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Effects of Condensed Tannin Content on Digestibility and Determination of Nutritive Value of Selected Some Native Legumes Species

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Abstract: The aim of this study, was to assess the nutritive value of some legumes forage species commonly used for feeding livestock in Turkey using the chemical composition including tannin, *in vitro* gas production and *in vitro* Dry Matter (DM) digestibility. There was wide variation in tannin content ranging from low, moderate to high especially in CO, CPW, DP, A, LCT, LA, LCV. A cumulative gas production, kinetics of gas production, ME, OMD, NH_3-N and VFA concentrations were determined. The CP was ranged on average from 132 -193 g kg^{-1} DM. As for ash content, the highest value was observed with Lotus corniculatus (130.2 g kg^{-1} DM) and the lowest value was 62.5 g kg^{-1} DM for DP. The highest ($p<0.05$) value of potential degradability (a+b) was estimated with LCV. The lowest ($p<0.05$) value of a+b was observed by LA. There was no significant ($p>0.05$) difference among all speices roughages for VFA concentration in rumen fluid. ME, OMD, CT content, WSC, NH_3 -N values were significantly ($p<0.05$) different among all legumes species, but there was no significantly ($p>0.05$) difference for pH among all legumes in rumen fluid. The present study concluded that different legumes of Turkey as good quality roughages and has potential as alternative animal feed resources for ruminants based on chemical composition, gas production and OMD, the legumes species forages have high potential nutritive value especially as protein sources. However, the presence of tannins in some of the legume species forages may adversely affect their potential nutritive value. Because significiant negative correlations between content of condensed tannin and gas production (total gas production mL/144 h) and OMD, ME showed significant negative correlations (r^2: -0.867; r^2: -0.883; r^2: -0.882, $p<0.001$), respectively observed.

Key words: Condensed tanin, gas production, legumes forage, nutritive value

INTRODUCTION

Legumens forages is a plant containing condensed tannin which is well known to improve the efficiency of protein digestion in ruminants. Legume forage species produce forage with high protein content. However, a large part of these proteins are degraded in the rumen, inducing a low efficiency of the proteins of the diet, nitrogen losses detrimental to the environment and risks of bloat when the forages are grazed. Legumes have gained interest and importance in the recent past as feeding resources in ruminant livestock diets in area of Turkey. The tannins are also known to reduce the dry matter digestibility and the palatability (Lowry *et al*., 1996). Condensed tannins are present in the birdsfoot trefoil (*Lotus corniculatus*) and other *Lotus* sp., in various concentrations depending on the species, the cultivar and the growing conditions (McMahon *et al*., 2000).

Legumes generally contain more protein and less fiber than grasses at similar stages of growth and can be an excellent feed source. Although, forage legumes generally have a high concentration of nitrogen (N), the critical nutrient in the dry season a major limitation is the presence of secondary plant compounds, such as hydrolysable and condensed tannins, which can depress feed intake andutilization by animals (Dube, 1993; Norton, 1994). Tannins in low to moderate concentrations (i.e.<55 g CT kg^{-1}) can have beneficial effects in ruminants, including improved amino acid absorption, reduced bloat and increased postruminal protein absorption. Condensed tannins are also classified as a quantitative plant defense that reduce digestibility of

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nutrients following ingestion. Condensed tannins have been primarily sought for use in the area of ruminal protein escape in ruminants. Leguminous proteins are often poorly utilized by ruminants due to extensive microbial degradation in the Rumen. Rapid degradation of protein in browse has led researchers to explore means to increase protein use efficiency in goats (Broderick, 1995; Gebrehiwot *et al*., 2002).

There is a lack of local information on the nutritional quality of legume species that have recently been introduced in Turkey. This information is necessary for the efficient incorporation of these forage legume species into the current ruminant feeding.

