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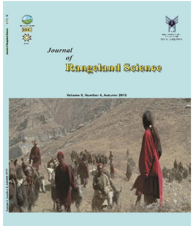

Research and Full Length Article:

# Comparing Mineral and Chemical Compounds, *in vitro* Gas Production and Fermentation Parameters of some Range Species in Torbat-e Jam, Iran

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**Abstract.** Plants growable in rangelands play an important role in the feeding of ruminants; hence, the nutritive value of four plants (*Falcaria vulgaris*, *Malva neglecta*, *Chenopodium album*, and *Polygonum aviculare*) was determined by different laboratory methods. The plant samples were randomly collected in vegetative phase from different rangelands of Torbat-e Jam, Iran in spring 2018. The range of dry matter (125 to 184 g/kg), neutral detergent fiber (252 to 358 g/kgDM), acid detergent fiber (155 to 258 g/kgDM), crude protein (172 to 275 g/kgDM), ether extract (9 to 41 g/kgDM), crude fiber (135 to 185 g/kgDM), ash (140 to 252 g/kgDM), acid detergent lignin (41 to 123 g/kgDM), nitrogen-free extract (300 to 496 g/kgDM), and non-fiber carbohydrates (183 to 356 g/kgDM) were different between the studied plants. The mineral composition was also different between treatments and they were containing reasonable minerals as compared to some other plants commonly used as forage feed. The highest *in vitro* Organic Matter Digestibility (OMD: 876 g/kgDM) and *in vitro* Dry Matter Digestibility (DMD: 828 g/kgDM) were observed in *Polygonum aviculare*. The other fermentation parameters ($NH_3$-N, total volatile fatty acids: TVFA, and pH) were also different among the plant species when incubated in the laboratory medium. There was a strong positive correlation between 24 h gas production with OMD, DMD and TVFA and negative correlation between 24 h gas production with crude protein, $NH_3$-N and ether extract. The results showed that each of the four studied plants can be considered as a potential source of feedstuff for the alleviation of problems associated with lack of forage in Iran. According to these reported data, it seems that the nutritional value of *Falcaria vulgaris* and *Polygonum aviculare* is higher than the other two plants.

**Key words:** Forage, Nutritive value, Laboratory methods, Ruminants

## Introduction

Traditionally, more than 60% of the ruminant feeds in Iran come from annual forages, rangelands, and pastures. On the other hand, there were a lot of range plants that their nutritional value is still unknown to most animal husbandries. Several studies have shown that range plants have a high potential nutritional value for ruminants (Kazemi *et al.,* 2009; Kazemi *et al.,* 2012; Ezzat *et al.,* 2018; Dehghani Bidgoli, 2018). Dry matter and organic matter digestibility of some range plants were determined by other researchers (Shadnoush, 2014; Naseri *et al.,* 2017). Compared to the *in vitro* techniques, measurement of digestibility of forage according to *in vivo* procedure is time-consuming and more expensive, thus replacing *in vivo* with other common laboratory methods (*in vitro*) can be more efficient and less expensive. Less-costly *In Vitro* Gas Production (IVGP) and the ANKOM methods can be used as a rapid evaluation to assess the nutritional value of forages (Getachew *et al.,* 2004). Many researchers have used the IVGP technique to evaluate the nutritional value of feeds and forages (Al-Masri, 2009; Abdalla *et al.,* 2012). The total surface area of Iran is about 1.6×106 $km^2$, which is situated within the dry belt of Asia and it has a rich botanical flora (Noroozi *et al.,* 2008). Many of these plants are traditionally used as forage feed for ruminants. *Falcaria vulgaris* (with a local name of Ghazzyaghi or Poghazeh), a member of *Umbelliferae* family, grows like a weed forage in some zones of Iran (Khazaei and Salehi, 2006). Turan *et al.* (2003) reported that *Falcaria vulgaris* is rich in minerals (phosphorus, magnesium, calcium, sulfur, and sodium) and ash. *Malva neglecta* (*Malvaceae* family) is mostly used as a medicinal plant to cure common cold in Iran (Seyyednejad *et al.,* 2010). The amount of zinc (as an immune system booster) available in *Malva neglecta* is abundant (Turan *et al.,* 2003). *Chenopodium album* is an annually weed plant which belongs to the *Amaranthaceae* (Adedapo *et al.*, 2011). Both young shoots and mature parts of *Chenopodium album* are rich in major minerals (Gqaza *et al.,* 2013). It has been reported that the leaves of *Chenopodium album* have an acceptable percentage of protein, carbohydrates, minerals, and fiber; and its toxic substances are low (Adedapo *et al.*, 2011). *Polygonum aviculare* (*Polygonaceae* family) is an annual plant also grazed by livestock in Iran. Forage plants belonging to *Polygonaceae* family are known to produce a lot of secondary metabolites such as steroids, anthraquinones, alkaloids, and flavonoids (Marjorie, 1999). Due to lack of sufficient information about the nutritional value of the four range plants (*Polygonum aviculare*, *Chenopodium album*, *Falcaria vulgaris* and *Malva neglecta*), several laboratory methods are useful in collecting the formulation of ruminant diet, hence the aim of this experiment was to determine the mineral and chemical compounds, dry matter and organic matter digestibility, and several fermentation parameters. The relationship between IVGP parameters, *in vitro* dry matter digestibility (DMD) and *in vitro* organic matter digestibility (OMD) as well as relationships among some chemical composition, IVGP, $NH_3$-N, and TVFA were also determined.

