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RESEARCH PAPER

Phenological effects on forage quality of *Salvia limbata* in natural rangelands

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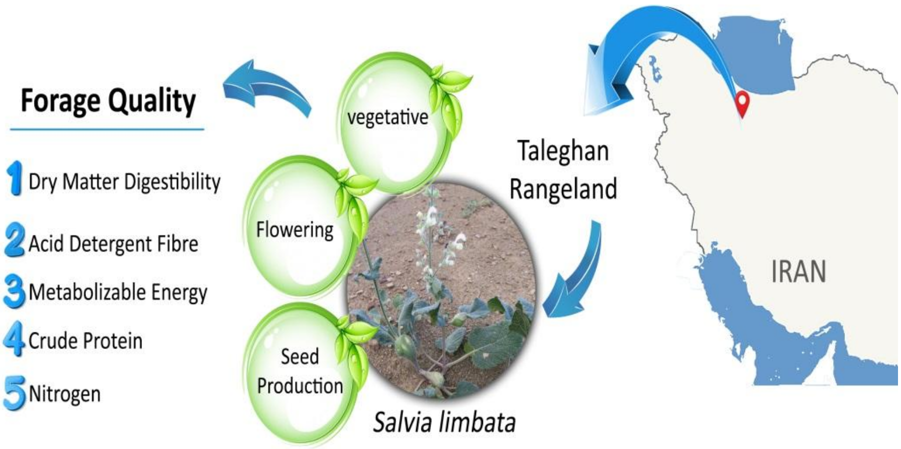
Highlights

• Forage quality of Salvia limbata was affected by phenological stages and altitudes.
• The highest percentage of N, CP, ME, and DMD were achieved in the vegetative stage.
• The highest ADF was obtained in in the seed ripening one.
• vegetative stage in 2500 m was determined as the best time for forage utilization of *Salvia limbata*.

Graphical Abstract



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Abstract

Salvia is a genus native to the Mediterranean regions and belongs to Lamiaceae family. This family are known as that has flowering plants which are called mint or sage family. They widely used around the world and the famous genus are basil, menthe, rosemary, sage, savoury, oregano, hyssop, thyme, lavender, and perilla. Some species are shrubs, trees or rarely vines. Forage quality of Salvia limbata at three growth stages (vegetative, flowering, and seed production) at different altitudes (1500, 2000, and 2500 m above sea level) has been studied in Taleghan rangeland. Aerial parts of the plant were sampled with five replications. Then samples were dried and milled. Five forage quality traits, including DMD (Dry matter digestibility), ADF (Acid detergent fibre), ME (Metabolizable energy), CP (Crude protein), and N were measured. Data were analysed in completely randomized design analysis of variance and means were compared by Duncan's test at 1% level. The results indicated that the forage quality of Salvia limbata was affected by phenological stages and altitudes. N (2.5%), CP (15.7%), ME (9.69%), and DMD (68.8%) have been obtained in higher amount in vegetative stage and ADF (55.60%) was achieved in the seed ripening. Due to the high percentage of CP, N, ME, and DMD, the vegetative stage at 2500 m was determined for animals to graze this plant.

