



# Nutritional values of *Cyperus rotundus* L. at three growth stages under *in vitro* and standard laboratory studies

Mohsen Kazemi

Department of Animal Science, Faculty of Agriculture and Animal Science, University of Torbat-e Jam, Torbat-e Jam, Iran.

Corresponding author: [phd1388@gmail.com](mailto:phd1388@gmail.com), [m.kazemi@tjamcaas.ac.ir](mailto:m.kazemi@tjamcaas.ac.ir)

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## Abstract:

*Cyperus rotundus* L. (*C. rotundus*) is a medicinal plant and a species of sedge (*Cyperaceae* family) that grows in different world rangelands, especially in Iran. The nutritional aspects of this plant have not been scientifically investigated by animal science nutritionists. Therefore, the *C. rotundus*'s nutritional potential at three growth stages by different standard laboratory methods was investigated. Different amounts of chemical- mineral compositions were observed among the various growth stages of *C. rotundus*. The dry matter (18.86 – 25.15 of fresh weight), crude protein (10.51 – 14.40 of Dry Matter (DM)), neutral detergent fiber (27.42 – 33.40 %), acid detergent fiber (18.75 – 24.83 %), ash (10.95 – 13.40 %), and non-fiber carbohydrates (41.01 – 45.14 %) contents of *C. rotundus* differed among three growth stages ( $P < 0.05$ ). The different contents of minerals (sodium: 2.37 – 2.88 g/kg of DM; calcium: 4.19 – 4.77 g; phosphorus: 1.20 – 1.36 g; magnesium: 2.50 – 2.99 g; potassium: 27.10 – 29.33 g; manganese: 45.13 – 54.12 mg/kg of DM; iron: 470 – 527 mg; and zinc: 13.40 – 18.07 mg) were also observed among three growth stages of *C. rotundus*. The highest content of potential gas production (47.66 mL/200 mg of DM) was observed in *C. rotundus* at the vegetative stage ( $P < 0.05$ ). The amounts of 24 h dry matter digestibility (47.97), 24 h organic dry matter digestibility (43.30), metabolizable energy (4.54 MJ/kg of DM), net energy for lactation (2.14 MJ/kg of DM), and dry matter intake (4.38 of body weight) were the highest in the vegetative stage of *C. rotundus*. Regarding *C. rotundus*'s relatively favorite potential nutritional, it was concluded that it can meet some of the nutrient requirements of small ruminants at the maintenance level. The vegetative stages exhibited better nutritional values compared with the other two growth stages. This plant can be nutritionally comparable to corn silage as a commonly used forage in small ruminants' feeding.

**Keywords:** *Cyperus rotundus*; Nutritional value; Growth stage; Rangeland plant

## Introduction

The nutritional aspects of different rangeland plants have been investigated by various researchers (Kazemi and Valizadeh, 2019; Kazemi, 2019). One of the plants that can extensively grow in Iran's rangelands is *Cyperus rotundus* L. (*C. rotundus*). The *C. rotundus* is also famous for other names such as coco-grass, Java grass, nut grass, purple nut sedge, purple nut sedge, red nut sedge, and Khmer kravanh chruk (Rajabi et al., 2021). In Iran, because of the high growth rate, it is commonly famous as Alaf-e salam aleik. The *C. rotundus* is also a perennial plant whose height may

reach up to 140 cm and is used as a traditional medicinal herb in treating various ailments (Kumar et al., 2014). The plant has an extensive network of creeping underground rhizomes with a bulbous base, which arise from a single tuber of 1 – 3 cm length (Bryson and DeFelice, 2009). Its tubers are usually oval- rectangular in shape and black to brown in color, with a reddish- white interior and a specific aroma (Lawal and Oyedeji, 2009). The *C. rotundus* has a long history as an herbal medicine in several countries, and accordingly, it has been collected in indigenous medicine systems in different countries and provinces (Xue et al., 2023). The *C. rotundus*'s antioxidant activity has been

