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Original article

# Effect of species on nutritive value and anti-methanogenic potential of vetch hays grown in native pasture in Turkey

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**Summary**. The aim of the current experiment was to determine the effect of species on chemical composition, *in vitro* gas production, methane production, metabolizable energy (ME), organic matter digestibility (OMD), true substrate digestibility (TSD), partitioning factor (PF), microbial protein yield (MPY) and efficiency of microbial protein yield (EMP) of vetches grown in native pasture in Turkey. The species had a significant effect on the chemical composition, *in vitro* gas production, methane production, ME, OMD, TSD, PF, MPY and EMPY of vetch hays. Crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), gas production, methane production, ME, OMD, PF, MPY, EMPY and TSD ranged from 17.2 to 26.6 %, 41.3 to 61.5 %, 23.3 to 39.2 %, 89.5 to 125.0 ml, 14.6 to 19.4 ml, 7.8 to 10.0 MJ (kg /DM), 60.2 to 74.1 %, 3.2 to 4.0, 107.1 to 173.5 mg, 31.9 to 44.2 % and 60.4 to 82.1 % respectively. There is considerable amount of variation among vetch hay samples in terms of chemical compositions, gas production, CH4 production, MPY, EMPY and the other estimated parameters such as ME and OMD. The vetch hays studied in the current experiment have a potential to meet the CP, ME and fiber requirement of ruminant animals for growth and lactation. Based on the chemical composition and fermentation parameters, *Vicia villosa* can be recommended for hay production since it has a high CP, MPY and EMPY. However, before large implication the biomass yield of vetch species should be tested.

**Key words:** Chemical composition, digestibility, methane production, microbial protein yield, partitioning factor, vetches

## Introduction

In Turkey, the most of ruminant animals meet their requirement from natural pastures which consist of legumes, grasses and other species. However, the lack of information on the nutritive value of plants in pasture prevents to make sound decision on maintaining high quality pasture for ruminant animals during grazing. Protein is one of the important limiting nutrients for the growth and milk production of ruminant animals. Legumes species with high protein content have a high potential to overcome this limitation during grazing in pasture (1). Vetch is annual forage legume widely adapted to the most parts of world providing with protein with cheaper price compared to concentrates especially in developing countries (2). Although previous studies were conducted mainly on the adaptation and biomass yield of vetch species, there is limited information on the chemical composition, fermentation parameters, methane production, microbial protein synthesis and relationship between chemical composition and fermentation parameters. Recently chemical analysis in combination with *in vitro* gas production technique has been used to evaluate the

potential nutritive value and anti-methanogenic potential of previously less investigated plant species (3-7). The aim of the current experiment was to determine effect of species on chemical composition, *in vitro* gas production, methane production, TSD, PF, MPY and EMP and the relationship between chemical composition and fermentation parameters of vetches grown in native pasture in Turkey.

## Materials and methods

*Collection of vetch samples grown in native pasture*

Vetch species (*Vicia hybrida, Vicia pregrina, Vicia narbonensis, Vicia pannonica, Vicia sativa, Vicia lutea, Vicia villosa, Vicia lens, Vicia sylvatica* and *Vicia tenuifolia*) were harvested at flowering stage in Kahramanmaras, Turkey in 2019 and dried in shade. After drying, samples of vetches were ground to pass through 1 mm sieve size and transferred into nylon bags for subsequent chemical analysis and *in vitro* gas production.

*Chemical analysis of vetch samples*

Dry matter, crude ash, crude protein and ether extract of vetch hay samples were analyzed using the method of AOAC (8). NDF and ADF contents of vetches were analyzed with the method suggested by Van Soest et al. (9).

