



# Nutritional characteristics of some medicinal-range plant species grazed by small ruminants in Torbat-e Jam region of Iran

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ORIGINAL RESEARCH

## Abstract:

Rangeland plants provide most of the nutritional requirements of small ruminants in Iran. Data about the nutritional value of some range plants are scarce. Hence, we investigated the nutritional characteristics of four range plants (*Pulicaria gnaphalodes*, *Amaranthus blitoides*, *Heliotropium europaeum*, and *Solanum nigrum*) through different laboratory analyses. In 2021, twenty samples of each plant species were randomly collected in the flowering stage from mountain pastures of Torbat-e Jam, Iran. We observed different ranges of dry matter (15.98–45.23%), crude protein (8.59–23.31%), ether extract (1.88–4.5%), neutral detergent fiber (31.52–64.71%), acid detergent fiber (26.97–47.05%), ash (6.09–16.06%), and non-fiber carbohydrates contents (13.99–31.55%) among the range plants species. The mineral compounds including calcium (33.28 g/kg DM), phosphorus (2.28 g/kg DM), magnesium (13.61 g/kg DM), potassium (25.83 g/kg DM), iron (501.89 mg/kg DM), and manganese (35.70 mg/kg DM) were the highest in *H. europaeum*, *P. gnaphalodes*, *H. europaeum*, *S. nigrum*, *H. europaeum*, and *S. nigrum*, respectively. *Solanum nigrum* exhibited the lowest potential gas production (23.50 ml/200 mg DM). *P. gnaphalodes* and *A. blitoides* had the highest values of metabolizable energy (6.89 and 6.77 MJ/kg DM, respectively). It is concluded that the studied plants can easily supply the maintenance requirements of small ruminants. Based on the present results, it seems that the nutritional value of *P. gnaphalodes* and *A. blitoides* was superior to *H. europaeum* and *S. nigrum*. Excessive consumption of *H. europaeum* due to its toxic effects should be done with more caution.

**Keywords:** Pasture; Plant; Ruminant; Nutritional value

## 1. Introduction

Rangeland plants play an important role in meeting the nutritional requirements of small ruminants. Most of the nutritional requirements of sheep and goats depend on the rangeland forages of Torbat-e Jam. Several types of research indicated that some range plants of Torbat-e Jam [1–4] or other range plants or forages have a relatively high nutritional potential for small ruminants [5–9]. The *in vitro* gas production and dry matter digestibility of some range plant species have been reported by other researchers [10, 11]. The *in vitro* techniques can be a cost-effective alternative to the expensive *in vivo* techniques. *Pulicaria* species is extensively famous as “kak kosht” and “Shabang” in Iran and is commonly used as a flavoring agent, herbal tea, and medicinal plants [12]. *Pulicaria gnaphalodes* (Vent.) Boiss. is a

persistent plant of about 10–30 cm high, with gold-yellow flowers, which grow on sandy, stony places in the Arabian Peninsula (Saudi Arabia), Afghanistan, Iran, Turkestan, and western Tibet [13]. Razavi Khorasan of Iran is the primary origin of this plant. There are no general reports on the nutritional potential of *P. gnaphalodes*. About 60 – 75 genera of *Amaranthus* have been identified until now and it contains the largest pantropical genus in *Amaranthaceae* [14]. *Amaranthus* is a common annual broadleaf genus of tropical origin, widely distributed worldwide, especially in tropical, subtropical, and mild regions of America, and with fewer species in Australia, Africa, and Eurasia [14]. *Amaranthus* spp. (Pigweeds) grows naturally in open areas with full sun and disturbed soils. They are annual plants that grow rapidly in disturbed areas and produce thousands of seeds per plant [15–17]. These habitat preferences enable them to

grow well in the rangelands or other farmlands.

The *Heliotropium* (Boraginaceae) genus with 250 species is found in warm regions worldwide. *Heliotropium europaeum* L., also known as the common heliotrope, European heliotrope, barooga weed, and caterpillar weed, is an herbaceous annual plant, native to Europe, Asia, North Africa, Australia, and Northern America [18]. *Heliotropium europaeum* contains a wide range of biological activities including antibacterial, antifungal, antitumor, anti-inflammatory, insecticidal, antispasmodic, cholagogue, emmenagogue, antipyretic, and anthelmintic effects, and is extensively used to treat warts and to promote wound healing [18–22]. *Heliotropium europaeum* is known to contain pyrrolizidine alkaloids such as heliotrine, heleurine, supinine, europine, and lasiocarpine, which are linked to the reported hepatotoxic, teratogenic, pulmotoxic, and mutagenic effects [22, 23]. Several cases of *H. europaeum* poisoning in sheep have been reported when it's hyperconsumption [24–26].