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1. Introduction

Rangeland managers are always looking for a way to increase livestock performance, and one of the best ways is to know the nutritional value of forage in phenological stages (Arzani et al., 2004). Recognizing the nutritional value of plants will be needed to determine rangeland capacity, harvest time, and nutrient deficiencies (Asaadi and Yazdi, 2001; Mosaffaei et al., 2020; Pourmohammad et al., 2020). Temporal changes in rangeland forage quality are important to obtain the best forage quality. Forage quality is the potential of forage to provide nutritious food that meets animal's food preferences the desired animal needs (Ball et al., 2001). Forage quality is influenced by several factors including pests, leaf-to-stem ratio, harvesting, species, climate, soil agents, and diseases (Arzani et al., 2004; Hilario et al., 2017). In addition, phenological stages are assumed as the most important phenomena which influence the quality of forage for grazing on rangeland plants species. Crude Protein (CP) is one of the main items in forage quality. Absorbable protein in the rumen can be increased by reducing the protein degradation process in it. Herbage maturity is the most important feature influencing the forage quality is (Mischkolz et al., 2013). The other study shown that the effect of plant maturity is the most serious factor related to plant environmental condition, including temperature and growth stages. Also reported that the digestibility of plants depends on their growth stage and increases in early spring, reaching about 80%, and after that it declines (Hoffman et al., 2003). With maturity of plant, digestible energy, metabolizable energy, and nutritional value of plant decrease. Digestibility of plant with maturity, decrease. However, fibre and lignin develop Digestible energy, metabolizable energy, digestibility of forage decrease, fiber, and lignin increase with maturity of plants (Delogu et al., 2002).

Another study found that decreased of CP and digestibility of forage happen when plants are mature, but the percentages of acid detergent fiber (ADF) and neutral detergent fiber (NDF) increase (Fulgueira et al., 2007). ADF is one of the best indicators of forage quality in various plant species (Iwaasa et al., 2012). The quality of these factors varies within a plant (Barre et al., 2015). The genus *Salvia* from the *Lamiaceae* family has about 900 species, which are extensively distributed throughout world (Calvo et al., 2009). Species such as *S.urmiensis*, *S. sahendica*, *S. hypoleuca,* and *S. persepolitana* are found in Iran. Many *Salvia* species are well-studied and widely used in traditional medicine. Fifty-eight species of this genus are found in Iran, and seventeen (29%) are endemic (Salimpour et al., 2011). Some species of the genus *Salvia* such as *Salvia limbata* are important as flavouring agents in perfumery, seasoning, and cosmetology (Asllani, 2000). Many medicinal uses have been recorded for sage including spasmolytic, antiseptic, astringent, antibiotic, creaminess, and bleeding (Barnes et al., 2002). This study aims to find out different phenological stages which cause a significant difference in forage quality. Identification and evaluation of forage quality at different growth stages in diverse habitats can be useful for planning livestock food supply in critical circumstances (Buxton et al., 1995; Crowe and White, 2001).

Salvia limabta is foraged in Alborz province (Taleghan rangeland). The nutritional values of the species have not been studied before, thus determining the quality of this forage can be crucial to use the rangeland. The aim of this study was to define the forage quality of *Salvia limbata* in phenological stages at different altitudes. There is also some anthropogenic impact that has an impact on the quality of plant species in rangeland including; agricultural activities, improper use of water resources by-products and wood, fire, overgrazing, crossing roads and coal mining.

2. Materials and Methods

2.1. Study area

This study was conducted in Taleghan rangeland (semi-humid) which is located in Alborz province of Iran (Fig. 1). In this region, mean relative humidity is about 12% and the average annual temperature has been recorded as 11.4 °C. In Taleghan rangeland, the annual number of freezing days is 150 and annual precipitation is 446 mm. Taleghan rangeland, one of the *Salvia limbata's* main sites, is a prohibited hunting region. Analysis of forage quality is crucial for livestock and wildlife management in this area. Thus this region has been chosen to evaluate forage quality of *Salvia limbata* to protect environmental values (Ellis and Porter-Bolland, 2008).

