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Determination of Nutritive Value of Wild Chicory (*Cichorium intybus*) Forage Harvested at Different Maturity Stage Using *in vitro* and *in situ* Measurements

[1]D. Ozturk, [1]M.A. Bal, [2]A. Erol, [1]M. Sahin, [1]C.O. Ozkan, [2]E. Karakas, [1]M. Ata and [1]P. Karabay
[1]Department of Animal Nutrition, [2]Department of Crop Science, Faculty of Agriculture,
Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey
[3]Bursa Uludag University, Yenisehir Vocational School, Bursa, Turkey

Abstract: The nutritive values of wild chicory, *Cichorium intybus* hays harvested at vegetative, flowering, late maturity stages were evaluated by chemical composition, *in situ* nylon bag and *in vitro* gas production techniques. *In situ* Dry Matter (DM), Crude Protein (CP) disappearance and *in vitro* gas production were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times and their kinetics were described using the equation $y = a + b(1-e^{-ct})$. Maturity had a significant effect on the chemical composition. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Condensed Tannin (CT) contents increased with increasing maturity whereas Crude Protein (CP) and ash contents decreased. Maturity stage had also a significant ($p<0.001$) effect on *in situ* DM, CP disappearance, *in vitro* gas production and estimated parameters. Effective Dry Matter (EDMD), Effective Crude Protein Degradability (ECPD) and most of the estimated parameters of wild chicory harvested at vegetative stage were significantly ($p<0.001$) higher than those obtained at flowering and late maturity stages. Crude protein, EDMD, ECPD and Metobolizable Energy (ME) content wild chicory decreased as maturity advanced. Therefore wild chicory should be harvested at vegetative stage to obtain higher quality forage.

Key words: Wild chicory, nutritive value, maturity stage, degradability, gas production, metabolizable energy

INTRODUCTION

Forages are the major part of diet for ruminant animals and provide energy, proteins and minerals. Although wild chicory, *Cichorium intybus* is one of commonest roadside flowers, literally grows like a weed which is grazed by the ruminant animals or collected and dried for winter forage for ruminant animals in most parts of Turkey there is no previous report on the nutritive value of wild chicory forage. In Turkey feed prices have been continuously increasing but the animal products such as milk and meat are relatively cheap. In this respect there is now significant move to look for new sources for ruminants livestocks from naturally grown plants to reduce the cost of diets (Kamalak *et al*., 2005). Accurate prediction of forages 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 rate and extent of degradation in the rumen are important characteristics of forage digestion in ruminants. Such characteristics can be used to predict the nutritive value more accurately and compare the utility of forages in diets for ruminants (Mehrez and Orskov, 1977). *In situ* nylon bag technique has been used for many years to estimate both the rate and the extent of DM and CP degradation. *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; Menke and Steingass, 1988).

The aim of this study was to determine the effect of maturity stage on the nutritive value of wild chicory, *Cichorium intybus* forage in terms of chemical composition, *in situ* DM, CP degradability and *in vitro* gas production.

MATERIALS AND METHODS

Forage sample: Wild chicory forage samples were obtained at vegetative, flowering and late maturity stages in 2004. Wild chicory 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

Corresponding Author: C.O. Ozkan, Department of Animal Nutrition, Faculty of Agriculture,
Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey
Tel: 00 90 344 2237666/394 Fax: 00 90 344 2230048

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. 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: After drying forages samples were milled through a 1 mm sieve for chemical analysis. DM 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). CP was calculated as N X 6.25. Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF) content were determined by the method of AOAC (1990). Condensed tannin was determined by butanol-HCl method as described by Makkar *et al.* (1995). Mimosa tannin (MT; Hodgson, England) was used as an external standard. All chemical analyses were carried out in triplicate.

