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Comparison of *in vitro* Gas Production, Nutritive Value, Metabolizable Energy and Organic Matter Digestibility of some Chickpea Varieties

Research Article

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ABSTRACT

This study was carried out to determine the nutritional value of different chickpea varieties using *in vitro* gas production technique. As a result, significant variations in terms of chemical composition, gas production rate and metabolizable energy, net lactation energy and digestible organic matter were found among the 8 different chickpea varieties. The crude protein contents of chickpea varieties ranged from 15.26 to 18.52% DM; the crude fat contents of chickpea varieties ranged from 4.14 to 5.33% DM; ash content of chickpea seeds varied from 2.69 to 3.46% DM. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of chickpea varieties varied from 12.46 to 17.29% DM and 3.80 to 4.87% DM, respectively. 24 h total *in vitro* gas production of varieties ranged from 58.67 to 81.66 mL/200 mg dry matter (DM). The calculated metabolizable energy (ME) and digestible organic matter (DOM) contents of chickpea varieties ranged from 10.25 to 13.83 MJ/kg DM and 68.69 to 91.64%, respectively. In conclusion, chickpea can be used successfully for ruminant feeding and also Çağatay and Izmir 92 varieties are better than the other varieties studied according to their nutritive values.

KEY WORDS chickpea variety, digestibility, *in vitro* gas production, nutritive value.

INTRODUCTION

Chickpea (*Cicer arietinum*) is the most grown plant after lentils and dry beans with edible grain legumes cultivated in Turkey due to its resistance to heat and drought. Chickpea is relatively high in the content of crude protein (CP) (16.4-31.12%) and carbohydrate (50-74%), compared to the some other legume grains and it is commonly used as food and feed materials (Düzdemir *et al.* 2007; Polesi *et al.* 2011; Uzun *et al.* 2012; Sharma *et al.* 2013; Man *et al.* 2015; Cobos *et al.* 2016; Ware, 2017). It was previously reported that the average protein content of chickpea as feed material is around 21.7% (Ayaşan, 2010).

Chickpea has an average composition of 16-21% protein, 3% ash, 3-7% lipids, 5-13% crude fibre and 59-67% carbohydrates (Polesi *et al.* 2011). Peas and lentils are sodium-free and chickpeas are very low sodium and peas, lentils and chickpeas are gluten-free ingredients (Miller, 2013). Protein, carbohydrate, fat and cellulose contents of chickpea grains were 16.4-31.2%; 38.1-73.3; 1.5-6.8% and 1.6-9.0%, respectively (Doğan *et al.* 2015). Chickpea proteins are rich in essential amino acids, especially isoleucine, lysine, tryptophan, amine and total aromatic amino acids. It is rich in aspartic acid and glutamic acid (Alajaji and El-Adawy, 2006; Namvar *et al.* 2011; Aguilar-Raymundo and Vélez-Ruíz, 2016).

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The same authors, Alajaji and El-Adawy (2006) reported that different processing methods affected the vitamin B content significantly (P<0.05) and decreased (P<0.05) riboflavin, thiamine, niacin and pyridoxine levels of grain chickpea due to chemical degradation and infiltration. Haytowitz and Matthews (1983) stated that cooking of grain chickpea in hot water led to losses of about 24, 15 and 8% in potassium, copper and iron contents, respectively. The seeds of chickpea are large in size, salmon white in color, and contain high levels of carbohydrate (41.10-47.42%) (Arab *et al.* 2010). Chickpeas contain about 6% fat that is important in the vegetarian diets of resource-poor consumers (Hirdyani, 2014). Recently, the emergence of high temperatures and droughts due to global warming in Turkey has led to major reductions in the chickpea production. In order to prevent this decrease, intensive researches are being conducted to obtain resistant, redundant and high quality chickpea varieties by research institutes affiliated to the General Directorate of Agricultural Research and Policies, as well as by universities. Sharma *et al.* (2013) showed that chickpea cultivars can be an economic and alternative protein source that could alleviate protein malnutrition in developing countries and improve overall nutritional status of functional food in the developed countries. This study was carried out to determine and to compare the feed values of different chickpea varieties by chemical analysis and *in vitro* gas production technique.