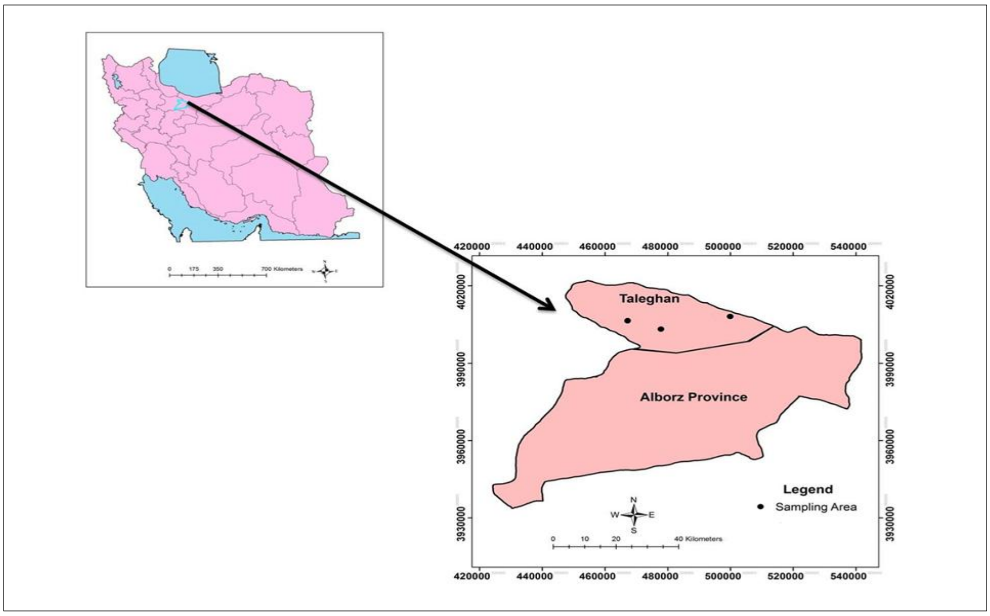

Fig. 1. Taleghan rangeland (semi-humid) which is located in Alborz province of Iran.

Since in our study area *Salvia limbata* was the only palatable species the aerial parts of *S.limbata* were collected during growth stages in June, July, and August in 2017 at (1500, 2000, and 2500 m) altitudes. In our study area the rangeland were rented to ranchers so the only use of this area is livestock grazing. The voucher specimens have been deposited at the Herbarium of the Department of Rehabilitation of Arid and Mountainous Regions, University of Tehran and department of plant science, University of California, Davis. This study was performed with five replications in each area. To evaluate the forage quality (nutritional value), the samplings from the areal organs were conducted during the whole growth period. The Samples were oven-dried at 78 °C for 24 hours. Finally, samples were ground by a mill and passed through a 0.5 mm sieve for chemical analysis.

Five samples in five replications at three phenological stages were sent to the Laboratory of Forage Quality Analysis at University of Tehran and department of Plant Science at University of California, Davis. Five forage quality traits were examined using NIR device (Near Infrared Spectroscopy) model INFRAMATIC8620 (Jafari et al., 2003), as follows, DMD (Dry matter digestibility), ME (metabolizable energy), CP (crude protein), ADF (acid detergent fiber), and %N. Effects of altitudes and phenology were investigated by Multivariate Test and to compare means, the Duncan test was applied.

3. Results and Discussion

The results illustrated a significant effect of phenological traits and altitudes on all factors ($P< 0.01$) (Table 1). Comparing means of ME, N, DMD, and CP showed that they had the highest values at vegetative stage and the lowest one at seed production (Table 1), except for ADF, which was the lowest in the vegetative stage and highest in seed production. Table 1 indicates that ME, N, DMD and, CP values were decreased by advancing plant growth to seed maturity but the reverse results were for ADF. Table 2 presents the analysis of variance for the qualitative traits of *S. limbata*. Table 2 reveals that the forage qualitative traits varied remarkably at growth stages at 1% level. Table 3 illustrates that soil factors except for lime were not significant. Therefore, the reasons for changes in forage qualities have been phenology, altitudes, and climate condition. According to the results of this study, the forage quality of *Salvia limbata* was different in growth stages. When plant age advances, CP, ME, N, and DMD decrease (Figs 2, 3, 5, and 6). These results are reversed for ADF (Fig. 4). Based on the results the lowest and highest ADF have obtained for vegetative and seed production stages respectively. The results illustrated that the lowest values of ADF have been obtained in vegetative 30.11 (1500 m), 28.11 (2000 m) and 29.62 (2500 m) and the highest amounts have been achieved in seed production 53.77 (1500 m), 51.79 (2000 m) and 55.60 (2500 m).

