Effect of species on chemical composition, metabolizable energy, organic matter digestibility and methane production of some legume plants grown in Turkey

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ABSTRACT
The aim of the current experiment was to evaluate the chemical composition, metabolizable energy (ME), organic matter digestibility (OMD), gas and methane production of some legume plants estimated by in vitro gas production technique, and to determine the relationship among chemical composition, ME, OMD, gas and methane production of some legume plants. Although species has a significant effect on the chemical composition, gas production, ME and OMD species has no effect on methane emission of legume hays. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) ranged from 22.86% to 42.93% and 20.24% to 37.16% respectively. Crude protein (CP) contents of legume hays ranged from 13.61% to 20.55%. Neutral detergent fibre and ADF contents of legume plants are negatively correlated with gas production, ME and OMD. It can be concluded that legume plant studied in the current experiment showed a wide nutritional diversity and will provide not only protein but also fibre for livestock. They may exhibit complementary role for ruminant animals grazing on poor quality forages. Legume species studied in the current experiment had a no anti-methanogenic properties.

1. Introduction
As in many parts of world, pasture is a very important feed source used to meet the requirements of ruminant animals in Turkey. However, despite the importance of pasture in ruminant nutrition, there is a lack of information chemical composition and nutritive value of plants in pasture. So far, except for several plants, ME, OMD and methane production of legume plants in pasture has not been determined due to lack of laboratory facilities and the forage quality assessment in Turkey has been largely relied on the chemical composition such as crude protein, ether extract cellulose etc. (Kiraz 2011). Recently comparison of potential nutritive value of previously limited or uninvestigated feedstuffs has been made in terms of chemical composition, in combination with in vitro gas production, OMD and ME and methane production of plants and trees (Kamalak et al. 2005; Kamalak et al. 2010; Kamalak and Canbolat 2010). Therefore the aim of the current experiment was to evaluate the chemical composition, ME, OMD, gas and methane production of some legume plants estimated by in vitro gas production technique, and to determine the relationship among chemical composition, ME, OMD, gas and methane production of some legume plants in Turkey.

2. Material and methods
2.1. Legume hays
Legume hays collected from 3 replicate plots established in the experiment field at flowering stage from 13 different legume species namely, Vicia narbonensis, Medicago sativa, Lathyrus sativus, Medicago orbitalis, Vicia sativa, Trigonella kotschii, Medicago lupulina, Trifolium repens, Trigonella foenum-graecum, Trifolium incarnatum, Trigonella sipicata, Trifolium campestre and Trifolium purpureum in 2015 in Turkey were dried in 65°C until a constant weight. Legume hays were then milled to pass a 1 mm sieve for chemical analysis and in vitro gas production assay.

2.2. Chemical analysis
Dry matter (DM), crude ash (CA), crude protein (CP) and ether extract (EE) contents of legume hays were analysed according to AOAC (2005). Neutral detergent fibre (NDF) and ADF contents of legume plants using the method described by Van Soest and Wine (1967) and Van Soest (1963), respectively. All chemical analyses were carried out in triplicate.

2.3. In vitro gas production
Approximately 200 mg of legume hays in 100 mL calibrated glass syringes were subjected to anaerobic fermentation in triplicate for 24 h in a water bath set at 39°C with buffered rumen fluid of three fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%) according to method described by Menke and Steingass (1988). Three syringes with only 30 ml buffered rumen fluid incubated and considered as blank. Total net gas productions of legume hays were determined after correction for blank incubation.
2.4. Statistical analyses

Data associated with chemical composition ME, OMD, gas and methane production were subjected to one-way analysis of variance (ANOVA) to determine the effect of species on legume hays. Duncan multiple range test was used to identify the differences between means. 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 legume hays were given in Table 1. Species had a significant effect on the chemical composition of legume hays. Crude ash content ranged from 5.99% to 11.37% with the highest being for $T. repens$ hay and lowest for $T. campestre$ hay. NDF ranged from 22.86% to 42.93% with the highest being for $T. purpureum$ hay and lowest for $T. foenum-graecum$ hay. Acid detergent fibre ranged from 20.24% to 37.16% with the highest being for $T. purpureum$ hay and lowest for $T. foenum-graecum$ hay. Crude protein contents of legume hays ranged from 13.61% to 20.55% with the highest being for $M. sativa$ hay and lowest for $T. purpureum$ hay. Ether extracts contents of legume hays ranged from 1.30% to 2.75% with the highest being for $T. campestre$ and lowest for $T. incarnatum$.

