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RESEARCH ARTICLE

Effect of Maturity Stages on Potential Nutritive Value, Methane Production and Condensed Tannin Content of *Sanguisorba minor* Hay

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Summary

The current trial was conducted to study the effect of maturity on the potential nutritive value, methane production and condensed tannin of *Sanguisorba minor* hay. *Sanguisorba minor* hay harvested at three different maturity stages (pre- flowering, flowering and seeding stages). *Sanguisorba minor* hay was shade dried and analyzed for chemical composition. Gas and methane productions of *Sanguisorba minor* hay were determined at 24 h incubation time. Maturity had a significant effect (P<0.05) on the chemical composition, gas production, methane production metabolisable energy (ME) and organic matter digestibility (OMD). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) of *Sanguisorba minor* hay increased with advancing maturity whereas crude protein (CP) and ash content decreased. The CP contents of *Sanguisorba minor* hay varied between 6.7 and 20.7%. The NDF and ADF contents of *Sanguisorba minor* hay ranged from 36.2 to 54.5 and 17.4 to 36.2 % respectively. The condensed tannin content of *Sanguisorba minor* hay varied with maturity between 0.4 and 1.6 % and decreased (P<0.05) with increasing maturity. The gas and methane production at 24 h incubation ranged from 32.2 to 43.5 ml and 4.6 to 6.5 ml respectively and decreased (P<0.05) after flowering. The ME and OMD of *Sanguisorba minor* ranged from 7.0 and 9.3 MJ/kg DM and 46.9 to 63.2% respectively and decreased (P<0.05) with each increment of the maturity. In conclusion, maturity had a significant effect on the nutritive value of *Sanguisorba minor* hay. The nutritive value of *Sanguisorba minor* decreased with increased maturity. It can be suggested that *Sanguisorba minor* should be grazed or harvested at pre-flowering and flowering stage since these stage provides hay with high ME and CP for ruminant.

Keywords: *Sanguisorba minor hay, Nutritive value, Condensed tannin, In vitro gas production, Methane production*

Olgunlaşma Döneminin Çayır Düğmesi Otunun Potansiyel Besin Değerine, Metan Üretimine ve Kondense Tanen İçeriğine Etkisi

Özet

Yürütülen bu çalışmanın amacı, olgunlaşma döneminin çayır düğmesi otunun potansiyel besleme değerine, metan üretimine ve kondense tanen içeriğine olan etkisini araştırmaktır. Çayır düğmesi otu üç faklı olgunlaşma döneminde (çiçeklenme öncesi, çiçeklenme ve tohum bağlama) hasat edilip, gölgede kurutularak kimyasal kompozyonu için analizler yapılmıştır. Yirmi dört saatlik ünkibasyon sonunda çayır düğmesi otunun gaz ve metan üretimi belirlenmiştir. Olgunlaşma dönemi, çayır düğmesi otunun kompozisyonuna, gaz üretimine, metan üretimine, metabolik enerji ve organik madde sindirim derecesine önemli derecede (P<0.05) etki etmiştir. Olgunlaşma dönemin ilerlemesiyle nötral deterjan fiber ve asit deterjan fiber oranı artarken ham protein ve kül içeriği azalmıştır. Çayır düğmesi otunun ham protein içeriği %6.7 ile 20.7 arasında değişmiştir. Çayır düğmesi otunun nötral deterjan fiber ve asit deterjan fiber içeriği sırasıyla %36.2 ile 54.5 ve %17.4 ile 36.2 arasında değişmiştir. Çayır düğmesi otunun kondense tanen içeriği ise %0.4 ile 1.6 arasında değişmiş olup, olgunlaşma döneminin ilerlemesiyle birlikte azalmıştır. Çayır düğmesi otunun olgunlaşma döneminin ilerlemesiyle birlikte yirmi dört saatlik gaz ve metan üretimi azalmıştır. Yirmi dört saatlik gaz ve metan üretimi sırasıyla 32.2 ile 43.5 ml ve 4.6 ile 6.5 ml arasında değişmiştir. Çayır düğmesi otunun metabolik enerji ve organik madde sindirim derecesi sırasıyla 7.0 ile 9.3 MJ/kg kuru madde ve %46.9 ile 63.2 arasında değişmiştir. Sonuç olarak, olgunlaşma dönemi, çayır düğmesinin besleme değerini önemli derecede etkilemiştir. Besleme değeri olgunlaşma döneminin ilerlemesiyle birlikte düşmüştür. Ham protein ve metabolik enerji içeriği yüksek olmasından dolayı çayır düğmesi otunun çiçeklenme öncesi ve çiçeklenme döneminde otlatılması veya hasat edilmesi önerilebilir.