reported by Kumar et al. (2014). El–Wakil et al. (2023) reported that *C. rotundus* 90% MeOH extract and its derived fractions had anti-trichinellosis activity. Different chemical constituents for this herb have been reported such as essential oils, flavonoids, terpenoids, sesquiterpenes, sitosterol, cyperene, cyperol, nootkatone and valencene (Sonwa and König, 2001; Tsoyi et al., 2011) which are the responsible for several therapeutic, pesticidal, fungicidal, and insecticidal properties. In traditional Chinese medicine, its rhizomes are often used to treat liver disease, stomachache, breast tenderness, dysmenorrheal and menstrual irregularities (Xue et al., 2023). This plant is native to southern Asia, Africa, and southern and central Europe; furthermore, it has been commonly adapted well in many tropical and subtropical regions of the world (Srivastava et al., 2013; Peerzada et al., 2015). This is probably the reason why grass like *C. rotundus* is generally grazed, is related to its small size and tender herbaceous texture (Shaheen et al., 2020). It is reported that *C. rotundus* as a grass forage can be useful for the control of helminths in ruminants (Mpofu, 2022). According to the available information, no scientific report about the nutritional value of *C. rotundus* has been published for small ruminants worldwide.

Therefore, this research aimed to investigate the nutritional value of *C. rotundus* at three growth stages. Also, the chemical- mineral compositions, *in vitro* gas production parameters, and some ruminal fermentative- digestive parameters of *C. rotundus* were determined.

## Material and Methods

### Plant collection

The whole parts of *Cyperus rotundus* (*C. rotundus*) were randomly collected at vegetative (May 2022), flowering (June 2022), and seeding (July 2022) stages from the natural rangeland of Torbat–e Jam city, Iran. This area is 8184 square kilometers, from 60° 15' E to 60° 30' E, and 34° 35' N to 35° 47' N. The average elevation is 950 m above sea level and the climate is cold and dry, with an average annual precipitation of about 100.6 mm. The samples were cut from 2 cm of the ground surface, stored in nylon bags and immediately transferred to the central laboratory of Torbat–e Jam University.

### Chemical and mineral composition determination

The Dry Matter (DM) content of the samples was determined following drying in a forced air oven at 60 ° C (AOAC, 2005). The ash content was determined after burning the sample in an electrical furnace at 550 ° C for 4 h. The concentrations of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) were determined by ANKOMTechnology (2005, 2006a, 2006b). The ether extract concentration was determined using a Soxhlet extractor and hexane solution (AOAC, 2005). The concentration of Crude Protein (CP) was determined using the Kjeldahl device. The content of Non–Fiber Carbohydrates (NFC) was calculated by subtracting CP, NDF, fat, and ash from total DM (Sniffen et al., 1992). The minerals including sodium, calcium, magnesium, potassium, manganese, iron, and zinc were determined by an atomic

absorption apparatus spectrophotometer (SavantAA, GBC, Australia). A simple spectrophotometric method has been developed for the determination of phosphorus (Kazemi et al., 2023).

### The *in vitro* rumen microbial fermentation and gas test

The method suggested by Menke and Steingass (1988) was employed for running gas test technique. Ruminal liquor was gathered from three fistulated sheep (40±4 kg) which were fed on a fodder basal ration at the maintenance level. The samples of Ruminal liquor were gathered 2 h after the morning feeding, strained through four layers of silky cloth and kept immediately at 39 °C under continuous flushing with CO<sub>2</sub> until the start of the experiment. The samples as well as rumen liquor, and artificial saliva (30 mL, 1:2 ratio) were moved to the 100– mL lubricated syringes. The end of each glass syringe was plumped by a plastic clip to prevent gas leakage. All glass syringes were slowly flicked and moved to a water bath at a temperature of 39 °C for times of 3, 6, 9, 12, 24, 48, 72, and 96 h (Menke and Steingass, 1988; Kazemi and Valizadeh, 2019; Kazemi et al., 2023). A media similar to that prepared for the gas test was used for the determination of Total Volatile Fatty Acids (TVFA), pH, ammonia nitrogen, *in vitro* organic matter digestibility (*in vitro* OMD), and *in vitro* DM digestibility (*in vitro* DMD) after 24 h incubation (Kazemi and Ghasemi Bezdi, 2021). The method of Getachew et al. (2004) was employed for sampling the culture medium for TVFA determination. Markham's device (Markham, 1942) was used to determine ruminal TVFA based on Barnett and Reid (1957) method. The method of Komolong et al. (2001) was employed for ammonia nitrogen determination.

