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CHEMICAL COMPOSITION, METABOLISABLE ENERGY, ORGANIC MATTER DIGESTIBILITY AND METHANE PRODUCTION OF SOME TANNIN CONTAINING FORAGES

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Abstract: The aim of the current experiment was to evaluate the chemical composition, gas, methane production, metabolisable energy (ME), organic matter digestibility (OMD) of some tannin containing hays. There are significant variations among hays in terms of the chemical composition. Crude protein contents of hays ranged from 14.3 to 23.5% with the highest being for Marrubium supinum hay and lowest for Anthyllis circinata hay. Neutral detergent fiber contents of hays ranged from 40.6 to 57.7% with the highest being for Polygonum aviculare hay and lowest for Scorpinus muricatus hay. Acid detergent fiber contents of hays ranged from 22.5 to 32.9% with the highest being for Lotus corniculatus hay and lowest for Scorpinus muricatus hay. Condensed tannin contents of hays ranged from 0.7 to 7.3% with the highest being for Polygonum aviculare hay and lowest for Marrubium supinum hay. Gas production of tannin containing hays ranged from 77.5 and 105.5 ml/0.5 g DM with the highest being for Anthyllis circinata and Scorpinus muricatus, and lowest for Marrubium supinum. Metabolisable energy content of legume hays varied between 7.6 and 9.1 MJ/kg DM with the highest being for Scorpinus muricatus hay and lowest for Cichorium intybus, Bituminaria bituminosa and Marrubium supinum hays. Organic matter digestibility of legume hays varied between 58.2 and 72.4% with the highest being for Scorpinus muricatus hay and lowest for Bituminaria bituminosa hay. The tannin containing hays investigated in the current experiment will provide not only protein but also fiber for ruminant animals. In addition they had low anti-methanogenic potential. The current experiment will provide information for the nutritionist to prepare well balanced diets for ruminants animals. However further in vivo experiments are required to determine the feed intake and anti-methanogenic potential of hays.

Keywords: Forage, Chemical composition, Tannin, Digestibility, Metabolisable energy, Methane emission

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1. Introduction

Forage will provide not only nutrients but also fiber for ruminant animal to meet their requirement. There are some forages in pasture which contains considerable amount of condensed tannin which may have potential on mitigation of enteric methane production when they are consumed by ruminant animals. Although there are a lot of studies involved in chemical composition of forages there is a lack of information about condensed tannin and anti-methanogenic potential of forages. *In vitro* gas production technique is widely used to evaluate forages in terms of potential nutritive value, metabolisable energy and organic matter digestibility for ruminant animals (Kamalak et al., 2004; Kamalak et al., 2005; Ozturk et al., 2006; Kamalak et al., 2010; Kamalak and Canbolat, 2010; Kamalak et al., 2011; Ozkan et al., 2017; Atalay et al., 2018; Boga et al., 2020; Kamalak et al., 2021). It is well known that some anti-nutritive factors such as tannin and saponin decrease the enteric methane production from ruminant animals. Therefore the aim of the current experiment was to evaluate the chemical composition, ME, OMD, gas and anti-methanogenic potential of tannin containing forages using in vitro gas production technique.

2. Material and Methods

2.1. Tannin Containing Hays

Hays obtained from 3 replicate plots established in the experiment field at flowering stage from7 different plant species namely, *Anthyllis circinata*, *Cichorium intybus*, *Scorpinus muricatus*, *Lotus corniculatus*, *Bituminaria bituminosa*, *Polygonum aviculare*, *Marrubium supinum* in 2019 in Turkey were dried in 65 °C until a constant weight. Hay samples were then milled to pass a 1 mm sieve for chemical analysis and *in vitro* gas production assay.

2.2. Chemical Analysis of Hays

Dry matter (DM), crude ash (CA), crude protein (CP) and ether extract (EE) contents of hay samples were analyzed according to AOAC (2005). Neutral detergent fiber (NDF) and ADF contents of hay samples using the method described by Van Soest and Wine (1967) and Van Soest

(1963) respectively. Condensed tannin contents of forages were determined by the Butanol –HCL method (Makkar, 1995). All chemical analyses were carried out in triplicate.

2.3. *In Vitro* Gas Production of Hays

Approximately 500 mg of hay samples were incubated in 100 mL calibrated glass syringes in triplicate for 24 h in a water bath set at 39 °C with buffered rumen fluid of three fistulated for anaerobic fermentation. Rumen fluid used in vitro gas production was obtained from slaughter house in Kahramanmaras. In vitro gas production trial of hay samples was carried out according to the method described by Menke et al (1979).

