

POTENTIAL NUTRITIVE VALUE OF ASTRAGALUS SPECIES HARVESTED AT THREE DIFFERENT MATURITY STAGES

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Abstract. The present study was conducted to investigate the effects of maturity stages on the chemical composition, in vitro gas and methane production, metabolic energy and organic matter digestibility for eleven *Astragalus* species widely encountered over the rangelands and intensely grazed by ruminants. *Astragalus* samples were collected from the rangelands at three different stages namely as before flowering, flowering and bear fruit stages. Dried samples were then subjected to various chemical analysis. Effects of *Astragalus* species and maturity stages on chemical composition, in vitro gas and methane production, metabolic energy and organic matter digestibility were found to be quite significant ($P < 0.001$). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) ratios increased, while the crude protein, crude ash, crude oil, condensed tannin contents, gas and methane production levels decreased with the progress of maturity stage. It can be suggested that grazing was more favorable at before flowering or flowering stages of *Astragalus* species because of high crude protein and metabolic energy content of plant at these stages. Also, although all *Astragalus* species were considered as a quality feed source for ruminants, endemic *Astragalus ocephalus* and *Astragalus longifolius* species were prominent with their superior nutritive properties.

Keywords. *chemical composition; in vitro gas production; metabolic energy; digestibility; condensed tannin content; grazing*

Introduction

Astragalus with about 2000 species is a member of legumes family and commonly composed of herbaceous and small shrubs. They are used in animal feeds, medicines and pharmaceuticals, in erosion prevention, bee pastures, dye and textile industries (Gruenwald et al., 1998). *Astragalus* species are widespread in steppes with low precipitation levels and Alpines with low temperatures and commonly grazed by

ruminants. There is limited information about the nutritional properties and grazing periods of *Astragalus* species.

Harvest or grazing periods greatly influence nutritive values of feeds. Studies about harvest or grazing periods of these species are of great significant for yield and quality of feed and also for sustainable use of natural resources. Despite these kinds of investigations on several species, there are still a lot of different plant species to be researched (Kamalak and Canbolat, 2010; Kaplan et al., 2014a).

Chemical composition, metabolic energy and digestible nutrients are the significant quality indicators for animal feeds (Canbolat, 2012). For this purpose, in vitro gas production technique developed by Menke et al. (1979) is widely used in recent years to determine nutritional values of feeds under in vitro conditions since the technique is a fast, easy and cheap method (Kaplan et al., 2014b). The gas production technique is also used to determine the methane reduction potential of feeds affecting global warming (Lin et al., 2013). The present study was conducted to investigate the effects of maturity stages on chemical composition, in vitro gas and methane production of different *Astragalus* species.

Materials and methods

Plant materials

Eleven different *Astragalus* species widespread in Bingol province of Turkey and commonly grazed by ruminants were harvested from the rangelands and natural areas at three different maturity stages (before-flowering, full-flowering, fruit-set). *Astragalus gummifer* Labill, *Astragalus cinereus* Willd, *Astragalus compactus* Lam, *Astragalus lineatus* Lam. var. *longidens*, *Astragalus ocephalus* Boiss subsp. *stachyophorus*, *Astragalus amblelepis*, *Astragalus declinatus* Wild, *Astragalus lineatus* Lam. var. *lineatus*, *Astragalus longifolius* Lam., *Astragalus aureus* Willd and *Astragalus onobrychis* species were used as the plant materials of the study. From these species *Astragalus ocephalus* Boiss subsp. *stachyophorus* and *Astragalus longifolius* Lam. are endemic species.

Climate data

Climate data for Bingol province were received from the nearest meteorological station of Directorate of Meteorology. According the meteorological data of research area, the long-term (2000-2015) monthly average temperature is 12.3 °C, total precipitation is 917.8 mm and relative humidity is 56.6%. Average temperature, precipitation and relative humidity of the research period (August 2015-August 2016) were quite close to long-term averages (13.9 °C, 923.7 mm and 53.4%, respectively). Soil structure of the Bingol province is clay-loam and loamy (Ates and Turan, 2015).

