-0.360^{NS}	0.377^{NS}	0.979^{z}	-0.920^{z}	-0.940^{z}
12	-0.408^{NS}	0.255^{NS}	0.942^{z}	-0.859^{y}	-0.902^{z}
24	-0.302^{NS}	0.828^{y}	0.851^{y}	-0.909^{z}	-0.891^{y}
48	-0.397^{NS}	0.436^{NS}	0.987^{z}	-0.948^{z}	-0.965^{z}
72	-0.352^{NS}	0.557^{NS}	0.958^{z}	-0.944^{z}	-0.950^{z}
96	-0.385^{NS}	0.573^{NS}	0.982^{z}	-0.970^{z}	-0.979^{z}
c	-0.332^{NS}	0.095^{NS}	0.699^{x}	-0.562^{NS}	-0.626^{NS}
a	-0.134^{NS}	0.407^{NS}	0.006^{NS}	-0.076^{NS}	-0.033^{NS}
b	-0.354^{NS}	0.612^{NS}	0.964^{z}	-0.959^{z}	-0.963^{z}
a+b	-0.350^{NS}	0.599^{NS}	0.968^{z}	-0.960^{z}	-0.966^{z}
ME	-0.342^{NS}	0.757^{y}	0.918^{z}	-0.954^{z}	-0.944^{z}
OMD	-0300^{NS}	0.832^{y}	0.848^{z}	-0.907^{z}	-0.888^{z}

Means within the same row wit differing superscripts are significantly different.
$^{x, y, z}$ p<0.05, p<0.01 and p<0.001 respectively.
NS: Non-significant. SEM = Standard error mean.
Sig = Significance level.
a = the gas production from the immediately soluble fraction (ml).
b = the gas production from the insoluble fraction (ml).
(a+b) = Potential gas production.
c = the gas production rate constant for the insoluble fraction (b).
ME = Metabolizable energy (MJ/kg DM).
OMD: Organic matter digestibility (%).

Gas production and estimated parameters

Gas production data are given in Table 4. Gas produced after 96 h incubation ranged between 64.7 and 81.5 ml per 0.200 g of substrate and decreased (p<0.001) with each increment of maturity.

At all incubation times except 3 h, gas production for wild mustard hays harvested at early flowering stage was significantly (p<0.001) higher than those harvested at mid-flowering and late maturity stages.

These results are in agreement with Zinash et al. (1996) and Lee et al. (2000). They also found a decrease in gas production as the forage growing period was prolonged. The estimated parameters are also given in Table 4. There were significant (p<0.001) differences between wild mustard hays in terms of gas production from the insoluble fraction (b).

The gas production from the insoluble fraction (b) of wild mustard hays harvested at early flowering stage was significantly higher than those harvested at mid-flowering and late maturity stages. The potential gas production (a+b) of wild mustard hays harvested at early flowering stage was also significantly higher than those harvested at mid-flowering and late maturity stages. Metabolizable energy and OMD values of wild mustard hay harvested at early flowering and mid-flowering stages were significantly (p<0.001) higher that harvested at late maturity stage.

It can be clearly seen from Table 1 that NDF and ADF content increased with increased maturity. An increase in

NDF and ADF resulted in the low gas production from the insoluble fraction (b).

It can be seen from Table 5 the gas production at all incubation times and some estimated parameters (a, b (a+b), ME and OMD) were negatively correlated with NDF and ADF. This result is consistent with findings reported by Ndlovu et al. (1997), Larbi et al. (1998), and Abdulrazak et al. (2000).

Estimated parameters (c, b, (a+b) ME and OMD) were also significantly ($p<0.001$) correlated with CP protein which is one of the limiting factors for microbial growth. This result is in agreement with findings of Tolera et al. (1997) and Larbi et al. (1998).

Gas production is associated with volatile fatty acid (VFA) production following fermentation of substrate so the more fermentation of a substrate the greater the gas production, although the fermentation end products do correlate more closely with gas production (Blummel and Orskov, 1993). Differences between total gas productions could be explained by the differences in total VFA production and molar proportion of VFA (Bevunik and Spoelstra, 1992). Doane et al. (1997) found a significant correlation between gas production and VFA production.

At all incubation times gas production and nylon bag technique did allow discrimination between hays harvested at different maturity stages. Therefore both methods can be used to evaluate the nutritive value of wild mustard hays.

It is well established that animal production is impaired as the quality of forage declines with plant development and maturity over the growth period (Castle, 1982; Steen, 1992). Generally as plant mature, CP decreases, fibre increases, while digestibility and energy content declines. These responses are relatively well known, and the obvious means to minimize the effects of maturity is to harvest at optimum maturity.

The reduction in DM degradation obtained *in vitro* and *in situ* techniques may be due to factors such as increased fibre concentration in plant tissues (Wilson et al., 1991), increased lignification during plant development (Morrison, 1980) and decreased leaf:stem ratio (Hides et al., 1983). Terry and Tilley (1964) reported that at early stages of growth, all parts of plants are highly digestible, but that during stem elongation and flowering there is a more rapid decline in the digestibility of stem than of leaf.

IMPLICATIONS

The nutritive value of wild mustard continually declined as it matured. However wild mustard, harvested at the proper stage of maturity offers considerable potential as a high quality forage for ruminant during winter feeding period. Wild mustard should be harvested at early flowering stage since wild mustard forage contained a higher amount of protein but lower fiber content (NDF and ADF) resulting in higher digestibility and metabolizable energy to obtain higher quality forage.

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