Metabolisable energy (ME, MJ/kg DM) and organic matter digestibility (OMD) of hay samples were determined using equations suggested by Menke and Steingass (1988) (equation 1 and 2).

$$\text{ME (MJ/kg DM)} = 2.20 + 0.1357GP + 0.057CP + 0.002859EE^2 \quad (1)$$

$$\text{OMD (\%)} = 14.51 + 0.88490GP + 0.448CP + 0.686CA \quad (2)$$

Where; GP= 24 h net gas production (ml/200 mg), CP= Crude protein (%), EE= Ether extract (%), CA= Ash content (%), Methane content (%) of total gas produced after 24 hour fermentation were determined using an infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (Goel et al., 2008). The amount of methane (ml) was calculated using the formula given below (equation 3).

$$\text{Methane production (mL)} = \text{Total gas production (mL)} \times \text{Percentage of methane (\%)} \quad (3)$$

2.4. Statistical Analysis

The effect of species on chemical composition gas production, methane production, ME and OMD of tannin containing hays. Differences between means were identified by Tukey test (Genç and Soysal, 2018). 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.

3. Results and Discussion

The effects of species on the chemical composition of tannin containing hays were given in Table 1. Species had a significant effect on the chemical composition of tannin containing hays.

Table 1. Species had a significant effect on the chemical composition of tannin containing hays.

Hays	DM	CA	CP	EE	NDF	ADF	CT
Anthyllis circinata	92.8^{bc}	10.4^{d}	14.3^{g}	3.5^{b}	49.6^{c}	28.2^{e}	1.3^{bc}
Cichorium intybus	93.7^{b}	17.3^{a}	18.1^{e}	3.5^{b}	56.7^{a}	32.1^{b}	1.3^{bc}
Scorpinus muricatus	91.6^{c}	13.3^{b}	20.2^{c}	4.7^{ab}	40.6^{d}	22.5^{f}	1.6^{bc}
Lotus corniculatus	94.4^{a}	7.7^{e}	19.4^{d}	4.2^{ab}	51.1^{b}	32.9^{a}	2.3^{b}
Bituminaria bituminosa	89.0^{d}	6.8^{f}	15.2^{f}	4.2^{ab}	42.2^{d}	29.6^{d}	1.1^{c}
Polygonum aviculare	94.8^{a}	12.4^{c}	21.4^{b}	4.9^{a}	57.7^{a}	30.7^{c}	7.3^{a}
Marrubium supinum	93.2^{b}	12.0^{c}	23.5^{a}	4.9^{a}	44.8^{cd}	27.1^{e}	0.7^{c}
SEM	0.353	0.194	0.207	0.347	1.611	0.493	0.326
P	***	***	***	***	***	***	***

^{ab}Column means with common superscripts do not differ (P>0.05), SEM= standard error mean, DM= dry matter (%), CA= crude ash (%), CP= crude protein (%), EE= Ether extract (%), NDF= neutral detergent fiber (%), ADF= acid detergent fiber (%), CT= condensed tannin (%).

Crude ash contents of hays ranged from 6.8 to 17.3% with the highest being for Cichorium intybus hay and lowest for Bituminaria bituminosa hay. Crude protein contents of hays ranged from 14.3 to 23.5% with the highest being for Marrubium supinum hay and lowest for Anthyllis circinata hay. CP contents of forages used ruminant diets should be higher than 8% of DM to meet maintenance requirement (Norton, 1994). In addition, CP contents of forages used ruminant diets should not be less than 10% to avoid low dry matter intake (Ranjhnan, 2001). As can be seen from Table 1 hays investigated in the current study had a CP contents that higher than those requested for maintenance and proper food intake, which can be used as a protein supplement for poor quality forages to improve productivity of ruminant animals.

Ether extract contents of hays ranged from 3.5 to 4.9% with the highest being for *Polygonum aviculare* and *Marrubium supinum* hays, and lowest for *Anthyllis circinata* and *Cichorium intybus* hay. Neutral detergent fiber contents of hays ranged from 40.6 to 57.7% with the highest being for *Polygonum aviculare* hay and lowest for *Scorpinus muricatus* hay. Acid detergent fiber contents of hays ranged from 22.5 to 32.9% with the highest being for *Lotus corniculatus* hay and lowest for *Scorpinus muricatus* hay. Condensed tannin contents of hays ranged from 0.7 to 7.3% with the highest being for *Polygonum aviculare* hay and lowest for *Marrubium supinum* hay. Except for *Polygonum aviculare*, the CT contents of forages investigated is not likely detrimental on the digestibility and animal performance.