MATERIALS AND METHODS

Samples

Three (Inci, Seckin and Hasanbey) cultivars of *Cicer arietinum* were obtained from fields of East Mediterranean Agricultural Research Institute (36° 51'18" North, 35° 20'49" East) and other cultivars (ILC-482, Gokce, Izmir 92, Azkan and Cagatay) were obtained from different Research Institutes of the Ministry of Food, Agriculture and Livestock of Turkey. All the eight cultivars were sown in randomized block design with 4 replicas and samples were harvested from every block. Harvested samples were dried at 70 °C in an oven for 24 hours to find out the dry matter (DM) ratio.

Chemical analyses

Dry chickpea samples were ground using a lab mill to pass a 1-mm screen. Standard methods as described in AOAC (1990) were used for the determination of ash, ether extract, crude fibre and nitrogen (N) contents. Crude protein levels were calculated using the equation N × 6.25.

The acid detergent fibre (ADF) and neutral detergent fibre (NDF) content were determined according to Van Soest *et al.* (1991). All chemical analyses were carried out in triplicate.

In vitro gas production technique

0.2 g of samples were used for gas production analysis according to Menke and Steingass (1988). Rumen digestive juice was collected from a ruminally-fistulated sheep fed *ad libitum* prairie grass, fresh water and mineral block. Rumen digestive juice was collected before the morning feeding and immediately transported to the laboratory for use. The medium was prepared by mixing 500 mL distilled H_2O, 0.1 mL micro-mineral solution, 200 mL buffer solution, 200 mL macro-mineral solution and 1 mL resazurin solution (0.1%). The buffer solution contained 35 g $NaHCO_3$ in 1 L of distilled water. The macro-mineral solution contained 9.45 g $Na_2HPO_4.12H_2O$, 6.2 g KH_2PO4 and 0.6 g $MgSO_4.7H_2O$ in 1 L of distilled water. These were prepared freshly before use. The micro mineral solution contained 13.2 g $CaCl_2.2H_2O$, 10.0 g $MnCl_2.4H_2O$, 1 g $COCl_2.6H_2O$ and 8 g $FeCl_3.6H_2O$ in 1 L of distilled water.

The micro-mineral and resazurin solutions were prepared beforehand and stored in the dark at 48 °C until required. The samples were placed in glass tubes containing 10 mL of rumen digesta and 20 mL of medium. The syringes were pre-warmed to 39 °C before the injection of 30 mL rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39 °C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Readings of gas production (GP) were recorded before incubation (0) and 24 h after incubation. Total gas values were corrected for blank and hay standards with known gas production (Goel *et al.* 2008). Metabolizable energy (ME), net energy lactation (NE_L) and organic matter digestibility (OMD) were calculated through *in vitro* gas production (GP) values for 24 h using formulas shown below (Menke *et al.* 1979):

$$ME = (2.20+0.1136\times GP+0.0057\times CP+0.00029\times CF^2) / 4.184$$

$$NE_L = (1.64+0.269\times GP+0.00078\times GP^2+0.0051\times CP+0.01325\times CF) / 4.186$$

Where:
GP: 24 h net gas production (mL/200 mg).
CP: crude protein (%).
CF: crude fat (%).

The organic matter digestibility (DOM) was calculated using the equations of Menke *et al.* (1979) as follows:

$$DOM\ (\%) = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times CA$$

Where:
GP: 24 h net gas production (mL/200 mg).
CP: crude protein (%).

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CA: ash content (%).

Statistical analyses

All data was analyzed using analysis of variance (ANOVA) and means were compared using Duncan's multiple range test and least significant difference test at $P < 0.05$ if ANOVA showed a significant effect (SPSS, 2011).