*Determination of gas and methane production of vetch hay samples*

*In vitro* gas production technique (10) was employed to determine the gas production, methane production, true dry matter digestibility of vetch hays, partitioning factor, microbial protein yield and efficiency of microbial protein synthesis. Three fistulated Awassi sheeps were fed with alfalfa hay (800g) and barley (400g) to obtain rumen fluid for *in vitro* fermentation. The rumen fluid obtained from three fistulated Awassi sheeps were transferred with thermo flask to laboratory, then filtered with four layered cheesecloth under flushing with $CO_2$. Approximately 500 mg vetch samples were weighted into 100 ml of glass syringes in quadruplicate. Then 40 ml of the buffered rumen fluid (1:2 V/V) was added into glass syringes containing vetch samples and transferred in water bath set at 39 °C for 24 h incubation. The same amount of the buffered rumen fluid was added into four glass syringes without substrate to obtain the blanks. The gas and $CH_4$ production of GP samples were measured after 24 h incubation. The percentages of $CH_4$ of total gas production of vetch samples after 24 h incubation were determined using infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) (11). The methane productions of vetch hay samples as mL were calculated as follows:

$CH_4$ production (ml) = Total gas production (ml) X Percentage of $CH_4$ (%)

The metabolizable energy and organic matter digestibility of vetch hay samples were estimated with equations suggested by Menke and Steingass (12)

ME (MJ/kg DM) = 0.72 + 0.1559GP + 0.068CP + 0.249EE
OMD (%) = 15.38 + 0.8453GP + 0.595CP + 0.675CA
GP: gas production of 200 mg sample at 24 h incubation (ml)
CP: Crude protein (%)
EE: Ether extract (%)
CA: Crude ash (%)

*Determination of truly degraded substrate*

After gas and methane measurements, the contents of the syringes were completely transferred into a beaker by using 50 ml of NDF solution and boiled for 1 h and the residue was filtered through a pre-weighed sintered glass crucible. The crucibles containing residues of unfermented vetch samples were transferred into oven set 65 °C for overnight. After drying TDS, PF, MPY and EMPY were calculated using the equations suggested by Blummel et al. (13) as follows.

TDS (mg) = Substrate incubated (mg) – the residue (mg).
PF = (TDS / Gas Production)
MPY (mg) = (TDS – (2.2 x gas production))
EMPY = ((TDS – (2.2 x gas production))/TDS)*100

The *in vitro* experimental protocols were approved by the Animal Experimentation Ethics Committee of University of Kahramanmaras Sutcu Imam, Faculty of Agriculture (Protocol No: 2020/03-04).

## Statistical analyses

One-way analysis of variance (ANOVA) was used to determine the effect of species on chemical composition, *in vitro* gas production, methane production, ME, OMD, TSD, PF, MPY and EMPY of vetches. Differences (P<0.05) among the mean of vetch species were determined with Tukey's multiple range tests.

## Results and discussion

*Effect of species on the chemical composition of vetch hays*

The effect of species on the chemical composition of vetch hays grown in native pasture in Turkey is presented in Table 1. The species had a significant effect on the chemical composition of vetch hays.

Crude ash contents of hays ranged from 6.3 to 10.1 %. The crude ash contents of hay obtained from *Vicia hybrida* and *Vicia pannonica* were significantly higher than the other. Crude protein content of vetch hays ranged from 17.1 to 26.6 %. Crude protein contents of hays from *Vicia narbonensis* and *Vicia villosa* were significantly higher than the others. Generally ruminant animals require forages with CP contents of 10.0 to 17.0 % of DM (1, 14, 15). As can be seen from Table 1, vetch hays studied in the current experiment tend to be at the upper, end of or above this range.