Chemical analysis

The *Astragalus* species were harvested at before flowering, flowering and bear fruit stages. Samples were dried at 70 °C for 48 h, ground and sieved through 1 mm sieve. Crude ash content of samples was determined by ashing the samples at 550 °C for 8 h in an ash oven. Soxhlet extracting system was used to determine the crude oil content of the samples by ether extraction method (AOAC, 1990). Kjeldahl method was used to determine nitrogen (N) content of the *Astragalus* species and crude protein level was

calculated by multiplying N content by 6.25 constant (AOAC, 1990). NDF (Van Soest and Wine, 1967) and ADF (Van Soest, 1963) analysis were performed by using ANKOM 200 Fiber Analyzer (ANKOM Technology Corp. Fairport, NY, USA). Condensed tannin content analysis of the samples was performed by using Butanol-HCl technique (Makkar et al., 1995).

The in vitro total gas and methane production

In vitro gas production technique developed by Menke et al. (1979) was used to determine in vitro gas and methane production of the samples. The rumen liquid was collected from three fistula installed hogget fed with a mixture 60% alfalfa and 40% barley. Liking blocks and clean water was supplied ad-libitum to the hogget. The rumen liquid was collected before feeding in the morning, filtrated through 6-layer cheesecloth to remove solid particles and mixed with twice as much synthetic saliva solution. Approximately 200 mg ground samples were placed in 100 ml glass syringes. The samples were weighed in triplicates. The sample syringes were then supplemented with 30 ml buffered rumen liquid. Three syringes including only buffered rumen liquid as control group and the syringes including samples and buffered rumen liquid were placed into a water bath set at 39 °C. The net gas production was calculated by subtracting gas production of control group from the gas production of sample group. Total gas production was determined as mL after incubation of *Astragalus* samples at 39 °C for 24 h. The methane ratio of the gas was obtained by using an Infrared methane analyzer (Sensor Europe GmbH, Erkrath, Germany) and the following equation (Eq. 1) was used to calculate methane production levels (Goel et al., 2008):

$$\text{Methane production (mL)} = \text{Total gas (mL)} \times \text{Methane ratio (\%)} \quad (\text{Eq. 1})$$

Metabolic energy (ME) and organic matter digestibility (OMD)

Metabolic energy content of *Astragalus* samples was calculated in accordance with the following equation (Eq. 2) using some parameters related to 24 h gas production and chemical composition of samples (Menke and Steingass, 1988). Organic matter digestibility (%) of the samples was determined by using Eq. 3 as suggested by Menke et al. (1979):

$$\text{ME (MJ kg}^{-1}\text{DM)} = 2.20 + 0.136 \text{ GP} + 0.057 \text{ CP} + 0.002859 \text{ CP}^2 \quad (\text{Eq. 2})$$

$$\text{OMD (\%)} = 14.88 + 0.889 \text{ GP} + 0.45 \text{ CP} + 0.0651 \text{ CA} \quad (\text{Eq. 3})$$

In these equations; DM: Dry matter, GP: Net gas production after 24 h (mL), CP: Crude protein ratio (%), CO: Crude oil ratio (%), CA: Crude ash ratio (%) and OMD: Organic matter digestibility (%).

Statistical analysis

The experimental design was completely randomized design with 3 replications. Variance and correlation analysis were performed by using Jump SAS (2009) statistical software. Tukey test was used to determine the difference between the means. As a complement of ANOVA procedure, Biplot Analysis was performed using nutritive

value and gas production parameters as variables and *Astragalus* species as classification criterion (Yan and Kang, 2003).

Results

Chemical composition

The effects of harvest time on nutritional characteristics of different *Astragalus* species were investigated in this study. Chemical composition of *Astragalus* samples is provided in Table 1. The effects of *Astragalus* species, harvest time and *Astragalus* x harvest time interaction on chemical composition were found to be highly significant ($P \leq 0.01$). ADF and NDF ratios increased and crude protein, crude ash, crude oil and condensed tannin contents decreased with the progress of harvest time. Crude protein ratios varied between 11.65-32.79%, ADF ratios between 16.22-48.54%, NDF ratios between 35.62-66.08%, crude oil contents between 0.40-3.43%, crude ash contents between 3.14-11.54% and condensed tannin contents between 0.32-1.00%.