***In situ* DM and CP degradation:** The *in situ* DM and CP degradation analysis was carried out according to the procedure described by Mehrez and Orskov (1977). Three gram wild chicory samples dried and milled through 3 mm were weighed into nylon bags and incubated in three rumen fistulated sheep for 3, 6, 12, 24, 48, 72 and 96 h. The sheep were fed twice a day on a 60% alfalfa hay and 40% concentrate diet. On removal the nylon bags were thoroughly washed with running cold water until no further coloured liquid could be extruded, and dried at 60°C for 48 h. DM losses for each incubation time were determined. The DM and CP degradation data were fitted to the equation:

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

where:

y = DM or CP disappearance in rumen at time t
a = The rapidly soluble fraction
b = The potentially degradable fraction
c = The constant rate of degradation of b (percentage per h)

The degradation kinetics was estimated using a computer package programme called Fig P (Biosoft, Cambridge, UK). Effective DM degradability (EDMD) was calculated applying the equation of Orskov and McDonald (1979) :

$$EDMD \text{ or } ECPD = a + (bc/(c+k))$$

Where a, b and c the same as in (1) and k is the rumen outflow rate of 2% per h which is at the maintenance level.

***In vitro* gas production:** Wild chicory samples milled through a 1 mm sieve were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). 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 1mL Resazurin solution. CO_2 gas was bubbled through the solution until the colour turned pink/purple or for 3 h. Two hundred gram dry weight of samples 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 of Orskov and McDonald (1979):

$$y = a + b\,(1 - e^{-ct}) \quad (2)$$

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 wild chicory 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 wild chicory hay was calculated using the equation of Menke *et al.* (1979) as follows:

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

Where XA ash content (%)

Potential Dry Matter Intake (DMI) of wild chicory hay for steers was calculated using the equation of Blümmel and Orskov (1993) as follows:

$$DMI\ (kg\ day^{-1}) = 1.66 + 0.49a + 0.0297b - 4c$$

Statistical analysis: Data of chemical composition, *in situ* DM and CP degradation and *in vitro* gas production were subjected to standard analysis of variance using General Linear Model (GLM) of statistica for windows Release 4.3 (1993). Significance between individual means were 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. The relationships between chemical composition and *in situ* degradation parameters or *in vitro* gas production parameters were obtained by simple correlation analysis.

RESULTS AND DISCUSSION

Chemical composition: There were significant ($p<0.001$) differences between chemical compositions of wild chicory hays harvested at different maturity stages (Table 1).

Crude protein and ash contents of wild chicory decreased with increased maturity. Crude protein and ash contents ranged from 8.56 to 13.73% and 9.58 to 10.70%, respectively. Crude protein and ash contents of wild chicory harvested at vegetative stage was significantly ($p<0.001$) higher than those for 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.* (2005). The average decreases in CP concentration with advance in maturity for several forages averaged 1 g kg^{-1} d^{-1} in data reported by Minson (1990).

A wild chicory hay of 13% CP would meet most protein sheep requirements except for early weaned lambs and ewes suckling twins in the first 6-8 weeks of lactation, which is in agreement with NRC (1985)nutrient concentration standards. Mean CP concentration of wild chicory hay harvested at vegetative stage (13.73%) over this limiting value.

On the other hand NDF, ADF and CT contents of wild chicory were significantly ($p<0.001$) increased with advancing maturity. NDF, ADF and CT contents ranged from 42.03 to 47.00%, 32.11 to 40.57% and 0.63 to 0.81%, 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.* (2005) who found that cell wall contents (NDF and ADF) increased with increased maturity but CP decreased with increased maturity.

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 the leaves of *Cichorium intybus* at three cutting stages were lower than this range. The CT of *Cichorium intybus* does not seem to have a detrimental effect on digestion of nutrients in ruminants. Therefore, supplementation of Polyethylene Glycol (PEG) can not be 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 *et al.*, 2003).