## Materials and Methods

### Plant sampling, chemical and mineral compositions

The plants samples (*Polygonum aviculare, Chenopodium album, Falcaria vulgaris,* and *Malva neglecta)* were randomly collected in vegetative phase from natural rangelands of Torbat-e Jam (Iran) in spring 2018. Whole samples of plants after collecting were immediately transferred to the laboratory, dried in an oven (Behdad Co.) at 60° C for 48 h, ground through a 1-mm mesh screen in a Wiley Mill and were used for chemical analysis, *in vitro* gas production (IVGP), *in vitro* organic matter digestibility (OMD) and *in vitro* dry matter

(DMD) digestibility (Getachew *et al.*, 2004). The Kjeldahl method (AOAC, 1999, ID 984.13) is applied to crude protein (CP) determination. For Dry Matter (DM) determination, a sample of each plant was oven dried at 135◦C for 4 h (AOAC, 1999, ID 930.5). The crude fiber (CF; Komarek *et al.*, 1996), Acid Detergent Lignin (ADL), Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) contents were measured by the ANKOM technology (ANKOM Technology, 2005; ANKOM Technology, 2006a; ANKOM Technology, 2006b) using solutions described by Van Soest *et al.* (1991). Fat content was determined using ether extraction in the Soxhlet device (AOAC, 1999, ID 954.02). The Markham device (1942) based on steam distillation was used for Total Volatile Fatty Acids (TVFA) determination according to the protocol described by Barnett and Reid (1957). The Non-Fiber Carbohydrate (NFC) content of samples was calculated by subtracting CP, NDF, fat, and ash from total DM (Sniffen *et al.*, 1992). The Nitrogen-Free Extract (NFE) was determined by Arshadullah *et al.* (2009) calculated as $\mathrm{NFE} = 100 - (\mathrm{CP} + \mathrm{EE} + \mathrm{Ash} + \mathrm{CF})$. Some of the minerals (S, Mg, Fe, and Zn) were measured by atomic absorption spectrometry (AOAC, 1990). Phosphorus was determined using the molybdate-vanadate method (spectrophotometer). Other macro elements (Calcium, Sodium, and potassium) were measured by a flame photometer.

### *In vitro* gas production, DMD and OMD

Rumen fluid was collected before the morning feed from three fistulated Baluchi male sheep fed by alfalfa hay and corn silage twice (7:30 and 18:30 h) a day. The taken rumen fluid via fistula was filtered through four layers of cheesecloth, flushed with $CO_2$, transferred into pre-warmed thermos flask and then transferred to the laboratory for the next experiments. Plant samples of 200 mg were weighed into 120 ml glass bottles. Before filling the bottles, the buffered-mineral solution was kept in a water bath at 39 °C under anaerobic conditions with $CO_2$ infusion. By the use of a dispenser, the glass bottles were filled with rumen fluid and artificial saliva solution (V: V, 1:2) prepared (30 ml) by Menke and Steingass (1988) procedure. Afterward, each glass bottle was plumped with rubber and aluminum caps, then gently shaken and placed in a water bath at 39 ◦C. The pressure and volume of gas production were recorded simultaneously at 0, 3, 6, 9, 12, 24, 48, 72, 96 and 120 h of incubation (Theodorou *et al.*, 1994). The gas produced during 24 h incubation was used to estimate the Metabolizable Energy (ME) and Net Energy for Lactation (NEL) according to equations of Menke and Steingass (1988). A medium similar to one developed for gas production was used to measure TVFA, pH, and $NH_3$-N. About 10 ml from bottle content were centrifuged at 1000×g for 20 min. Also, 5ml of the supernatant was transferred into a 10 ml plastic tube containing 1 ml of 25% metaphosphoric acid, centrifuged at 1000×g and then conserved in 18 °C until VFA determination (Getachew *et al.*, 2004). The pH of the media was measured by a pH meter after 24 h incubation. After 24 h incubation, the contents of each glass bottle were discharged, and strained through four layers of cheesecloth and then 10 ml of strained rumen fluid was acidified by 10 ml of 0.2 N HCl for determination of $NH_3$-N using the distillation method (Komolong *et al.*, 2001). The technology of ANKOM (F57 filter bag) was used for determination of DMD and OMD (Getachew *et al.*, 2004). Before incubation, the polyester bags were rinsed with acetone and then oven-dried at 60 ◦C for 48 h. The amount of 500 mg sample was transferred to each bag (50 mm×55 mm) heat sealed and then inserted in the incubation jars. After 24 h incubation, the bags were removed, rinsed with distilled water and oven-dried at 60 °C for 48 h.

The residual sample in each bag was used for OMD. The protocol of Menke and steingass (1988) was used to the preparation of the incubation media.

## Gas test equation and statistical analysis

All data were subjected to one-way analysis of variance using the GLM of SAS (2002). Duncan's multiple-range test was used to compare means (Kazemi *et al.*, 2012). Correlation coefficient among chemical composition, IVGP parameters and other fermentation parameters was calculated using SAS (2002). Constant rate (c) and potential gas production (b) was determined for each plant by fitting gas production data to the nonlinear equation $Y = b(1 - e^{-ct})$ (Ørskov and Mcdonald, 1979) where Y is the volume of gas produced at time t, b is the potential gas production (ml/200 mg DM), and c is the constant rate of gas production (ml/h).

## Results

## Chemical compound

The results indicated that the chemical compositions of four plants were different. The highest ADF (258 g/kgDM), NFC (356 g/kgDM), ADL (123 g/kgDM) and NFE (496 g/kgDM) also belonged to *Falcaria vulgaris* and the lowest NDF (252 g/kgDM) and ADF (155 g/kgDM) were both related to *Chenopodium album*. The highest CP (275 g/kgDM) and EE (41 g/kgDM) were also related to *Chenopodium album* (Table 1).