MATERIALS AND METHODS

Experimental forages: Forage from seven legumens species which are; Coronilla Orientalis (CO), Coronilla Parviflora (Wild) (CPW), Dorycnium Pentaphyllum (DP), Arygrolobium (A), Lotuscorniculatus (LCT) (Trabzon), Lotus Corniulatus Vartenofolius (LCV), Lotus Langustissimus (LA). The forage samples were collected of different in Turkey country which Coronilla Orientalis (CO) from Adiyaman Golbasi; Coronilla Parviflor (CP) from K. Maras-Zeytin; Dorycnium Pentaphyllum (DP) From K.Maras-Menzelet; Arygrolobium crotalarioides from Gaziantep-Nizip; Lotus corniculatus var. Corniculatus From Trabzon-Uzungol; Lotus corniulatus var. tenuifolius (LCV) from Adiyaman-Golbasi; Lotus angustissimus (LA) from Hatay-Kirikhan. The areas is located at N 36°:30′-N 41°:00′, W 36°: 21′- W 39°-43′ between altitude- longitude. Above sea level of these areas were of 900, 580, 580, 510, 1800, 900 and 190 m and temperature of these areas were 14.45, 17.0, 17.05, 18.20, 14.45 and 19.25°C, respectively. Average annual rainfall of these areas were 692, 727, 727, 380, 831, 692 and 562 mm, respectively. Legumes were hand harvested at flowering stage from at least each species selected at random in 4 locations within flowering and fructification. The harvested samples were then pooled for each individual legumes species and then at dried during roomtemperature in unlighted room at room temperature for 5 day to constant weight and ground to pass through a 1.0 mm sieve in laboratory mill. The samples were then sub-sampled to obtain 3 samples for each tree species and used for the laboratory analysis. However, for the analysis of phenolics and *in vitro* gas production experiments, the forages were further ground to pass through a 1.0 mm.

***In vitro* experiments:** *In vitro* analyses were perform at the Institute of Grassland and Environmental Research, animal research laboratory (IGER), United Kingdom. In Experiment; Coronilla Orientalis (CO), Coronilla Parviflora (Wild) (CPW), Dorycnium Pentaphyllum (DP), Arygrolobium (A), Lotus corniculatus (LCT) (Trabzon), Lotus Corniulatus Vartenofolius (LCV), Lotus Langustissimus (LA) were tested. Forages were tested for *in vitro* ruminal fermentation characteristics with the gas pressure transducer technique as described by Theodorou *et al*. (1994). One gram of forage DM was weighed into serum bottles with 150 mL capacity. Bottles were filled with 85 mL of a mixture of mineral and buffer solution and 4 mL of reducing agent (Menke and Steingass, 1988). After adding 10 mL of rumen fluid, 3 replications of each treatment were incubated in a pre-heated water bath at 39°C. Additionally, 6 blanks without plant material but with buffer solution, reducing agent and rumen fluid were incubated in each experiment. The mixed rumen fluid was collected from two rumen cannulated sheeps grazing on a pasture dominated by grass (medium feed quality). The serum bottles were sealed by means of a rubber stopper and the gas pressure was set to zero using a transducer and a LED digital readout voltmeter. Gas pressure and volume were recorded at 3, 6, 10, 16, 19, 24, 30, 37, 47, 60, 72, 96 and 144 h after the inoculation. All chemical analyses were carried out in triplicate. The gas production characteristics were estimated by fitting the mean gas volumes to the exponential equation ($G = a+b\ (1-e^{-ct})$) of Ørskov and McDonald (1979). The metabolizable energy (MJ kg^{-1} DM) and OMD of sample was calculated using equations of Menke *et al*. (1979).

After the last measurement, the bottles were stored at 4°C until further processing. An amount of 30 mL of the fermentation fluid was centrifuged on ice for 15 min at 30.000×g. Subsamples of the supernatant were taken for subsequent determination of pH, redox potential and Volatile Fatty Acid (VFA) concentration. For VFA determination, 1.6 mL of fermentation fluid was deproteinised with 0.4 mL methaphosphoric acid (250 g L^{-1}). Subsequently, samples were stored at -20°C until later processing.

Chemical analyses: Chemical analyses were perform at the Institute of Grassland and Environmental Research, chemical analyses laboratory (IGER), United Kingdom. Individual forages were subjected to analysis samples were stored at -20°C prior to subsequent analysis. DM content of the samples for chemical analysis determined by freeze-drying. Ash was measured by igniting samples in a muffle furnace at 550°C for 16 h. Total Nitrogen (TN) concentrations were determined using a Leco FP 428 nitrogen analyzer and expressed as CP (TN×6.25) (AOAC 1995). Concentrations of WSC were determined spectro-photometrically using anthrone in sulphuric acid

on an auto analyser (Thomas, 1977). Volatile Fatty Acids (VFAs) were analyzed by gas chromatography as described by Zhu *et al.* (1996), Ammonia-N was analyzed according to the method of Bremner and Keeney (1965). Determinations of NDF and ADF concentrations were carried out according to the method of Van Soest *et al.* (1991). Contents of extractable and bound CT in individual forages were determined as suggested by Terrill *et al.* (1992) and Makkar *et al.* (1995). Purified tannins, extracted from each material by the procedure of Hagerman and Butler (1980), were used as standards. Analyses of pH (combination pH-electrode, model 8102 ross, Orion Research Inc., Beverly, MA, USA.