*Solanum nigrum* is a medicinal plant which belonged to the family Solanaceae. Its common name in Iran is Tajrizi. Two varieties of *S. nigrum* have been identified one with black color fruit and the other is reddish-brown color fruit. The leaves, whole plant, and roots of *S. nigrum* are used from health points of view [27].

There were no nutritional data about the present rangeland plants (*P. gnaphalodes*, *A. blitoides*, *H. europaeum*, and *S. nigrum*) worldwide, so we investigated the chemical-mineral compositions and nutritional value of four medicinal-rangeland plants by common in vitro and laboratory methods.

## 2. Materials and methods

### 2.1 Sampling method and laboratory analysis

The whole samples (containing stem, leaf, and flower) of *Pulicaria gnaphalodes* (perennial herb), *Amaranthus blitoides* (annual herb), *Heliotropium europaeum* (annual herb), and *Solanum nigrum* (annual herb) were randomly collected in the flowering stage from mountainous rangelands of Torbat-e Jam (Revenj village, Iran) in September 2021. These species were grouped in the herbal plants. This area (steppe with rock outcrops and semi-arid climate) was located at 35° 18' 22" N and 60° 19' 41" E, 1321 m above sea level. Twenty samples of each plant species (containing whole plant without root) were harvested simultaneously, and mixed together and a sub-sample was used for the subsequent analysis. The collected samples were immediately moved 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 (Arthur Thomas Company, Philadelphia, PA), and were preserved in polyethylene bags for the next analysis. A fresh sample of each plant was also dried in an air-forced oven at 135°C for 4 h for dry matter (DM) determination [28]. The acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the ANKOM technology [29, 30] using solutions described by Van Soest et al. [31]. The ether extract content (EE) was determined using ether extraction in the Soxhlet device [28]. The Kjeldahl method (AOAC, [28]) was employed for crude protein

(CP) determination. The mineral contents (calcium, sodium, potassium, magnesium, manganese, iron, and zinc) were determined by atomic absorption spectrometry (SavantAA, GBC, Australia). Phosphorus (P) was determined by a spectrophotometer (UV-Vis array Spectrophotometer, Photonix-Ar-2017, Iran) using the molybdovanadate method. The Non-Fiber Carbohydrate (NFC) content of samples was calculated by subtracting CP, NDF, fat, and ash from total DM [32].

### 2.2 Gas production and digestibility

The method described by Menke and Steingass was employed for gas production test [33]. All laboratory analyses were conducted at the central laboratory at the University of Torbat-e Jam, Iran. The rumen fluid was collected from three rumen fistulated lambs (two years old, 40 kg body weight) fed with alfalfa hay and a commercial concentrate twice (7:0 am and 18:0 pm) a day at the maintenance level with 10% CP. The rumen fluid was strained via four layers of cheesecloth, flushed with CO<sub>2</sub>, transferred into a pre-warmed thermos flask, and then, transferred to the laboratory for the next analysis. The amount of 200 mg from each plant (whole dried and ground with a 1-mm mesh Wiley mill) sample was weighed into the 100 ml glass syringes. The artificial saliva was prepared according to Menke and Steingass procedures [33]. Each glass syringe was filled with rumen fluid and artificial saliva solution with a ratio of 1:2. Afterward, each syringe outlet was closed by a plastic clip, then gently shaken and placed in a water bath at 39°C. The gas volume was recorded continuously at 3, 6, 9, 12, 24, 48, 72, and 96 h of incubation [33]. The 24 h gas production, CP, and EE were employed to estimate the metabolizable energy (ME) and net energy for lactation (NEI) according to the equations described by Menke and Steingass [33]. A medium similar to the gas test was used to determine total volatile fatty acids (TVFA), pH, and NH<sub>3</sub>-N concentrations. The sampling for TVFA determination was conducted according to the methods described by Getachew et al. [34]. The Markham device based on steam distillation was used for TVFA determination according to the protocol described by Barnett and Reid [35, 36]. The 24 h nutrient digestibility (DM and OM) of samples was conducted according to Kazemi and Ghasemi Bezdi procedure [37]. The method of Komolong et al. was used for NH<sub>3</sub>-N determination [38]. The buffering capacity parameters were determined according to Jasaitis et al. [39]. Briefly, 0.5 g DM of sample was weighed into a beaker, added to 50 ml distilled deionized water and then, stirred continuously with a magnetic stir bar. Buffering capacity was determined by addition of acid (0.1 N HCl) or base (0.1 N NaOH) until the pH was decreased to 4 or increased to 9, respectively. Initial pH and all further measurements were recorded when the solution reached the equilibration point after 3 min.