Table 1. Mean comparisons of forage quality traits in *Salvia limbata.*

Altitude	Growth stages	ME (%)	N (%)	ADF (%)	DMD (%)	CP (%)
1500 m	S1	9.32 ^{a}	2.5 ^{b}	30.11 ^{c}	66.57 ^{a}	16 ^{b}
	S2	7.1 ^{b}	1.47 ^{d}	42.85 ^{b}	53.15 ^{b}	10.8 ^{d}
	S3	5.2 ^{cd}	0.9 ^{f}	53.77 ^{a}	41.55 ^{c}	5.5 ^{f}
2000 m	S1	8.95 ^{a}	3.02 ^{a}	28.11 ^{c}	65.35 ^{a}	18.5 ^{a}
	S2	7.43 ^{b}	1.8 ^{c}	40.34 ^{b}	55.46 ^{b}	11.5 ^{cd}
	S3	5.7 ^{c}	1.2 ^{c}	51.79 ^{a}	44.67 ^{c}	7.38 ^{e}
2500 m	S1	9.69 ^{a}	2.5 ^{b}	29.62 ^{c}	68.60 ^{a}	15.7 ^{b}
	S2	7.5 ^{b}	1.8 ^{c}	40.33 ^{b}	53.36 ^{b}	12.4 ^{c}
	S3	4.7 ^{d}	0.9 ^{f}	55.60 ^{a}	40.30 ^{d}	5.6 ^{f}
F		99.91 **	195.87 **	99.09 **	134.42 **	168.41 **

** P< 0.01, common letter do not differ significantly (S1: Vegetative stages; S2: Flow), ** P< 0.01, common letter do not differ significantly (S1: Vegetative stages; S2: Flowering stage; S3: Seed ripening stage).

Table 2. Results of analysis of variances for *S. limbata* forage quality.

Source	Forage quality traits	Sum of squares	Df	Mean square	F
Growth stages	Me	68.72	2	34.361	336.13 **
	N	12.07	2	6.038	649.67 **
	ADF	2505.16	2	1252.58	322.41 **
	DMD	2542.26	2	1271.13	463.82 **
	CP	466.56	2	233.28	637.11 **
Altitudes	Me	0.1	2	0.054	0.52 ^{ns}
	N	0.6	2	0.32	34.89 **
	ADF	24.57	2	12.28	3.16 **
	DMD	9.1	2	4.56	1.66 ^{ns}
	CP	13.62	2	6.81	18.61 **
Growth stage*Altitude	Me	2.08	4	0.52	5.08 ^{ns}
	N	0.2	4	0.05	5.42 ^{ns}
	ADF	16.97	4	4.24	1.09 ^{ns}
	DMD	43.84	4	10.96	4 ns
	CP	10.34	4	2.58	7.06 ^{ns}

ns: non-significant, **: P< 0.01

Table 3. Comparison of soil factors of *Salvia limbata* in altitudes.

Soil factors (%)	1500 m	2000 m	2500 m	F
OM	1.81 ^{a}	2.06 ^{a}	1.64 ^{a}	0.1 ^{ns}
N	4.6 ^{a}	6.9 ^{a}	4.6 ^{a}	1.9 ^{ns}
P	0.15 ^{a}	0.72 ^{a}	0.1 a	3.2 ^{ns}
K	372.33 ^{a}	448.16 ^{a}	298.83 ^{a}	0.1 ^{ns}
pH	12.5 ^{a}	12.2 ^{a}	7.4 ^{a}	0.4 ^{ns}
EC	0.35 ^{a}	0.39 ^{a}	0.21 ^{a}	0.5 ^{ns}
Cl	0.7 ^{a}	0.7 ^{a}	0.4 ^{a}	0.5 ^{ns}
Lime	7.6 ^{a}	3.3 ^{a}	13.1 ^{a}	6.2 ^{ns}
Clay	4.41 ^{a}	2.92 ^{a}	3.04 ^{a}	0.4 ^{ns}
Silt	12.62 ^{a}	15.4 ^{a}	9.7 ^{a}	0.6 ^{ns}
Sand	85.52 ^{a}	81.6 ^{a}	86.3 ^{a}	0.8 ^{ns}
Texture	Sandy loam	Loamy sand	Sandy	

ns: non-significant.