Table 1: The effect of maturity stage on chemical composition of wild chicory

Constituents	Maturity stage				
(%)	Vegetative	Flowering	Late maturity	SEM	Sig
DM	89.21	89.87	90.27	0.529	NS
CP	13.73^{a}	11.23^{b}	8.56^{c}	0.280	***
NDF	42.03^{a}	44.09^{b}	47.00^{c}	0.313	***
ADF	32.11^{a}	35.37^{b}	40.57^{c}	0.544	***
Ash	10.70^{a}	10.05^{b}	9.58^{c}	0.140	***
CT	0.63^{a}	0.73^{b}	0.81^{c}	0.002	***

Means within the same row with various superscripts are significant, DM = Dry Matter, CP = Crude Protein, NDF = Neutral Detergent Fibre, ADF = Acid Detergent Fibre, CT = Condensed tannin content, SEM = Standard Error Mean, NS = Non-significant, Sig = Significance level, ***$p<0.001$

Table 2: The effect of maturity stage on *in situ* fermentation kinetics of wild chicory when incubated in the rumen and rumen fluid

Parameters DM	Maturity stage				
	Vegetative	Flowering	Late maturity	SEM	Sig
c_{dm}	0.026	0.028	0.032	0.002	NS
a_{dm}	35.02^{a}	30.87^{b}	25.19^{c}	0.249	***
b_{dm}	43.13^{a}	38.24^{b}	39.80^{b}	0.919	***
EDMD	60.26^{a}	53.46^{b}	48.70^{c}	0.304	***
CP					
c_{cp}	0.043^{a}	0.031^{b}	0.031^{b}	0.001	***
a_{cp}	41.53^{a}	36.87^{b}	31.48^{c}	0.748	***
b_{cp}	34.97^{a}	31.45^{b}	26.68^{c}	0.596	***
ECPD	65.47^{a}	56.12^{b}	47.79^{c}	0.549	***

Means within the same row with various superscript are significantly different, DM: Dry Matter c_{dm} = rate of dry matter disappearance, a_{dm} = the rapidly soluble dry matter fraction, b_{dm} = the potentially degradable dry matter fraction, EDMD: Effective Dry Matter Degradability calculated at outflow rate of 0.02 h^{-1}, CP: Crude Protein, c_{cp} = rate of crude protein disappearance, a_{cp} = the rapidly soluble crude protein fraction, b_{cp} = the potentially degradable crude protein fraction, ECPD: Effective Crude Protein Degradability calculated at outflow rate of 0.02 h^{-1}

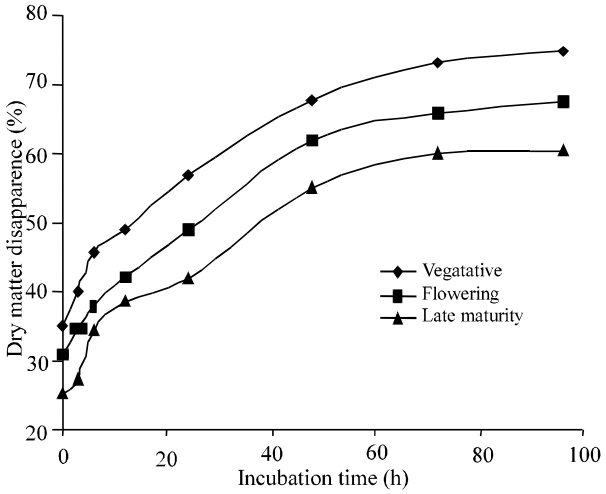

Fig. 1: The effect of maturity stage on *in situ* dry matter disappearance of wild chicory when incubated in the rumen at different incubation time