### 2.3 Statistical analysis and equations

Each plant species was considered as a treatment. Four replicates were considered for each measured parameter. The gas test was replicated in two runs. The parameters related to the gas production including fractional rate ( $c_{gas}$ , %/h) and potential gas production ( $b_{gas}$ , ml/h) were deter-

mined by a nonlinear equation  $[P=b(1-e^{-ct})]$ , [40] where P is the volume of gas produced at time t. All data were analyzed in a completely randomized design using the GLM procedure of SAS [41]. The statistical differences between means of treatments were determined by Duncan's multiple range test.

### 3. Results

#### 3.1 Chemical compositions

The chemical compositions of four range plant species are presented in Table 1. Different results for chemical compositions were observed among treatments. The highest ADF (47.05%), NDF (64.71%), DM (45.23%), and EE (4.50%) concentrations belonged to *P. gnaphalodes*. The highest CP (23.31%) was related to *H. europaeum*. The highest NFC content was observed in *A. blitoides* (31.55%) and *S. nigrum* (29.36%). The lowest ash content (6.09%) was related to *P. gnaphalodes*.

#### 3.2 Mineral compositions

The mineral compositions of four range plant species are shown in Table 2. The highest content of calcium (33.28 g/kg DM), magnesium (13.61 g/kg DM), and iron (501.89 mg/kg DM) was related to *H. europaeum*. *Solanum nigrum* had the highest content of potassium (25.83 g/kg DM), manganese (35.70 mg/kg DM), and zinc (34.76 mg/kg DM) compared to the other plant species. *Pulicaria gnaphalodes* exhibited the highest phosphorus content (2.28 g/kg DM). There was no significant difference in sodium content among plant species.

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

The estimated gas production parameters related to four plant species are shown in Table 3. *S. nigrum* exhibited the lowest potential gas production (23.50 ml/200 mg DM), 24 h (11.63 ml/200 mg DM), 48 h (18.83 ml/200 mg DM), and 72 h (20.57 ml/200 mg DM) gas production compared to other plant species. *P. gnaphalodes* exhibited the highest  $c_{gas}$  (0.066%/h) and 72 h gas production (39.80 ml/200 mg DM) among the plant species. There was no significant difference for  $b_{gas}$  among *P. gnaphalodes* (38.54 ml/200 mg DM), *A. blitoides* (36.89 ml/200 mg DM), and *H. europaeum* (37.32 ml/200 mg DM) species.

Some in vitro fermentation parameters after incubation of four plant species in the culture medium are presented in Table 4. The highest ammonia nitrogen (16.84 mg/dl) in the culture medium was observed in *H. europaeum*. The lowest TVFA was observed in *S. nigrum* (30.30 mmol). We found no significant difference in the pH of the culture medium among the plant species following the incubation. Among the plant species, *S. nigrum* exhibited the lowest ME (4.74 MJ/kg DM) and NEI (2.29 MJ/kg DM). The highest 24 h OMD (65.75%) and 24 h DMD (62.27%) were observed in *A. blitoides*.

#### 3.4 Buffering capacity parameters

The pH of plant in extract and buffering capacity parameters of four range plant species are presented in Table 5. We found different buffering capacity parameters among the plant species. *H. europaeum* exhibited the highest titratable acidity ( $637.33 \text{ mEq} \times 10^{-3}$ ) and extract pH (8.68) among the plant species. The highest acid-buffering capacity ( $143.78 \text{ mEq} \times 10^{-3}$ ), base-buffering capacity ( $57.37 \text{ mEq} \times 10^{-3}$ ), and acid-base-buffering capacity ( $201.15 \text{ mEq} \times 10^{-3}$ ) were observed in *A. blitoides*. The lowest acid-buffering capacity ( $79.49 \text{ mEq} \times 10^{-3}$ ) and acid-base buffering capacity ( $118.94 \text{ mEq} \times 10^{-3}$ ) were observed in *P. gnaphalodes*.

