

Determination of Potential Nutritive Value of *Trigonella kotschi* Fenzl Hay Harvested at Three Different Maturity Stages

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Summary

The objective of this experiment was to determine the potential nutritive value of *Trigonella kotschi* Fenzl hay harvested at three maturity stages using chemical composition and *in vitro* gas production technique. *In vitro* gas productions of *Trigonella kotschi* hay were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times and their gas production kinetics were described using the equation $y = A(1 - \exp(-ct))$. Maturity had a significant effect on the chemical composition, *in vitro* gas production, metabolisable energy (ME) and organic matter digestibility (OMD). Cell wall contents (neutral detergent fiber (NDF), acid detergent fiber (ADF)) of *Trigonella kotschi* hay increased with maturity whereas crude protein (CP) content decreased. The CP contents of *Trigonella kotschi* hay ranged from 13.22 to 22.56%. The NDF and ADF contents of *Trigonella kotschi* hay ranged from 26.56 to 44.72 and 19.65 to 35.30% respectively. The condensed tannin content of *Trigonella kotschi* hay ranged from 0.69 to 1.06 % and decreased with increasing maturity. The Ca, K and Fe contents of *Trigonella kotschi* hay harvested at flowering stage were significantly higher than those harvested at pre-flowering and seeding stages. The P, K and Cu contents of *Trigonella kotschi* hay harvested at pre-flowering stage were significantly higher than those harvested at flowering and seeding stages. The potential gas production and estimated parameters decreased with increasing maturity of *Trigonella kotschi* hay. The potential gas production (A) ranged from 59.26 to 70.46 ml. The ME and OMD ranged from 9.25 and 10.85 MJ/kg DM and 62.63 to 74.08% respectively. In conclusion, although the nutritive value of *Trigonella kotschi* plant dramatically changed with increasing maturity it appears that wild *Trigonella kotschi* plant could be grazed or harvested at these more advanced stages and still provides forage of an acceptable quality for ruminant animals during summer and winter periods.

Keywords: *Trigonella kotschi* hay, Nutritive value, Chemical composition, Condensed tannin, *In vitro* gas production, Digestibility

Üç Farklı Dönemde Hasat Edilen *Trigonella kotschi* Otunun Potansiyel Besleme Değerinin Belirlenmesi

Özet

Bu çalışmanın amacı, üç farklı dönemde hasat edilen *Trigonella kotschi* otunun potansiyel besleme değerini kimyasal kompozisyonu ve *in vitro* gaz üretim tekniğini kullanarak belirlemektir. *In vitro* gaz ölçümleri fermentasyonun başlamasından sonra 3, 6, 12, 24, 48, 72 ve 96 saatlerinde yapılmıştır. Gaz üretimine ait parametreler $y = A(1 - \exp(-ct))$ fonksiyonu kullanılarak belirlenmiştir. Hasat zamanı *Trigonella kotschi* otunun kimyasal kompozisyonuna, *in vitro* gaz üretimine, metabolik enerji (ME) ve organik madde sindirim derecesine (OMSD) parametrelerini önemli derecede etkilemiştir. *Trigonella kotschi* bitkisi olgunlaştıkça hücre duvarını oluşturan unsurlar (nötral deterjan fiber (NDF) ve asit deterjan fiber (ADF)) ve kuru madde içeriği yükselmiş diğer taraftan ham protein içeriği azalmıştır. *Trigonella kotschi* otunun ham protein içeriği %13.22 ile 22.56 arasında değişmiş olup NDF ve ADF içerikleri sırasıyla %26.56 ile 44.72 ve 19.65 ile 35.30 arasında değişmiştir. *Trigonella kotschi* otunun kondense tanen içeriği % 0.69 ile 1.06 arasında değişmiş olup, bitki olgunlaştıkça kondense tanen içeriği azalmıştır. Çiçeklenme hasat edilen *Trigonella kotschi* otunun Ca, K ve Fe içerikleri, çiçeklenme öncesi ve tohum bağlama döneminde hasat edilenlerden yüksek bulunmuştur. Çiçeklenme öncesinde hasat edilen *Trigonella kotschi* otunun P, K ve Cu içerikleri, çiçeklenme ve tohum bağlama döneminde hasat edilenlerden daha yüksek bulunmuştur. Potansiyel gaz üretimi ve tahmin edilen parametrelerde *Trigonella kotschi* bitkisinin olgunlaşmasıyla birlikte azalmalar meydana gelmiştir. Potansiyel gaz üretimi 59.26 ile 70.46 ml arasında olup metabolik enerji (ME) ve organik madde sindirim derecesi (OMSD) 9.25 ile 10.85 MJ ve %62.63 ile 74.08 arasında değişmiştir. Sonuç olarak, *Trigonella kotschi* otunun besleme değeri bitkinin olgunlaşmasına bağlı olarak dramatik bir şekilde azalmasına rağmen *Trigonella kotschi* otu ilerlememiş dönemlerde bile, yazın otlayan ruminant hayvanlar için kabul edilebilir kalitede ot sağlayabileceği gözükmektedir.