Ether extract of vetch hays ranged from 0.7 to 2.3 %. Ether extract of hay from Vicia villosa was significantly higher than those for *Vicia tenuifolia, Vicia lens, Vicia lutea, Vicia sativa* and *Vicia narbonensis*. NDF and ADF contents of vetch hay ranged from 41.3 to 61.5 % and 23.3 to 39.2 % respectively. NDF and ADF contents of hay from *Vicia tenuifolia* were significantly higher than the others. The CA, CP, NDF and ADF contents of *Vicia sativa*, *Vicia villosa* and *Vicia narbonensis* were similar to those reported by Gezahagn et al. (16) who showed that species and their accessions had a great effect on the chemical composition. The CP content of *Vicia narbonensis*, *Vicia pregrina* were

**Table 1.** Effect of species on chemical composition of vetch hays grown in native pasture

| Species | DM | CA | CP | EE | NDF | ADF |
|---|---|---|---|---|---|---|
| *Vicia hybrida* | 21.1$^{d}$ | 10.1$^{a}$ | 18.9$^{e}$ | 2.0$^{ab}$ | 52.5$^{b}$ | 28.3$^{cd}$ |
| *Vicia narbonensis* | 27.1$^{c}$ | 7.5$^{c}$ | 23.2$^{b}$ | 1.7$^{bcd}$ | 50.3$^{bc}$ | 29.4$^{c}$ |
| *Vicia pregrina* | 20.4$^{de}$ | 9.2$^{b}$ | 21.9$^{cd}$ | 1.9$^{abc}$ | 46.8$^{bcd}$ | 26.5$^{de}$ |
| *Vicia pannonica* | 20.2$^{de}$ | 10.1$^{a}$ | 22.4$^{c}$ | 2.0$^{ab}$ | 46.1$^{bcd}$ | 26.0$^{e}$ |
| *Vicia sativa* | 26.4$^{c}$ | 7.6$^{c}$ | 17.5$^{f}$ | 0.7$^{e}$ | 48.6$^{bcd}$ | 26.0$^{e}$ |
| *Vicia lutea* | 20.9$^{d}$ | 7.7$^{c}$ | 17.1$^{f}$ | 1.3$^{dce}$ | 41.3$^{d}$ | 25.0$^{ef}$ |
| *Vicia villosa* | 16.7$^{e}$ | 9.6$^{ab}$ | 26.6$^{a}$ | 2.0$^{ab}$ | 42.2$^{d}$ | 25.4$^{ef}$ |
| *Vicia lens* | 30.8$^{b}$ | 7.6$^{c}$ | 17.7$^{f}$ | 0.8$^{e}$ | 42.8$^{cd}$ | 23.3$^{f}$ |
| *Vicia sylvatica* | 16.0$^{e}$ | 9.3$^{a}$ | 21.1$^{d}$ | 2.3$^{a}$ | 52.8$^{b}$ | 32.4$^{b}$ |
| *Vicia tenuifolia* | 35.8$^{a}$ | 6.3$^{d}$ | 17.2$^{f}$ | 1.2$^{de}$ | 61.5$^{a}$ | 39.2$^{a}$ |
| SEM | 0.819 | 0.190 | 0.234 | 0.170 | 2.159 | 0.622 |
| Sig. | *** | *** | *** | *** | *** | *** |

$^{a\,b\,c}$ *Column means with common superscripts do not differ (P>0.05). SEM: standard error mean. DM: Dry matter (%), CA: Crude Ash (% of DM), CP: Crude protein (% of DM), EE: Ether extract (% of DM), ADF: Acid detergent fiber (% of DM), NDF: Neutral detergent fiber (% of DM), *** P<0.00.*

similar to those reported by Basbag et al. (17) whereas NDF and ADF contents of *Vicia narbonensis* and *Vicia pregrina* were considerable higher than those reported by Basbag et al. (17). The differences obtained in two studies are possibly associated with differences in their accessions where vetches grow. The CP content of *Vicia sativa* was considerably lower than that obtained by Basbag et al. (17) whereas the NDF and ADF content were significantly higher than those obtained by Basbag et al. (17).