Anahtar sözcükler: *Çayır düğmesi otu, Besin değeri, Kimyasal kompozisyon, Kondense tanen, İn vitro gaz üretimi, Metan üretimi, Sindirim derecesi*

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INTRODUCTION

There are several factors affecting the nutritive value of forages. The maturity stage at harvesting is one of important factors affecting the nutritive value of forages. The pastures provide important forage for ruminant. Rangeland is commonly grazed goat and sheep to meet some nutrient requirement depending on quality of rageland as well as provide fibre to ruminant for chewing and rumination in Turkey [1]. *Sanguisorba minor* a perennial plant of the family Rosaceous grow up to a length of 50-70 cm and is widely distributed the natural pasture of Turkey providing early grazing forage [2]. Although the nutritive value of many types of forages in the pasture harvested at different maturity is well established [3-6], the information about the nutritive value of *Sanguisorba minor* hay with maturity is scarce. Accurate prediction of nutritive value of forages at different maturity stages allows nutritionist to meet specific animal requirements [7].

Recently *in vitro* gas production technique with chemical composition have been widely used to evaluate the potential nutritive value of previously uninvestigated forages since *in vitro* gas production technique is quick, cheap, less time consuming [3-6]. In addition, *in vitro* gas production technique was used to screen the feedstuffs in terms of their methane reduction potential [8-11]. Methane production during rumen fermentation is one of important contributors to global warming [12].

The current trial was conducted to study the effect of maturity stage on the potential nutritive value, methane production and condensed tannin of *Sanguisorba minor* hay.

MATERIAL and METHODS

The experiment was carried out in University of Kahramanmaras Sutcu Imam, Faculty of Agriculture, and Department of Animal Science. The experimental protocols were approved by the Animal Experimentation Ethics Committee of University of Kahramanmaras Sutcu Imam, Faculty of Agriculture (Protocol No: 2013/03-3).

Sanguisorba minor plants were hand harvested at three maturity stages (pre-flowering (13.03.2013), flowering (15.04.2013) and seeding stages (07.05.2013) from three plots that established in completely randomized block design in 10x2 m plots in the experimental field in 2013 in Kahramanmaras, Turkey. Plant 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.

Dry matter (DM) content of *Sanguisorba minor* hay was analyzed by oven drying at 105°C 24 h. Ash content was determined by igniting the samples of *Sanguisorba minor* hay in muffle furnace at 525°C for 8 h. Nitrogen (N) content of *Sanguisorba minor* hay was measured by the Kjeldahl method [13]. Crude protein of *Sanguisorba minor* hay was calculated as N X 6.25. Neutral detergent fiber (NDF) of *Sanguisorba minor* hay was determined according to Van Soest and Wine [14] and ADF content of *Sanguisorba minor* hay were determined by the method of Van Soest [15]. Condensed tannin was determined by butanol-HCl method as described by Makkar *et al.*[16]. All chemical analyses were carried out in triplicate.

Sanguisorba minor hay samples that were also milled through a 1 mm sieve were incubated *in vitro* rumen fluid in 100 ml calibrated glass syringes following the procedures of Menke *et al*.[17]. Rumen fluid was obtained from three fistulated Awassi sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%) with a free access to water and mineral block. Rumen fluid was collected before morning feeding and filtered through four layers of cheesecloth under flushing with CO_2. The rumen fluid was combined with buffered solution in the ratio of 1:2 respectively. Approximately 0.200 gram air dried samples of *Sanguisorba minor* hay samples was weighed into calibrated glass syringes which were prewarmed at 39°C. Then 30 mL rumen fluid-buffer mixture was transferred into each syringe. The glass syringes containing samples and rumen fluid-buffer mixture were placed in a water bath at 39°C. Gas production was measured at 24 h after incubation and corrected for blank and hay standard (University of Hohenheim, Germany).

