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Determination of Nutritive Value of Soybean Varieties Using *in vitro* Methods and Gas Production Technique

Research Article

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ABSTRACT

This study was conducted to determine the nutritive value of soybean varieties using *in vitro* methods and gas production technique. *In vitro* gas productions and gas production kinetics of soybean variety were determined at 0, 3, 6, 12, 24, 48, 72 and 96 h incubation times. This study revealed that there were variations among five different soybean varieties in terms of chemical composition and gas production rate. The crude protein (CP) contents of soybean variety ranged from 34.02 to 37.13%. Ash content of soybean seeds ranged from 5.42 to 5.59%. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents ranged from 16.4 to 25.0% and 13.0 to 21.6%, respectively. The ether extract (EE) ranged from 18.72 to 21.00%. The gas production rate (c) ranged from 0.110 to 0.150%. The highest gas production rate of soybean variety were found in Adasoy and Nazlıcan varieties (P<0.05). The metabolisable energy (ME) and organic matter digestibility (OMD) contents of soybean variety ranged from 7.053 to 7.383 MJ/kg DM and 71.953 to 75.320%, respectively. As a result of this study, soybean variety can be successfully used for ruminant feeds. There are considerable differences in the CP, NDF and ADF between different soybean varieties. Türksoy and Nazlıcan varieties are recommended for use in ration because of higher protein and digestibility.

KEY WORDS chemical composition, digestibility, *in vitro* gas production, nutritive value, soybean variety.

INTRODUCTION

Soybean (*Glycine max*) is one of the most important food plants of the world, and seems to be growing in importance. It is an annual crop, fairly easy to grow, that produces more protein and oil per unit of land than almost any other crop, and soybeans are a source of high value animal feed. The average nutritional composition of this leguminous crop reveals that it is extremely high in protein content. Soybean seed quality is often determined by seed protein, oil, fatty acid and mineral content. Therefore, improving soybean seed quality is key to improving human and animal nutrition. The soy protein has a high biological value and contains all the essential amino acids.

Soybean seed protein concentration consists of 341 to 568 g/kg of total seed weight, with a mean of 421 g/kg and oil concentration ranges from 83 g/kg to 279 g/kg (Bellaloui *et al.* 2010; Bellaloui *et al.* 2014; Lui, 2014). Siulapwa and Mwambungu (2015) pointed out that soybeans processed by steaming could be used as a protein and energy source at the village level as the method is easier and cheaper to use while it maintains most of the nutrients and removes the anti-nutrient factors. Siulapwa and Mwambungu (2015) found that the soybean seeds contained 48.9% CP; 2.7% ether extract and 4.4% ash. Another study found that soybean seeds contained up to 40% crude protein (CP) and about 20% fat, crude ash ranged from 4.5 to 6.4%; neutral detergent fiber (NDF), from 10 to 14.9%; acid detergent fiber (ADF), from 9 to 11.1%.

Soybean is used for soybean silage too. Soybean silage includes, on the average, 18.3% CP, 35% dry matter (DM), 43.3% NDF, 32.3% ADF and 6.7% ADL (Ayaşan, 2011). Soybean can also be grown as a silage crop in pure culture or intercropped with corn, sorghum or sudangrass (Ayaşan, 2011). Kökten *et al.* (2013) also found that soybean silage contained moderate levels of CP (15.48%). CP content of Türksoy silage (17.20%) was higher than that of Nazlıcan variety (16.21%) (Kökten *et al.* 2013).

Chemical composition in combination with *in vitro* gas production, chemical composition, organic matter digestibility (OMD) and metabolizable energy (ME) concentration are widely used to determine the potential nutritive value of soybean (Karaaslan, 2011; Eren *et al.* 2012). Gas production technique was first developed and introduced by Menke and Steingass (1988) and measured the amount of gas produced from the rumen fermentation of feedstuff in 24 h to estimate the ME.

Metabolizable energy contents of feedstuffs can be estimated by some equations using gas produced in *in vitro* gas production technique. However, there are some different equations for estimation of ME content and huge differences can be found between these equations for similar feedstuffs (Kılıc and Gulboy, 2015). Gas production is usually a good index of fermentability and digestibility (Salem *et al.* 2014).

However, little is known regarding the effects of soybean varities on *in vitro* gas production. This study was conducted to determine the nutritive value of soybean varieties using *in vitro* methods and gas production technique.

MATERIALS AND METHODS

The experiment was conducted in the Faculty of Agriculture Research Farm at Cukurova University (Turkey). Soybean seeds obtained from five different varieties were shade-dried, sent to the laboratory and milled in a hammer mill, and sieved through a 1 mm sieve for subsequent analysis.

The soybean seed varieties were produced during the second crop growing season in summer 2013 at the Eastern Mediterranean Agricultural Research Institute (36° 51'18" latitude N. 35° 20'49" longitude E) in Adana Province. All measurements in this section are based on 100% DM with three replications.

Dry matter contents of soybean seeds were determined by drying the samples at 105 ˚C overnight and ash contents were determined by igniting the samples in muffle furnace at 525 ˚C for 8 h. Nitrogen (N) contents of soybean seeds were determined by the Kjeldahl method (AOAC, 1990). Crude protein contents of soybean seeds were calculated as N × 6.25. Neutral detergent fiber contents of soybean seeds was determined by the method: described by Van Soest and Wine (1967) and ADF contents of soybean seeds were determined by the method of Van Soest (1963).

Soybean varieties milled through a 1 mm sieve were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke *et al.* (1979). Rumen fluid was obtained from eight year old and approximately 550 kg body weight of one fistulated Holstein infertile cow after a one-week adjustment period of a diet. The cow was fed twice daily with a diet containing rough feed (60%) and concentrate (40%). Basal diet (corn silage and concentration feed) was included at 2500 kcal/kg ME and content of 16% CP. Rumen fluid was collected before morning feeding and squeezed through four layers of cheesecloth. The fluid was flushed with CO_2 and then was added to buffered mineral solution in ratio of 1:2 respectively.