Crude ash, EE and CP content of $T. repens, V. sativa, M. sativa, T. incarnatum$ and $M. lupulina$ hays were similar to those reported by Kiraz (2011) whereas NDF and AF contents of $T. repens, V. sativa, M. sativa, T. incarnatum$ and $M. lupulina$ hays were lower than those reported by Kiraz (2011). The differences between two studies in terms of cell wall contents may be associated with growing conditions and maturity at harvest. In the current experiment legume hays were obtained in late March whereas Kiraz (2011) harvest the legume hays in late May. Therefore, the lower cell wall contents of $T. repens, V. sativa, M. sativa, T. incarnatum$ and $M. lupulina$ is reasonable when compared with those Kiraz (2011). Kaplan et al. (2014) showed that cell wall contents increased with increasing maturity. It was also reported that temperature at growing areas has a significant effect on the NDF content of forages. Even at the same maturity, NDF contents of forages will be high when growth takes place at high temperature, compared with cool temperature (Linn and Martin, 1999). The CP, EE, CA and ADF contents of $M. sativa$ were also similar to those reported by Gungor et al. (2008). The chemical composition of $M. sativa$ was also comparable with that reported by Boga et al. (2014).

The CP in forages is one of the very important nutrients. The level of CP of diet affect the animal performance since CP content of diet should be higher than 8% of DM to meet maintenance requirement (Norton 1994). The intake of forage is limited when CP content of forages is less than 10% (Raanjhman 2001). As can be seen from Table 1 all of the legume hays studied contain reasonable content of CP and can be used as a supplement for poor quality forages to improve the productivity of ruminant animals.

There is wide variation in chemical composition of legume plants studied in the current experiment even if they were grown in the same environmental conditions and harvested at the similar maturity. The differences in chemical composition among legume species can be explained by the inherent characteristics of each legume species associated with the ability to extract and accumulate nutrients from the soil and fix nitrogen from the atmosphere (Yusuf and Muritala 2013). There would be some differences in leaf: stem ratio among legume plants studied in the current experiment. These differences in leaf: steam ratio might have resulted in differences in

Table 1. The effect of species on the chemical composition of legume plants.

| Species          | DM (%) | CA (%) | NDF (%) | ADF (%) | CP (%) | EE (%) |
|------------------|--------|--------|---------|---------|--------|--------|
| $V. narbonensis$ | 18.51   | 8.27   | 42.58   | 32.16   | 15.34  | 2.15   |
| $M. sativa$      | 18.65   | 9.96   | 35.50   | 26.65   | 20.55  | 2.26   |
| $L. sativus$     | 19.37   | 8.34   | 37.64   | 24.83   | 21.51  | 2.00   |
| $M. orbitalis$   | 18.92   | 8.11   | 35.44   | 26.65   | 13.76  | 2.33   |
| $V. sativa$      | 23.61   | 9.26   | 31.54   | 27.69   | 19.91  | 2.66   |
| $T. kotschyi$    | 19.49   | 10.35  | 30.70   | 29.86   | 14.82  | 2.53   |
| $M. lupulina$    | 18.31   | 10.82  | 32.35   | 22.57   | 16.54  | 2.46   |
| $T. repens$      | 21.50   | 11.37  | 26.67   | 21.51   | 17.29  | 1.41   |
| $T. foenum-graecum$ | 25.27  | 9.86   | 22.86   | 20.24   | 19.70  | 1.49   |
| $T. incarnatum$  | 16.67   | 10.30  | 33.46   | 24.43   | 15.93  | 1.30   |
| $T. sipicate$    | 16.02   | 9.52   | 34.24   | 33.43   | 15.76  | 1.57   |
| $T. campestre$   | 15.40   | 5.99   | 34.73   | 25.77   | 14.89  | 2.75   |
| $T. purpureum$   | 17.54   | 8.27   | 42.93   | 37.16   | 13.61  | 2.32   |
| $P$              | 1.816   | 0.287  | 0.998   | 0.913   | 0.441  | 0.208  |

Notes: *a, b, c* Column means with common superscripts do not differ ($P > 0.05$); **SEM**: standard error mean; **DM**: Dry matter(%); **CA**: Crude ash (%); **NDF**: Neutral detergent fibre (%); **ADF**: Acid detergent fibre (%); **CP**: Crude protein (%); **EE**: Ether extract(%).
of legume hays. This result is in line with correlations between gas production and NDF or ADF contents. As can be seen from Table 3 there are negative compositional differences of legume hays, especially NDF and ADF contents such as tannin and saponin (Jayanegara et al. 2014; Kondo et al. 2014). However, the presence of secondary metabolites was not determined in the current experiment.