## 4. Discussion

#### 4.1 Chemical compositions

Data of the present study can be suitable for animal nutritionists in preparing a suitable and balanced diet when animals graze in the pastures. Recently, it has been reported that some plant species in Torbat-e Jam rangelands because of their potential nutritional value and favorite chemical compositions can easily meet the maintenance requirements of small ruminants when grazing. Chemical compositions can affect the nutritional potential of plants. We found suitable chemical contents in the presented rangeland plants. The CP content of *H. europaeum* (23.31%) was relatively similar to that reported by Kazemi and Valizadeh for alfalfa (24.95%) [1]. The ash (11.44%) and DM (16.01%) contents of *S. nigrum* were similar to those (10.18% and 15.3%, respectively) reported by Akubugwo et al. [42]. Higher content of CP (25.35%) for *A. blitoides* has been reported by

**Table 1.** Chemical compositions (% of DM) of four range plant species.

(Means within columns followed by the same letter are not different. DM (% of fresh weight): dry matter; NDF: neutral detergent fiber; ADF: acid detergent fiber; CP: crude protein; EE: ether extract; NFC: non-fiber carbohydrate.)

Plant	DM	NDF	ADF	CP	EE	Ash	NFC
<i>P. gnaphalodes</i>	45.23 <sup>a</sup>	64.71 <sup>a</sup>	47.05 <sup>a</sup>	8.59 <sup>d</sup>	4.5 <sup>a</sup>	6.09 <sup>c</sup>	16.11 <sup>b</sup>
<i>A. blitoides</i>	16.92 <sup>b</sup>	31.52 <sup>c</sup>	26.97 <sup>c</sup>	19.55 <sup>b</sup>	1.88 <sup>c</sup>	15.49 <sup>a</sup>	31.55 <sup>a</sup>
<i>H. europaeum</i>	15.98 <sup>c</sup>	44.47 <sup>b</sup>	30.77 <sup>b</sup>	23.31 <sup>a</sup>	2.17 <sup>bc</sup>	16.06 <sup>a</sup>	13.99 <sup>b</sup>
<i>S. nigrum</i>	16.01 <sup>c</sup>	43.07 <sup>b</sup>	30.25 <sup>bc</sup>	13.54 <sup>c</sup>	2.58 <sup>b</sup>	11.44 <sup>b</sup>	29.36 <sup>a</sup>
SEM	0.18	0.84	1.01	0.10	0.18	0.26	0.91
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

**Table 2.** Mineral compositions of four range plants species.

(Means within columns followed by the same letter are not different. Ca (g/kg DM): calcium; P (g/kg DM): phosphorus; Na (g/kg DM): sodium; K (g/kg DM): potassium; Mg (g/kg DM): magnesium (g/kg DM); Mn (mg/kg DM): manganese; Fe (mg/kg DM): iron; Zn (mg/kg DM): zinc.)

Species	Ca	P	Na	K	Mg	Mn	Fe	Zn
<i>P. gnaphalodes</i>	6.75 <sup>c</sup>	2.28 <sup>a</sup>	1.20	7.45 <sup>c</sup>	4.28 <sup>d</sup>	27.76 <sup>b</sup>	463.78 <sup>ab</sup>	34.36 <sup>a</sup>
<i>A. blitoides</i>	13.02 <sup>b</sup>	0.92 <sup>b</sup>	1.02	18.61 <sup>b</sup>	11.05 <sup>b</sup>	17.43 <sup>d</sup>	405.85 <sup>b</sup>	17.11 <sup>b</sup>
<i>H.europaeum</i>	33.28 <sup>a</sup>	1.10 <sup>b</sup>	1.15	21.0 <sup>b</sup>	13.61 <sup>a</sup>	23.68 <sup>c</sup>	501.89 <sup>a</sup>	12.67 <sup>b</sup>
<i>S. nigrum</i>	7.06 <sup>c</sup>	0.71 <sup>b</sup>	1.42	25.83 <sup>a</sup>	7.88 <sup>c</sup>	35.70 <sup>a</sup>	460.06 <sup>ab</sup>	34.76 <sup>a</sup>
SEM	0.62	0.19	0.15	1.14	0.26	0.77	20.79	2.16
P-value	<0.0001	<0.0001	0.34	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Nwaogu et al. rather than in the present study (19.55%) [43]. The reported fat content of *A. blitoides* by Nwaogu et al. was comparable with the present study [43]. The NDF (64.71%) and ADF (47.05%) contents of *P. gnaphalodes* were higher than those reported for alfalfa (NDF: 36%, ADF: 23.27%) by Kazemi and Valizadeh [1]. It has been reported that the lowest required CP to meet the maintenance requirements of a single animal unit is about 7% [44, 45]. Therefore, four present plants can easily meet the protein requirements of a single animal unit when grazing. Part of the high EE content (4.5%) in *P. gnaphalodes* can be attributed to its high essential oils contents reported by other researchers [12, 46]. The high differences for DM between *P. gnaphalodes* and other studied plants can be related to its fibrous characteristics.