Table 1. Chemical composition of *Astragalus* species harvested at different stages

<i>Astragalus</i> species	Stages	CP	ADF	NDF	CT	CO	CA
<i>Astragalus gummifer</i> Labill.	Before flowering	21.7 e	26.05m	41.14mn	0.62g-j	1.98cd	6.39i-l
	Flowering	19.42 gh	28.14l	42.39lm	0.52l-o	1.31g-k	5.62lmn
	Bear fruit	14.05 r	32.45j	45.01k	0.40pq	0.92lmn	5.36m-p
	Average	18.39 I	28.88 H	42.85 G	0.51 G	1.40 D	5.79 D
<i>Astragalus cinereus</i> Willd.	Before flowering	24.82c	23.18o	36.43p	0.87b	2.36b	8.58cde
	Flowering	22.41de	28.63kl	39.32o	0.66fg	1.5fgh	7.65efg
	Bear fruit	19.9fg	33.69i	41.80m	0.49mno	0.87mno	6.93g-k
	Average	22.37 C	28.50 I	39.19 I	0.68 C	4.58 C	7.72 B
<i>Astragalus compactus</i> Lam	Before flowering	21.91e	42.43d	52.93f	0.55j-m	1.13kl	5.14n-q
	Flowering	18.23i-l	46.19c	57.3e	0.46op	0.82nop	4.58o-r
	Bear fruit	17.26lmn	47.29b	63.04b	0.39q	0.59pq	3.60 rst
	Average	19.13 H	45.30 A	57.76 B	0.47 H	0.85 G	4.44 F
<i>Astragalus lineatus</i> Lam. var. <i>longidens</i>	Before flowering	22.11e	33.23ij	42.17m	0.98a	1.71ef	7.96def
	Flowering	19.32ghi	37.23g	47.95j	0.76cde	1.14jkl	7.23f-i
	Bear fruit	17.99jkl	39.97e	51.28gh	0.54k-n	0.93lmn	6.4i-l
	Average	19.81 F	36.81 D	47.13 E	0.76 B	1.26 E	7.20 C
<i>Astragalus oocephalus</i> Boiss subsp. <i>stachyophorus</i>	Before flowering	32.79a	24.63n	35.62p	0.70ef	1.71ef	8.68cd
	Flowering	26.64b	28.91kl	39.76no	0.56i-l	1.40g-j	6.64h-k
	Bear fruit	22.28de	37.99fg	49.53i	0.32r	1.15jkl	5.58l-o
	Average	27.24 A	30.51 F	41.64 H	0.53 G	1.42 D	6.96 C
<i>Astragalus amblolepis</i>	Before flowering	16.18nop	26.94m	58.79d	0.8cd	2.21bc	4.28qrs
	Flowering	14.55qr	32.87ij	62.06b	0.56i-m	1.7ef	3.14t
	Bear fruit	11.65s	35.86h	66.08a	0.37qr	1.23i-k	3.36st
	Average	14.13 K	31.89 E	62.31 A	0.58 E	1.71 B	3.60 F

<i>Astragalus declinatus</i> Willd.	Before flowering	22.53de	27.08m	36.2p	0.67fg	3.43a	11.54a
	Flowering	20.57f	29.44k	40.37no	0.57i-l	1.48f-i	10.15b
	Bear fruit	17.41klm	33.46ij	47.81j	0.52l-o	0.90lmn	9.14c
	Average	20.17 E	30.00 G	41.46 H	0.58 E	1.94 A	10.28 A
<i>Astragalus lineatus</i> Lam. var. <i>lineatus</i>	Before flowering	22.55de	35.73h	45.72k	0.76cde	2.21bc	7.34f-i
	Flowering	20.24fg	43.06d	50.37hi	0.65fgh	1.22ijk	7.14f-j
	Bear fruit	18.5h-k	45.6c	52.17fg	0.52l-o	0.83nop	6.58h-l
	Average	20.43 D	41.46 B	49.42 D	0.65 D	1.42 D	7.02 C
<i>Astragalus longifolius</i> Lam.	Before flowering	26.43b	28.74kl	36.71p	1.00a	1.82de	7.7d-g
	Flowering	23.27d	35.84h	43.60l	0.75de	0.74nop	6.98f-k
	Bear fruit	19.3ghi	48.54a	52.17fg	0.59h-k	0.59pq	6.04k-n
	Average	23.00 B	37.70 C	44.16 F	0.78 A	1.05 F	6.91 C
<i>Astragalus aureus</i> Willd.	Before flowering	18.61hij	24.56n	52.00fg	0.83bc	1.52fg	5.12n-q
	Flowering	16.62mno	28.42kl	57.28e	0.47no	1.11klm	4.52pqr
	Bear fruit	15.34pq	38.42f	60.64c	0.35qr	0.40q	4.14q-t
	Average	16.85 J	30.47 F	56.64 C	0.55 F	1.01 F	4.59 E
<i>Astragalus onobrychis</i>	Before flowering	22.56de	16.22r	45.32k	0.75de	1.25h-k	7.45fgh
	Flowering	20.07fg	18.31q	47.14j	0.63ghi	0.63opq	7.05f-j
	Bear fruit	15.97op	20.53p	49.58i	0.58h-l	0.43q	6.17j-m
	Average	19.54 G	18.35 J	47.32 E	0.65 D	0.77 H	6.89 C