**Effect of species on gas, methane, metabolizable energy, digestibility, partitioning factor and microbial yield of vetch hays**

Effect of species on gas, methane, metabolizable energy, digestibility, partitioning factor and microbial yield of vetch hays was given in Table 2. Species had a significant effect on gas, methane, ME, OMD, TSD, PF, MPY and EMPY of vetch hays. Gas and methane production ranged from 89.5 to 125.0 ml, 14.6 to 19.4 ml. The gas production of hay from *Vicia lutea* was significantly higher than the others. The methane production of has from *Vicia sylvatica* and *Vicia tenuifolia* were significantly lower than those for *Vicia lens, Vicia lutea, Vicia sativa, Vicia pannonica* and *Vicia pregrina.*

The percentage of $CH_4$ of vetch hays ranged from 14.4 to 16.4 %. Lopez et al (18) suggested that feedstuffs may have an anti-methanogenic potential if percentage of $CH_4$ of gas produced after 24 h incubation is lower than 14 %. As can be seen from Table 2, the percentage of $CH_4$ of vetch hays was higher than 14 %. Therefore, vetch hays studied in the current experiment is not likely to have an anti-methanogenic potential.

Metabolizable energy contents of hays ranged from 7.8 to 10.0 MJ (kg /DM). Metabolizable energy content of *Vicia lutea* was significantly higher than those for *Vicia hybrida, Vicaia narbonensis, Vicia sativa, Vicia sylvatica* and *Vicia tenuifolia.*

Organic matter digestibility of vetch hays ranged from 60.2 to 74.1 %. Except for *Vicia pregrina* and *Vicia lutea*, OMD of hays from *Vicia pannonica* and *Vicia villosa* were significantly higher than the others. True digestible substrate of vetch hays ranged from 304.0 to 414.5 mg. Except for *Vicia pregrina, Vicia pannonica* and *Vicia villosa,* the TDS of hay from *Vicia lutea* was significantly higher than the others.

GP, $CH_4$, ME and OMD values of *Vicia narbonensis* and *Vicia sativa* were similar to those obtained by Uslu et al (7) who collected the vetch hay samples from similar natural pasture. On the other hand, ME and OMD of *Vicia villosa* hay was considerable higher than those reported by Gürsoy and Macit (19) but lower than those reported by Canbolat and Karaman (20). The differences in ME and OMD values are possible associated with chemical composition of *Vicia villosa* hay. The NDF and ADF contents of *Vicia villosa* hay obtained current experiment was lower than those reported by Gürsoy and Macit (19) but only NDF content was lower than that reported by Canbolat and Karaman (20). It is well known that cell wall contents of hays are generally negatively associated with ME and OMD of hays.

Partitioning factors of vetch hays ranged from 3.2 to 4.0. Except for *Vicia villosa,* the PF of *Vicia sylvatica* was significantly higher than the others. Microbial yield of vetch hays ranged from 107.1 to 173.5 mg. Except for *Vicia narbonensis*, *Vicia pregrina* and *Vicia sylvatica,* MY of hay from *Vicia villosa* was significantly higher than the others. Efficiency of microbial yield ranged from 31.9 to 44.2 %. Except for *Vicia villosa*, EMY of hay from *Vicia sylvatica* was significantly higher than the others. True digestibility of vetch hays ranged from 60.4 to 82.1 %. Except for *Vicia pregrina* and *Vicia pannonica,* TD of hay from *Vicia villosa* was significantly higher than the others.

Correlation coefficient (r) of relationship of chemical composition with gas, methane production and estimated parameters was given in Table 3. NDF and ADF contents of vetch hays were negatively correlated with GP, $CH_4$ (ml), ME, OMD and TDS. On the other hand, PF, MY and EMPY were positively correlated with CP and EE contents of vetch hays. It was reasonable that less GP and $CH_4$ production occurs when cell wall contents of feedstuffs increase at the expense of more fermentable substrate. Total GP and $CH_4$ production are associated with the amount of fermentable organic matter in feedstuffs during fermentation. The type of volatile fatty acid produced during fermentation also affects the GP and $CH_4$ production (21). The contribution of protein and fat in