#### 4.2 Mineral compositions

We determined the mineral composition of four range plant species with the aim of finding how much they can meet the mineral requirement of small ruminants when grazing. The present plants had a comparable mineral content to those range plants reported by Kazemi and Valizadeh [1]. For example, calcium which is essential for the skeletal system and muscle development was the highest in *H. europaeum* (33.28 g/kg DM). The recommended ratio of calcium to phosphorus should be kept around 2:1 to prevent urinary calculi but animals can tolerate 7:1 as long as phosphorus is

adequate [47]. In the present plants except *P. gnaphalodes*, phosphorus levels were lower than critical level (1.6 g/kg) that can be restrictive for small ruminants. In this regard, the daily requirement of calcium is 5.5 g [48] for a 40 kg non-lactating ewes, so the use of approximately 165 g DM of *H. europaeum* per day can easily meet their calcium requirements. Zinc is a trace mineral that is important for the normal immune response of the body, and it is also abundant in *S. nigrum* (34.76 mg/kg DM) and *P. gnaphalodes* (34.36 mg/kg DM), respectively. In hemoglobin formation and many cellular processes, iron is necessary but its deficiency can lead to anemia [49]. Therefore, the use of each of the present plants with the appropriate level of iron (405.85–501.89 mg/kg DM) can probably prevent anemia during grazing feeding. The deficiency of dietary magnesium can lead to grass tetany in grazing and lactating ewes [50]. Hence, the suitable levels of Mg in *H. europaeum* (13.61 g/kg DM) and *A. blitoides* (11.05 g/kg DM) may reduce the problems related to Mg deficiency in the small ruminants. Lower calcium (0.17 vs. 7.06 g/kg DM), sodium (0.03 vs. 1.42 g/kg DM), and potassium (0.43 vs. 25.83 g/kg DM) contents in the study of Akubugwo et al. for *S. nigrum* have been reported compared to the present study [42]. Lower sodium (0.65 vs. 13.02 g/kg DM), potassium (0.12 vs. 18.61 g/kg DM), and magnesium (0.23 vs. 11.05 g/kg DM) contents in the study of Nwaogu et al. for *A. blitoides* have been reported compared to the

**Table 3.** The estimated gas production parameters related to four plant species.

(Means within columns followed by the same letter are not different.  $b_{\text{gas}}$ : potential gas production;  $c_{\text{gas}}$ : fractional rate of gas production; gas 12, 24, 48, and 72 h: in vitro gas production after 12, 24, 48, and 72 h incubation.)

Plant	$c_{\text{gas}}$ (%/h)	$b_{\text{gas}}$	gas 12 h	gas 24 h (ml/200 mg DM)	gas 48 h	gas 72 h
<i>P. gnaphalodes</i>	0.066 <sup>a</sup>	38.54 <sup>a</sup>	21.17 <sup>a</sup>	26.63 <sup>a</sup>	35.03 <sup>a</sup>	39.80 <sup>a</sup>
<i>A. blitoides</i>	0.049 <sup>b</sup>	36.89 <sup>a</sup>	16.47 <sup>b</sup>	24.70 <sup>a</sup>	31.43 <sup>a</sup>	35.83 <sup>b</sup>
<i>H.europaeum</i>	0.023 <sup>c</sup>	37.32 <sup>a</sup>	7.53 <sup>c</sup>	17.53 <sup>b</sup>	26.57 <sup>b</sup>	30.20 <sup>c</sup>
<i>S. nigrum</i>	0.034 <sup>c</sup>	23.50 <sup>b</sup>	8.53 <sup>c</sup>	11.63 <sup>c</sup>	18.83 <sup>c</sup>	20.57 <sup>d</sup>
SEM	0.003	1.23	0.89	0.82	1.18	1.03
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