Volatile Fatty Acids (VFA) analysis: Volatile fatty acids analyses were perform at the Institute of Grassland and Environmental Research, chemical analyses laboratory (IGER), United Kingdom. Volatile Fatty Acids (VFAs) in Rumen fluids were analyzed by gas chromatography as described by Zhu *et al.* (1996). Fermentation end-products, Acetic (Ac), Butyric (Bu), Propionic (Pr), Valeric (Va), Iso-butyric (IBu) and Isovaleric (IVa) were measured by gas chromatography. Culture fluid (1 mL) was acidified with 5 mL of orthophosphoric acid and stored at -4°C until analysis. Prior to analysis, acidified samples were defrosted and centrifuged at 3500 g for 3 min. Internal standard (0.2 mL; 15 mM 2-methylvaleric acid in 0.15 Morthophosphoric acid) was added to the supernatant. A calibration mixture consisting of the following VFA: 15.0 mM Ac, 5.0 mM Pr, 0.2 mM IBu acid, 2.0 mM Bu, 2.0 mM IVa acid and 2.0 mM Va in 0.15 M orthophosphoric acid, was used as the external standard. VFA quantification was carried out using a Chrompack 9000 chromatograph fitted with an automatic sampler (Chrompack 911) and linked to an IBM PC.

Determination of condensed tannins: Condensed tannin analyses were perform at the Institute of Grassland and Environmental Research, plant research laboratory (IGER), United Kingdom. Total condensed tannins were determined as the sum of extractable (acetone-soluble) and bound fractions on 20±30 mg samples of freeze-dried leaf and stem using a modification of the butanol±HCl method outlined by Terrill *et al.* (1992) and Carter *et al.* (1997). The samples were mixed and extracted with 4 mL of 70% aqueous acetone and 2 mL diethyl ether, vortex-mixed for 2 min then centrifuged at 2500 rpm for 5 min resulting in three phases. The upper solvent phase, containing chlorophyll pigments and lipids, was discarded and the clear aqueous phase containing acetone-soluble condensed tannins was decanted and retained. The residue containing bound condensed tannins was then re-extracted as above. The combined aqueous phases were concentrated, adjusted to 2.5 mL with distilled water and 0.5 mL aliquots hydrolyzed in 3.5 mL of BuOH: HCl (95:5 v/v) for 1 h at 100°C, followed by rapid cooling. The residue was dried in a stream of air to remove traces of solvent and directly hydrolyzed in 4 mL of BuOH: HCl. The hydrolysates were then scanned between 400 and 700 nm by visiblespectrophotometer and condensed tannin concentration calculated from the peak height at 550 nm using an E1% 550 value of 150, derived from a standard curve of L corniculatus condensed tannin extracted from whole plant by the method of Terrill *et al.* (1992) following purification on Sephadex LH20.

Calculations and statistical analysis: All the data was subjected to Analysis Of Variance (ANOVA) using the General Linear Model procedure (SAS/Statview, 1999) and significance between means tested using least significant difference.

RESULTS AND DISCUSSION

Chemical analysis: The chemical composition and phenol compounds of legumes (Coronilla Orientalis (CO), Coronilla Parviflora (Wild) (CPW), Dorycnium Pentaphyllum (DP), Arygrolobium (A), Lotus Vorniculatuc (LCT) (Trabzon), Lotus Corniulatus Vartenofolius (LCV), Lotus Langustissimus (LA)) were shown in Table 1. The ash, CP, NDF, ADF, WSC and CT contents were highly variable and also significant differences detected among the various species ($p<0.05$). The ash (g kg^{-1} DM) ranged from 62.5 (DP) to 123.8 (LCV). The CP content (g kg^{-1} DM) range was 132.2 (A) to 192.8 (CPW).

Table 1 shows CP had the lowest A while highest rate of CP had CPW. DP had the highest NDF and ADF contents while LTC had the lowest NDF and ADF contents. The value of protein among the legumes, legumes and browse plants was within the reported ranges (Bamikole *et al.*, 2004). Many studies have shown that in roughages and in legumes, CP cannot be considered a uniform fraction because some nitrogen can be found either as soluble non-protein nitrogen or bound to cell walls (Shayo and Udén, 1999).