RESULTS AND DISCUSSION

Table 1 shows the results of analysis of variance indicating the level of significance differences in the contents of crude ash, CP, CF, ADF and NDF between several varieties of chickpea grain. The crude ash contents ranged from 2.69% (ILC-482) to 3.46% (Çağatay). The highest CP content was obtained from the variety of ILC-482 with a value of 18.52%, whereas the lowest value was obtained from the Azkan variety with a value of 15.26%. Crude fat contents varied from 4.14% (İnci) to 5.33% (İzmir92).

The lowest and highest ADF values were obtained from Çağatay (3.80%) and İzmir92 (5.13%) varieties, respectively. The lowest and highest NDF contents were obtained from Çağatay (12.46%) and İzmir92 (17.29%) varieties, respectively.

The *in vitro* gas production (GP) expressed as mL/200 mg DM and methane gas (CH4) at the end of the 24 hour incubation period of chickpea varieties are given in Table 2. As can be seen in Table 2, the differences in gas and methane production and percentage of total gas methane significantly ($P<0.01$) differed between the varieties. The highest GP value was obtained from Çağatay variety with a value of 81.66 mL. It was followed by the variety of İzmir92 with a value of 81.33 mL. The lowest GP value of 58.67 mL was seen in the Gökçe variety. The highest methane production of 14.82 mL was obtained from the Çağatay variety. The lowest methane value of 8.55 mL was observed in the Gökçe variety. The percentages of methane in total *in vitro* gas production at the end of the 24-hour incubation period varied from a value of 14.56% (Gökçe) to a value of 18.15% (Çağatay).

The calculated ME, NEL and DOM contents of the chickpea varieties are shown in Table 3. When Table 3 was examined, it could be seen that the differences in ME, NEL and DOM between chickpeas were significant ($P<0.01$). The lowest and highest values of ME contents were determined to be 10.25 MJ/kg DM (Gökçe) and 13.83 MJ/kg DM (Çağatay).

The highest NEL value was obtained from the Çağatay variety with a value of 9.16 MJ/kg DM and this was found in the Gökçe variety with a minimum value of 6.53 MJ/kg DM. DOM values significantly varied between 68.69% (Gökçe) and 91.64% (Çağatay).

In this study, the CP contents (15.26-18.52%) for the analyzed chickpea varieties were relatively lowered than the values reported by some other researchers (Hulse, 1991; Şanlı, 2007; Erdin and Kulaz, 2014). Akçin (1988) reported highest CP values of feed grade chickpea ranging from 22.2 to 23.9% and the values of food grade chickpea varied from 21.5 to 21.9%. An extensive search of the literature showed a large range of the CP contents such as 14.3-27.0% (Singh *et al.* 1990); 15.8-31.6%, (Sepetoğlu, 1994) and 21.9-24.6% (Şanlı, 2007).

Alajaji and El-Adawy (2006) found that the effect of different processing methods can insignificantly alter the CP content of chickpea seeds: the values of 23.64, 23.21, 23.15 and 23.16% were found for raw, heated, autoclaved and microwave cooked chickpeas, respectively.

Wood and Grusak (2007) reported that CP content of chickpea peas were ranged from 10.6 to 16.63%, whereas there was another study, where the highest and lowest CP values of İnci variety were 26.16 and 21.36%, respectively (Aydoğan *et al.* 2011). The same researchers (Aydoğan *et al.* 2011) have determined the protein contents of Gökçe, İnci, Çağatay and ILC-482 varieties as 22.54, 21.36, 23.08 and 24.29%, respectively. Jukanti *et al.* (2012) calculated the protein content of chickpea as 17-22% with shell, and 25.3-28.9% after the shell separated. Results of the nutrient composition are not in line with Sharma *et al.* (2013) who have reported mean values for protein content of 24.79% for chickpea cultivars.

Erdin and Kulaz (2014) stated that the CP in grain chickpea was 21.03% for ILC-482. Ghribi *et al.* (2015) determined the protein content in grain chickpea between 20.29 and 24.51%.