**Table 4.** Some in vitro fermentation parameters after incubation of four plant species in the culture medium. (Means within columns followed by the same letter are not different. NH<sub>3</sub>-N: ammonia nitrogen; TVFA: total volatile fatty acid; OMD: organic matter digestibility; ME: metabolizable energy; NEL: net energy for lactation; DMD: dry matter digestibility.)

Plant species	NH <sub>3</sub> -N (mg/dL)	TVFA (mmol/L)	pH 24 h	OMD 24 h (%)	ME (MJ/kg DM)	NEL (MJ/kg DM)	DMD 24 h (%)
<i>P. gnaphalodes</i>	15.27 <sup>b</sup>	36.75 <sup>a</sup>	6.88	62.13 <sup>ab</sup>	6.89 <sup>a</sup>	3.77 <sup>a</sup>	58.48 <sup>ab</sup>
<i>A. blitoides</i>	15.67 <sup>b</sup>	35.92 <sup>a</sup>	6.85	65.75 <sup>a</sup>	6.77 <sup>a</sup>	3.71 <sup>a</sup>	62.27 <sup>a</sup>
<i>H.europaeum</i>	16.84 <sup>a</sup>	32.84 <sup>b</sup>	7.12	59.03 <sup>bc</sup>	6.04 <sup>b</sup>	3.19 <sup>b</sup>	55.13 <sup>ab</sup>
<i>S. nigrum</i>	15.12 <sup>b</sup>	30.30 <sup>c</sup>	7.07	57.65 <sup>c</sup>	4.74 <sup>c</sup>	2.29 <sup>c</sup>	54.65 <sup>b</sup>
SEM	0.26	0.35	0.12	1.25	0.13	0.09	2.10
P-value	0.005	<0.0001	0.06	<0.0001	<0.0001	<0.0001	<0.0001

present study [43]. The contents of potassium, calcium, magnesium, iron, manganese, sodium, and phosphorus for *Pulicaria undulata* (belonging to the Asteraceae family and similar to *P. gnaphalodes*) have been reported about 19.69, 32.41, 4.26, 3.66, 1.52, 2.09, and 2.04 g/kg DM, respectively, which is not consistent with our results [51]. In the present study, the concentrations of calcium, potassium, and magnesium determined for *H. europaeum* were 33.28, 21, and 13.61 g/kg DM, respectively, which calcium and magnesium were higher than those for alfalfa, and potassium was lower (Calcium: 13.29, Potassium: 28.15, Magnesium: 4.50 g/kg DM) [1].

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

In vitro gas production is an easy and low-cost technique for assessing the nutritional potential of feedstuffs compared to in vivo methods. We found different gas production among the plant species. There are no data about the gas production of the present plants, so we had to compare them with alfalfa as a common forage. We found lower 24 h gas production (11.63–26.63 ml/200 mg DM),  $b_{\text{gas}}$  (23.50–38.54 ml/200 mg DM), and  $c_{\text{gas}}$  (0.023–0.066%/h) in the present plants than those reported for alfalfa (24 h gas production: 49.25 ml/200 mg DM,  $b_{\text{gas}}$ : 59.58 ml/200 mg DM, and  $c_{\text{gas}}$ : 0.091%/h) by Kazemi and Valizadeh [1]. When a feedstuff is incubated in an in vitro medium, the carbohydrates are fermented to microbial cells, short-chain fatty acids, and gases (mainly CO<sub>2</sub> and CH<sub>4</sub>). The produced gas

