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Determination of Nutritional Value and Digestibility of Twigs in Four Tree Species through Chemical and Gas Production Methods

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Abstract: This study has been conducted in order to determine nutritional value and digestibility and degradability of twigs in four tree species including *Zelkova carpinifolia*, *Gleditchia Caspica*, *Populus deltoids* and *Quercus castanaefolia* through chemical and gas production method using 3 fistulated sheep in National Research Institute for Animal Science, Iran. The experiment conducted based on Randomized Complete Design and obtained data were analyzed by software SAS and Neway. Chemical compounds (crude protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Ether Extract (EE), Ash, Crude Fiber (CF), NFC (Non Fiber Carbohydrate), Nitrogen Free Extract (NFE) and organic material (OM) and degradation were determined at the times of 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h in gas production method and parameters such as (OMD-SCFA-ME-NEL-CP-DMD) were determined. Amount of crude protein for 4 species are as follow (*Z. carpinifolia* (11%), *G. caspica* (15.4%), *P. deltoids* (10.3%) and *Q. castanaefolia* (9.5%)), also amount of crude fiber in these trees are respectively 32.7, 18.6, 13.9 and 22.9%. The most and the least fermentation and gas production was observed respectively in *G. caspica* and *Z. carpinifolia*. The most mount of gas production belonged to *G. caspica* with 54 mL/g of dry matter at 96th h.

Key words: Chemical, digestibility, gas production, nutritional value, tree, twigs

INTRODUCTION

One of the most important issues for human is creating food safety especially about protein and diaries. Livestock production has been effective in social and economic welfare of most rural and tribal societies and it is also effective as a strategic supply in family stability and agricultural system, especially in developing countries (FAO and World Food Summit Tehran, 1996). About 70% of livestock cost has been allocated to forage resources, therefore, identifying and exploiting new forage resources is very important to provide a complete and inexpensive food ration. *P. deltoids* has occupied more than 80% (150,000 ha) of non-forest arboriculture (Modir, 2008). Most of the natives use twigs of species *G. caspica, Z. carpinifolia* and *Q. castanaefolia* in cold season as they are diffused in Iran (Kelagry *et al*., 2008). Ruminants utilize wooden materials, Cellulose and Hemicellulose included in tree twigs through their 4-part digestive system and micro organism of their rumen (Ensiminger *et al*., 1986). Torres (1983) also, represented tree and shrub twigs as key resource of protein for farm animals along a year. Tree twigs have high nutritional value and the compensate a considerable part of shortage in forage resources in Africa and Savanna (Eyog-Matigo and Obel-Lawsone, 2002), (Larbi *et al*., 2005), (Gautier *et al*., 2005), (Draogo-kone *et al*., 2006) and (Petit and Mallet, 2001). Marker and Singh (1991) reported that leaves of different species of *Q. castanaefolia*, *P. deltoids* and other such trees are parts food intake by ruminants during shortage of forage in winter in areas of Himalaya, India, Nepal, China and some other countries. In a survey about chemical compound of twigs of tree species in west of Africa, SalifouOue *et al*. (2008) reported that their amount of crude protein is 12%. In a study about 6 tree species for livestock through gas production method, Gofoon and Abdel Nasir (2007) stated that high value obtained by gas production method shows better bioavailability for microorganism of rumen. Akinfemi *et al*. (2009) dealt with evaluation of agricultural waste as one of an accurate, quick, inexpensive and also a proper method to predict nutritional value of foods. Lamers and Khamzina (2010) Szekai *et al*. (1998) and Ayers *et al*. (1996) reported that *P. deltoids'* leaves have a proper quality so that its crude protein is between 12.7 to 15.6% and its crude fibers is between 18.9 to 25.6%. Kamlak *et al*. (2005) reported that chemical compounds of leaves of *P. deltoids'* five species and BenSalem *et al*. (2003)

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Singh and Doel (1985) and Matinzaeh *et al*. (2006) reported that nutritional value of *P. deltoids'* fruit and twig is relatively high. The study four species are cultivated in wide area of Iran and are used for feeding livestock. the research was conducted to determine nutritional value and digestibility of these species through chemical method and gas production.