The condensed tannin content of CO, CPW, DP, A, LCT, LA and LCV respectively were 3.78, 103.78, 18.41, 0.75, 22.19, 55.58 and 55.74 g kg^{-1} DM, respectively. DP had the highest of condensed tannin content while A had lowest condense tannin content. The Water Soluble Carbohydrate (WSC) contents of CO, CPW, DP, A, LCT, LA and LCV, respectively were 4.87, 2.15, 4.29, 2.81, 3.25, 2.54 and 3.22 g kg^{-1} DM, respectively. CO had value of highest of WSC while DP had value of lowest of

Table 1: The tannin content and chemical composition of legumes species harvested at flowering stage

	CO	DP	CPW	A	LTC	LA	LCV	
	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	Sig.
Cp	16.63±0.13^{b}	14.38±0.13^{dc}	19.28±0.28^{a}	13.22±0.04^{d}	15.94±0.07^{b}	13.75±0.18^{dc}	14.59±0.84^{c}	**
Wsc	4.87±0.15^{a}	2.15±0.04^{e}	4.29±0.08^{b}	2.81±0.12^{d}	3.25±0.03^{c}	2.54±0.01^{d}	3.22±0.05^{c}	**
Dm	90.38±0.08^{e}	92.76±0.15^{a}	92.18±0.05^{b}	91.60±0.08^{c}	91.60±0.08^{c}	91.07±0.13^{d}	92.83±0.13^{a}	**
Ash	12.08±0.13^{b}	6.25±0.04^{f}	8.94±0.02^{c}	7.51±0.05^{d}	13.02±0.10^{a}	6.83±0.09^{e}	12.38±0.27^{b}	**
NDF	34.39±0.36^{de}	46.64±0.46^{a}	35.50±0.52^{d}	40.11±0.74^{b}	33.17±0.13^{e}	37.87±0.52^{c}	35.90±0.10^{d}	**
ADF	31.16±0.55^{c}	43.16±0.93^{a}	29.51±0.01^{c}	30.14±0.13^{c}	26.61±0.31^{d}	36.91±0.82^{b}	36.91±0.82^{b}	**
CT mg dm^{-1}	3.78±0.39^{de}	103.78±0.54^{a}	18.41±2.71^{c}	0.75±0.19^{e}	22.19±3.33^{c}	55.58±4.37^{b}	8.9774±0.54^{d}	**

significant ($p<0.05$); ** significant ($p<0.01$); ***significant ($p<0.001$); $\bar{x}$:means; sem: standard error means, DM: Dry Matter; CP, Crude Protein; NDF, Neutral Detergent Fiber; ADF, Acid Detergent Fiber; WSC: Water Soluble Carbohydrate; CT, Condensed Tannin

Table 2: Value of ME, OMD and *In vitro* gas production and fermentation characteristics of different legumes species forages harvested at flowering stage