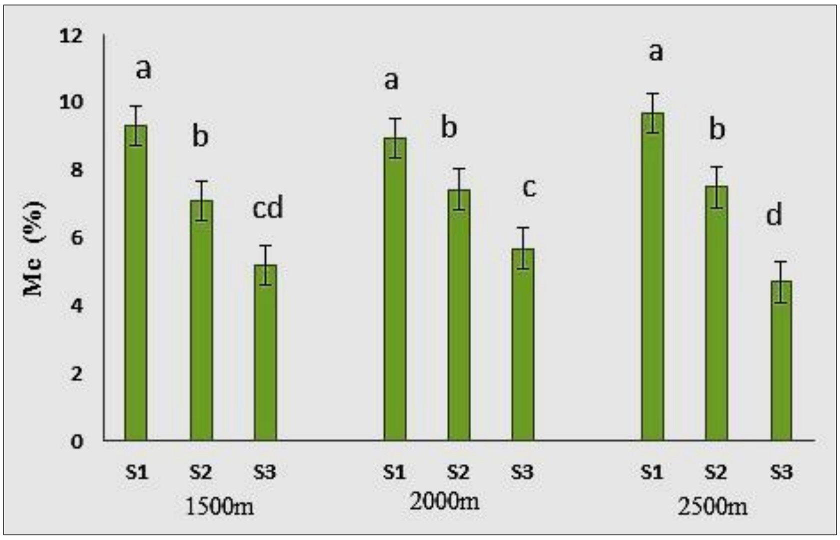

Fig. 2. Average percentage of ME in phonological stages and altitudes (S1: Vegetative stage; S2: Flowering stage; S3: Ripening stage).

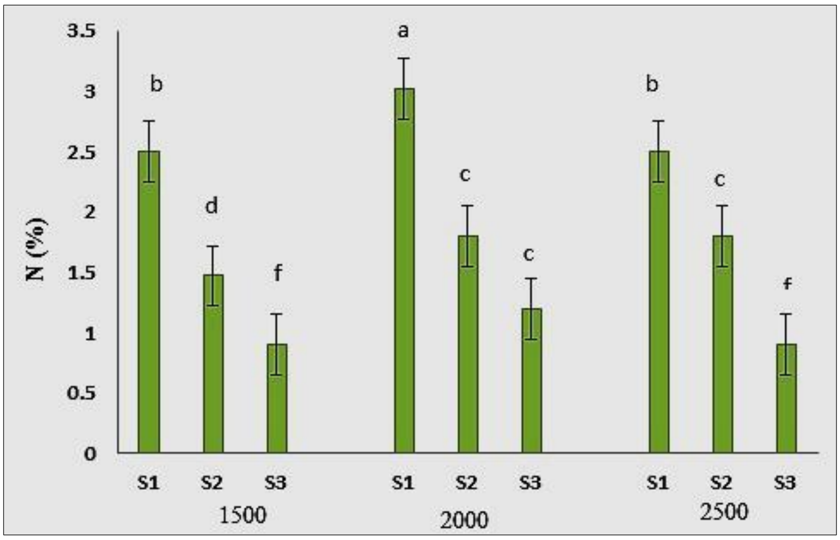

Fig. 3. Average percentage of N in phonological stages and altitudes (S1: Vegetative stage; S2: Flowering stage; S3: Ripening stage).