### Data Analysis

The equation of Ørskov and McDonald (1979) [ $Y = b(1 - e^{-c(t-lag)})$ ] was employed for determination of *in vitro* gas production parameters. In that Y is the gas production at time t, b is the potential of gas production ( $b_{gas}$ , mL/200 mg of DM), c is the fractional rate of gas production ( $c_{gas}$ , %/h), and lag denotes the time elapsed to the onset of fermentation. The values obtained from the laboratory and *in vitro* protocols were analyzed in a completely randomized design using the GLM procedure of SAS (2002). All parameters were repeated five times. The gas test was repeated in two runs. The equations proposed by Menke and Steingass (1988) were used for the determination of Metabolizable Energy (ME) and Net Energy for Lactation (NEL). Means were compared using Tukey's test. Dry Matter Intake (DMI) was estimated by the equation of Sanson and Kercher (1996).

## Results

### Chemical and mineral materials

Chemical compositions (% of DM) of *C. rotundus* collected at three growth stages are presented in Table 1. A different range of chemical compositions among the three growth stages of *C. rotundus* was observed. The contents of chemical compositions differed from 18.86 – 25.15 of fresh weight for DM, 10.95 – 13.4 of DM for ash, 18.75 – 24.83 of DM for ADF, 27.42 – 33.40 of DM for NDF, 5.50 – 8.05

**Table 1.** Chemical compositions (% of DM) of *C. rotundus* collected at three growth stages

Growth stages	DM	Ash	ADF	NDF	ADL	EE	CP	NFC
Vegetative	18.86 <sup>c</sup>	10.95 <sup>c</sup>	18.75 <sup>c</sup>	27.42 <sup>c</sup>	5.50 <sup>c</sup>	2.10 <sup>a</sup>	14.40 <sup>a</sup>	45.14 <sup>a</sup>
Flowering	21.78 <sup>b</sup>	12.07 <sup>b</sup>	21.83 <sup>b</sup>	30.40 <sup>b</sup>	6.87 <sup>b</sup>	1.92 <sup>a</sup>	12.51 <sup>b</sup>	43.11 <sup>ab</sup>
Seeding	25.15 <sup>a</sup>	13.40 <sup>a</sup>	24.83 <sup>a</sup>	33.40 <sup>a</sup>	8.05 <sup>a</sup>	1.68 <sup>b</sup>	10.51 <sup>c</sup>	41.01 <sup>b</sup>
SEM	0.83	0.37	0.92	0.90	0.38	0.06	0.59	0.72
P-value	<0.0001	0.001	0.0006	0.0004	0.0005	0.002	0.001	0.03

Within columns, means followed by the same letter are not significantly different according to Tukey's test. DM (of fresh weight): dry matter; ADF: acid detergent fiber; NDF: neutral detergent fiber; ADL: acid detergent lignin; EE: ether extract; CP: crude protein; NFC: non-fiber carbohydrate; SEM: standard error of the mean.

of DM for ADL, 1.68 – 2.10 of DM for EE, 10.51 – 14.40 of DM for CP, and 41.01 – 45.14 of DM for NFC, respectively (Table 1). The vegetative stage of *C. rotundus* exhibited the lowest NDF, ADF, ADL, Ash, and DM contents, but the highest EE, CP, and NFC.

Mineral compositions of *C. rotundus* collected at three growth stages are exhibited in Table 2.