Anahtar sözcükler: *Trigonella kotschi* otu, Besleme değeri, Kimyasal kompozisyon, Makro ve mikro mineral, Kondense tanen, *In vitro* gaz üretimi, Sindirim derecesi



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INTRODUCTION

It is well known that forages have an important role in ruminant nutrition in terms of providing energy, protein and minerals. Forages also provide fiber to ruminant animals for chewing and rumination. In the Mediterranean areas pastures represent the most important forage resource. *Trigonella kotschi* Fenzl is one of the most important weeds in grazing area in summer in the South of Turkey. Sheep and goat preferred to consume, *Trigonella kotschi* hay. Although the nutritive value of many types of forages obtained at different harvest maturity is well established¹⁻⁴ there is limited information about the nutritive value of *Trigonella kotschi* Fenzl hay obtained at different maturity stages. However there is also no information on macro and micro mineral contents of *Trigonella kotschi* Fenzl hay obtained at different maturity stages. Therefore it is also very important to investigate the potential of *Trigonella kotschi* hay in terms of mineral content to meet the small animal requirements. 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 requirements⁵. Recently some researches have used the *in vitro* gas production technique for evaluation of feedstuffs and determination of essential oil on microbial fermentation parameters⁶⁻⁹. Chemical composition, in combination with *in vitro* gas production, OMD and ME content were widely used to determine the potential nutritive value of forages which are previously limited or uninvestigated¹⁻⁴.

The objective of this experiment was to determine the potential nutritive value of *Trigonella kotschi* Fenzl hay harvested at three maturity stages using chemical composition and *in vitro* gas production technique.

MATERIAL and METHODS

Trigonella kotschi Fenzl plants were harvested at three maturity stages (pre- flowering, flowering and seeding stages). *Trigonella kotschi* Fenzl plants were hand harvested from at least three replicate plots of 10 x 2 m established in the experimental field in April-May, 2010 in Kahramanmaras, Turkey. 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) 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 method¹⁰. Crude protein was calculated as N X 6.25. Neutral detergent fiber (NDF) was determined by the method van Soest and Wine¹¹ and ADF were determined by the method of van Soest¹². Condensed tannin was determined by butanol-

HCl method as described by Makkar et al.¹³.

Plant samples were digested with HNO₃/HClO₄ mixture for P, K, Ca, Mg, Fe, Mn, Zn and Cu¹⁴. The total P was determined by colorimetric vanadomolybophosphoric acid method¹⁵ and the total concentrations of K, Ca, Mg, Fe, Mn, Zn and Cu were determined using atomic absorption spectrophotometer. All chemical analyses were carried out in duplicate.