Percentage of methane production after 24 h anaerobic fermentation can be assessed to rank the feedstuffs in terms of anti-methanogenic potential. Generally the percentage methane of usual feeds such as hay, concentrate or a mixture of hay and concentrate range from 16% to 20%. The methane reduction potential can be categorized into three groups in terms of the methane reduction potential, namely low potential (% methane in gas between >11% and ≤14%) (Lopez et al. 2010). As can be seen from Table 2 the percentage of methane of legume hays studied in the current study ranged from 15.94% to 18.37%. Therefore the legume hays studied in the current study has no anti-methanogenic potential since their methane percentage fell into the range of 16–20% of usual feeds.

The metabolizable energy content of legume hays varied between 8.98 and 10.30 MJ/kg DM with the highest being for V. sativa hay and lowest for T. purpureum hay. OMD of legume hays varied between 64.21% and 72.79% with the highest being for T. repens hay and lowest for T. purpureum hay. Although cell contents of legume hays provide fibre to proper rumen function, it has a dilution effect on OMD and ME content of legume hays. As can be seen from Table 3 cell wall contents (NDF or ADF) are negatively correlated with OMD and ME content of legume hays. Therefore the fibre content after CP content of legume hays is the second important factor affecting the hay quality.

4. Conclusion

The species has a significant effect on the chemical composition and potential nutritive value of legume hays. It can be concluded that legume plant studied in the current experiment showed a wide nutritional diversity and will provide not only protein but also fibre for livestocks. They may exhibit complementary role for ruminant animals grazing on poor quality forages. Legume species studied in the current experiment had a no anti-methanogenic properties.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

AOAC. 2005. Official methods of analysis, 18th ed. Arlington, VA: Association of Official Analytical Chemists.

Table 2. The effect of species on the gas production, methane production, metabolizable energy and organic matter digestibility.

| Species                        | GP (ml) | CH₄ (ml) | CH₄ (%) | ME (%) | OMD (%) |
|-------------------------------|---------|----------|---------|--------|---------|
| Vicia narbonensis             | 45.02    | 7.65     | 17.00   | 9.41   | 67.17   |
| Medicago sativa,              | 44.93    | 8.23     | 18.32   | 9.76   | 70.54   |
| Lathyrus sativus              | 47.97    | 8.40     | 17.53   | 10.25  | 72.60   |
| Medicago angularis            | 51.42    | 8.66     | 16.85   | 10.25  | 72.06   |
| Vicia sativa                  | 48.29    | 8.44     | 17.49   | 10.30  | 72.79   |
| Trigonella kotschii           | 47.71    | 8.26     | 17.29   | 9.79   | 70.67   |
| Medicago lupulina             | 46.03    | 7.36     | 15.99   | 9.64   | 70.27   |
| Trifolium repens              | 49.33    | 8.26     | 16.80   | 9.92   | 73.91   |
| T. foemum-graecum             | 47.82    | 8.78     | 18.37   | 9.98   | 72.87   |
| Trifolium incarnatum          | 48.98    | 8.72     | 15.94   | 9.70   | 72.35   |
| Trigonella siphatic          | 48.30    | 8.77     | 18.15   | 9.70   | 71.00   |
| Trifolium campestus           | 49.47    | 8.44     | 17.07   | 10.21  | 69.45   |
| Trifolium purpureum           | 42.56    | 7.69     | 18.03   | 8.98   | 64.21   |
| SEM                           | 1.239    | 0.455    | 0.785   | 0.183  | 1.144   |
| P                             | <.001    | .072     | .058    | <.001  | <.001   |

Notes: GP: Gas production (ml); CH₄: Methane production; ME: Metabolizable energy (MJ/kg DM); OMD: Organic matter digestibility (%).

Table 3. Correlation coefficient (r) of relationship of chemical composition with in situ dry matter degradation or estimated parameters.

| Parameters | GP | CH₄ (ml) | CH₄ (%) | ME | OMD |
|------------|----|----------|---------|----|-----|
| DM         | 0.145 | 0.106 | 0.007 | 0.278 | 0.429 |
| CA         | −0.016 | −0.105 | −0.117 | −0.125 | −0.42 |
| NDF        | −0.453 | −0.352 | −0.030 | −0.439 | −0.704 |
| ADF        | −0.453 | −0.163 | 0.195 | 0.550 | −0.697 |
| CP         | −0.039 | 0.181 | 0.243 | 0.389 | 0.481 |
| EE         | −0.139 | −0.086 | 0.016 | 0.152 | −0.312 |

Notes: DM: Dry matter(%); CA: Crude ash (%); NDF: Neutral detergent fibre (%); ADF: Acid detergent fibre (%); CP: Crude protein (%); EE: Ether extract(%); GP: Gas production (ml); ME: Metabolizable energy(MJ/kg DM); OMD: Organic matter digestibility (%). NS: Non-significant. *P < .05; **P < .01; ***P < .001.
Arelovich HM, Abney CS, Vizcarra JA, Galyean M. 2008. Effects of dietary neutral detergent fiber on intakes of dry matter and net energy by dairy and beef cattle: analysis of published data. Prof Anim Sci. 24:375–383.