The contents of minerals differed from 2.37 – 2.88 g/kg DM for sodium, 4.19 – 4.77 g/kg DM for calcium, 1.20 – 1.36 g/kg DM for phosphorus, 2.50 – 2.99 g/kg DM for magnesium, 27.10 – 29.33 g/kg DM for potassium, 45.13 – 54.12 mg/kg DM for manganese, 470 – 527 mg/kg DM for iron, and 13.40 – 18.07 mg/kg DM for zinc, respectively (Table 2). The vegetative stage of *C. rotundus* exhibited the highest mineral contents (Na, Ca, P, Mg, K, Mn, Fe, and Zn) compared with the seeding stage (Table 2).

#### The *in vitro* rumen microbial fermentation and gas test

The *in vitro* gas production parameters of *C. rotundus* collected at three growth stages are shown in Table 3. The amounts of *in vitro* gas production parameters differed from 40.87 – 47.66 mL/200 mg DM for  $b_{gas}$ , 0.0122 – 0.0136 /h for  $c_{gas}$ , 4.90 – 5.24 h for lag time, 2.92 – 4.22 mL/200 mg DM for 12 h gas production, 7.73 – 10.32 mL/200 mg DM for 24 h gas production, 17.97 – 22.68 mL/200 mg DM for

48 h gas production, and 22.23 – 27.65 mL/200 mg DM for 72 h gas production, respectively. The vegetative stage of *C. rotundus* showed the highest  $b_{gas}$ ,  $c_{gas}$ , and 12, 24, 48, and 72 h gas production parameters.

The estimated parameters and *in vitro* fermentative –digestive parameters of *C. rotundus* collected at three growth stages is presented in Table 4. The amounts of some parameters differed from 53.73 – 58.16 mmol/L for TVFA, 40.33 – 47.97 % of DM for *in vitro* 24 h DMD, 35.47 – 43.30 % of DM for *in vitro* 24 h OMD, 3.93 – 4.54 MJ/Kg of DM for ME, 1.73 – 2.14 MJ/kg of DM for NEL, and 3.59 – 4.38 % of live weight for DMI, respectively. The parameters including NH<sub>3</sub>-N, and 24 h pH remained unchanged among three growth stages.

## Discussion

Chemical analysis provides absolute values and therefore differs from most laboratory methods that merely rank feeds. However, without the involvement of the host animal, the nutritional value estimate is inferred by statistical correlation (Mould, 2003). Since the ideal method of evaluating the nutritional value of a feed, i.e. presenting it to the appropriate class of animal and recording the resulting production response, is neither practical nor economical, a wide range

**Table 2.** Mineral compositions of *C. rotundus* collected at three growth stages

Growth stages	Na	Ca	P	Mg	K	Mn	Fe	Zn
Vegetative	2.88 <sup>a</sup>	4.77 <sup>a</sup>	1.36 <sup>a</sup>	2.99 <sup>a</sup>	29.33 <sup>a</sup>	54.12 <sup>a</sup>	527.57 <sup>a</sup>	18.07 <sup>a</sup>
Flowering	2.56 <sup>ab</sup>	4.55 <sup>ab</sup>	1.30 <sup>ab</sup>	2.76 <sup>ab</sup>	28.03 <sup>ab</sup>	49.57 <sup>ab</sup>	494.67 <sup>b</sup>	16.30 <sup>a</sup>
Seeding	2.37 <sup>b</sup>	4.19 <sup>b</sup>	1.20 <sup>b</sup>	2.50 <sup>b</sup>	27.10 <sup>b</sup>	45.13 <sup>b</sup>	470.0 <sup>b</sup>	13.40 <sup>b</sup>
SEM	0.09	0.09	0.03	0.08	0.37	1.54	8.87	0.001
P-value	0.04	0.01	0.01	0.006	0.01	0.02	0.002	0.003

Within columns, means followed by the same letter are not significantly different according to Tukey's test. Na (g/kg DM): Sodium; Ca (g/kg DM): calcium; P (g/kg DM): phosphorus; Mg (g/kg DM): magnesium; K (g/kg DM): potassium (g/kg DM); Mn: manganese (mg/kg DM); Fe (mg/kg DM): Iron; Zn (mg/kg DM): Zinc. SEM: standard error of the mean.