MATERIALS AND METHODS

Gleditchia Caspica is family of *Laguminosae*, compound leaves with 15 to 25 cm long along and large elongated maroon fruit. Stems have large thorns with relatively 15 cm in length.

Zelkova Carpinifoli is a tree growing to 20 to 35 meters tall; its leaves are 4 to 10 cm long and 5.2 to 6 cm wide which are rough, alternative, deciduous, ovate, dentate and pulpy. Its flowers is non-flourished which have green smooth and rough level.

Populus deltoids is family of *Salicaceae* and grows fast, culture is known as wood farming, mostly it needs light and deep soil and water.

Quercus castanaefolia is family of *Fagaceae* and just grows in lands with deep and heavy soil which has suitable moisture (Sabeti, 2003).

Sampling were completely conducted randomly from twigs of the four species with three replications (each replication were a compound of 5 samples of each species) in north of Iran. Plant samples were dried in oven with 70ºC for 72 h and then they were milled. In this research 3 adult castrated sheep with similar weight (Average weight of 65±2.6) were used which were Talyshiyan species and they were fistulated. This research was done in the Research Institute of Animal Science of Iran (April, 2011). In order to have a proper growth and concentration of microbial population and also to make a habit in animals, two weeks before each test (daily at 8 a.m and 15 p.m), forage including 60% of tree twigs and 40% concentrate were given to animals to the extent of their appetite and they were allowed to have rock salt and water as much as they want (Orskov and McDonald, 1979). The experiment conducted based on randomized complete design and data were analyzed by software SAS and Neway.

Chemical compounds including dry matter, crude protein (Kjeldahl), crude ash (furnace), Fibre (Fibrotic), Ether Extract (Soxhlet extractor) amount of cell wall without hemicellulose (ADF) and cell wall (NDF) determined based on common method of Van Soest (1991) and AOAC (2005).

Methods of gas production conducted based on relation between produced gas and amount of forage fermentation in especial syringe as proposed by Menk *et al*., (1979). Required solutions include solution of main elements, micronutrients, buffer or artificial saliva, Rizorazin and reducer were used. Feeding was done to the extent of treatment and rumen fluid was provided before giving food in the morning. 200 mg of each sample was put in 100-mL/g syringe and 30 mL/L of cleared rumen fluid including buffer was added to syringes. Amount of gas production were measured and recorded for each treatment and replications at the time of 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h.

DMD% = 83.54-0.824 (ADF%)+2.626 (N%) (Oddy *et al*., 1983)

$NE_{(Mcal/lb)}$ = (2.20+(.0272*Gas)+(.057*CP) +149*CF))/14.64 (Menke *et al*., 1979)

OMD (%) = 0.9991 (G_{24h}) + 0.0595 (CP) + 0.0181 (CC) + 9 (Menke and steingass, 1987)

$ME_{(MJ/kgDM)}$ = 0.157 (G_{24h}) + 0.0084 (CP) + 0.022 (EE) – 0.0081(CC) + 1.06 (Menke and steingass, 1987).

$SCFA_{(mmol)}$ = 0.0222(G_{24h}) – 0.00425 (Makkar, 2005)

where G_{24h} is 24h net gas production (ml/g DM), CC, CP, EE and CF are crude ash, crude protein, ether extract and fat respectively (% DM).

RESULTS

Results of survey chemical compounds of four species showed that the most amount of crude protein is in *G. caspica* and the least amount of it is in *Q. castanaefolia* and ADF and NDF is the least amount of *G. caspica* and *Q. castanaefolia* has the most amount of it (Table 1).

Results of gas production showed that when time of fermentation increases, amount of gas production in all treatments has on an uptrend and it increases. Also amount of produced gas in tree twigs at all times of incubation has significant differences ($p \leq 0.05$) (Table. 2, Fig. 1). The most amount of gas production rate in amount of insoluble, soluble and fixed parts of produced gas belongs to *G. caspica* (Table 2).

Amount of OMD (Organic Matter Digestible), SCFA (Short Chain Fatty Acid), ME (Metabolism Energy, DMD (Dry Matter Digestibility) and NEL (Net Energy for Lactating) of twigs represented a significant statistical differences ($p \leq 0.05$). *G. caspica* has the most amount of SCFA, ME, DMD and NEL and *P. deltoids* has the most amount of OMD (Table 3).