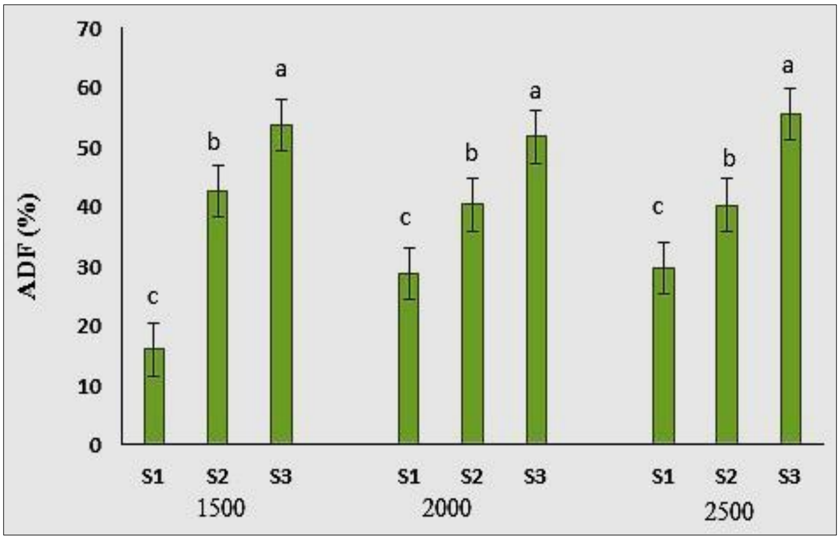

Fig. 4. Average percentage of ADF in phonological stages and altitudes (S1: Vegetative stage; S2: Flowering stage; S3: Ripening stage).

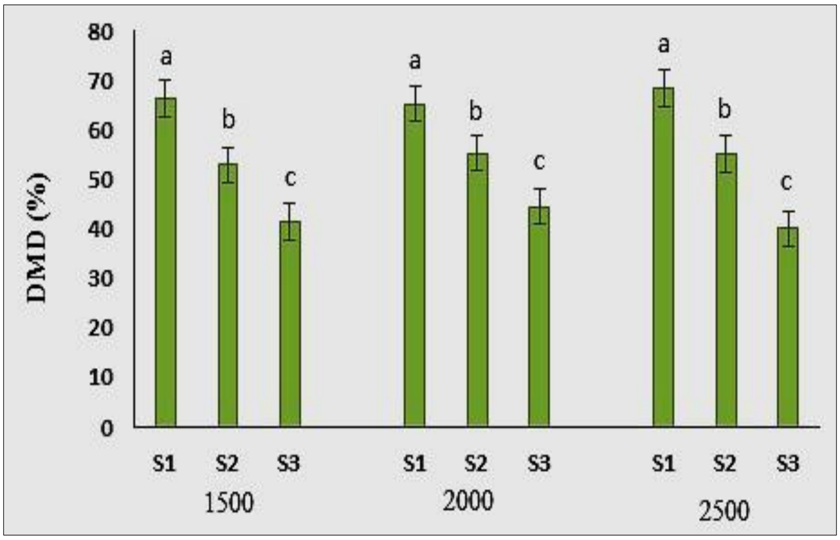

Fig. 5. Average percentage of DMD in phonological stages and altitudes (S1: Vegetative stage; S2: Flowering stage; S3: Seed ripening stage).