	CO	DP	CPW	A	LTC	LA	LCV	
	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	Sig.
3	36.50±3.50^{b}	21.50±1.50^{e}	32.00±2.00^{b}	33.00±2.00^{b}	38.50±0.50^{b}	24.00±0.00^{e}	47.50±1.50^{a}	**
6	73.50±0.50^{b}	37.50±1.50^{d}	62.50±1.50^{c}	68.00±4.00^{bc}	68.50±0.50^{bc}	42.00±0.00^{d}	89.00±2.00^{a}	**
10	108.50±4.50^{b}	55.50±1.50^{e}	95.50±6.50^{b}	107.50±6.50^{b}	101.50±0.50^{b}	61.00±0.00^{e}	139.00±2.00^{a}	**
16	137.00±5.00^{bc}	70.00±1.00^{e}	130.50±6.50^{c}	147.50±6.50^{b}	136.50±0.50^{bc}	84.50±0.50^{d}	176.00±2.00^{a}	**
19	154.00±5.00^{bc}	80.50±1.50^{e}	149.50±6.50^{c}	167.50±6.50^{b}	153.50±0.50^{bc}	97.00±0.00^{d}	192.50±1.50^{a}	**
24	170.00±6.00^{bc}	92.50±3.50^{d}	164.50±6.50^{c}	184.00±8.00^{b}	168.00±0.00^{bc}	107.00±0.00^{d}	206.50±1.50^{a}	**
30	184.00±6.00^{bc}	104.00±4.00^{d}	179.00±6.00^{c}	199.00±7.00^{b}	182.00±0.00^{c}	116.00±0.00^{d}	221.00±2.00^{a}	**
37	200.00±7.00^{bc}	116.00±6.00^{d}	194.00±6.00^{c}	213.50±6.50^{b}	196.50±0.50^{bc}	126.00±0.00^{d}	235.00±2.00^{a}	**
47	213.00±7.00^{bc}	132.50±5.50^{d}	208.00±6.00^{c}	228.00±6.00^{b}	210.50±0.50^{c}	132.00±1.00^{d}	247.00±1.50^{a}	**
60	224.00±7.00^{b}	155.00±3.00^{e}	217.00±6.00^{b}	245.00±11.50^{a}	220.50±0.50^{b}	154.00±1.00^{e}	256.00±1.00^{a}	**
72	230.00±8.00^{b}	168.50±0.50^{e}	224.00±6.00^{b}	255.00±11.00^{a}	227.50±0.50^{b}	169.00±0.00^{e}	260.50±0.50^{a}	**
96	236.00±8.00^{b}	180.00±0.00^{e}	230.50±5.50^{b}	262.50±11.50^{a}	232.50±0.50^{b}	182.00±0.00^{e}	265.00±0.00	**
144	242.00±8.00^{b}	190.50±0.50^{e}	237.00±5.00^{b}	269.00±12.00^{a}	238.50±0.50^{b}	193.00±0.00^{e}	270.00±1.00^{a}	**
Estimated parameters								
c	0.05±0.00^{bc}	0.02±0.00^{e}	0.05±0.00^{bc}	0.05±0.00^{c}	0.05±0.00^{b}	0.03±0.00^{d}	0.07±0.00^{a}	**
a	4.88±2.04^{bc}	7.36±0.39^{ab}	0.96±0.12^{d}	1.47±1.25^{d}	4.16±0.43^{bcd}	9.60±0.14^{a}	2.37±0.28^{cd}	**
b	230.60±9.76^{b}	189.45±2.40^{e}	230.23±4.94^{b}	260.79±9.60^{a}	228.40±0.56^{b}	179.42±0.11^{e}	259.83±0.25^{a}	**
a+b	235.48±7.73^{b}	196.81±2.79^{e}	231.19±4.82^{b}	262.04±11.06^{a}	232.56±0.13^{b}	189.02±0.03^{e}	262.20±0.52^{a}	**
ME	10.53±0.21^{ab}	8.80±0.03^{c}	10.82±0.09^{ab}	10.77±0.33^{ab}	10.33±0.02^{b}	8.79±0.03^{c}	11.00±0.09^{a}	**
OMD	65.38±1.36^{ab}	54.83±0.15^{c}	65.48±0.77^{ab}	67.81±2.68^{ab}	64.49±0.09^{b}	55.03±0.09^{c}	69.46±0.19^{a}	**

* significant ($p<0.05$); ** significant ($p<0.01$); ***significant ($p<0.001$); $\bar{x}$:means; sem: standard error means, OMD, Organic Matter Digestibility; ME, Metabolic Energy, a: gas production (mL) from quickly soluble fraction, b: gas production (mL) from insoluble but degradable fraction, c: gas production rate (%)

WSC. The chemical compositions of the forages were within the range reported in the literature for browse forages from Kenya (Osuga *et al*., 2005; Abdulrazak *et al*., 2000).

Variations in chemical composition among different studies on species of legumes foliages may be partly due to genotypic factors that control accumulation of forage nutrients. Accumulation of nutrients in plants is a property of species (Minson, 1990) and varies among species and genera. Differences in CP, NDF, ADF, WSC, CT and Ash could similarly be due to species genotypic differences in factors. CP content differed among plant species and t among ash, NDF and ADF content (Haddi *et al*., 2003), Ash content, which is characteristically in legumes forage such as herbaceous and lotus ssp. was ranged between 6.25 and 13.02% DM. Ash content in legumes species which tested in the current study is in agreement with the their results.. The legumes forages had low to moderate content of fiber. This is a positive attribute of the browse forages since DM digestibility are dependent of the cell wall constituents (fiber) especially the NDF and lignin (Bakshi and Wadhwa, 2004). In addition, the fiber of legumes species forages has been shown to be more digestible (Hassan *et al*., 2000) than that of grasses and crop residues. The variation in the various chemical compositions evaluated may be due to several factors such as species, soil, stage of maturity and harvesting (Singh *et al*., 2005).

***In vitro* gas production:** Cumulative gas production profiles, corrected for blank are shown in Table 2. The cumulative gas production values during 144 h of incubation were 242.00, 190.50, 237.00, 269.00, 238.50 193.00 and 270.00 mL^{-1} mg DM for Coronilla Orientalis (CO) Coronilla Parviflora (wild) (CPW), Dorycnium Pentaphyllum (DP), Arygrolobium (A), Lotus Corniculatus (LCT), Lotus Angustissimus (LA), Lotus Corniulatus Vartenofolius (LCV, respectively. The forages significantly ($p<0.05$) differed in the gas production and

fermentation characteristics in 144 h of incubation The high cumulative gas production was observed in LCV. Lotus corniulatus vartenofolius which followed by A and CO. The lowest value of total tannins in A. LCV could be a positive factor for increasing the microbial activity, resulted in enhancing the gas production.