DISCUSSION AND CONCLUSION

Conclusions showed that the most amount of protein belongs to *G. caspica* (15.4%) and the least amount of it belongs to *Q. castanaefolia* (9.5%). Amount of protein in *Populus deltoids* is 10.3% and in *Z. carpinifolia* is 11%.

Table 1: Comparing chemical compound of species through chemical analysis (based on DM)

	%OM	%NFE	%NFC	%NDF	%ADF	%Ash	%CF	%EE	%CP	%DM	Hemi	%WSC
Z. carponifolia	82.9c	48.7b	27.5c	36.4b	32.7a	17.1a	15.3a	8.0a	11.0b	93.5a	3.7a	19.1c
G. caspica	90.1b	54.0a	39.0a	27.4c	18.6c	9.9b	12.4a	8.4a	15.4a	93.3a	8.8a	27.4b
P. deltoids	87.1b	56.7a	37.7a	33.3a	24.6b	12.9b	13.9ab	6.0b	10.3b	93.2a	8.7b	26.1b
Q. castanaefolia	96.3a	55.7a	34.5b	43.4a	31.7a	3.7c	22.9a	8.8a	9.5b	92.1a	117a	29.2a
SEM	0.42	0.43	1.13	0.91	0.00	0.22	0.27	0.39	0.22	0.58	0.89	0.55
Sig	**	**	**	**	**	**	**	*	**	ns	**	**

Different letters in each column show significant differences. *, ** is significant respectively in levels of 1 and 5%

Table 2: Cumulative gas production in vitro (mL/200 mg DM) from four trees twigs after incubation for 96 h and the characteristics of fermentation obtained by fitting data for gas production after 2, 4, 6, 8, 12, 24, 48, 72 and 96 h incubation to the equation $P = a + b\,(1 - e^{-ct})$

	Incubation time (h)								Exponential equation				
	2	4	6	8	12	24	48	72	96	a	b	a+b	c
Z. carponifolia	3.4c	6.1c	9.1c	10.6c	10.7d	18.6c	21.4c	24.4c	25.1c	1b	23.5c	24.5c	0.05b
G. caspica	9.7a	19.4a	27.1a	32.1a	32.1a	43.3a	49.2a	51.8a	54.1a	1.5a	49.4a	50.9a	0.1a
P. deltoids	4.2c	7.3c	9.8c	11.8c	15.2c	19.8c	22.7c	25.5c	26.5c	0.85c	24.3c	25.2c	0.07b
Q. castanaefolia	7.2b	13.3b	18.3b	21.9b	27.7b	35.4b	41.3b	44.9b	46.1b	1.1b	43.2b	44.3b	0.09a
SEM	0.26	0.39	0.65	0.65	0.62	0.85	0.79	0.63	0.55	0.11	0.53	0.47	0.09
Sig	**	**	**	**	**	**	**	**	**	**	**	**	**

a, b and c are constants in the exponential equation P= a + b (1 – e–ct). a, initial gas production; b, gas production during incubation; a + b, potential gas production; c, rate of gas production, Different letters in each column show significant differences.*, ** is significant respectively in levels of 1 and 5%

Table 3: Comparison of digestibility of nutrients of tree species (Gas test)

	OMD	SCFA	ME	DMD	NEL
Z. carponifolia	36.4c	4.5c	5.7c	38.4c	3.0b
G. caspica	60.4b	10.4a	8.5a	64.3a	4.9a
P. deltoids	87.1a	4.8c	5.6c	36.6c	2.9b
Q. castanaefolia	91.7a	8.5b	7.8b	55.9b	4.4a
SEM	0.88	0.26	0.4	0.63	0.25
Sig	**	**	**	**	**

Different letters in each column show significant differences, *, ** is significant respectively in levels of 1 and 5%