**Table 3.** The *in vitro* gas production parameters of *C. rotundus* collected at three growth stages

Growth stages	$b_{gas}$	$c_{gas}$	LT	gas 12 h	gas 24 h	gas 48 h	gas 72 h
Vegetative	47.66 <sup>a</sup>	0.0136 <sup>a</sup>	4.90 <sup>b</sup>	4.22 <sup>a</sup>	10.32 <sup>a</sup>	22.68 <sup>a</sup>	27.65 <sup>a</sup>
Flowering	44.11 <sup>b</sup>	0.0128 <sup>ab</sup>	5.16 <sup>a</sup>	3.39 <sup>b</sup>	8.89 <sup>b</sup>	20.01 <sup>b</sup>	24.64 <sup>b</sup>
Seeding	40.87 <sup>c</sup>	0.0122 <sup>b</sup>	5.24 <sup>a</sup>	2.92 <sup>c</sup>	7.73 <sup>c</sup>	17.97 <sup>c</sup>	22.23 <sup>c</sup>
SEM	1.01	0.0002	0.057	0.19	0.38	0.70	0.80
P-value	0.0002	0.02	0.01	<0.0001	<0.0001	0.0002	0.0001

Within columns, means followed by the same letter are not significantly different according to Tukey's test.  $b_{gas}$ : potential gas production (mL/200 mg of DM);  $c_{gas}$ : fractional rate of gas production (%/h); LT: lag time (hour); gas 12, 24, 48, and 72 h: the gas produced at times 12, 24, 48, and 72 h incubation (mL/200 mg of DM); SEM: standard error of the mean.

of feed evaluation techniques have been developed (Mould, 2003). All feed evaluation techniques attempt to identify the extent to which the feed contributes to the ruminant nutritional requirements. The evaluation of the data related to the chemical composition of *C. rotundus*, especially in the vegetative stage, showed that this plant can meet the nutritional needs of small ruminants at the maintenance level. For example, the CP content (14.40 % of DM) of *C. rotundus* at the vegetative and flowering stages is similar to that reported for late-cut alfalfa (12–15 % of DM) by Foster et al. (2009). The fiber and CP are two important quality attributes of forages, and their accurate estimation in different weather conditions is very important to produce quality forage. In this regard, *C. rotundus* has a relatively good CP and fiber content compared with the common forages used in feeding small ruminants. Unlike other estimates of feed quality, chemical analysis is the only one that provides absolute values (e.g., the exact amount of nitrogen or ash present) (Mould, 2003). Consistent with the results of the current study, it has been reported that the concentrations of DM, NDF, ADL, and ADF increase and the

concentration of CP decreases as the plant matures (Kazemi and Valizadeh, 2020). Also, by passing from the vegetative stage to the flowering stage, the nutritional value of two plants (*Salvia hydrangea* and *Sophora alopecuroides*) decreased (Kazemi and Valizadeh, 2020). In several studies, the nutritional potential of many rangeland plants grown in Iran has been reported by researchers (Kazemi and Valizadeh, 2019; Kazemi, 2019, 2020, 2021; Dehghani Bidgoli, 2022). The DM content of *C. rotundus* (18.86–25.15 % of fresh weight) is close to that reported (14.88 % of fresh weight for *Centaurea cyanus* to 20.62 % for *Vicia sativa*) for some rangeland plants by Kazemi and Valizadeh (2019). The content of EE (1.68–2.10 % of DM) was comparable with that reported for *Medicago sativa* (1.76 % of DM) by Kazemi and Valizadeh (2019).