The effect of tannin on the gas production, dry matter digestibility and ME content of legumes species harvested at flowering stage is given in Table 2. The variation in gas production and potential of gas production between the species forages can be attributed to compositional differences of the forages, especially CP, fiber, nature and concentration of polyphenolics and may be other anti-nutritional components.

In the present study, CO, CPW, A, LTC and LCV higher increase higher in gas production than the other species. This demonstrates that the tannins in the legumes species forages would adversely affect their nutritive value. This could probably be due to lower tannin activity of tannins in the species or presence of some other anti-nutritional factors not measured in this study.

The same trend was observed. Condensed tannins was different from 0.75-103.78 mg kg^{-1} DM, thus the gas production increased from 190.50 -270.00 mL^{-1} g DM at 144 h of incubation for CO, CPW, DP, A, LCT, LA and LCV, respectively. The low values of gas production were observed with DP and LA which is due to the high tannins content in legumes. Total VFA was not statically different in Rumen fluid concentration, but DP and LA had highest condensed tannin which numerically were showed least VFA and WSC (44.24 and 44.86), (2.15 and 2.54). The low values of VFA were observed with DP and LA which is due to the high tannins content in legumes, The present results of gas production are in agreement with the previous finding, although, the proportion of individuals VFA (acetic, butyric and propionic acids) were determined. Gas is produced mainly when substrate is fermented to acetate and butyrate. Substrate fermentation to propionate yields gas only from buffering of the acid and, therefore, relatively lower gas production is associated with propionate production. Rapidly fermentable carbohydrates yield relatively higher propionate as compared to acetate and the reverse takes place when slowly fermentable carbohydrates are incubated. Many workers found more propionate and thus lower acetate to propionate ratio in the ruminal fluid. If fermentation of feeds leads to a higher proportion of acetate, there will be a concomitant increase in gas production compared with a feed with a higher proportion of propionate (Blümmel *et al*., 1997; Blümmel and Orskov, 1993).

The data of degradation kinetics of roughages using gas production confirmed the results of gas production (Table 2). The highest ($p<0.05$) value of potential degradability (a+b) was estimated with LCV and followed by A, LTC and CO. The low ($p<0.05$) value of a+b was observed by LA followed by (Table 2). Production rate constant for gas production rate c were 0.05, 0.02, 0.05, 0.05, 0.05, 0.03 and 0.07 mL h^{-1} for CO, DP, CPW, A, LTC, LA and LCV, respectively. These results suggest that gas production profiles are not necessarily linearly related to degradation or fermentation of feedstuffs according to the results of the chemical composition of feedstuffs. Mathematical descriptions of gas production profiles allows analysis of data, evaluation of substrate- and media related differences and fermentability of soluble and slowly fermentable components of feeds (Getachew *et al*., 1998; Liu *et al*., 2002; Makkar, 1993; Makkar, 2005), which is in agreement and confirmed by the current study.

Rumen fermentation, energy contents and organic matter digestibility: The predicted metabolizable energy (ME, MJ kg^{-1} DM) and Organic Matter Digestibility (OMD) from gas production after 144 h incubation for CO, DP, CPW, A, LTC, LA and LCV are presented in Table 4. There were ($p<0.001$) significantly differences among all roughages in ME and OMD. . The highest value was 69.46 g kg^{-1} OMD for LCV and which followed by 67.81, 65.48, 65.38, 64.49, 55.03 and 54.83 g kg^{-1} OMD for A, CPW, CO, LTC, LA and DP, respectively, however there were significantly differences between them. These results suggest that there are positive correlation between gas production and ME (r: 0.944) and also between gas production and OMD (r: 0.981). Deaville and Givens (1998) suggested that it is preferable to use prettied silages to avoid interference from any indirect gas produced. The result indicates that fermentation acids in forages do not yield direct gas and therefore provide little energy for rumen microbial growth and the contribution of fermentation acids to diet/feed ME content should be subtracted during the calculation of fermented ME.