It could be thought possibility, these differences among the chemical compositions of the chickpea varieties originated from differences in regions, genotyping, planting, frequency of planting, heating processes, irrigation and applied methods.

The CF ratio in our study varied between the types of chickpeas; the lowest CF level was observed in Inci with 4.14% and the highest one was seen in İzmir92 variety with 5.33%. Alajaji and El-Adawy (2006) reported that the different processing methods did not statistically alter the CF content of chickpea seeds ($P>0.05$), while the CF content of raw chickpea (DM-based) was 6.48%; the content of grain chickpeas in autoclaved was 6.12%; the content of cooked in microwave was 6.21% and the content of CF in heated chickpea granules was 6.22%. It was observed that the CF content values of the other researchers were higher than the values we found in our research.

Wood and Grusak (2007) reported that the CF content between different chickpea varieties differs from each other (3.40-8.83% in Kabuli and 2.90-7.42% in Desi varieties).

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Table 1 The composition of chickpea varieties (DM %)

Varieties	Crude ash, %	Crude protein, %	Crude fat, %	ADF, %	NDF, %
ILC-482	2.69^{e}	18.52^{a}	4.94^{abc}	4.87^{ab}	12.48^{d}
Gökçe	3.42^{ab}	17.14^{abc}	5.03^{ab}	4.13^{bcd}	14.29^{bc}
Hasanbey	3.29^{bc}	18.03^{ab}	4.29^{bc}	4.53^{abcd}	14.46^{b}
İnci	2.92^{d}	16.69^{bc}	4.14^{c}	4.67^{abc}	13.47^{bcd}
İzmir92	3.38^{ab}	17.60^{abc}	5.33^{a}	5.13^{a}	17.29^{a}
Azkan	3.20^{c}	15.26^{d}	4.71^{abc}	3.93^{cd}	13.51^{bcd}
Seçkin	3.29^{bc}	17.36^{abc}	4.37^{bc}	4.33^{abcd}	13.35^{cd}
Çağatay	3.46^{a}	16.49^{cd}	4.39^{bc}	3.80^{d}	12.46^{d}
SEM	0.054	0.237	0.108	0.118	0.317
P-value	0.001	0.004	0.029	0.026	0.001

DM: dry matter; ADF: acid detergent fiber and NDF: neutral detergent fiber.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 2 *In vitro* gas and methane production (mL/200 mg DM) of chickpea varieties after 24 h incubation

Varieties	Gas production	Methane production (MP)	Total methane production (MP), %
ILC-482	66.33^{c}	9.96^{cd}	15.01^{bc}
Gökçe	58.67^{d}	8.55^{e}	14.56^{c}
Hasanbey	77.33^{ab}	12.25^{b}	15.83^{bc}
İnci	75.00^{b}	12.15^{b}	16.18^{b}
İzmir92	81.33^{a}	14.42^{a}	17.74^{a}
Azkan	66.33^{c}	10.75^{c}	16.20^{b}
Seçkin	60.00^{d}	8.81^{de}	14.68^{c}
Çağatay	81.66^{a}	14.82^{a}	18.15^{a}
SEM	1.848	0.480	0.294
P-value	0.001	0.001	0.001

DM: dry matter.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effect of chickpea variety on the metabolizable energy (ME), net energy for lactation (NE_L) and organic matter digestibility (OMD)

Varieties	ME, MJ/kg DM	NE_L, MJ/kg DM	DOM, %
ILC-482	11.52^{c}	7.42^{c}	76.43^{c}
Gökçe	10.25^{d}	6.53^{d}	68.69^{d}
Hasanbey	13.18^{ab}	8.67^{ab}	87.39^{ab}
İnci	12.83^{b}	8.40^{b}	84.98^{b}
İzmir92	13.82^{a}	9.14^{a}	91.37^{a}
Azkan	11.45^{c}	7.40^{c}	76.24^{c}
Seçkin	10.45^{d}	6.68^{d}	70.04^{d}
Çağatay	13.83^{a}	9.16^{a}	91.64^{a}
SEM	0.289	0.212	1.846
P-value	0.001	0.001	0.001