ME (MJ/kg DM) content of *Sanguisorba minor* hay samples was calculated using equation of Menke *et al*.[17] as follows:

ME (MJ/kg 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 *Sanguisorba minor* hay samples was calculated using equation of Menke *et al*.[17] as follows:

OMD (%) = 14.88 + 0.889GP + 0.45CP +0.0651 XA, where XA: ash content (%)

Methane gas content of total gas produced at 24 h fermentation was measured using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) [18]. 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 (%)

All data obtained were subjected to analysis of variance (ANOVA) using the randomized completed block design.

Significance between individual means was identified using the Tukey's multiple range tests. Mean differences were considered significant at P<0.05.

RESULTS

As shown in *Table 1* the maturity stage had a significant effect on the chemical composition of *Sanguisorba minor* hay. The NDF and ADF contents of *Sanguisorba minor* hay increased (P<0.05) whereas CP, ash and CT contents were decreased (P<0.05) with each increment of maturity stage. The DM content was similar (P>0.05) at fre-flowering and flowering stage, but was higher (P<0.05) at seeding stage. The DM, NDF and ADF contents varied between 22.6 and 33.7%, 36.2 and 54.5% and 17.4 and 36.2% respectively. On the other hand, CP, ash and CT contents varied between 6.7 and 20.7 and 0.5 and 1.6 % respectively.

As shown in *Table 2* the maturity stage had a significant effect on the gas production, methane production, ME and OMD of *Sanguisorba minor* hay. The gas and methane production at 24 h incubation ranged from 32.2 to 43.5 ml and 4.6 to 6.5 ml respectively and decreased (P<0.05) after flowering. The ME and OMD of *Sanguisorba minor* ranged from 7.0 and 9.3 MJ/kg DM and 46.9 to 63.2% respectively and decreased (P<0.05) with each increment of the maturity.

DISCUSSION

The marked decrease in CP and increase in DM, NDF and ADF with advancing maturity was in accordance with the findings of others studies with various forage spices. Similar changes with maturity were also observed by Kamalak *et al.*[3] in *Gundelia tournefortii* hay, Kamalak *et al.*[4] in *Sinapsis arvensis* hay, Kamalak and Canbolat [6] in *Trifolium angustifolium* hay, Kamalak *et al.*[19] in *Trigonella kotschi* hay, Canbolat [1] in *Convolvulus arvensis* hay. Decrease in CP content of hays due to advancing maturity is possibly due to a combination effect of decrease of CP in leaves and increase of stem content at the expense of leaves of hay samples advancing maturity. The protein content of stem is lower than that of leaves [20]. Daily reduction in CP was calculated by the difference between CP of hay obtained at pre-flowering and seeding stages, divided by the time (days) required to reach from pre-flowering to seeding stage. In the current study the reduction in CP content of *Sanguisorba minor* hay was approximately 2.54 g/kg/day. On the other hand the increase in NDF and ADF contents of *Sanguisorba minor* hay were 3.32 and 3.41 g/kg/day respectively.

CP content of *Sanguisorba minor* hay obtained in the current study was comparable with finding of Asaadi and Yazdi [21] who reported that CP ranged from

Table 1. *The effect of maturity stage on the chemical composition of Sanguisorba minor hay*
Tablo 1. *Hasat zamanının küçük çayır düğmesi otunun kompozisyonuna etkisi*

Nutrients (%)	Maturity Stages			SEM	Significance
	Pre-flowering	Flowering	Seeding		
DM	22.6^{b}	24.9^{b}	33.7^{a}	1.06	***
CP	20.7^{a}	13.7^{b}	6.7^{c}	0.37	***
Ash	8.7^{a}	7.5^{b}	6.5^{c}	0.15	***
NDF	36.2^{c}	49.2^{b}	54.5^{a}	0.93	***
ADF	17.4^{c}	29.8^{b}	36.2^{a}	0.82	***
CT	1.6^{a}	0.9^{b}	0.4^{c}	0.03	***

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

Table 2. *The effect of maturity stage on the gas production kinetics, metabolisable energy and organic matter digestibility of Sanguisorba minor hay*
Table 2. *Hasat zamanın Sanguisorba minor otunun gaz üretim parametrelerine, metabolik enerji ve organik madde sindirim derecesine etkisi*

