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Original article

Potential nutritive value of *Trigonella spicata* hay harvested at different maturity stages

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Abstract: The aim of the current experiment was to determine the effects of harvesting maturity on the chemical composition, dry matter digestibility (DMD), dry matter intake (DMI) and relative feed value (RFV), gas production, methane production, metabolisable energy (ME) and organic matter digestibility (OMD) of *Trigonella spicate* hay. Harvesting maturity had a significant (P<0.001) effect on chemical composition, DMD, DMI and RFV, gas production, methane production, ME and OMD of *Trigonella spicate* hay. Crude protein (CP), ether extract (EE), DMD, DMI and RFV, gas production, methane production, ME and OMD of *Trigonella spicate* hay decreased with increasing maturity whereas the cell wall contents (NDF and ADF) increased with increasing maturity. In conclusion, maturity had a significant effect on the nutritive value of *Trigonella spicate* hay. It seems to reasonable that *Trigonella spicate* hay will provide more nutrient to ruminant animals when harvested or grazed at preflowering and flowering stages.

Key words: *Trigonella spicata* hay, maturity, chemical composition, organic matter digestibility, metabolisable energy and methane production

Introduction

Native pasture has an important role in Mediterranean areas in terms of providing energy, protein and minerals for ruminant animals. Native pasture also provide fiber to ruminant animals for chewing and rumination. The legume plants in native pasture are one of the very important components of pasture since they are rich in protein when compared with other plant species (1). *Trigonella spicata* is one of the annual legume plants in native pasture in Turkey.

Small ruminant animals such as sheep and goat consume *Trigonella spicata* plants distributed in native pasture. Although the chemical composition, nutritive value, organic matter digestibility and metabolisable energy of many types of plant species obtained at different maturity stages were well established (1, 2, 3, 4) there is limited information about the chemical composition, organic matter digestibility and metabolisable energy of *Trigonella spicata* plant obtained at different maturity stages. 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 (5).

Although the relative feed value of hays can be estimated from chemical composition (6) recently the potential nutritive value, OMD and ME of previously uninvestigated plants have been estimated from chemical composition and *in vitro* gas production technique (1, 2, 3, 4).

Therefore, the aim of the current experiment was to determine the effects of maturity stage on the chemical composition, DMD, DMI and RFV, GP, methane production, ME and OMD of *Trigonella spicata* hay.

Material and Methods

Harvesting of Trigonella spicata plants

Trigonella spicata plants were hand harvested at three maturity stages [preflowering (19.04.2015), flowering (09.05.2015) and seeding stages (01.06.2015)] from at least three replicate plots of 10 m X 2 m established in completely randomized block design in the experimental field. Samples were shade dried and representative dry samples from each plot was taken to laboratory and milled in a hammer mill through a 1 mm sieve for subsequent analysis.

Determination of chemical composition and relative feed value of Trigonella spicata hays

All chemical analyses were carried out in triplicate in the laboratory of Department of Animal Science, Faculty of Agriculture, Kahramanmaras Sutcu Imam University, Turkey. Dry matter (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 (7). Crude protein was calculated as N X 6.25. Neutral detergent fiber (NDF) was determined by the method of Van Soest and Wine (8) and ADF were determined by the method of Van Soest (9). Relative feed value (RFV) was calculated from the estimates of dry matter digestibility (DMD, %) and dry matter intake (DMI, % of BW) (6).

$$DMD\ (\%) = 88.9 - (0.779 \times ADF)$$
$$DMI = 120 / NDF$$
$$RFV = (DMD \times DMI) / 1.29$$

Determination of in vitro gas, methane, metabolisable energy and organic matter digestibility of Trigonella spicata hays.

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. (10). Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60 %) and concentrate (40%). Approximately 0.200 gram dry weight of samples was weighed in triplicate into calibrated glass syringes of 100 mL. 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. Gas production was recorded at 24 h after incubation and corrected for blank incubation. Organic matter digestibility and ME of *Trigonella spicata* hay were estimated using equation suggested by Menke and Steingass (11).

$$ME\ (MJ\ kg^{-1}\ DM) = 1.68 + 0.1418GP + 0.73CP + 0.217EE - 0.028CA$$

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

GP: Gas production at 24 h incubation, CP: Crude protein (%), EE: Ether extract (%), CA: Crude ash (%).