Yusuf and Muritala (2013) suggested that wide variation in chemical composition can be expected among forages even if they were grown in the same environmental conditions and harvested at the similar maturity due to the inherent characteristics of forages associated with ability to extract and accumulate nutrients from soil and fix nitrogen from atmosphere. Some of differences among forages in terms of chemical composition may be associated with differences in leaf: stem ratio, which may results in differences in chemical composition, especially in NDF and CP contents of forages.

As can be seen Table 1, forages with high cell contents investigated in the current experiment will provide not only CP but also fiber for ruminant animals. NRC (1989) recommends that dairy cow ration should contain of 25% NDF of DM with 75% of the NDF from forages whereas feed intake of dairy cattle decreased with increasing NDF content of diets ranging from 22.5 to 45.8% (Arelovich et al., 2008).

The gas production, methane production, metabolisable energy and organic matter digestibility of tannin containing hays were given in Table 2. Species had a significant effect on the gas production, methane production, ME and OMD of tannin containing hays. Gas production of tannin containing hays ranged from 77.5 and 105.5 ml/0.5 g DM with the highest being for *Anthyllis circinata* and *Scorpinus muricatus*, and lowest for *Marrubium supinum*. The differences among hays in terms of gas production might be associated to compositional differences of hays, especially cell contents and CT contents. The extent of total gas production depends on the available carbohydrate for fermentation of rumen micro-organism (Blümmel and Orskov, 1993). However, the presence of secondary metabolites such as tannin and saponin in hay may affect the extent of gas produced during fermentation (Kondo et al., 2014; Jayanegara et al., 2014).

Generally, the percentage methane of usual feeds such as hay, concentrate or mixture of hay and concentrate range from 16 to 20%. Feedstufs can be classified in terms of anti-methanogenic potential using percentage of methane production after 24 h anaerobic fermentation (Lopez et al., 2010) According to this classification, most of hay samples had a low anti-methanogenic potential since the percentage of methane fell into the range of >11% and ≤14%. Metabolisable energy content of legume hays varied between 7.6 and 9.1 MJ/kg DM with the highest being for *Scorpinus muricatus* hay and lowest for *Cichorium intybus*, *Bituminaria bituminosa* and *Marrubium supinum* hays. Organic matter digestibility of legume hays varied between 58.2 and 72.4% with the highest being for *Scorpinus muricatus* hay and lowest for *Bituminaria bituminosa* hay.

Table 2. The gas production, methane production, metabolisable energy and organic matter digestibility of tannin containing hays

Hays	Gas	CH_4 (%)	CH_4 (ml)	ME	OMD
Anthyllis circinata	105.5^{a}	14.9^{b}	12.7^{c}	8.7^{ab}	66.6^{c}
Cichorium intybus	80.75^{d}	12.7^{c}	14.1^{b}	7.6^{c}	65.5^{bc}
Scorpinus muricatus	105.2^{a}	14.1^{bc}	12.0^{c}	9.1^{a}	72.4^{a}
Lotus corniculatus	100.7^{ab}	16.8^{a}	14.9^{b}	8.8^{ab}	66.5^{bc}
Bituminaria bituminosa	86.2^{cd}	14.2^{bc}	14.8^{b}	7.8^{c}	58.2^{d}
Polygonum aviculare	92.2^{bc}	13.4^{bc}	13.0^{c}	8.5^{b}	68.2^{b}
Marrubium supinum	77.5^{d}	14.0^{bc}	16.2^{a}	7.8^{c}	64.1^{c}
SEM	0.328	0.337	0.501	0.179	1.115
P	***	***	***	***	***

^{ab}Column means with common superscripts do not differ (P>0.05), SEM= Standard error mean, GP= gas production (ml), CH_4= methane production, ME= metabolisable energy (MJ/kg DM) , OMD= organic matter digestibility(%).

4. Conclusion

There are significant variation among hay samples in terms of chemical composition and potential nutritive value. The tannin containing hays investigated in the current experiment will provide not only protein but also fiber for ruminant animals. In addition they had low anti-methanogenic potential. The current experiment will provide information for the nutritionist to prepare well balanced diets for ruminants animals. However further in vivo experiments are required to determine the feed intake and anti-methanogenic potential of hays.

Author Contributions

All authors have equal contribution and the authors reviewed and approved the manuscript.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Approval

Ethical approval is not required, because this article does not contain any studies with human or animal subjects.

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