DM: dry matter.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Değirmenci *et al.* (2009) stated that the level of CF in the chickpea was 4.5-5.5%. The USDA (2009) identified 6.0% of the crude fat content in chickpea. According to Silva-Cristobal *et al.* (2010), chickpea seeds have relatively higher CF (5.18%) than lentil (0.98%) and black bean (1.59%). Jukanti *et al.* (2012) expressed that the CF content in the chickpea varies between 2.70 and 6.48%. Nobile *et al.* (2013) indicated that the CF content varied between 5.68 and 9.01 g/100 g. Ghribi *et al.* (2015) determined the CF content in the chickpea between 5.20 and 6.54%. The NDF and ADF levels of chickpea varieties were highest in İzmir92 and the lowest in Çağatay variety. Low levels of proportions of the ADF and NDF that make up the cell wall components are desirable.

This difference between all chickpea varieties may be related to the leaf/stem ratio and developmental status of the plants. Crude cellulose content of chickpea is 18-22 g/100 g on raw chickpea (Jukanti *et al.* 2012). Ghribi *et al.* (2015) determined the content of crude cellulose in the chickpea between 18.73 and 21.86%.

In this study, the crude ash content varied between 2.69 and 3.46% in the chickpea cultivars (P<0.001). Alajaji and El-Adawy (2006) reported that different methods of processing changed the ash content of chickpea seeds and that these changes were statistically significant (P<0.05). 3.72% of ash content was found in row chickpea (based on DM), 3.52% in heated chickpea granules; 3.56% in autoclaved, and 3.51% in cooked chickpeas in the microwave. In an-

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other study, 4.05% ash content has been recorded (Silva-Cristobal *et al.* 2010). Nobile *et al.* (2013) reported that the ash content varied between 3.55 and 4.46 g/100 g among different chickpea varieties. During the harvesting and processing, the soil mixing can cause the crude ash content to be higher.

In addition, some factors such as fertilization and irrigation, soil structure, climate, harvest time, differences in drying and storage conditions, harvest time and vegetation period may affect the crude ash content. The ash and fat contents of the chickpea varieties (Table 1) were in agreement with values found in the literatüre (Sharma *et al.* 2013). The ash content ranged from 2.13 to 3.52% in seeds of the chickpea genotypes (Kahraman *et al.* 2015).

In this study, Çağatay variety had the highest energy level with 13.83 MJ/kg DM when the metabolizable energy values of chickpea varieties were examined. This may be due to the fact that the content of NDF and ADF in Çağatay variety is lower than in other varieties. This result was supported by previous results, suggesting that ME content is inversely related to cell wall components and is directly proportional to CP content (Mountousis *et al.* 2008; Tan and Serin, 2011).

When the digestible organic matter levels were examined among the varieties, the lowest ratio was obtained from Gökçe variety with 68.69%. The highest value was found in Çağatay variety with 91.64%. Alajaji and El-Adawy (2006) reported that *in vitro* protein digestibility was affected by heating, so *in vitro* digestibility of treated chickpea seeds were higher than that of raw chickpea seeds ($P<0.05$). This improvement in digestibility can be attributed to the degradation of trypsin inhibitor, decrease tannins and phytic acid, or protein denaturation. In the same researches, *in vitro* protein digestibility of chickpea seeds were 83.6, 88.52; 89.96 and 89.40% in raw, heating, autoclave and microwave cooking methods, respectively (Alajaji and El-Adawy, 2006). Jukanti *et al.* (2012) stated that the *in vitro* protein digestibility of raw chickpeas varied between 34 and 76%; Chitra *et al.* (1995) found this value between 65.3-79.4%.

CONCLUSION

The results from this study demonstrated that the grain chickpeas, especially the Çagatay and İzmir92 varieties, can be successfully used in ruminant feeds due to their high nutritional values in terms of CP, ME and DOM content.

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