The imbalance of ration minerals in marginal pastures has challenged the precise mineral supplementation of sheep (Stewart et al., 2021). Knowing the mineral status of plants can facilitate the prediction of mineral deficiency. The content of two major minerals including calcium (4.19–4.77 g/kg DM) and phosphorus (1.20–1.36 g/kg DM) was lower

**Table 4.** The estimated parameters and *in vitro* fermentative–digestive parameters of *C. rotundus* collected at three growth stages

Growth stages	NH <sub>3</sub> -N	TVFA	<i>In vitro</i> 24 h DMD	<i>In vitro</i> 24 h OMD	ME	NEI	24 h pH	DMI
Vegetative	16.81	58.16 <sup>a</sup>	47.97 <sup>a</sup>	43.30 <sup>a</sup>	4.54 <sup>a</sup>	2.14 <sup>a</sup>	6.80	4.38 <sup>a</sup>
Flowering	16.16	55.97 <sup>b</sup>	44.40 <sup>b</sup>	41.13 <sup>b</sup>	4.22 <sup>b</sup>	1.93 <sup>b</sup>	6.74	3.95 <sup>b</sup>
Seeding	15.71	53.73 <sup>c</sup>	40.33 <sup>c</sup>	35.47 <sup>c</sup>	3.93 <sup>c</sup>	1.73 <sup>c</sup>	6.75	3.59 <sup>c</sup>
SEM	0.24	0.67	1.12	1.19	0.09	0.06	0.02	0.12
P-value	0.17	0.0007	<0.0001	<0.0001	0.0002	0.0002	0.32	0.0005

Within columns, means followed by the same letter are not significantly different according to Tukey's test. NH<sub>3</sub>-N (mg/dL): ammonia nitrogen; TVFA (mmol/L): total volatile fatty acids; *in vitro* 24 h DMD (%): *In vitro* 24 h dry matter digestibility; *In vitro* 24 h OMD (%): *in vitro* 24 h organic matter digestibility; ME (MJ/kg of DM): metabolizable energy; NEI (MJ/kg of DM): net energy for lactation; 24 h pH: the pH measured in the culture medium after 24 h incubation; DMI: dry matter intake (% of body weight); SEM: standard error of the mean

than those reported for *Medicago sativa* (Kazemi and Valizadeh, 2019); Ca: 13.29 g/kg DM, P: 2.61 g/kg DM) as a common forage used in small ruminant feeding. The concentrations of iron (470 – 527 mg/Kg DM) and sodium (2.37 – 2.88 g/Kg DM) for *C. rotundus* were higher than those reported for *Medicago sativa* (iron: 260 mg/Kg DM, sodium: 1.30 g/Kg DM), but with a similar potassium content (27.10 – 29.33 g/kg DM vs. 28.15 g/Kg DM). In line with the present results, several mineral contents decreased when the plant matured (Kazemi and Ghasemi Bezdi, 2021). It has been reported that the iron, manganese, and zinc requirements of sheep are about 30 – 50, 20 – 40, and 20 – 33 mg/kg DM, respectively (Moniello et al., 2005). Therefore, the sheep should use 57 – 64, 370 – 444, and 1111 – 1538 g DM of *C. rotundus* per day at each growth stages to provide minimum iron, manganese, and zinc requirements, respectively. Range of macro-mineral required for sheep are reported about 0.09 – 0.18 sodium, 0.20 – 0.82 calcium, 0.16 – 0.38 phosphorus, 0.12 – 0.18 magnesium, 0.50 – 0.80 g/kg DM potassium (Moniello et al., 2005). Therefore, the sheep should consume at least 31 – 38, 42 – 48, 118 – 133, 40 – 48, and 17 – 18 g DM of *C. rotundus* per day to meet the minimum sodium, calcium, phosphorus, magnesium, and potassium requirements, respectively.