**Table 1.** Dry matter (g/kg) and chemical compound (g/kgDM) of Four different plants

| Plant | DM | NDF | ADF | CP | EE | CF | Ash | ADL | NFE | NFC |
|---|---|---|---|---|---|---|---|---|---|---|
| *Falcaria vulgaris* | 140[c] | 279[c] | 258[a] | 172[d] | 24[b] | 168[ab] | 140[c] | 123[a] | 496[a] | 356[a] |
| *Malva neglecta* | 184[a] | 307[b] | 237[b] | 225[b] | 15[c] | 144[bc] | 252[a] | 114[ab] | 364[c] | 201[c] |
| *Chenopodium album* | 125[c] | 252[d] | 155[c] | 275[a] | 41[a] | 135[c] | 249[a] | 41[c] | 300[d] | 183[d] |
| *Polygonum aviculare* | 164[b] | 358[a] | 256[a] | 186[c] | 9[c] | 185[a] | 191[b] | 81[b] | 429[b] | 255[b] |
| SEM | 5.22 | 2.98 | 2.05 | 2.00 | 2.84 | 7.77 | 2.71 | 6.16 | 5.20 | 5.06 |
| P value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.0002 | 0.007 | <0.0001 | 0.004 | <0.0001 | <0.0001 |

Means within columns followed by the different letter are significantly different (P<0.05).
DM: dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: Crude Protein; EE: Ether Extract; CF: Crude Fiber; ADL: acid detergent lignin; NFE: nitrogen-free extract; NFC: non-fiber carbohydrate.

## Mineral compound

The highest and the lowest Ca were related to *Malva neglecta* (355.4 mg/100gDM) and *Chenopodium album* (30.1 mg/100gDM), respectively (Table 2).

**Table 2.** Mineral compound of four different plants (mg/100gDM)

| Plant | Ca | P | K | Mg | Na | S | Fe | Zn |
|---|---|---|---|---|---|---|---|---|
| *Falcaria vulgaris* | 48.5[c] | 45.1[b] | 881.7[b] | 151.7[b] | 7.1[c] | 61.0[a] | 6.4[c] | 2.3[c] |
| *Malva neglecta* | 355.4[a] | 9.1[d] | 657.2[c] | 196.0[a] | 49.3[a] | 45.3[b] | 9.9[b] | 3.6[b] |
| *Chenopodium album* | 30.1[d] | 17.8[c] | 333.0[d] | 20.57[d] | 11.8[b] | 47.3[b] | 2.3[d] | 0.5[d] |
| *Polygonum aviculare* | 318.2[b] | 171.7[a] | 1635.0[a] | 96.8[c] | 7.3[c] | 24.8[c] | 67.7[a] | 4.8[a] |
| SEM | 2.7 | 3.2 | 7.7 | 3.0 | 0.6 | 1.6 | 0.7 | 0.1 |
| P value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Means, within columns, followed by the different letter are significantly different (P<0.05).
Ca: Calcium; P: Phosphorus; K: Potassium; Mg: magnesium; Na: Sodium; S: Sulfur; Fe: Iron; Zn: Zinc.

The amount of P, K, Fe, and Zn in *Polygonum aviculare* (171.7, 1635.0, 67.7, 4.8 mg/100gDM, respectively) was the highest; however, *Malva neglecta* had higher Mg (196.0 mg/100gDM) and Na (49.3 mg/100gDM) contents than other plants. The amount of S for *Falcaria vulgaris* (61 mg/100gDM) was also higher than other plants.

## *In vitro* gas test and other fermentation parameters

The IVGP after 24, 48 and 72 h of incubation as well as potential gas production (bgas) and constant rate of gas production (cgas) changed when four different plants were incubated in the media (Table 3). Despite having a higher bgas, gas12, 24, 48 and 72 h compared to other plats, there was also no significant difference for these parameters between *Falcaria vulgaris* and *Polygonum aviculare*.

**Table 3**. The cumulative gas production after 12, 24, 48 and 72 h of incubation and estimated gas (b and c) parameters

| Plant | bgas (ml/200mgDM) | cgas (ml/h) | gas 12 h (ml/200mgDM) | gas 24 h (ml/200mgDM) | gas 48 h (ml/200mgDM) | gas 72 h (ml/200mgDM) |
|---|---|---|---|---|---|---|
| *Falcaria vulgaris* | 64.5[a] | 0.08[a] | 41.3[a] | 53.5[a] | 59.7[a] | 64.1[a] |
| *Malva neglecta* | 52.3[b] | 0.07[b] | 30.5[b] | 41.3[b] | 47.3[b] | 51.4[b] |
| *Chenopodium album* | 35.1[c] | 0.03[c] | 11.0[c] | 21.0[c] | 24.5[c] | 29.7[c] |
| *Polygonum aviculare* | 65.6[a] | 0.07[b] | 39.6[a] | 52.5[a] | 60.5[a] | 64.4[a] |
| SEM | 2.1 | 0.002 | 1.5 | 1.9 | 2.1 | 2.0 |
| P value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Means within columns followed by the different letter are significantly different (P<0.05).
bgas: potential gas production; cgas: constant rate of gas production; gas 12, 24, 48 and 72 h: *in vitro* gas production after 12, 24, 48 and 72 h incubation.

The cgas was the highest for *Falcaria vulgaris*. The amounts of TVFA (34.2 mmol/L), ME (9.6 MJ/kgDM) and NEL (5.7 MJ/kgDM) were the highest in *Falcaria vulgaris* and lowest in *Chenopodium album* (26.6 mmol/L, 5.2 and 2.7 MJ/kgDM, respectively). The lowest $NH_3$-N (11.8 mg/dL) and pH (6.6) were produced when *Falcaria vulgaris* was incubated in the media. The lowest OMD (765 g/kgDM) and DMD (711 g/kgDM) were both observed in *Chenopodium album* (Table 4).