These results may due to the highest values of them for CP, WSC and ME. Also, there are significant a negatif correlation between *in vitro* gas measurement and Tannin content. But, There was a positive correlation between ME and OMD (Table 3). The present study found that amount of tannin content decreased OMD and value of ME content in between them. In addition, the gas production of LCV, CPW, A and CO were statically same as OMD and ME, but were than different of DP, LTC. Also, LCV has the best potential degradability and a faster degradability rate with shorter lag time than of

Table 3: Ruminal ammonia-N, pH and concentration of total volatile fatty acids (VFA) in the liquid fraction of the culture media at the end of the incubation from seven legumes

	CO	DP	CPW	A	LTC	LA	LCV	
Item	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±sem	$\bar{x}$ ±se	$\bar{x}$ ±sem	sig
pH	7.01±0.03	6.93±0.01	6.93±0.01	6.93±0.01	6.93±0.01	6.93±0.01	6.96±0.03	ns
NH_3N mg dL^{-1}	78.40±1.69^{a}	62.73±0.72^{abc}	75.70±11.29^{a}	56.71±0.63^{bc}	68.13±1.38^{ab}	51.65±0.58^{c}	68.67±3.79^{ab}	*
VFA mg 100 mL^{-1}	52.23±0.88	44.24±0.32	49.70±2.44	51.42±0.36	49.65±1.18	44.86±1.69	52.76±5.25	ns
Acetic	32.59±0.26	29.82±0.41	31.43±1.71	34.37±0.34	32.65±0.90	30.79±1.14	34.77±3.61	ns
propionic	10.90±0.20	9.33±0.04	10.43±0.46	10.37±0.03	9.98±0.27	9.03±0.43	10.95±1.12	ns
i-Bütüric	0.79±0.08^{a}	0.53±0.01^{cd}	0.67±0.02^{b}	0.55±0.00^{bcd}	0.57±0.00^{bc}	0.44±0.00^{d}	0.67±0.04^{b}	ns
n-Butiric	5.64±0.18^{a}	2.91±0.09^{e}	4.61±0.18^{b}	4.45±0.02^{b}	4.29±0.00^{b}	3.29±0.07^{c}	4.19±0.30^{b}	**
i-Valeric	1.16±0.05^{a}	0.85±0.05^{cd}	1.02±0.02^{ab}	0.91±0.03^{bc}	1.02±0.01^{ab}	0.74±0.01^{d}	1.10±0.09^{a}	**
n-Valeric	1.17±.12^{b}	0.81±0.02^{c}	1.56±0.07^{a}	0.77±0.01^{c}	1.14±0.00^{b}	0.60±0.03^{c}	1.09±0.09^{b}	**

ns: not significant ($p<0.05$); * significant ($p<0.05$); ** significant ($p<0.01$); ***significant ($p<0.001$); $\bar{x}$:means; sem, standard error means; VFA, Volatile Fatty acid

Table 4: Correlation coefficient (r) relationship of chemical composition with in vitro gas production, condensed tannin and estimated parameters

	DM	CP	NDF	ADF	ASH	WSC	CT
24 h	0.029	0.183	-0.626*	-0.817***	0.699**	0.505	-0.906***
144 h	0.063	0.069	-0.469	-0.748**	0.566*	0.400	-0.867 ***
Estimated parameters							
c	0.054	0.297	-0.728**	-0.813***	0.817***	0.574*	-0.860***
a	-0.252	-0.354	0.354	0.657**	-0.363	-0.454	0.686 **
b	0.142	0.079	-0.388	-0.705 **	0.525*	0.372	-0.816***
a+b	0.128	0.044	-0.386	-0.697 **	0.534*	0.356	-0.815***
ME	0.040	0.392	-0.589*	-0.825***	0.628*	0.615*	-0.882***
OMD	0.051	0.165	-0.557*	-0.801***	0.641*	0.528*	-0.883***
CT	0.342	-0.251	0.753**	0.885***	0.625*	-0.648*	

*significant ($p<0.05$); ** significant ($p<0.01$); ***significant ($p<0.001$); $\bar{x}$:means; sem: standart error means, OMD, Organic matter digestibility; ME, metabolic energy, ; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC: Water Soluble Carbohdyrate;CT, condensed tannin; a: gas production (mL) from quickly soluble fraction, b: gas production (mL) from insoluble but degradable fraction, c: gas production rate (%)

forages sp. which tested in the present study. Therefore, the current study concluded that legumes ssp. could be used by ruminant animals form as good quality roughages and an alternative animal feed resources for ruminant feeding especially in dry season or when roughage sources availability is low.

The low OMD and ME value of LA and DP foliage is due to the presence of phenolic compounds, especially tannins (Makkar *et al*., 1995). Tannins can reduce the nutritive value of feedstuffs more than lignin and they can also affect the health of the animal. The level of tannins varied substantially with season and area of plantation. The present results indicated the tannins content in different legumes species which might be leaded to an increment of bacterial activity during eating as a result of decreasing of chemical composition of different legumes and fiber fractionations and increasing in gas production and OMD and ME. The forages had moderate to high *in vitro* DM and DM degradability. This demonstrates the high nutritive value of the browse forages when used in ruminant feeding. Getachew *et al*. (2000) demonstrated that the browse forages are better used as protein than poor quality roughages such as hay and straws.