is principally a result of the fermentation of carbohydrates to acetate, propionate, and butyrate [52–54]. The amount of gas produced from protein fermentation is relatively small as compared to carbohydrate fermentation [52]. We found higher 24 h gas production (26.63 and 24.70 ml/200 mg DM) as well as higher TVFA (36.75 and 35.92 mmol/L, respectively) in *P. gnaphalodes* and *A. blitoides*. Higher gas production in the two plants can be attributed to the higher TVFA in the culture medium. Among the plant species, higher NH<sub>3</sub>-N as a result of *H. europaeum* incubation in the culture medium can be attributed to its higher CP content. We estimated the ME and NEL from 24 h gas production, CP, and fat contents. So, plants (*P. gnaphalodes* and *A. blitoides*) with higher 24 h gas production had higher ME and NEL. We estimated a lower ME (4.74–6.89 MJ/kg DM) and NEL (2.29–3.77 MJ/kg DM) than those reported for *Medicago sativa* as a common forage (ME: 9.03 MJ/kg DM, NEL: 5.36 MJ/kg DM) by Kazemi and Valizadeh [1]. Data about DMD and OMD are scarce; however, we found that *A. blitoides* (DMD: 62.27%, OMD: 65.75%) and *P. gnaphalodes* (DMD: 58.48%, OMD: 62.13%) had approximately values near to alfalfa (DMD: 64.08%, OMD: 67.77%).

#### 4.4 Buffering capacity parameters

The buffering system in ruminants is controlled by three important mechanisms including the 1) salivary buffer system, 2) the buffering capacity of the feed consumed and 3) the dietary additive buffers [55]. The buffering capacity of some

**Table 5.** The pH in plant extract and buffering capacity (mEq × 10<sup>-3</sup>) parameters of four range plant species. (Means within columns followed by the same letter are not different.)

Plant	pH	Titrateable acidity	Acid-buffering capacity	Titrateable alkalinity	Base-buffering capacity	Acid-base buffering capacity
<i>P.gnaphalodes</i>	5.24 <sup>d</sup>	106.00 <sup>d</sup>	79.49 <sup>c</sup>	148.33 <sup>b</sup>	39.45 <sup>c</sup>	118.94 <sup>d</sup>
<i>A. blitoides</i>	5.32 <sup>c</sup>	200.33 <sup>c</sup>	143.78 <sup>a</sup>	211.33 <sup>a</sup>	57.37 <sup>a</sup>	201.15 <sup>a</sup>
<i>H.europaeum</i>	8.68 <sup>a</sup>	637.33 <sup>a</sup>	136.21 <sup>ab</sup>	17.00 <sup>d</sup>	52.63 <sup>b</sup>	188.84 <sup>b</sup>
<i>S.nigrum</i>	6.67 <sup>b</sup>	357.00 <sup>b</sup>	133.21 <sup>b</sup>	93.33 <sup>c</sup>	40.01 <sup>c</sup>	173.22 <sup>c</sup>
SEM	0.016	5.12	2.32	1.35	0.63	2.32
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

protein sources and leguminous fodder has been reported to be higher than  $85 \text{ mEq} \times 10^{-3}$  [56], which is consistent with our study. In this study, the highest acid and also acid-base buffering capacity in *A. blitoides* indicated more acid needed to change in pH of the sample dissolved in water, and high control of this plant in ruminal pH balance. Initial pH and titratable acidity have been reported to be the most important determinants of rumen fluid pH. In the present study, the highest titratable acidity was observed for *H. europaeum* ( $637.33 \text{ mEq} \times 10^{-3}$ ), indicating high resistance to acidification. Feed ingredients can play an effective role in the buffering capacity of the diet. So, by evaluating pH and buffering capacity of these plants, we can predict the buffers needed to control and maintain rumen pH [57]. Except for *H. europaeum*, other plants had near neutral pH and therefore, their consumption could not lead to rumen pH reduction. It is reported that the amount and composition of minerals in the ash have a particular buffering effect on the plant's initial pH [58]. Due to the different ash content of the plant species studied in this study (6.09–16.06%), their buffering capacity was also different.

## 5. Conclusion

Though the plants studied here exhibited lower nutritional value compared to values reported by other researchers for alfalfa; however, the present data can be useful for animal nutritionists in preparing a balanced diet for small ruminants especially when grazing. Among the plant species, it seems that the nutritional potential of *P. gnaphalodes* and *A. blitoides* was superior to *H. europaeum* and *S. nigrum*. Also, excessive consumption of *H. europaeum* due to its toxic effects should be done with more caution. Generally, other in vivo and in vitro studies are needed to assess their anti-nutritional factors and their effects on animal performance.

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### Conflict of interest statement:

The authors declare that they have no conflict of interest.

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