**Table 4.** *In vitro* dry matter digestibility (DMD), *in vitro* organic matter digestibility (OMD), metabolizable energy (ME), net energy for lactation (NEL) and fermentation parameters of different plants measured in the culturemedia

| Plant | $NH_3$-N (mg/dL) | TVFA (mmol/L) | pH | OMD (g/kgDM) | ME (MJ/kgDM) | NEL (MJ/kgDM) | DMD (g/kgDM) |
|---|---|---|---|---|---|---|---|
| *Falcaria vulgaris* | 11.8[c] | 34.2[a] | 6.6[b] | 849.0[b] | 9.6[a] | 5.7[a] | 825.0[a] |
| *Malva neglecta* | 13.9[b] | 28.1[b] | 6.7[ab] | 803.0[c] | 7.9[b] | 4.6[b] | 789.0[b] |
| *Chenopodium album* | 16.0[a] | 26.6[b] | 6.7[ab] | 765.0[d] | 5.2[c] | 2.7[c] | 711.0[c] |
| *Polygonum aviculare* | 12.3[c] | 33.7[a] | 6.8[a] | 876.0[a] | 9.4[a] | 5.6[a] | 828.0[a] |
| SEM | 0.2 | 0.8 | 0.03 | 8.0 | 0.2 | 0.2 | 9.0 |
| P value | <0.0001 | 0.0002 | 0.06 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Means within columns followed by the different letter are significantly different (P<0.05).
$NH_3$-N: Ammonia nitrogen; TVFA: total volatile fatty acid; OMD: *in vitro* organic matter digestibility; ME: metabolizable energy; NEL: net energy for lactation; DMD: *in vitro* dry matter digestibility.

## Correlation coefficient between different parameters

The IVGP after 12, 24 and 48 h as well as b and c were positively correlated with OMD, DMD, TVFA, NFE and NFC and they were negatively correlated with $NH_3$-N, CP, and EE (Table 5).

**Table 5.** Correlation coefficient among some chemical compositions, *in vitro* gas production (IVGP) parameters and other fermentation products

| | gas 12 h | gas 24 h | gas 48 h | B | c | OMD | DMD | TVFA | $NH_3$-N | CP | EE | NFE | NFC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| gas 12 h | 1.0 | 0.99**** | 0.99**** | 0.98**** | 0.96**** | 0.93**** | 0.99**** | 0.89**** | -0.99**** | -0.97** | -0.73** | 0.93**** | 0.72** |
| gas 24 h | | 1.0 | 0.99**** | 0.99**** | 0.94**** | 0.94**** | 0.99**** | 0.90**** | -1.0**** | -0.97**** | -0.72** | 0.92**** | 0.72** |
| gas 48 h | | | 1.0 | 0.99**** | 0.93**** | 0.93**** | 0.99**** | 0.90**** | -0.99**** | -0.96**** | -0.75** | 0.92**** | 0.69** |
| bgas | | | | 1.0 | 0.91**** | 0.93**** | 0.98**** | 0.92**** | -0.99**** | -0.95**** | -0.72** | 0.89**** | 0.70** |
| cgas | | | | | 1.0 | 0.85*** | 0.94**** | 0.76** | -0.94**** | -0.93**** | -0.71** | 0.95**** | 0.70** |
| OMD | | | | | | 1.0 | 0.94**** | 0.94**** | -0.94**** | -0.95**** | -0.50* | 0.85*** | 0.87*** |
| DMD | | | | | | | 1.0 | 0.90**** | -0.99**** | -0.96**** | -0.69** | 0.91**** | 0.74** |
| TVFA | | | | | | | | 1.0 | -0.90**** | -0.90**** | -0.50 | 0.74** | 0.74** |
| $NH_3$-N | | | | | | | | | 1.0 | 0.96**** | 0.72** | -0.92**** | -0.72** |
| CP | | | | | | | | | | 1.0 | 0.66* | -0.90**** | -0.82*** |
| EE | | | | | | | | | | | 1.0 | -0.70** | -0.19 |
| NFE | | | | | | | | | | | | 1.0 | 0.66* |
| NFC | | | | | | | | | | | | | 1.0 |

Gas 12, 24 and 48 h: *In vitro* gas production after 12, 24 and 48 h incubation (ml/200mgDM); bgas: Potential gas production (ml/200mgDM); cgas: Constant rate of gas production (ml/h); OMD: *in vitro* organic matter digestibility (g/kgDM); DMD: *in vitro* dry matter digestibility (g/kgDM); TVFA: total volatile fatty acid (mmol/L); $NH_3$-N: ammonia nitrogen (mg/dL); CP: crude protein (g/kgDM); EE: ether extract (g/kgDM); NFE: nitrogen-free extract(g/kgDM); NFC: non-fiber carbohydrate (g/kgDM). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

## Discussion

### Chemical compound

This article has provided valuable information on the nutritive value of four plants for use as the feedstuff in ruminants. The obtained data may be useful for animal nutritionists in preparing an appropriate forage feed for ruminants in Iran. Some weed plants in good growth conditions usually supply a good nutritional value for livestock (Kazemi *et al.*, 2009; Kazemi *et al.*, 2012). The results indicated the evaluated four plants had different chemical compositions. The contents of OM, CP, EE, NDF, and ADF were reported as 870, 151.9, 24.6, 311.6 and 283.7 g/kgDM, respectively (Kulivand and Kafilzadeh, 2015) which are almost close to those reported for *Falcaria vulgaris* in the present study. The results of Gqaza *et al.* (2013) indicated that young shoots and mature plants of *Chenopodium album* have good nutritional value. The higher CP was founded in the young shoots (322 g/kgDM) and mature plants (292 g/kgDM) of *Chenopodium album* compared to the present study (275 g/kgDM). Adedapo *et al.* (2011) reported that *Chenopodium album* had relatively high ash (232.5 g/kgDM) and CP (264.4 g/kgDM) contents which are in agreement with our results. The ash (114.9 g/kgDM) and ADF (300.2 g/kgDM) contents of *Polygonum aviculare* reported by Kamalak (2010) were respectively lower and higher than those of present study. Some nutrients may be transported through the soil to the plant tissue which will produce plants with different chemical and nutrient components (Cook and Stubbendieck, 1986). Different plants may also vary in susceptibility to leaching or may be consisted of different proportions of stems, leaves, and flower stalks at various stages of maturity which will be effective in the nutritional value of the plant when harvesting (Arzani *et al.*, 2004). The *Chenopodium album* (275 g/kgDM) and *Malva neglecta* (225 g/kgDM) were high in CP, and they can be compared with alfalfa as forage feed. Generally, all four plants had high CP (172-275 g/kgDM), more above the requirement for optimal rumen function (Van Soest, 1994).