CP: crude protein (%); **ADF:** acid detergent fiber (%); **NDF:** Neutral detergent fiber (%); **CT:** condense tannin (%); **CO:** crude oil (%); **CA:** crude ash (%); small letters show significant differences between the interactions of *Astragalus* species and harvest stage; capital letters show significant differences between the means of *Astragalus* species

Gas and methane production, metabolic energy and organic matter digestibility

Average gas production, methane production, metabolic energy and organic matter digestibility of *Astragalus* species harvested at different vegetation stages are provided in Table 2. The effects of *Astragalus* species, harvest times and *Astragalus* x harvest time interaction on gas and methane production, ME and OMD levels were found to be highly significant ($P \leq 0.01$). Gas and methane production, ME and OMD decreased with the progress of harvest time. Gas production values varied between 31.50-50.00 ml, methane productions between 3.31-6.54 ml, metabolic energy contents between 7.19-10.43 MJ kg⁻¹ DM and organic matter digestibility levels between 50.47-69.52%.

Table 2. Gas and methane production, metabolic energy and organic matter digestibility of *Astragalus* species harvested at different stages

<i>Astragalus</i> species	Stages	GP	CH ₄	ME	OMD
<i>Astragalus gummifer</i> Labill.	Before flowering	46.50 cde	6.00 b	9.98 d	66.40 bc
	Flowering	39.00 jk	4.69 f	8.64 j	58.65 j
	Bear fruit	34.00 op	3.78 i	7.48 op	51.78 qr
	Average	39.83 D	4.82 EF	8.70 EF	58.94 EF

<i>Astragalus cinereus</i> Willd.	Before flowering	41.50 hi	5.41 cd	9.58 e	63.50 d-g
	Flowering	37.00 k-n	4.28 g	8.62 jk	58.35 jk
	Bear fruit	33.00 pq	3.64 I	7.76 no	53.62 opq
	Average	37.17 F	4.44 G	8.65 F	58.49 F
<i>Astragalus compactus</i> Lam	Before flowering	50.00 a	6.22 ab	10.32 ab	69.52 a
	Flowering	45.50 def	5.30 cd	9.37 efg	63.83 def
	Bear fruit	38.00 jkl	3.76 I	8.22 lm	56.67 klm
	Average	44.50 AB	5.12 BC	9.30 BC	63.34 B
<i>Astragalus lineatus</i> Lam. var. <i>longidens</i>	Before flowering	49.50 ab	6.35 a	10.37 a	69.35 a
	Flowering	44.00 fg	5.11 de	9.30 e-h	63.16 e-h
	Bear fruit	37.00 k-n	4.26 g	8.19 lm	56.29 lmn
	Average	43.50 BC	5.24 AB	9.29 BC	62.93 B
<i>Astragalus</i> <i>oocephalus</i> Boiss subsp. <i>stachyophorus</i>	Before flowering	41.50 hi	5.24 cd	10.05 bcd	67.09 bc
	Flowering	39.00 jk	4.72 f	9.19 f-I	61.97 f-i
	Bear fruit	35.50 mno	3.68 I	8.34 kl	56.83 j-m
	Average	38.67 E	4.55 G	9.19 C	61.97 C
<i>Astragalus amblelepis</i>	Before flowering	43.00 gh	5.37 cd	9.12 ghi	60.66 i
	Flowering	39.50 ij	4.83 ef	8.42 jkl	56.75 klm
	Bear fruit	35.00 nop	3.90 hi	7.50 o	51.46 r
	Average	39.17 DE	4.70 F	8.35 G	56.29 G
<i>Astragalus declinatus</i> Wild	Before flowering	45.50 def	5.51 c	10.15a-d	66.22 c
	Flowering	44.00 fg	5.15 de	9.45 ef	63.91 de
	Bear fruit	39.00 jk	4.13 gh	8.42 jkl	57.98 jkl
	Average	42.83 C	4.93 CD	9.34 B	62.70 BC
<i>Astragalus lineatus</i> Lam. var. <i>lineatus</i>	Before flowering	48.00 abc	6.38 a	10.29 abc	68.18 ab
	Flowering	37.50 j-m	4.65 f	8.48 jkl	57.79 jkl
	Bear fruit	33.50 opq	3.86 hi	7.72 no	53.10 pq
	Average	39.67 DE	4.96 D	8.83 DE	59.80 E
<i>Astragalus longifolius</i> Lam.	Before flowering	47.50 bcd	5.96 b	10.43 a	69.50 q
	Flowering	44.50 efg	4.69 f	9.59 e	65.37 cd
	Bear fruit	42.50 gh	4.32 g	8.99 I	61.74 ghi
	Average	44.83 A	4.99 DC	9.67 A	65.54 A
<i>Astragalus aureus</i> Willd.	Before flowering	49.00 ab	6.54 a	10.01 cd	67.15 bc
	Flowering	43.50 fgh	5.49 c	9.03 hi	61.32 hi
	Bear fruit	36.50 lmn	3.74 I	7.83 n	54.50 nop
	Average	43.00 C	5.26 A	8.96 D	60.99 D
<i>Astragalus</i> <i>onobrychis</i>	Before flowering	41.50 hi	5.13 de	9.21 f-i	62.41 e-i
	Flowering	35.00 nop	4.23 g	8.01 mn	55.48 mno
	Bear fruit	31.50 q	3.31 j	7.19 p	50.47 r
	Average	36.00 G	4.22 H	8.14 H	56.12 G