Methane gas content of total gas produced at 24 h fermentation was measured using an infrared methane

Table 1. Legume, grass and legume-grass mixture quality standards

Quality standard [a]	CP, % of DM	ADF, % of DM	NDF, % of DM	RFV [b]
Prime	>19	<31	<40	>151
1	17–19	31–40	40–46	151–125
2	14–16	36–40	47–53	124–103
3	11–13	41–42	54–60	102–87
4	8–10	43–45	61–65	86–75
5	<8	>45	>65	<75

[a] Standard assigned by Hay Market Task Force of American Forage and Grassland Council

[b] Relative feed value (RFV)- Reference hay of 100 RFV contains 41 % ADF and 53 % NDF

DM – Dry matter (%), CP – Crude protein(%), NDF – Neutral detergent fiber (%), ADF – Acid detergent fiber (%).

analyzer (Sensor Europe GmbH, Erkrath, Germany) (12). After measuring gas produced at 24 h incubation, gas samples was transferred into inlet of the infrared methane analyzer using the plastics syringe. The infrared methane analyzer displays methane as percent of total gas. Methane production (mL) was calculated as follows.

Methane production (mL) = Total gas production (mL) X Percentage of methane (%)

Statistical analysis

One-way analysis of variance (ANOVA) was carried out to determine the effect of maturity stage on the chemical composition, DMD, DMI and RFV, gas production, methane production, ME and OMD of *Trigonella spicata* hay. Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at $P<0.05$.

Results and Discussion

The effect of maturity stage on the chemical composition of *Trigonella spicata* hay was given in Table 2. Maturity had a significant effect on the chemical compotion of *Trigonella spicata* hay. The DM, NDF and ADF content of *Trigonella spicata* hay increased with increasing maturity whereas CP and EE contents decreased increased with increasing maturity. These results are in consistent with findings of Kamalak et al. (1), Kamalak and Canbolat (2), and Kaplan et al. (3, 4) who found the similar trends in *Trifolium angustifolium, Trigonella kotschi, Sanguisorba minor and Onobrychis caput-galli* hays harvested at different maturity stages.

CP contents of *Trigonella spicata* hay ranged from 7.74 to 17.45 %. NDF and ADF ranged from 32.81 to 51.85 %, and 26.85 to 39.60 % respectively. On the other hand EE contents ranged from 1.11 to 2.65 %. Daily reduction in CP was estimated based on the difference between CP of hay obtained at preflowering and seeding stages, divided by the time (days) required to reach from flowering to seeding stage. In the current study the reduction in CP content of *Trigonella kotschi* hay was approximately 2.43 g kg^{-1} day^{-1}. The reduction obtained in the current experiment was consistent with findings of Kamalak et al. (1) and Kaplan et al. (3) who reported that the average decline in crude protein concentration with advancing maturity for *Trigonella kotschi* and *Sanguisorba minor* hay was 2.34 and 2.54 g kg^{-1} day^{-1} respectively. On the other hand the reduction obtained in the current experiment was considerably higher than that reported by Kamalak and Canbolat (2) who reported by average decline in crude protein concentration with advancing maturity for *Trifollium angustifolium* hay was 0.84 g kg^{-1} day^{-1}. The differences in reduction of CP of plant with advancing maturity is possible associate with differences in plants and climatic conditions where plants growth (3). It was suggested that ewes require 7-9 % CP for the maintenance and 10-12 % CP for the lactation (13). As can be seen from Table 2, it is likely that the hay obtained at preflowering and flowering stages will meet the reuirement of sheep for maintenance and lactation whereas the hay obtained at seeding stage will meet the requirement of sheep for maintenance.

Daily increases in NDF and ADF were estimated based on the difference between NDF or ADF of hay obtained at preflowering and seeding stages, divided by the time (days) required to reach from flowering to seeding stage. In the current study the daily increases in NDF and ADF contents of *Trigonella kotschi* hay were approximately 4.76 and 3.18 g kg^{-1} day^{-1} respectively.

The daily increases in NDF obtained in the current experiment was higher than that reported by Kaplan et al. (3) who reported that the avarege daily increase in NDF content with advancing maturity for *Sanguisorba minor* hay was 3.32 g kg^{-1} day^{-1}. On the other hand the daily increases in ADF obtained in the current experiment was lower than that reported by Kaplan et al. (3) who reported that the daily increases in ADF content with advancing maturity for *Sanguisorba minor* hay was 3.41 g kg^{-1} day^{-1}. Decrease in CP and increase in NDF or ADF of *Trigonella spicata* hay are possibly associated with decrease in leaves:stem ratio with advancing maturity. Stavarache et al (14) showed that leaves:stem ratio decreased from 0.59 to 0.27. It is well known that the stem is poor in crude protein whereas it is very rich in NDF and ADF contents when compared with leaves. Therefore it is inevitable that the

NDF and ADF contents will increse with advancing maturity.

On the other hand, Stavarache et al (14) showed that CP content of leaves of alfalfa also decrease with advancing maturity. Therefore, the combination effect of decrease of CP in leaves and increase of stem content at the expense of leaves of hay with advancing maturity resulted in decrease in CP content and increase in NDF or ADF contents of hay.