### Mineral compound

One of the goals of this study was to assess and compare the mineral composition of four common plants that are widely consumed by the livestock in Iran. Most of the plants analyzed in this study were containing of suitable minerals. Present data show that four plants had a very high nutritional value, and their mineral content was greater than common vegetable plants reported by Turan *et al.* (2003). For example, calcium which is essential for skeletal system and muscle development was the maximum in *Malva neglecta* (355.4 mg/100g DM). Regarding that, the daily requirement of calcium is 5500 mg (NRC, 2007) for a non-lactating ewe (40 kg), the consumption of approximately 1.55 kg DM of *Malva neglecta* per day can meet their calcium requirements. Iron is needed for hemoglobin formation and many cellular processes, but its deficiency can cause anemia (Kaya and Incekara, 2000). Therefore, *Polygonum aviculare* with the appropriate level of iron (67.7 mg/100g DM) can prevent from anemia during feeding. Zinc, a trace mineral that is especially important for the normal immune response, was also abundant in *Polygonum aviculare* (4.8 mg/100g DM). The data about the magnesium (Mg) requirement of sheep is limited; however, its deficiency can lead to grass tetany in grazing and lactating ewes (McGrath *et al.*, 2015). Hence, the existence of high levels of magnesium in *Malva neglecta* (196 mg/100g DM) may reduce the problems of magnesium deficiency in the animals. It is reported the mineral component of *Chenopodium album* indicated high amounts of macro/microelements (Gqaza *et al.*,

2013) which is inconsistent with the present study. The consumption of *Chenopodium album* as vegetarian food in the diets of children and women can be useful in alleviating the problem of iron deficiency anemia in South Africa (Gqaza *et al.*, 2013).

## *In vitro* gas test and other fermentation parameters

The parameter estimated from the gas test was significantly different (P<0.05) among the plants. IVGP after 24 h incubation from *Falcaria vulgaris* was lower (53.5 ml/200mgDM) than that reported by Kulivand and kafilzadeh (2015); however, 24 h IVGP from *Polygonum aviculare* was similar to that reported by Kamalak (2010). It has been reported that gas production can be affected by external factors such as animal donor of rumen fluid, temperature, pH, sufficient buffering capacity and movement of gas production bottles (Getachew *et al.*, 1998). Therefore, the difference in the gas reported from various experiments may be related to these issues. The gas production information for *Malva neglecta* and *Chenopodium album* is limited. Among the four plants, *Polygonum aviculare* (65.6 ml/200mgDM) and *Falcaria vulgaris* (64.5 ml/200mgDM) had the highest gas production potential compared to two other plants. The constant rate of gas production, metabolism energy and organic matter digestibility of *Polygonum aviculare* reported by Kamalak (2010) during pre-flowering were 0.09%, 12.33 MJ/kgDM, and 831.8 g/kgDM, respectively. The potential gas production for *Polygonum aviculare* was lower than that reported by kamalak (2010), which could be due to differences in chemical composition during harvesting or in the method of gas production measurement. For example, instead of measuring the gas pressure used in the current study, Kamalak (2010) recorded directly gas volume by the means of glass syringes. Two dominant gases ($CO_2$ and $CH_4$), VFA and microbial biomass 3were produced mainly in the IVGP technique (Mauricio *et al.*, 2001). Neutralization of VFA by buffers can affect the produced gas (Van Soest, 1994). Therefore, as well as fermentation substrates via incorporating to microorganisms, organic matter digestibility can also affect the gas production (Mauricio *et al.*, 2001). In line with previous assumptions, both *Falcaria vulgaris* and *Polygonum aviculare* produced higher IVGP as well as having higher OMD. At the present study, the ANKOM method has also caused different results (876 g/kgDM) to that of conventional *in vitro* methods in determining OMD (831.8 g/kgDM for *Polygonum aviculare*; Kamalak, 2010). Having a significantly higher TVFA in *Falcaria vulgaris* (34.2 mmol/L) or *Polygonum aviculare* (33.7 mmol/L) compared to *Chenopodium album* or *Malva neglecta* reflects basic differences in chemical composition of them. This may be due to higher OMD in *Falcaria vulgaris* (34.2 mmol/L) or *polygonum aviculare* as VFA is a by-product arising mainly from microbial fermentation of carbohydrates (France and Siddons, 1993). The OMD for *Polygonum aviculare* (876 g/kgDM), *Falcaria vulgaris* (849 g/kgDM), *Malva neglecta* (803 g/kgDM) and *Chenopodium album* (765 g/kgDM) was higher than that reported for different varieties of alfalfa (561.5 to 663.3 g/kgDM) by kamalak *et al.* (2005). A strong positive correlation ($P$<0.001) between 24 h IVGP and NFC was reported (Getachew *et al.*, 2004), hence higher 24 h IVGP found in *Falcaria vulgaris* (53.5 ml/200mgDM) and *Polygonum aviculare* (52.5 ml/200mgDM) seems to be associated with its higher content of NFC. It has been reported that true protein of feed can produce both peptides and amino acids in the rumen which they can produce ammonia nitrogen after deamination

(Bach *et al.,* 2005). The higher ammonia nitrogen for *Chenopodium album* in the media may be due to its higher crude protein content (275 g/kgDM). The VFA and lactic acid are principal metabolic intermediates produced from feed fermentation in the rumen (Dijkstra *et al.,* 2012). If rumen buffering cannot confront with the accumulation of these acids, they can accumulate and reduce ruminal pH (Plaizier *et al.,* 2009). It seems likely that higher value in TVFA of *Falcaria vulgaris* represents the pH reduction in the media.