The concentrations of ammonia (NH -N) were ranged from 51.65-78.40 mg dL^{-1} after 144 h incubation (Table 4). The values were different significantly ($p<0.05$) concentrations of ammonia (NH -N) of legumes species and this is due to the enhancement of breakdown of crude protein of them and also due to the amount of tannins content in the legumes.

The present results of NH-N concentration are in agreement with the results of Khattab (2007). He found that high NH-N concentration have lower ammonia (NH-N). Some legumes ssp could be due low tannin content, high non protein nitrogen and soluble nitrogen. The concentrations of Volatile Fatty Acids (VFA) were not significantly differences among all roughages (Table 3). The mean values of VFA concentrations of sampling times were 52.23, 44.24, 49.70, 51.42, 49.65,44.86 and 52.76 m.eq/100 mL R.L. for CO, DP, CPW, A, LTC, LA and LCV, respectively. The formation of undegradable complexes (Fall Toure *et al*., 1998). between Condensed Tannins (CT) and protein and/or carbohydrates may have reduced the amount of available substrate for fermentation. Woodward and Reed (1989), indicated that the low and steady 3 production of VFA and ruminal NH-N may resulted from high fiber content in legumes species and from inhibition of rumen microbes by tannins which lead to low rate of deamination.

Effects of condensed tannin on digestibility: The tannin contents were highly variable, with significant ($p<0.01$) differences among the seven legumes species. The differences may be attribute to variation in environmental

conditions, plant nutrition and stage of plant growth. In addition, the variations in tannin content may be due to different reactions of tannins or other compounds present in the forage legumes. The total CT content of legumes are comparable to that of LCV, CO and A of 8.97, 3.78 and 0.75 g kg^{-1} DM, respectively, but lower than that of DP, CPW, LTC and LA which were high and ranged from 103.38, 18.41, 22.19 and 55.58, respectively g kg^{-1} DM. The presence of high concentrations of tannins is reported to reduce N degradation in the rumen through formation of tannin-protein complexes which are stable at rumen pH but do cleave at the low gastric pH (2.5-3.5) of the abomasums and the relatively high pH (8-9) of the distal small intestines (Mangan, 1998). Ruminant nutrition studies with legumes species have indicated an optimal CT content in forage of 22 g CT kg^{-1} DM, while a range of 60-100 g CT kg^{-1} DM depresses intake and growth (Barry *et al*., 1986). Thus, the CT content of legumes in this study should be beneficial when included in ruminant diets.

These plants can be considered as good protein source in Turkey. Furthermore, the digestive utilization of nitrogenous compounds depends on the presence of tannins and other phenolic compounds (Rittner and Reed, 1992), commonly present in some legumes species. The legumes material of 7 legumes species studied herein can be considered of high digestibility based on the comparison of our results on OMD with other data reported in the literature for conventional grass and legume forages or for other browse plants (Hove *et al*., 2001; Khanal and Subba, 2001). Therefore, other factors may constrain the feeding value of these legumes sources, such as the presence of secondary compounds. The tannin content of legumes from some legume species is almost negligible (Ammar *et al*., 2004) and should not affect negatively its digestibility.

There are positive correlation between CT and NDF and ADF, The negative correlation CT between ME, OMD and total gas production (Table 4). The correlation between the change in gas production in the presence of condensed of legumes forages was consistent with those of Tolera *et al*. (1997). The negative correlation CT and ME (r^2: -0.882); OMD (r^2: -0.883); (r^2: -0.867) observed in the present study and that reported by others (Abdulrazak *et al*., 2000) could be due to the variation in structural and biological activity of tannins.

CONCLUSION

This study is attempt to characterise of *in vitro* dry matter digestible, ME and condensed tannin of some local *legumes* sp. in Turkey. It has been revealed a wide range variation in nutritive value of seven different legumes species to meet nutritional requirements of livestock. Results presented herein have shown that the crude protein content of the studied legumes sp. was sufficiently high to consider of these species as a potential forages resources. In comparison with previous data reported on digestibility of conventional forages, our results suggest that species from these seven leguminous plants can be considered highly digestible. In the seven species, contents of condensed tannin were tended to decrease at ME and OMD whereas legumes species less than 5% of content of tannin showed a tendency to increase ME and digestibility in seven legumes species. As compared with legumes species had a high nutritive value and were affected by content of condensed tannin.

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