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.¹⁶. Rumen fluid was obtained from three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Rumen fluid was collected before morning feeding and squeezed through four layers of cheesecloth. The rumen fluid was flushed with CO₂. The rumen fluid was added to buffered mineral solution in the ratio of 1:2 respectively. 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 3, 6, 12, 24, 48, 72 and 96 h after incubation and corrected for blank incubation. Cumulative gas production data were fitted to non-linear exponential model as: $Y = A (1 - \exp^{-ct})$

Where Y is gas production at time 't', A is the potential gas production (ml/200 mg DM), c is the gas production rate constant (h⁻¹) and t is the incubation time (h).

ME (MJ/kg DM) content of samples was calculated using equation of Menke et al.¹⁶ 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 samples was calculated using equation of Menke et al.¹⁶ as follows:

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

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

RESULTS

The effect of maturity stage on the chemical composition of *Trigonella kotschi* hay is given in Table 1. The maturity stage has significant effect on the chemical composition of *Trigonella kotschi* hay. The DM, NDF and ADF contents of

Trigonella kotschi hay significantly increased with maturity whereas CP, EE and Ash contents were significantly decreased. The DM and CP contents ranged from 20.59 to 31.13 and 13.22 to 23.84% respectively. On the other hand cell wall contents (NDF and ADF) of *Trigonella kotschi* hay ranged from 26.56 to 44.72 and 19.65 to 35.30% respectively.

The effect of maturity stage on the macro and micro mineral contents of *Trigonella kotschi* was given in Table 2. The maturity had a significant effect on the macro and micro mineral contents of *Trigonella kotschi* hay. The Ca, K and Fe contents of *Trigonella kotschi* hay harvested at flowering stage were significantly higher than those harvested at pre-flowering and seeding stages. The P and Cu contents of *Trigonella kotschi* hay harvested at pre-flowering stage were significantly higher than those

harvested at flowering and seeding stages.

The effect of maturity stages on gas production at different time intervals is given in Fig. 1. Except for early incubation times (3, 6 and 12 h), at all incubation times, gas production at pre-flowering stage was significantly higher than those of flowering and seeding stages.

The effect of maturity stage on gas production kinetics, ME, OMD of *Trigonella kotschi* hay is given in Table 3. The maturity had no significant effect on the gas production rate whereas A, ME and OMD of *Trigonella kotschi* hay decreased with maturity.

The potential gas production ranged from 59.26 to 70.46 ml. On the other hand, ME and OMD ranged from 9.25 to 10.85 MJ/kg DM and 62.63 to 74.08% respectively.

Table 1. The effect of maturity stage on the chemical composition of *Trigonella kotschi* hay

Tablo 1. Hasat zamanının *Trigonella kotschi* otunun kompozisyonuna etkisi

Nutrients (%)	Maturity Stages			SEM	Sig.
	Pre-flowering	Flowering	Seeding		
DM	20.59 ^c	23.84 ^b	31.13 ^a	0.317	***
CP	22.56 ^a	18.51 ^b	13.22 ^a	0.219	***
Ash	12.60 ^a	11.72 ^b	8.45 ^c	0.040	***
EE	2.64 ^a	1.82 ^{ab}	1.25 ^a	0.360	*
NDF	26.56 ^c	31.96 ^b	44.72 ^a	1.061	***
ADF	19.65 ^c	26.25 ^b	35.30 ^a	0.262	***
CT	1.06 ^c	0.91 ^b	0.69 ^a	0.062	***

^{a,b,c} Row means with common superscripts do not differ ($P>0.05$); **SEM**: standard error mean; **Sig.**: significance level; **DM**: Dry matter %, **CP**: Crude protein, **EE**: Ether extract, **NDF**: Neutral detergent fiber, **ADF**: Acid detergent fiber, **CT**: Condensed tannin

Table 2. The effect of maturity stage on the macro (% of DM) and micro (mg/kg DM) mineral of *Trigonella kotschi* hay

Tablo 2. Hasat zamanının *Trigonella kotschi* otunun makro (KM'nin %) ve mikro (mg/kg kuru madde) mineral içeriğine etkisi