## Correlation coefficient between different parameters

The strong correlation between cumulative gas production after 24 h incubation and chemical composition is inconsistent with Kazemi *et al.* (2012), Nsahlai *et al.* (1994) and Getachew *et al.* (2004). There was a strong negative correlation between CP and gas production parameters which is not agreed with the previous studies (Ndlovu and Nherera, 1997; Larbi *et al.,* 1998) but it is consistent with Getachew *et al.* (2004). A poor correlation between gas produced after 24 h and *in vitro* true digestibility of DM was reported by Getachew *et al.* (2004) that may be due to different feed compositions such as protein and fat, which play a little role in gas produced but are degraded in the media. The positive significant correlation (r=0.74, $P<0.01$) between NFC and TVFA production indicates that NFC fermentation contributes to VFA production. The positive correlation between 24 h gas production and VFA production (r=0.76) was strongly higher than that reported in a previous study on feed species (Getachew *et al.,* 2004). The VFA production will be effective in gas production. The truly digested substrate is incorporated to VFA, gas, and microbial biomass so gas measurements only account for the substrate that is consumed for VFA and gas production, not for substrate utilized for microbial growth (Getachew *et al.,* 2004). Although there was a correlation between gas production and amount of substrate used for VFA production, it has also been reported that gas production is positively related to microbial protein synthesis (Krishnamoorthy *et al.,* 1991) and feed intake (Blummel and Ørskov, 1993). The cause of correlation between 24 h gas and the VFA may be related to the production of more propionate as a result of the incubation of NFC-rich feed which is agreed with Getachew *et al.* (2004). A strong positive relationship was reported between Short Chain Fatty Acids (SCFA) and gas production (Blummel and Ørskov, 1993). There was a strong negative correlation between gas production parameters and $NH_3$-N ($p<0.0001$). Cone and Van Gelder (1999) found that high $NH_3$-N concentration due to its highly basic nature might prevent from the release of gas in the media. There was a positive correlation between DMD (r=0.98) and/or OMD (r=0.93) with potential gas production. Parissi *et al.* (2005) also reported a positive correlation ($P<0.01$) between gas 12, 24 and 48 h with OMD. Unlike of Kazemi *et al.* (2012), the CP was negatively correlated with 24 and 48 h gas production. It is reported the gas production from protein fermentation is relatively small rather than carbohydrate fermentation (Wolin, 1960). The amount of fat contribution in gas production is very low when 200 mg of coconut oil, palm kernel and/or soybean oil were added to the media, only 2.0 to 2.8 ml of gas was produced; however, when carbohydrates such as casein and cellulose were incubated, 23.4 and 80 ml gas were produced, respectively (Menke and Steingass, 1988; Getachew *et al.,* 1998). The negative correlation ($P<0.01$) was observed between EE and IVGP parameters. Unlike our report, a positive correlation was reported between $NH_3$-N and NFC by Tylutki *et al.* (2008).

## Conclusion

Having knowledge about the ME, NE, chemical composition and other nutritional values of different plants can also help the nutritionists to provide a suitable diet for ruminants. The present study showed that four plants had a very high nutritional value, and their nutritional value was equal or greater than that of other plants commonly used as forage in ruminants. In addition to being rich in nutrients, these low-cost plants can provide a good source of macro and micro minerals for ruminants. Generally, P, K, Fe and Zn contents in *Polygonum aviculare*, Ca, Mg and Na in *Malva neglecta* and S in the *Falcaria vulgaris* were the highest. According to fermentation parameters, it seems that *Falcaria vulgaris* and *Polygonum aviculare* have the highest nutritional value than the other plants. Generally, more *in vivo* and *in vitro* studies are required to assess the other nutritive values and their effects on animal performance.

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## References

Abdalla, A. L., Louvandini, H., Sallam, S., Bueno, I., Tsai S., Figueira A., 2012. *In vitro* evaluation, *in vivo* quantification, and microbial diversity studies of nutritional strategies for reducing enteric methane production. Trop. Anim. Health. Prod., 44, 953-964.

Adedapo, A., Jimoh, F., Afolayan, A., 2011. Comparison of the nutritive value and biological activities of the acetone, methanol and water extracts of the leaves of *Bidens pilosa* and *Chenopodium album*. Acta Pol. Pharm., 68, 83-92.

Al-Masri, M., 2009. An *in vitro* nutritive evaluation and rumen fermentation kinetics of *Sesbania aculeata* as affected by harvest time and cutting regimen. Anim. Health. Prod., 41, 1115-1126.

ANKOM Technology., 2005. Method for determining acid detergent lignin in Beakers method 8. Available at https://www.ankom.com/sites/default/files/document-files/Method_8_Lignin_in_beakers.pdf

ANKOM Technology., 2006[a]. Acid detergent fiber in feeds-filter bag technique method 12. Available at https://www.ankom.com/sites/default/files/document-files/Method_12_ADF_A2000.pdf

ANKOM Technology., 2006[b]. Neutral detergent fiber in feeds-filter bag technique method 6. Available at https://www.ankom.com/sites/default/files/document-files/Method_6_NDF_A200.pdf

AOAC., 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists Washington, DC, USA.

AOAC., 1999. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, DC, USA.

Arshadullah, M., Anwar, M., Azim, A., 2009. Evaluation of various exotic grasses in semi-arid conditions of Pabbi Hills, Kharian range. J. Anim. Plant Sci., 19(2), 85-89.

Arzani, H., Zohdi, M., Fish, E., Zahedi Amiri, G. H., Nikkhah, A., Wester, D., 2004. Phenological effects on forage quality of five grass species. J. Range Manage., 57(6), 624-629.

Barnett, A. J. G., Reid, R. L., 1957. Studies on the production of volatile fatty acids from grass in artificial rumen. 1. Volatile fatty acids production from fresh grasses. J. Agric. Sci., 48, 315-321.

Bach, A., Calsamiglia, S., Stern, M. D., 2005. Nitrogen metabolism in the rumen. J. Dairy Sci., 88, E9-E21.

Blummel, M., Ørskov, E. R., 1993. Comparison of *in vitro* gas production and nylon bag degradability of roughages in prediction of feed intake in cattle. Anim. Feed Sci. Technol., 40, 109-119.

Cone, J. W., Van Gelder, A. H., 1999. Influence of protein fermentation on gas production profiles. Anim. Feed Sci. Technol., 76, 251-264.