Estimate Parameters	Maturity Stages			SEM	Significance
	Pre-flowering	Flowering	Seeding		
Total Gas (*mL*)	43.5^{a}	43.2^{a}	32.2^{b}	0.80	***
CH4 (*mL*)	6.5^{a}	6.37^{a}	4.6^{b}	0.05	***
CH4 % of Total Gas	14.9	14.8	14.4	0.26	NS
ME (*MJ /Kg DM*)	9.3^{a}	8.8^{b}	7.0^{c}	0.11	***
OMD (%)	63.2^{a}	59.8^{b}	46.9^{c}	0.71	***

*$^{a, b, c}$ Row means with common superscripts do not differ (P>0.05); **S.E.M.:** standard error mean; **NS:** Non-significant, **c:** gas production rate (%); **A:** potential gas production (mL), **ME:** Metabolisable energy (MJ/kg DM); **OMD:** Organic matter digestibility %, *** P<0.001*

5.21 to 17.04% and decreased with maturity.

In the current study the reduction in CP of *Sanguisorba minor* hay estimated was considerably higher than those obtained by Minson [22] and Kamalak and Canbolat [6] who indicated that the average reduction in CP of several forages due to maturity ranged from 0.82 and 1 g/kg/day. On the hand, the reduction in the current experiment was in accordance with the findings of Kamalak *et al*.[19] who reported that decline in CP due to maturity was 2.34 g/kg/day.

Condensed tannin had an important role in forages depending on the amount. Low level tannin (2-3% of DM) may have beneficial effect since the level tannin in diets prevent the CP from extensive degradation through formation of protein-tannin complexes [23]. On the other hand, high tannin level (5% of DM) in diets may result in the increased indigested CP due to excessive formation of tannin-protein complexes [24].

As can be seen from *Table 1*, the observed condensed tannin levels of *Sanguisorba minor* hay harvested at three maturity stages were low magnitude. Therefore, low condensed tannin of *Sanguisorba minor* hay seems to have a potential for beneficial effect when included into ruminant diets as it can increase rumen undegradable crude protein without decreasing digestibility.

The marked decreases in gas production, methane production, ME and OMD due to maturity were closely associated with increase in less digestible cell contents (NDF and ADF) and decrease in CP of *Sanguisorba minor* hay with maturity. As can be seen from the equation suggested by Menke *et al*.[17] OMD and ME values were estimated using the gas production and CP. The gas production is closely associated with the amount of fermented substrate in diets [25]. Therefore, decrease in fermentable fraction in forage with maturity due to increased cell wall contents that consist of less digestible carbohydrates resulted in less gas production. As a result, the decrease in gas production with maturity was inevitable. Similar observation with maturity were also obtained by Kamalak *et al*.[3] in *Gundelia tournefortii* hay, Kamalak *et al*.[4] in *Sinapsis arvensis* hay, Kamalak and Canbolat [6] in *Trifolium angustifolium* hay, Kamalak *et al*.[19] in *Trigonella kotschi* hay Canbolat [1], in *Convolvulus arvensis* hay.

Metabolisable energy content of *Sanguisorba minor* hay obtained in the current study was comparable with finding of Asaadi and Yazdi [21] who reported that ME content ranged from 5.54 to 9.96 MJ/kg DM and decreased with maturity.

Lopez *et al*.[26] suggested that the methane reduction potential of any feedstuffs can be estimated from the percentage of methane of *in vitro* gas production and the feedstuffs can be arbitrarily divided in three groups, low potential (% methane in gas between >11% and ≤14%), moderate potential (% methane in gas between >6% and <11%), high potential (% methane in gas between >0% and <6%). Therefore *Sanguisorba minor* hay had no methane reduction potential since the percentage of methane for all three maturity stages is higher than %14.

In conclusion, maturity had a significant effect on the nutritive value of the forage of *Sanguisorba minor*. The nutritive value of *Sanguisorba minor* decreased with increased maturity. *Sanguisorba minor* should be grazed or harvested at pre-flowering and flowering stage since these stage provides hay with high ME and CP for ruminant.

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