Blümmel M, Orskov ER. 1993. Comparison of in vitro gas production and nylon bag degradability of roughages in predicting of food intake in cattle. Anim Feed Sci Technol. 40:109–119.

Boga M, Yurtsen S, Kilic U, Aydemir S, Polat T. 2014. Determination of nutrient contents and in vitro gas production values of some legume forages grown in the Harran plain saline soils. Asian Australas J Anim Sci. 27(6):825–831.

Goel G, Makkar HPS, Becker K. 2008. Effect of Sesbania sesban and Carduus pycnocephalus seeds and their extract on partitioning of nutrients from roughage-and concentrate-based feeds to methane. Anim Feed Sci Technol. 147(1-3):72–89.

Gungor T, Basalan M, Aydogan I. 2008. The determination of nutrient contents and metabolisable energy levels of some roughages produced in Kirikkale region. Ankara Univ Vet Fak Der. 55:111–115.

Jayanegara A, Wina E, Takahashi J. 2014. Meta-analysis on methane mitigating properties of saponin-rich sources in the rumen in vitro: influence of addition levels and plant sources. Asian-Australasian J Anim Sci. 27:1426–1435.

Kamalak A, Canbolat O. 2010. Determination of nutritive value of wild narrow-leaved clover (Trifolium angustifolium) hay harvested at three maturity stages using chemical composition and in vitro gas production. Trop Grassland. 44:128–133.

Kamalak A, Canbolat O, Atalay AI, Kaplan M. 2010. Determination of potential nutritive value of young, old and senescent leaves of Arbutus andrachne tree. J App Anim Res. 37:257–260.

Kamalak A, Canbolat O, Gurbuz Y, Erol A, Ozay O. 2005. Effect of maturity stage on chemical composition, in vitro and in situ dry matter degradation of tumbleweed hay (Gundelia tournefortii L). Small Rum Res. 58 (2):149–156.

Kaplan M, Kamalak A, Kasra AA, Güven I. 2014. Effect of maturity stages on potential nutritive value, methane production and condensed tannin content of Sanguisorba minor. Kafkas Univ Vet Fak Der. 20 (3):445–449.

Kiraz AB. 2011. Determination of relative feed value of some legume hays harvested at flowering stage. Asian J Anim Vet Adv. 6(5):525–530.

Kondo M, Hirano Y, Ikai N, Kita K, Jayanegara A, Yokota H. 2014. Assessment of anti-nutritive activity of tannins in tea by-products based on in vitro ruminal fermentation. Asian-Aust J Anim Sci. 27:1571–1576.

Linn JG, Martin NP. 1999. Forage quality tests and interpretations. University of Minnesota Extension service, Food and Environmental science. Available at: www.extension.umn.edu/distribution/…/DI2637.html.

Lopez S, Makkar HPS, Soliva CR. 2010. Screening plants and plant products for methane inhibitors. In: Vercoe PE, Makkar HPS, Schlink A, editors. In vitro screening of plant resources for extra-nutritional attributes in ruminants: nuclear and related methodologies. London: Springer; p. 191–231.

Menke KH, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim Res Dev. 28:7-55.

Norton BW. 1994. Tree legumes and dietary supplements. In: Gutteridge RC and HM Shelton, editor(s). Forages tree legumes in tropical agriculture. Wallingford (Oxon): CAB International; pp. 192-201.

NRC. 1989. Nutrient requirements of dairy cattle, 6th rev. ed. Washington, (DC): National Academy Press.

Raanjhman SK. 2001. Animal nutrition in the tropics. 5th ed. New Delhi: Vikas Publishing House; p. 593.

Van Soest PJ. 1963. The use of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fiber and lignin. J Assoc Offic Anal Chem. 46: 829–835.

Van Soest PJ, Wine RH. 1967. The use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. J Assoc Offic Anal Chem. 50:50–55.

Yusuf AO, Muritala RO. 2013. Nutritional evaluation and phytochemical screening of common plants used in smallholder farming system. Pac J Sci Technol. 14(2):456–462.