Cook, C. W., Stubbendieck, J., 1986. Range research: basic problems and techniques. society for range management, Colorado, 317 pp.

Dehghani Bidgoli, R., 2018. Forage quality of *Calligonum comosum* in three phenological growth stages (Case study: Kashan rangelands, Iran). J. Range. Sci., 8(3), 309-314.

Dijkstra, J., Ellis, J. L., Kebreab, E., Strathe, A. B., Lopez, S., France, J., Bannink, A., 2012. Ruminal pH regulation and nutritional consequences of low pH. Anim. Feed Sci. Technol., 172, 22-33.

Ezzat, S., Fadlalla, B., Ahmed, H., 2018. Effect of growth stage on the macro mineral concentrations of forbs and grasses in a semi-arid region of Sudan. J. Range. Sci., 8(1), 23-29.

France, J., Siddons, R. C., 1993. Volatile fatty acid production. In: Forbes, J.M., France, J. (ed.), Quantitative aspects of ruminant digestion and metabolism. CAB International, Wallingford, UK.

Gqaza, B. M., Njume, C., Goduka, N. I., George, G., 2013. Nutritional assessment of *Chenopodium album* L. (*Imbikicane*) young shoots and mature plant-leaves consumed in the Eastern Cape province of South Africa. In: international proceedings of chemical, biological and environmental engineering, Singapore. 53(19), 97-102.

Getachew, G., Blummel, M., Makkar, H. P. S., Becker, K., 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim. Feed Sci. Technol., 72, 261-281.

Getachew, G., Robinson, P. H., DePeters, E. J., Taylor, S. J., 2004. Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. Anim. Feed Sci. Technol., 111, 57-71.

Kamalak, A., 2010. Determination of potential nutritive value of *Polygonum aviculare* hay harvested at three maturity stages. J. Appl. Anim. Res., 38, 69-71.

Kamalak, A., Canbolat, O., Erol, A., Kilinc, C., Kizilsimsek, M., Ozkan, C.O., Ozkose, E., 2005. Effect of variety on chemical composition, *in vitro* gas production, metabolizable energy and organic matter digestibility of alfalfa hays. LRRD., 17(7).

Kaya, I., Incekara, N., 2000. Contents of some wild plant species consumed as food in Aegean Region. Turk. J. Weed Sci., 3, 56-64.

Kazemi, M., Tahmasbi, A. M., Valizadeh, R., Naserian, A. A., Moheghi, M. M., 2009. Assessment of nutritive value of four dominant weed species in range of khorasan distinct of Iran by *in vitro* and *in situ* techniques. J. Anim. Vet. Adv., 8, 2286-2290.

Kazemi, M., Tahmasbi, A.M., Naserian, A. A., Valizadeh, R., Moheghi, M. M., 2012. Potential nutritive value of some forage species used as ruminants feed in Iran. Afr. J. Biotechnol., 11, 12110-12117.

Khazaei, M., Salehi, H., 2006. Protective effect of *Falcaria vulgaris* extract on ethanol induced gastric ulcer in rat. Iran. J. Pharmacol. Ther., 5, 43-46.

Komarek, A. R., Manson, H., Theix, N., 1996. Crude fiber determinations using the ANKOM fiber system. ANKOM technology publication 102.

Komolong, M. K., Barber D. G., McNeill D. M., 2001. Post-ruminal protein supply and N retention of weaner sheep fed on a basal diet of lucerne hay (*Medicago sativa*) with increasing levels of *quebracho* tannins. Anim. Feed Sci. Technol., 92, 59-72.

Krishnamoorthy, U., Steingass, H., Menke K. H., 1991. Preliminary observation on the relationship between gas production and microbial protein synthesis *in vitro*. Arch. Anim. Nutr., 41, 521-526.

Kulivand, M., Kafilzadeh, F., 2015. Correlation between chemical composition, kinetics of fermentation and methane production of eight pasture grasses. Acta Sci., 37, 9-14.

Larbi, A., Smith, J. W., Kurdi, I. O., Adeknle, I. O., Raji, A. M., Ladipo, D. O., 1998. Chemical composition, rumen degradation, and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in the humid tropics. Anim. Feed Sci. Technol., 72, 81-96.

Marjorie, M. C., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12, 564-582.

Markham, R., 1942. A steam distillation apparatus suitable for micro-kjeldahl analysis. Biochem. J., 36, 790-791.

Mauricio, R. M., Owen, E., Mould, F. L., Givens, I., Theodorou, M. K., France, J., Davies, D. R., Dhanoa, M. S., 2001. Comparison of bovine rumen liquor and bovine faeces as inoculum for an *in vitro* gas production technique for evaluating forages. Anim. Feed Sci. Technol., 89, 33-48.

McGrath, S. R., Bhanugopan, M. S., Dove, H., Clayton, E. H., Virgona, J. M., Friend, M. A., 2015. Mineral supplementation of lambing ewes grazing dual-purpose wheat. Anim. Prod. Sci., 55, 526-534.

Menke, K. H. and 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.

Naseri, S., Adibi, M. A., Kianian, M. K., 2017. Forage quality of endangered species of *Astragalus fridae* Rech. F. in Semnan province, Iran. J. Range. Sci., 7(4), 387-399.

Ndlovu, L. R., Nherera F. V., 1997. Chemical composition and relationship to *in vitro* gas production of zimbabwean browsable indigenous tree species. Anim. Feed Sci. Technol., 69, 121-129.

Noroozi, J., Akhani, H., Breckle, S., 2008. Biodiversity and phytogeography of the alpine flora of Iran. Biodivers. Conserv., 17: 493-521.

NRC., 2007. Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids. 6rd ed. Washington: National Academy Press.

Nsahlai, I. V., Siaw, D., Osuji, P. O., 1994. The relationship between gas production and chemical composition of 23 browses of the genus *Sesbania*. J. Sci. Food Agric., 65, 13-20.

Ørskov, E. R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., 92, 499-503.