GP: gas production (ml); **CH₄:** methane (ml); **ME:** metabolic energy (MJ kg⁻¹ DM); **OMD:** organic matter digestibility (%); small letters show significant differences between the interactions of *Astragalus* species and harvest stage; capital letters show significant differences between the means of *Astragalus* species

Biplot analysis

Biplot analysis revealed that two principle components were able to explain 73.10% of total variation (41.80% by PC1 and 31.30% by PC2). As can be seen in Figure 1; crude ash, crude protein and condensed tannin parameters constituted one group; ME and OMD constituted one group and GP, methane and ADF constituted another group. Crude oil and NDF alone separately constituted different groups. *Astragalus longifolius* was prominent with ME and OMD, *Astragalus amblolepis* with NDF, *Astragalus ooecephalus* with crude protein, *Astragalus compactus* with ADF and *Astragalus aureus* was prominent with methane production (Table1, Table 2, Figure 1).

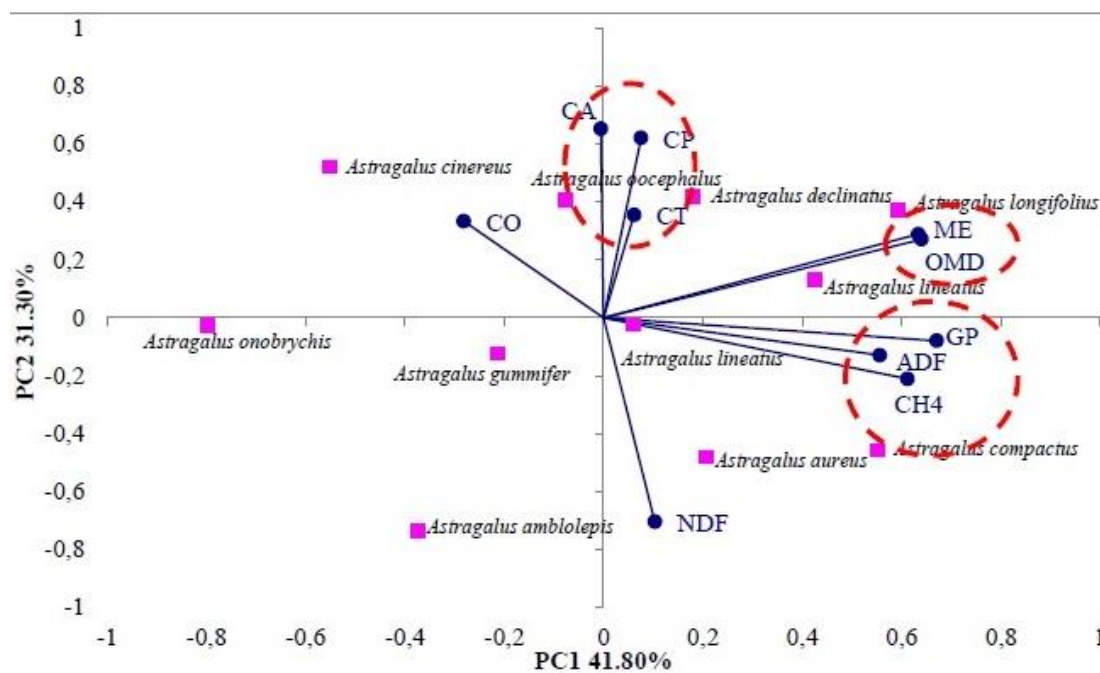


Figure 1. The biplot for nutritive values of *Astragalus* species

Discussion

Crude protein content is a significant indicator of feed quality (Assefa and Ledin, 2001). It was reported that differences in crude protein contents of different varieties can be resulted from genetics of the plants and such values can also vary depending on leaf, spike and stem ratios, ripening periods, fertilization, climate and soil conditions (Ball et al., 2011). Number of leaves and thus leaf/stalk ratios decrease with the progress of ripening. Ripening reduces number of leaves rich in crude protein and reduces crude protein ratios of the plants (Buxton, 1996). In present study, crude protein ratios of *Astragalus* species decrease with the progress of ripening and quite significant differences were observed among the species. The crude protein contents of the present study ranged within the values reported by Davis (1982) for 33 different *Astragalus* species. However, crude protein content (32.79%) of endemic *Astragalus ooecephalus* at before flowering period was higher than those reported values.

The differences in stem and leaf ratios in plants result in differences also in crude protein contents, ADF and NDF ratios. ADF and NDF levels, which are the cell wall components, increased with the progress of ripening. It was reported for several plant

species that increased ADF and NDF ratios reduced crude protein, crude oil and carbohydrate levels of the plants with the progress of vegetation period (Canbolat, 2012; Kaplan et al., 2014a, 2014b).