Macro (%)	Maturity Stages			SEM	Sig.
	Pre-flowering	Flowering	Seeding		
Ca	1.76 ^b	2.44 ^a	1.73 ^b	0.110	**
P	0.21 ^a	0.19 ^b	0.17 ^c	0.004	**
Mg	0.27 ^a	0.26 ^a	0.21 ^b	0.009	**
K	2.13 ^b	2.37 ^a	1.25 ^c	0.032	***
Micro (mg/kg DM)					
Zn	14.82 ^a	12.39 ^a	9.23 ^b	0.862	*
Fe	347.17 ^b	807.42 ^a	321.57 ^b	13.61	***
Mn	14.21 ^b	4.39 ^b	26.48 ^a	3.718	*
Cu	4.13 ^a	2.60 ^b	1.02 ^c	0.028	***

^{a,b,c} Row means with common superscripts do not differ ($P>0.05$); **SEM**: standard error mean; **Sig.**: significance level; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

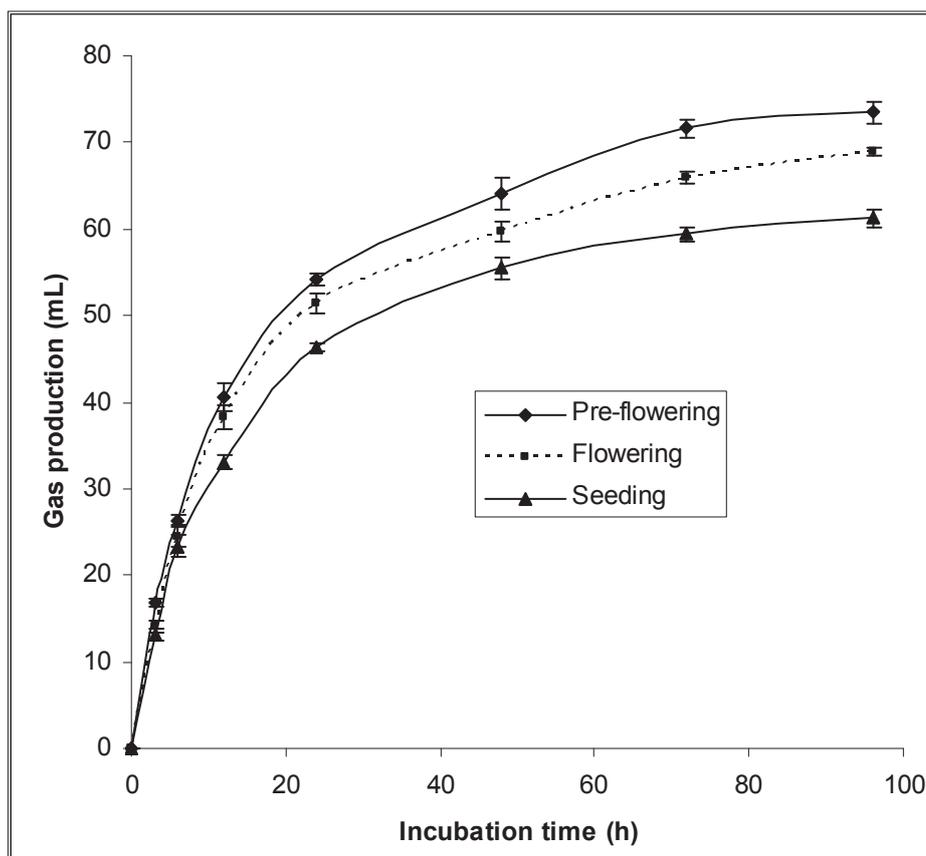


Fig 1. The effect of maturity stage on gas production of *Trigonella kotschi* hay

Şekil 1. Hasat zamanının *Trigonella kotschi* otunun gaz üretimine etkisi

Table 3. The effect of maturity stage on the gas production kinetics, metabolisable energy and organic matter digestibility of *Trigonella kotschi* hay

Table 3. Hasat zamanının *Trigonella kotschi* otunun gaz üretim parametrelerine, metabolik enerji ve organik madde sindirim derecesine etkisi