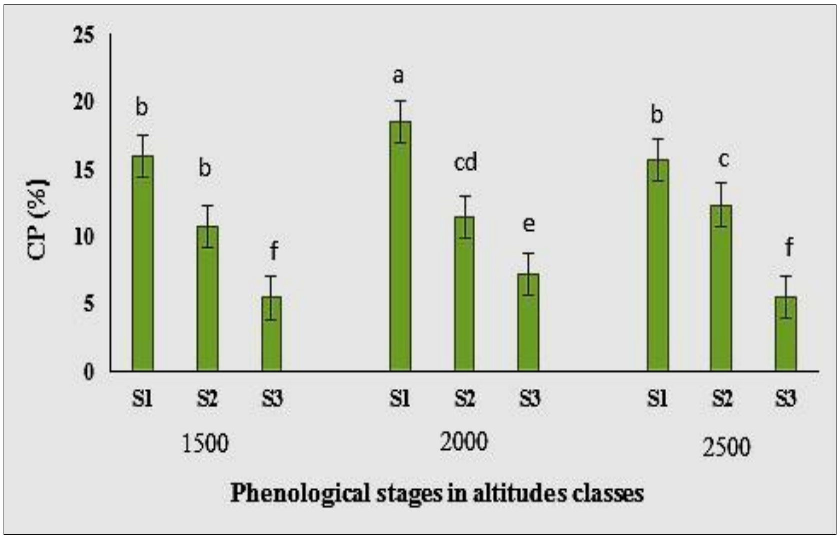

Fig. 6. Average percentage of CP in phonological stages and altitudes (S1: Vegetative stage; S2: Flowering stage; S3: Ripening stage).

Our results suggested that *Salvia limbata* contained a higher forage quality in the flowering and vegetative stages than the seed maturity. The results of this study indicated that the highest values of ME, CP, N and DMD (Those factors that are considered essential in forage quality) have been achieved in vegetative and flowering stage (at three altitudes). These results are consistent with those of other researchers (Delogu et al., 2002). Moreover, based on total indicators, the vegetative stage at 2500 m can be understood as the best altitude for grazing. This altitude can be appropriate for wildlife habitat protection purposes and also as an indicator of protected areas for herbivorous habitats and biodiversity (Sobhani et al., 2018).

The number of wildlife species and the quality of habitat are the main criteria for selection of sites and development of wildlife management concepts. Therefore, it is essential to know, which regions have suitable habitat characteristics for wildlife species, and also what quality and quantity of the characteristics of habitat are required to maintain a determined population size (Suchant et al., 2003; Barati et al., 2017; Aghajani et al., 2014; Jahani, 2017). Regarding the threats that herbivores face, it would be wise to take actions for conserve them for future generations by measures including restoring local herbal and animal populations and effectively protecting habitats. For example, elevation significantly affects the habitat selection for musk deer. Those selected regions with elevation ≥ 3529 m, have been taken into account as the most appropriate area for habitat of wild deer (Khadka and James, 2016).

Generally in this study, the best stage in terms of forage quality was considered for vegetative the lowest quality has measured for seed production and the highest was for vegetative. The rate of CP in plants in the vegetative stage was higher than the flowering and seed maturity (Amigot et al., 2006). Furthermore, according to Larbi et al., 2010, as the plant ages, the amount of ME and DMD decrease (Larbi et al., 2010). As plant growth increases, the amount of cellulose, hemicellulose and lignin in the plant increases. As a result, the amount of ADF increases and the concentration of CP decreases (Arzani et al., 2004; Hoffman et al., 2003).

It is crucial to know a plant's nutritional values in its phenological stages. With this knowledge, the best time of livestock grazing can be determined in accordance with forage quality (Hyder and Sneva, 1963). Modelling techniques and artificial intelligence would help to predict the most appropriate time for forage quality of *Salvia limbata* (Jahani, 2017; Jahani, 2019; Jahani et al., 2011). The result of the present study made clear that the vegetative stage was the best time for grazing this plant. The strength point of my study was that I investigated the forage quality of *Salvia limbata* for the first time in three phenological stage and altitudes. I recommend studying this plant in other areas with different conditions.

4. Conclusion

Phenological stages have a considerable influence on forage quality of varied parts of plants. With the development of *Salvia limbata*, CP, DMD, and ME were reduced. Higher forage quality was recorded for the first stage of growth. Based on the results, it is clear that *Salvia limbata* can be used both for production of essential oil and also can be effective for grazing in its vegetative stage. According to our results the grazing time of *Salvia limbata* was from June to August so it should be noted that in other areas what would be the best time to graze *Salvia limbata*. This research could be conducted in other areas with the same conditions. If other factors including human activities were studied carefully, the results would be more trustworthy.

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