It was reported that low condensed tannin levels (2-3%) had beneficial effects because they prevent extreme decomposition of proteins in rumen (Barry, 1987). Kumar and Singh (1984) reported that high condensed tannin levels had harmful effects because they decrease protein digestibility. In the current study, condensed tannin levels (0.32-1.00%) were lower than those specified ones and thus such levels had beneficial effects. Crude ash is the residual unburnt portion after ashing dry matter in an oven (Genctan, 1998). Since it cannot be synthesized by animal organisms, they should be taken externally.

Gas production of *Astragalus* species were different because of the differences in crude protein, crude oil, ADF and NDF ratios resulted from differences in their stem and leaf ratios. The increased crude oil and crude protein levels caused an increase in the metabolic energy. In vitro gas productions through fermentation in feeds are directly related to the fermentable carbohydrate content. The differences in ME and OMD levels of *Astragalus* species were mainly resulted from differences in their gas production, crude protein, crude ash and crude oil contents as well as harvest period-induced reductions in these parameters. Decreased fermentable carbohydrate contents with the progress of ripening and differences in stem and leaf ratios also decreased in vitro gas production levels (Blummel and Orskov, 1993). The gas production and thus carbohydrate levels decrease with the progress of ripening.

The feeds are classified according to the methane content after fermentation as low anti-methanogenic ($> 11\%$ and $\leq 14\%$), medium anti-methanogenic ($> 6\%$ and $< 11\%$) and high anti-methanogenic ($> 0\%$ and $< 6\%$) (Lopez et al., 2010). Accordingly, *Astragalus* species of the present study were classified as high anti-methanogenic.

Conclusion

It was concluded based on present findings that nutritive values of *Astragalus* species were quite different from each other and the progress of growing stages affected the nutritive profile of *Astragalus* samples significantly. Just because of high protein and ME contents, it was recommended that *Astragalus* species should be grazed in before flowering or full flowering stages. Among 11 *Astragalus* species investigated in this study, the endemic *Astragalus ocephalus* and *Astragalus longifolius* were found to be prominent with their high crude protein content and ME levels and low ADF and NDF ratios. It was observed that *Astragalus* species had superior properties for the animals. Further research is recommended on nutritive values of different *Astragalus* species with new in vivo and in vitro studies and also on the effects of *Astragalus* species on animal health.

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