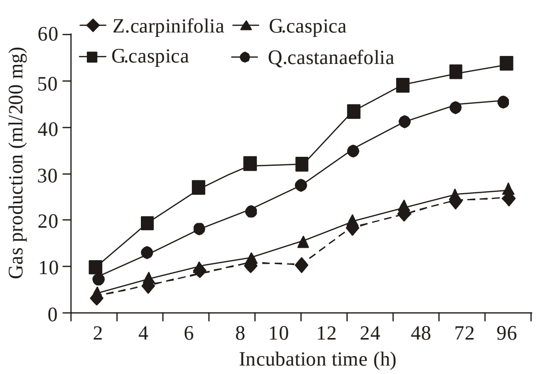

Fig. 1: Cumulative gas production (*in vitro*) over 96 h from the experimental Twigs in Four Tree Species

It considers that high protein in *G. caspica* in compare with other species is due to higher weight of leaves to stems in considered species as leaves occupy a greater part of the plant's weight. This species has compound leaves which own greater part of it. However, in *Z. carpinifolia* species, leaves are smaller and rougher and weight of leaves is less than stems. Also there is higher weight of stem to leaves in *Q. castanaefolia* and *P. deltoids* (Sabeti, 2003). Conclusions showed that there is an inverse relation between amounts of structural hydrocarbons and protein in considered species so that *Q. castanaefoli* has the most amount (ADF = 31.7% and NDF = 43.4%) and *G. caspica* has the least amount (ADF = 18.6% and NDF = 27.4%). Also, *Z. Carpinifolia* has 32.7% ADF and 36.4% NDF and *P. deltoid* has 24.7% ADF and 33.4% NDF. Conclusions obtained about *P. deltoid* are consistent with conclusions obtained by Kelagry *et al*. (2008) and Szekai *et al*. (1998) who reported that crude protein in *P. deltoid* is 7.9 to 13.5% and its crude fiber is 15.2 to 19.8%. But Ayers *et al*. (1996), reported that amounts of crude protein, NDF and ADF in leaves of *P. deltoid* are, respectively 14.8%, 42.1 and 29.3%. BenSalem *et al*. (2003); Singh and Doel (1985) and Matinzaeh *et al*. (2006) reported that nutritional value of *P. deltoid*'s fruit and twigs are relatively good which is consistent with conclusions of this research. However, Lowry *et al*. (1996) showed that adult leaves of *P. deltoids* naturally do not cause death for animals but damage their production. Indexes of ADF and NDF show extent of cellulose and lignin and these structural carbohydrates are mostly observed in plant cells (Arzani *et al*., 2006). Regarding obtain results, *G. caspica*'s twigs have higher nutritional values than *P. deltoids*, *Z. Carpinifolia* and *Q. castanaefolia* respectively. Kleinschmit *et al*., (2007). Results of the present study show that *G. caspica* (15.4%) provides 80% and three other species provide 70% of crude protein of Alfalfa for livestock. Also, Danieli *et al*. (2004) reported that ADF in Alfalfa is approximately 32%. According to the results amount of ADF in *Z. carpinifolia*, *Q. castanaefolia*, *P. deltoids* and *G. caspica* are respectively 32, 31.7, 24 and 18.6%. As it could be observed, *Z. carpinifolia* and *Q. castanaefolia* have almost equal ADF and ADF in *G. caspica* is half of ADF in Alfalfa which shows higher quality and digestibility of *G. caspica* to Alfalfa. These results show that, generally, considered species could be up to 70% replaced (*G. caspica* and a part of three other species*)* for ration and provide some parts of it. Gautier *et al*., (2005) also, stated that numbers of livestock could be increased or considerable parts of shortage in forage could be compensated through tree and shrub's twigs in north of Cameron. Of course, it should be considered that probably problems caused by *G. Caspica's* seeds overusing such as emphysema and diarrhea are not caused by using its twigs. Rip pods of *G. Caspica* fall down and absorb rainwater. When these watery pods included seeds are eaten by livestock, they find a proper condition for growth in rumen. Therefore, they, gradually, begin to sprout in animal's stomach and as they secrete toxin while sprouting and growing, they are not digestible but they become like a ball and they could not be excreted. Therefore, stomach's mechanical movement could not be performed and serious disorders cause in livestock's digestive system. It has been observed that in some advanced issues, all attempts by the animal to excrete this balls of seeds is useless and finally it removes after 2 to 3 months with lots of suffering (Khalatbari *et al*, 1997), while this problem has not observed when animals feed by twigs and it is another positive factor of using tree twigs which should be considered.