Estimated Parameters	Maturity Stages			SEM	Sig.
	Pre-flowering	Flowering	Seeding		
c	0.069	0.068	0.070	0.001	NS
A	70.46 ^a	65.79 ^b	59.26 ^c	0.868	***
ME	10.85 ^a	10.24 ^b	9.25 ^c	0.093	***
OMD	74.08 ^a	69.73 ^b	62.63 ^c	0.611	***

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

DISCUSSION

The current study has provided useful information on the likely decline in nutritive value of *Trigonella kotschi* hay with increasing maturity. It is well known that CP concentration and digestibility declined with age, while NDF and ADF concentrations of plant species increased. Buxton¹⁷ reported that the decline in protein concentration with increasing maturity was a reflection of a decrease in protein in leaves, while stems, which had a lower protein concentration, represented a larger proportion of the available herbage in more mature forages. Daily reduction

in CP was estimated based on the difference between CP of hay obtained at pre-flowering 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 2.34 g/kg/day. The reduction obtained in the current experiment was considerably higher than those obtained by Minson¹⁸ and Kamalak and Canbolat⁴ who reported that the average decline in crude protein concentration with advancing maturity for *Trifolium angustifolium* and several forages and averaged 1 and 0.82 g/kg/day respectively.

Low level of tannin (2-3% of DM) in ruminant diets

may have a beneficial effect through reduced protein degradation in the rumen as a result of the formation of protein-tannin complexes¹⁹. However, due to excessive formation of tannin-protein complexes, CP utilization could be restricted by high tannin level (%5 of DM) and protein may pass through the animal largely undigested²⁰. High level of tannins can adversely affect the microbial and enzyme activities²¹⁻²⁴. However, in this experiment, the condensed tannin levels of *Trigonella kotschi* hay harvested at three maturity stages were lower than those considered detrimental to ruminant animals.

For ewes that weight approximately 50 kg the daily macro minerals (Ca, P, Mg and K) requirements for maintenance ranges from 0.20 to 0.82, 0.16 to 0.38, 12 to 0.18 and 0.50 to 0.80% of DM respectively²⁵. As can be seen from [Table 2](#) *Trigonella kotschi* hays harvested at three maturity stages were adequate in terms of Ca, P, Mg and K minerals.

For ewes that weight approximately 50 kg the daily micro minerals (Fe, Cu, Mn and Zn) requirements for maintenance ranges from 30 to 50, 7 to 11, 20 to 40 and 20 to 33 mg /kg DM respectively²⁵. As can be seen from [Table 2](#) *Trigonella kotschi* hays harvested at three maturity stages were inadequate in term of Zn, Mn and Cu except that *Trigonella kotschi* hays harvested at seeding stage was adequate in terms of Mn. On the other hand, *Trigonella kotschi* hays harvested at three maturity stages were adequate in term of Fe. Therefore productivity of sheep grazing on the *Trigonella kotschi* plant could be improved through supplementation with micro mineral sources such as Zn, Mn and Cu.

The decline in potential gas production with maturity was also a reflection of declining quality and was in agreement with the findings of Kamalak³ and Kamalak and Canbolat⁴ who reported a decrease in gas production as the forage growing period was prolonged. With increasing maturity, the cell wall contents (NDF and ADF) increased, and these components were the more indigestible fractions of the plant. It is well known that 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 influence more closely with gas production²⁶. As a result, there was reduced gas production from the indigestible fractions with increasing maturity. With the reduction in CP concentration and increase in the fibre fraction, OMD and ME concentration also decreased with increasing maturity.

In conclusion, despite the decline in nutritive value of the forage of *Trigonella kotschi* hay with advancing maturity, even at the seeding stage, the forage had moderately high CP concentration and was quite digestible. It appears that wild *Trigonella kotschi* hay could be grazed or harvested at

these more advanced stages and still provides hay of an acceptable quality for ruminant animals during summer and the winter periods.

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