Parissi, Z. M., Papachristou, T. G., Nastis, A. S., 2005. Effect of drying method on estimated nutritive value of browse species using an *in vitro* gas production technique. Anim. Feed Sci. Technol. 123-124, 119-128.

Plaizier, J. C., Krause, D. O., Gozho, G. N., McBride, B. W., 2009. Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. Vet. J., 176, 21-31.

SAS Institute, Inc., 2002. SAS user's guide: Statistics. Version 9.1. SAS Institute, Inc., Cary, NC.

Seyyednejad, S. M., Koochak, H., Darab pour, E., Motamedi, H., 2010. A survey on *Hibiscus rosa-sinensis*, *Alcea rosea* L. and *Malva neglecta* Wallr as antibacterial agents. Asian Pac. J. Trop. Med., 3, 351-355.

Shadnoush, G., 2014. Chemical composition and *in vitro* digestibility of some range species in rangelands of Chaharmahal and Bakhtiari province, Iran. J. Range. Sci., 3(4), 343-352.

Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G., Russell, J. B., 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. J. Anim. Sci., 70, 3562-3577.

Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol., 48, 185-197.

Turan, M., Kordali, S., Zengin, H., Dursun, A., Sezen, Y., 2003. Macro and micro mineral content of some wild edible leaves consumed in eastern Anatolia. Acta Agric. Scand B., 53, 129-137.

Tylutki, T. P., Fox, D. G., Durbal, V. M., Tedeschi, L. O., Russell, J. B., Van Amburgh, M. E., Overton, T. R., Chase, L. E. and Pell, A. N., 2008. Cornell net carbohydrate and protein system: a model for precision feeding of dairy cattle. Anim. Feed Sci. Technol., 143, 174-202.

Van Soest, P. J., 1994. Nutritional ecology of the ruminant. Cornell university press, Ithaca, NY, USA.

Van Soest, P. J., Robertson, J. B., Lewiss, B. A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74, 3583-3597.

Wolin, M. J., 1960. A theoretical rumen fermentation balance. J. Dairy Sci., 43, 1452-1459.

# مقایسۀ ترکیبات شیمیایی و معدنی، تولید گاز به‌روش برون‌تنی و پارامترهای تخمیری برخی گونه‌های مرتعی در تربت‌جام، ایران

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**چکیده.** گیاهان قابل رویش در مرتع، نقش مهمی را در تغذیۀ حیوانات نشخوارکننده دارند، از این‌رو ارزش تغذیه‌ای چهار گونۀ گیاهی (شامل *Falcaria vulgaris*، *Malva neglecta*، *Chenopodium album* و *Polygonum aviculare*) با استفاده از روش‌های آزمایشگاهی مختلف، تعیین شدند. نمونه‌های گیاهان به‌صورت تصادفی قبل از گلدهی از مراتع مختلف تربت‌جام (ایران) در بهار سال ۱۳۹۷ جمع‌آوری شدند. دامنۀ ماده‌خشک (۱۲۵ تا ۱۸۴ گرم در کیلوگرم ماده‌خشک)، الیاف نامحلول در شویندۀ خنثی (۲۵۲ تا ۳۵۸ گرم در کیلوگرم ماده‌خشک)، الیاف نامحلول در شویندۀ اسیدی (۱۵۵ تا ۲۵۸ گرم در کیلوگرم ماده‌خشک)، پروتئین‌خام (۱۷۲ تا ۲۷۵ گرم در کیلوگرم ماده‌خشک)، چربی‌خام (۹ تا ۴۱ گرم در کیلوگرم ماده‌خشک)، فیبرخام (۱۳۵ تا ۱۸۵ گرم در کیلوگرم ماده‌خشک)، خاکستر (۱۴۰ تا ۲۵۲ گرم در کیلوگرم ماده‌خشک)، لیگنین نامحلول در شویندۀ اسیدی (۴۱ تا ۱۲۳ گرم در کیلوگرم ماده‌خشک)، عصارۀ عاری از نیتروژن (۳۰۰ تا ۴۹۶ گرم در کیلوگرم ماده‌خشک) و کربوهیدرات‌های غیرفیبری (۱۸۳ تا ۳۵۶ گرم در کیلوگرم ماده‌خشک) در بین گیاهان متفاوت بود. این گیاهان از مواد معدنی متفاوت، اما قابل قبول در برابر سایر گیاهان پرمصرف برخوردار بودند. بیشترین مقدار قابلیت هضم ماده‌آلی (۸۷۶ گرم در کیلوگرم ماده‌خشک) و ماده‌خشک (۸۲۸ گرم در کیلوگرم ماده‌خشک) مربوط به گونۀ *Polygonum aviculare* بود. سایر پارامترهای تخمیری (نیتروژن آمونیاکی، کل اسیدهای چرب فرار و pH) در بین گونه‌های گیاهی در اثر انکوباسیون در محیط کشت آزمایشگاهی، نیز متفاوت بودند. یک همبستگی مثبت قوی بین تولید گاز در زمان ۲۴ ساعت انکوباسیون با قابلیت هضم ماده‌آلی، قابلیت هضم ماده‌خشک و کل اسیدهای چرب فرار و نیز یک رابطه منفی بین تولید گاز در زمان ۲۴ ساعت انکوباسیون با پروتئین‌خام، نیتروژن آمونیاکی و چربی‌خام مشاهده شد. نتایج کلی نشان داد که هر یک از چهار گیاه مورد مطالعه را می‌توان به عنوان یک منبع بالقوۀ خوراکی و علوفه‌ای برای برآورده ساختن مسائل مربوط به کمبود علوفه در ایران استفاده نمود. بر طبق برخی از اطلاعات گزارش شده در این پژوهش، به‌نظر می‌رسد که گونه‌های *Falcaria vulgaris* و *Polygonum aviculare* از ارزش تغذیه‌ای بالاتری نسبت به دو گیاه دیگر برخوردار باشند.

**کلمات کلیدی:** علوفه، ارزش تغذیه‌ای، روش‌های آزمایشگاهی، نشخوارکنندگان