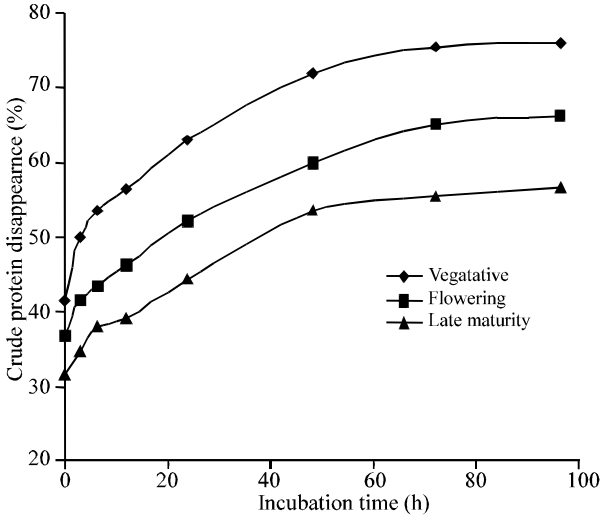

Fig. 2: The effect of maturity stage on *in situ* crude protein disappearance of wild chicory when incubated in the rumen at different incubation time

***In situ* DM and CP degradation:** The maturity stage had a significant ($p<0.001$) effect on DM and CP disappearance. DM and CP disappearance significantly ($p<0.001$) decreased with increasing maturity. DM and CP disappearance of wild chicory harvested at vegetative stage were significantly ($p<0.001$) higher than the other two stages (Fig. 1 and 2). This result is in agreement with findings of Gulsen *et al.* (2004) who found that DM disappearance of *Prangos uechritzii* reduced with advancing maturity. Long *et al.* (1999) also found that at 48 h incubation time *in situ* DM disappearance of grass and forbs decreased with increasing maturity. This result is also in agreement with findings of Kamalak *et al.* (2005) who found that DM disappearance of *Gundelia tournefortii* reduced with advancing maturity.

The estimated parameters were significantly ($p<0.001$) reduced with increasing maturity (Table 2). This result is in agreement with findings of Khazaal *et al.* (1993), Ayed *et al.* (2001), Gulsen *et al.* (2004) and Kamalak *et al.* (2005). It can be seen from Table 1 that cell wall contents (NDF and ADF) increased with increased maturity. An increase in cell wall ,contents resulted in low a, b and EDMD. There were significant ($p<0.001$) negative correlations between these parameters. This result is consistent with finding of Ayed *et al.* (2001) and Kamalak *et al.* (2005) who found that estimated parameters (a, b and EDMD) were negatively correlated with cell wall contents.

The decrease in CP degradability was usually associated with the change toward a higher proportion of nitrogen bound to the NDF as maturity increases (Sanderson and Wedin, 1989; Hoffman *et al.*, 1993). The increase in cell wall resistance to microbial attack and breakdown may also decrease CP degradation (Sanderson and Wedin, 1989).

Gas production and estimated parameters: The maturity had a significant ($p<0.001$) effect on the gas production. Gas production significantly ($p<0.001$) reduced with increasing maturity (Fig. 3). These results are in consistent with findings of Zinash *et al.* (1996) and Lee *et al.* (2000). They also found a decrease in gas production as the forage growing period was prolonged.

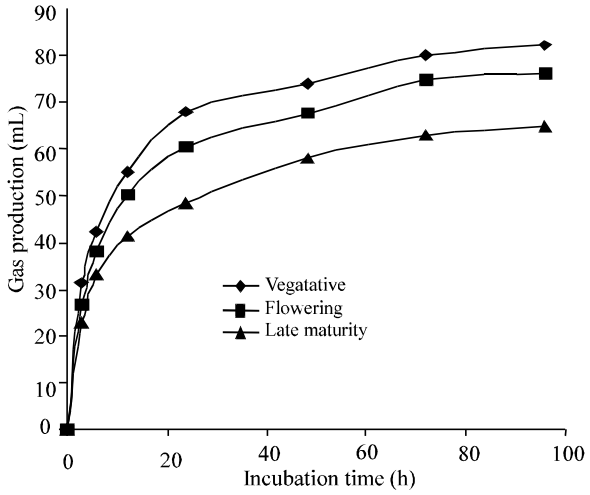

Fig. 3: The effect of maturity stage on *in vitro* gas production of wild chicory when incubated with rumen fluid at different incubation times

Table 3: The effect of maturity stage on *in vitro* fermentation kinetics of wild chicory when incubated in the rumen and rumen fluid