Due to its speed, availability, and potential to simulate fermentation kinetics in the rumen, the gas test technique has been widely used in the past decade to evaluate various pasture plants (Kazemi and Valizadeh, 2019; Kazemi, 2019, 2020; Kazemi and Valizadeh, 2020; Kazemi, 2021). Gas production is a reflection of the generation of short– chain fatty acids and microbial mass (Getachew et al., 1998). Incubation of *C. rotundus* at the vegetative stage compared with the other stages resulted in greater bgas and 12, 24, 48, 72 h gas production. This can be explained by the fact that the ruminal microorganisms can better digest a plant with lower fiber. A negative relation between bgas and the fiber content of some rangeland plants has been reported by Kazemi (2019). On the other hand, as a plant matures, its fiber content increases. It is well known that with increasing maturity, forages become more fibrous and lignified and their digestibility declines (Alibes and Tisserand, 1990). An increase in NDF and ADF resulted in the low cgas at the seeding stage. This result is in agreement with the findings of Kazemi (2019) who found that the cgas was negatively correlated with NDF and ADF of some rangeland plants. It can be seen from Table 3 that the gas production at all incubation times and some estimated parameters ( $b_{gas}$ ,  $c_{gas}$ , *in vitro* OMD, *in vitro* DMD, NEI, and ME) were negatively correlated with NDF and ADF. This result was consistent with findings reported by Abdulrazak et al. (2000), Kazemi and Valizadeh (2019), and Kazemi (2019). Estimated parameters ( $b_{gas}$ , and *in vitro* 24 h OMD) were also significantly correlated with CP which is one of the limiting factors for microbial growth. This result was in agreement with the findings of Larbi et al. (1998), Kazemi (2019), and Kazemi and Valizadeh (2019).

In line with the present study, it has been reported that digestibility of plants generally decreases with advancing ma-

turity. This reduction arises from the interaction of factors such as increased fiber concentration in plant tissues (Wilson et al., 1991; Kazemi and Valizadeh, 2019), increased lignification during plant growth (Morrison, 1980), and different leaf-stem ratios (Hides et al., 1983). A strong positive relation between TVFA and *in vitro* DMD and *in vitro* OMD has been reported by Kazemi and Valizadeh (2019). This shows *C. rotundus* at the vegetative stage because of lower NDF and ADF contents and higher *in vitro* DMD and OMD produce higher TVFA in the culture medium among the three growth stages. McSweeney et al. (1999) reported that *in vitro* DMD provide a poor indication of true fermentability. Instead, the authors recommended measuring end products of *in vitro* fermentation such as ammonia, short and branched chain fatty acids to better assess the nutritional value of forage plants. In this regards, the present study shows a reasonable levels of TVFA for *C. rotundus* at the vegetative stages. The higher *in vitro* OMD and TVFA in *C. rotundus* at the vegetative stage were higher than those in the other growth stage, possibly because *C. rotundus* at vegetative stage contains more fermentable carbohydrate which is a vital substrate for growth of ruminal microorganisms (Anele et al., 2009). The variation of the ME values among the three growth stages of *C. rotundus* (3.93 – 4.54 MJ/kg DM) was less than 1 MJ/kg DM. Estimation of ME values is valuable for ration formulation purposes and determining the economic value of feed for other purposes (Getachew et al., 2000). The ME (3.93 – 4.94 MJ/kg DM) and NEI (1.73 – 2.14 MJ/kg DM) of *C. rotundus* at three growth stages was lower than that reported for *Medicago sativa* (ME: 9.01 MJ/kg DM, NEI: 5.36 MJ/kg DM) by Kazemi and Valizadeh (2019).

## Conclusion

The nutritional value of *C. rotundus* decreased as the plant matured. The evaluation of *in vitro* fermentation parameters and chemical– mineral analysis suggests that *C. rotundus* at the vegetative stage is of higher nutritional value among the three growth stages. The nutritional potential of *C. rotundus* at three growth stages was lower than *Medicago sativa* as a common forage in ruminants feeding. Generally, *C. rotundus* as a pasture plant can meet the nutritional requirements of small ruminants such as sheep and goats at the maintenance level in the absence of common forages. The common *in vivo* studies is recommended for the dietary effects of *C. rotundus* in small ruminant feeding.

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**Authors contributions**

All the authors have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data (when applicable), as well as the writing of the manuscript.

**Availability of data and materials**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Conflict of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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