**Table 2.** Effect of species on gas, methane, metabolizable energy, digestibility, partitioning factor and microbial yield of vetch hays

| Species | GP(ml) | CH4(ml) | CH4(%) | ME (MJ) | OMD(%) | TDS(mg) | PF | MPY(mg) | EMPY(%) | TSD(%) |
|---|---|---|---|---|---|---|---|---|---|---|
| *Vicia hybrida* | 108.2d | 16.8ab | 15.6 | 9.2d | 70.0d | 356.7d | 3.3ef | 119.1de | 33.3ef | 71.0e |
| *Vicia narbonensis* | 107.0d | 17.4ab | 16.2 | 9.4d | 70.4d | 390.8bc | 3.6bc | 155.4abc | 39.7bc | 77.4cd |
| *Vicia pregrina* | 114.2c | 18.0a | 15.8 | 9.8ab | 73.2ab | 406.8abc | 3.6bcd | 157.1abc | 38.6bcd | 80.6abc |
| *Vicia pannonica* | 114,2c | 18.7a | 16.4 | 9.9ab | 74.1a | 402.5abc | 3.5bcd | 151.7bc | 37.6cde | 79.5abcd |
| *Vicia sativa* | 118.6c | 19.4a | 16.4 | 9.5cde | 71.0cd | 387.9c | 3.3f | 127.7de | 32.9f | 76.3d |
| *Vicia lutea* | 125.0a | 17.9a | 14.4 | 10.0a | 73.0ab | 414.5a | 3.3ef | 138.9cd | 33.5ef | 82.1a |
| *Vicia villosa* | 107.6d | 17.2ab | 16.0 | 9.7abc | 74.0a | 409.5ab | 3.8ab | 173.5a | 42.3ab | 81.7ab |
| *Vicia lens* | 120.8ab | 19.4a | 16.1 | 9.7abc | 71.9bc | 393.1c | 3.2f | 125.8de | 31.9f | 77.9bcd |
| *Vicia sylvatica* | 92.4e | 14.9b | 16.2 | 8.5f | 65.5e | 363.3d | 4.0a | 160.9ab | 44.2a | 71.8e |
| *Vicia tenuifolia* | 89.5e | 14.6b | 16.2 | 7.8g | 60.2f | 304.0e | 3.4def | 107.1e | 35.2def | 60.4f |
| SEM | 1.480 | 0.852 | 0.704 | 0.091 | 0.500 | 5.938 | 0.070 | 6.410 | 1.261 | 1.155 |
| Sig. | *** | *** | NS | *** | *** | *** | *** | *** | *** | *** |

[a b c] *Column means with common superscripts do not differ (P>0.05). SEM: Standard error mean, GP: Gas production (ml), $CH_4$: Methane emission (ml), ME: Metabolizable energy (MJ / kg DM), OMD: Organic matter digestibility (%), TDS: True digestible substrate (mg), PF: Partitioning factor, MPY: Microbial protein yield (mg), EMPY: Efficiency of microbial protein yield(%), TSD: True substrate digestibility (%) NS: Not significant, *** P<0.00*

**Table 3.** Correlation coefficient (r) of relationship of chemical composition with gas, methane production and estimated parameters

| | DM | CA | CP | EE | NDF | ADF |
|---|---|---|---|---|---|---|
| GP | -0.109NS | 0.062 NS | -0.168 NS | -0.418 NS | -0.824** | -0.898** |
| CH4 (ml) | 0.001 NS | 0.075 NS | -0.052 NS | -0.458 NS | -0.733* | -0.870** |
| CH4 (%) | 0.237 NS | 0.030 NS | 0.292 NS | 0.018 NS | 0.384 NS | 0259 NS |
| ME | -0.415 NS | 0.371 NS | 0.257 NS | 0.042 NS | -0.918** | -0.951** |
| OMD | -0.510 NS | 0.498 NS | 0,392 NS | 0.079 NS | -0.906** | -0.944** |
| TDS | 0.180 NS | -0.255 NS | 0.323 NS | 0.072 NS | 0.075 NS | 0.067 NS |
| PF | -0.607 NS | -0.407 NS | 0.729* | 0.753* | 0.089 NS | 0.242 NS |
| MY | -0.752* | 0.527 NS | 0.865** | 0.632* | -0.489 NS | -0,343 NS |
| EMP | -0,581 NS | 0.400 NS | 0.798** | 0.735** | 0.059 NS | 0.223 NS |
| TD | -0.553 NS | 0.380 NS | 0.427 NS | 0.087 NS | -0.943** | -0.899** |