Results related to gas production in twigs of the four tree species show that the process of gas production has on an uptrend when samples are more remained in fermentation condition but speed and process of gas production in the first 24 h was more and its slope and process decrease after passing time. Krishna krishnamoorthy *et al*. (1995) also stated that more than 80% of total produced gas is produced at the first 24 h and differences among foods in fermentation of digestible parts are only create at the first hours of incubation which it has in consistent with the present research. Fermentation activity of rumen fluid at the first hours increases gas producing from fermentation of the first hours and protein degrades. But as the time passes, protein finishes and hydrocarbons of cellulose and lignin which are less degradable, ferment less and with low speed Anker *et al*. (1990), Blummel and Orskov (1993). The most amount of produced gas after 96 h belongs to *G. caspica* and the less gas is produced by *Z. carpinifolia* and *P. deltoids* while *Q. castanaefolia* produces an average amount of gas production.

Regarding that some of studies connect more gas production to protein and some of them connect it to the amount of ADF, both conditions could be observed here so that with regard to results (Table 1) *G. caspica* has the most amount of protein (16%) and the least amount of ADF (18%) and *Q. castanaefolia* has the most amount of ADF (43%) while two other species have similar protein and cellulose structure. To determine amount of produced gas by leaves of five species of *Q. castanaefolia*, Kamlak *et al*. (2005) stated that after 12 h produced gas is more and also there is a high correlation between gas production and amount of crude fiber. BenSalem *et al*. (2003) also reported that low digestibility and high lignin in leaves of *Q. castanaefolia* could be effective in gas production which is consistent with the results of this research. Results of soluble and insoluble produced gas by twigs of the species show that *Q. castanaefolia* and *G. Caspica* are very similar and amount of *Z. carpinifolia* and *P. deltoids* are very similar too. Anaerobic digestion of cellulose and other fibers through microorganism of rumen, produces volatile fatty acids, CO_2, CH_4 and a little H_2.

Even in a living livestock or in in vitro condition, volatile fatty acids produced from fermentation, interacts with bicarbonate and so CO_2 exits so that there is stimulus production of gas and volatile fatty acids due to fibers digestion. Therefore, measuring gas production in in vitro condition could provide lots of information about speed and amount of cellulose digestion (Menke and steingass, 1987). Hatami represented that as NDF goes high in maturity stage of plant growth, rate of Acetate to Propionate in produce gas goes up and when Acetate gas decreases, produced CO_2 decreases too and lead to decrease in gas production. Results of other researchers show that digestibility of dry matter decreases when ADF increases due to decrease of soluble glusides and development of cell wall (Najafnejad, 2006) which is consistent with the present study. Singh and Doel (1985) stated that high produced gas is due to high metabolism energy and fermentable nitrogen and other required nutrients for microorganism activity.

Also, Cone *and* Van Gelder (1999) showed that in foods with high percentage of protein, carbon remains in the fluid and it does not exit so in gas production method, value of energy in these foods is shown less than what it is in real. Therefore, when percentage of protein is high in samples of foods, gas production should be corrected in samples with high protein. Various studies reported application of tree twigs as forage of livestock (Eyog-Matigo *and* Obel-Lawsone (2002); Larbi *et al*. (2005); Gautier *et al*. (2005); Draogo-kone *et al*. (2006) and Petit and Mallet (2001) which it consistent with this issue so that with regard to the results of gas production, the best condition of microbial fermentation is provided for *G. caspica*, *Q. castanaefolia*, *P. deltoids* and *Z. carpinifolia* respectively. Therefore these species could be replaced by alfalfa or they could be used to provide a part of forage of livestock especially in the regions with lower forage. Similar results have been reported in researches conducted by Marker and Singh (1991) for leaves of different species of *Q. castanaefolia* (Modir, 2008) and *P. deltoids* (Gofoon and Abdel Nasir, 2007) for 6 tree species and by Kamlak *et al*. (2005) for leaves of *Q. castanaefolia* so that the represented that tree twigs have high nutritional value for ruminants specially small livestock.

CONCLUSION

The results revealed that the *G. Caspica*, *Q. castanaefolia*, *P. deltoids* and *Z. carpinifolia*, respectively are a good source of forage livestock.

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