	Maturity stage				
Parameters gas	Vegetative	Flowering	Late maturity	SEM	Sig.
c_{gas}	0.113	0.111	0.095	0.019	NS
a_{gas}	4.03[a]	2.88[b]	2.43[b]	0.589	***
b_{gas}	73.38[a]	69.25[b]	58.24[c]	0.584	***
$(a+b)_{gas}$	77.41[a]	71.94[b]	60.07[c]	0.626	***
ME	11.52[a]	10.47[b]	8.84[c]	0.091	***
OMD	82.21[a]	74.23[b]	62.48[c]	0.664	***
DMI	5.36[a]	4.68[b]	4.20[c]	0.080	***

Means within the same row with various superscript are significantly different, c_{gas} = gas production rate, a_{gas} = gas production from rapidly soluble fraction (mL), b_{gas} =gas production from insoluble fraction, $(a+b)_{gas}$ = potential gas production (mL), ME: Metabolizable Energy content (MJ kg^{-1} dry matter), OMD: Organic Matter Digestibility (%), DMI: Dry Matter Intake (kg day^{-1})

Long *et al.* (1999) also found that at 24 h incubation time gas production of grass and forbs decreased with increasing maturity.

The estimated parameters (a, b, (a+b), ME OMD and DMI) of wild chicory hay harvested at vegetative stage were significantly ($p<0.001$) higher than those obtained at flowering and late maturity (Table 3).

Long *et al.* (1999), Gülsen *et al.* (2004) and Kamalak *et al.* (2005) found that a decrease in ME energy content estimated by *in vitro* gas production with advancing maturity. The reduction in the estimated parameters may be a result of reduction of microbial activity due to increased level of NDF and ADF with advancing maturity.

There were significant ($p<0.001$) negative correlations between cell wall contents (NDF and ADF). This result is consistent with findings of Ndlovu and Nherera (1997), Larbi *et al.* (1998) and Abdulrazak *et al.* (2000) and Kamalak *et al.* (2005).

The decrease in CP with advancing maturity may 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. There is a significant ($p<0.001$) positive correlation between CP and estimated parameters. This result is in agreement with findings of Tolera *et al.* (1997) and Larbi *et al.* (1998).

Condensed tannin content was negatively ($p<0.001$) correlated with *in situ* DM, CP degradation and *in vitro* gas production parameters. Condensed tannins are phenolic compounds and act within animal's digestive tract by binding to the substrate to be digested (usually proteins, carbohydrate, lipids), inhibiting digestive enzyme or exerting anti-microbial effects (Scalbert, 1991). Therefore condensed tannin might have contributed to the reduction in *in situ* DM, CP degradation and *in vitro* gas production parameters obtained in this experiment. However moderate level of tannins may prevent bloat and increase the bypass proteins for digestion in the small intestine (Salunkhe, 1990) and improve the utilization of the essential amino acids (Waghorn *et al.*, 1987). CT contents of forages in the range of 6-10% of DM depress intake and growth of animals (Barry *et al.*, 1984). The CT contents of wild chicory harvested at three stages were lower than these values.

As plant mature, most of nutritional characteristics are changed. Decreased digestibility leads to low ME content, decreased DM and CP contents provide fewer nutrients to the animals and low intake potential may cause suboptimal feed intake. The use of forages for ruminant nutrition is essentially limited by it low digestibility and voluntary intake. The major factor affecting the voluntary food intake of ruminants is the cell wall content and digestibility of forages (Buxton, 1996). 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 ang Tilley, 1964).

Maturity at harvest had a large impact on chemical composition, *in situ* dry matter, crude protein degradability, *in vitro* gas production, dry matter intake and digestibility of wild chicory forage. Crude protein, effective dry matter and crude protein degradability and metobolizable energy content wild chicory decreased as maturity advanced. Our results indicate that it is inappropriate to assume fixed degradation characteristics for a forage without considering factors such as stage of maturity. Wild chicory should be harvested at vegetative stage to obtain higher quality forage.

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