The effect of maturity stage on the DMD, DMI and RFV of *Trigonella spicata* hay was given in Table 3. Maturity had a significant effect on DMD, DMI and RFV of *Trigonella spicata* hay. The DMD, DMI and RFV of *Trigonella spicata* hay decreased with increasing maturity. The RFV of *Trigonella spicata* hay ranged from 104.66 to 192.81. As can be seen from quality standards (Table 1) the RFV of *Trigonella spicata* hay harvested before flowering was prime whereas RFV of *Trigonella spicata* hay harvested at flowering and late maturity were 1 and 2. This results in agreement with finding of Canbolat et al (15) who showed that RFV of alfalfa hay decreased with advancing maturity. The reduction in DMD, DMI and RFV of *Trigonella spicata* hay is associated with increase in NDF and ADF contents with advancing maturity.

The effects of harvest stage on the gas production, methane production, metabolisable energy and organic matter digestibility of *Trigonella spicata* hay were given in Table 4. Maturity had a significant effect on the gas production, methane production, metabolisable energy and organic matter digestibility of *Trigonella spicata* hay. The metabolisable energy and organic matter digestibility of *Trigonella spicata* hay decreased with increasing maturity. These results are in consistent with findings of Kamalak et al. (1), Kamalak and Canbolat (2) and Kaplan et al. (3, 4) who found the similar trends in ME and OMD values of *Trifolium angustifolium, Trigonella kotschi,*

Table 2. The effect of harvest stage on the chemical composition of *Trigonella spicata* hay

	Harvest stage				
Parameters	Preflowering	Flowering	Seeding	SEM	Sig
DM	21.46^{c}	25.23^{b}	35.58^{a}	0.558	***
CA	10.62	9.91	10.75	0.523	NS
CP	17.45^{a}	14.57^{b}	7.74^{c}	0.577	***
NDF	32.81^{c}	37.00^{b}	51.85^{a}	0.629	***
ADF	26.85^{c}	29.05^{b}	39.60^{a}	0.600	***
EE	2.65^{a}	1.74^{b}	1.11^{b}	0.242	***

*$^{a\,b\,c}$ Row means with common superscripts do not differ (P<0.05); S.E.M. – standard error mean; Sig. – significance level; DM – Dry matter (%), CA: crude ash (%), CP – Crude protein (%), NDF – Neutral detergent fiber (%), ADF – Acid detergent fiber (%), CT – Condensed tannin (%) , NS: Non-significant, ***P<0.001*

Table 3. The effect of harvest stage on the chemical composition of *Trigonella spicata* hay

	Harvest stage				
Parameters	Preflowering	Flowering	Seeding	SEM	Sig
DMD	67.98^{a}	66.26^{b}	58.05^{c}	0.469	***
DMI	3.66^{a}	3.24^{b}	2.31^{c}	0.062	***
RFV	192.81^{a}	166.66^{b}	104.66^{c}	3.176	***

*$^{a\,b\,c}$ Row means with common superscripts do not differ (P<0.05); S.E.M. – standard error mean; Sig. – significance level; DMD: Dry matter digestibility (%), DMI : Dry matter intake (% of body weight), RFV: Relative feed value, ***P<0.001*

Table 4. The effect of harvest stage on the gas production, methane production, metabolisable energy and organic matter digestibility of *Trigonella spicata* hay

	Harvest stage				
Parameters	Preflowering	Flowering	Seeding	SEM	Sig
Gas	48.01[a]	44.53[b]	32.43[c]	0.853	***
CH_4(ml)	8.37[a]	7.86[a]	5.61[b]	0.230	***
CH_4(%)	17.44	17.66	17.32	0.422	NS
ME	10.03[a]	9.61[b]	6.78[c]	0.048	***
OMD	72.31[a]	67.46[b]	54.18	0.588	***

*[a b c] Row means with common superscripts do not differ (P<0.05); S.E.M. – standard error mean; Sig. – significance level; CH_4 – Methane emission (ml or %), ME: Metabolisable energy (MJ kg^{-1} DM), OMD: Organic matter digestibility (%), NS: Non-significant, ***P<0.001*

Sanguisorba minor and *Onobrychis caput-galli* hays harvested at different maturity stages. The reductions in gas production, ME and OMD are reflection of declining fermentable substrate content of hay samples with advancing maturity. As mentioned before, cell wall contents (NDF and ADF) increased at the expense of crude protein with advancing maturity. It is well know that cell wall contents are more indigestible fractions of hay. Increase in cell wall contents and reduction in CP of *Trigonella spicata* resulted in decrease in gas production, ME and OMD with advancing maturity.

Although the methane production (ml) decreased with advancing maturity of *Trigonella spicata*, the percentage (%) of methane production was not changed with advancing maturity.

Conclusion

In conclusion, maturity had a significant effect on the nutritive value of *Trigonella spicata* hay. It seems to reasonable that *Trigonella spicata* hay will provide more nutrient to ruminant animals when harvested or grazed at prefloweing and flowering stages.

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