**DM:** Dry matter (%), **CA:** Crude Ash (% of DM), **CP:** Crude protein (% of DM), **EE:** Ether extract (% of DM), **ADF:** Acid detergent fiber (% of DM), **NDF:** Neutral detergent fiber (% of DM), **GP:** Gas production (ml), $CH_4$: Methane emission (ml), **$CH_4$:** Methane emission (%), **ME:** Metabolizable energy (MJ / kg DM), **OMD:** Organic matter digestibility (%), **NS:** Not significant, ** P<0.01, *P<0.05

feedstuffs is small or negligible when compared with carbohydrate fermentation. Therefore, in the current experiment there is no correlation between CP and EE with GP and $CH_4$ production.

As can seen from Table 2, PF of vetch hays in the current experiment ranged from 3.2 to 4.0 which fell into the theoretical range of 2.74 to 4.65 reported by Blummel et al. (13). The partitioning factor indicated the proportion of nutrients which goes to the gas production and microbial protein. Higher the PF value higher is the partitioning of substrate to microbial mass and higher is the efficiency of microbial mass. Therefore, PF were positively correlated with microbial yield and efficiency of microbial protein synthesis in the current experiment.

## Conclusion

There is considerable amount of variation among vetch hay samples in terms of chemical compositions, gas production, $CH_4$ production, MP, EMPY and the

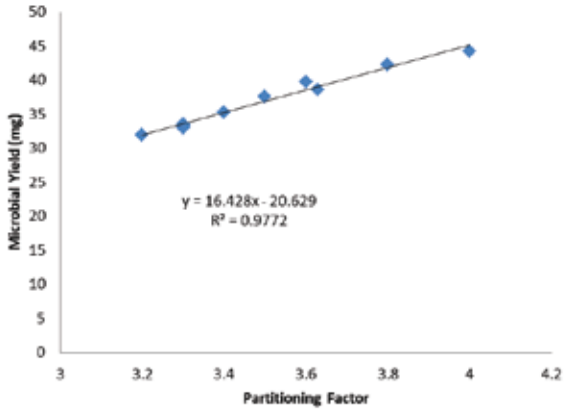


**Figure 1.** Relationship between partitioning factor and microbial yield

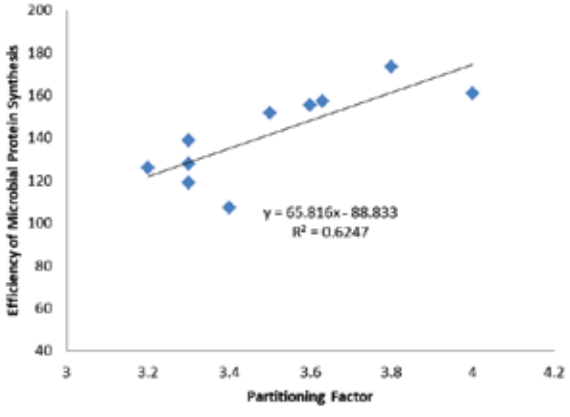


**Figure 2.** Relationship between partitioning factor and efficiency of microbial protein synthesis

other estimated parameters such as ME and OMD. The vetch hays studied in the current experiment have a potential to meet the CP, ME and fiber requirement of ruminant animals for growth and lactation. Based on the chemical composition and fermentation parameters, *Vicia villosa* can be recommended for hay production since it has a high CP, MPY and EMPY. However, before large implication the biomass yield of vetch species should be tested.

**Conflict of Interest Statement**

The author declares no conflicts of interest.

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