Approximately 0.200 g dry weight of soybean variety samples were weighed in triplicate into calibrated glass syringes of 100 mL. The syringes were pre-warmed at 39 ˚C before filling of each syringe with 30 ml rumen fluid-buffer mixture followed by incubation in a water bath at 39 ˚C. Gas production was recorded at 3, 6, 12, 24, 48, 72 and 96 h after incubation and corrected for blank incubation. Cumulative gas production data of soybean varieties were fitted to non-linear exponential model as (Orskov and McDonald, 1979):

$$Y = a + b\,(1 - \exp^{-ct})$$

Where:
Y: gas production at time't'.
a: potential gas production (mL/200 mg DM).
b: gas production from the insoluble fraction (mL).
c: gas production rate constant (h^{-1}) and t is the incubation time (h).
a + b: potential gas production (mL).

ME (MJ/kg DM) values of soybean seeds were estimated using the equation (Menke and Steingass, 1988) as follows:

$$ME\ (MJ/kg\ DM) = 1.06 + 0.157GP + 0.084CP + 0.22EE - 0.081\ XA$$

Where:

GP: 24 h net gas production (mL/0.200 g).
CP: crude protein (at 100% dry matter).
EE: ether extract (at 100% dry matter).
XA: ash content (at 100% dry matter).

Organic matter digestibility (%) values of soybean variety were calculated using the equation of Close and Menke (1986) as follows:

OMD (%)= 0.7602 GP + 0.6365 HP + 22.53

Where:
CP: crude protein (at 100% dry matter).
GP: net gas production (mL/200 mg DM) at 24 h of incubation.

One-way analysis of variance (ANOVA) was carried out to determine the effect of variety on the chemical composition, gas production kinetics, ME and OMD of soybean varieties. The significance between individual means was identified using the Tukey's multiple range tests (Pearse and Hartley, 1966). Mean differences were considered significant at P < 0.05.

RESULTS AND DISCUSSION

The effect of variety on the chemical compositions of soybean seeds is presented in Table 1. The variety was found to have significant effect on the chemical composition of soybean varieties (P<0.05). It is seen that CP contents are different in all five varieties of soybean.

Means of gas production at different times were presented in Table 2. Gas production was not significantly affected by soybean varieties (P>0.05).

The effect of variety on gas production of soybean varities at different time intervals are presented in Figure 1. Gas production at 9 h incubation of Adasoy variety was significantly higher than the others (P<0.05).

Gas production parameters of soybean varities are shown in Table 3. The effects of variety on gas production kinetics, except gas production rate, ME and OMD of soybean was not found to be statistically significant (P>0.05).

The variety was found to have significant effect on the chemical composition of soybean variety (P<0.05). The CP contents of soybean varieties were found to be 34.02% to 37.13%. While Nazlıcan variety was found to have the highest CP (37.13%); Yemsoy variety was found to have the lowest CP (34.02%). The findings of this study about CP contents of Nazlican (37.13%) are higher than findings (36.18%) of Eren *et al.* (2012) while lower than findings (38.55%) of Yetgin (2008) and Yetgin and Arıoglu (2009) and findings (39.90%) of Kınacı (2011). The CP content of Türksoy variety studied in this experiment (36.91%) was considerably lower than those obtained (43.59%) by Yetgin (2008).

Karaaslan (2011) also reported that CP of Türksoy variety was found to be 37.90%. The soybean seeds contain CP (37.9%), EE (2.34%), ash (4.4%), NDF (15.8%), ADF (6.4%) and acid detergent lignin (ADL) between 18.72% and 21.00% DM.

The NDF contents of soybean were altered from 16.40 to 25.0% DM and the ADF contents of soybean ranged from 13.0 to 21.6% DM. The Yeşilsoy variety had significantly higher ADF content than that of other groups. The soybean variety from Yeşilsoy and Yemsoy had significantly higher NDF contents than the others. As is known, the high NDF content of feed are related with digestibility negatively. In this study, NDF was not high. Yeşilsoy variety contained moderate levels of crude fat (7.66% DM). Crude fat content of Yeşilsoy was higher than that of Nazlıcan variety.

Gas production is generally a good indicator of digestibility, fermentability and rumen microbial protein production. The means of gas production of soybean varieties at different times are presented in Table 2 and Figure 1.

Dspite an inceased in cumulative gas production during the incubation period, the amount of cumulative gas production was not significantly affected by soybean varieties (P>0.05). At 9 h incubation, the gas production for Adasoy was higher than the others (P<0.05) due to low cell wall and high CP contents. After 48, 72 and 96 h incubation times, the gas produced were not affected significantly by soybean varieties. Gas produced after 96 h incubation varied between 37.45 and 40.87 mL based on the variety type.

Gas production parameters of soybean varities are shown in (Table 3). In this study, the effects of variety on gas production kinetics, except gas production rate, metabolisable energy and organic matter digestibility of soybean was not found to be statistically significant (P>0.05).

Analysing the characteristic of both gas production and rumen degradability, pH was not significantly affected by soybean varieties (P>0.05). The highest pH was in Türksoy variety but not significant, whereas Yemsoy variety had the lowest pH. The mean pH value was found to be 6.83.

The ME and OMD contents of soybean varied from 7.053 to 7.383 MJ/kg DM and 71.953 to 75.320%, respectively. The net energy for lactation (NE_L) contents of the soybean varities were between 8.397% and 8.537%. The differences of results obtained from many studies may depend on plant type, vegetation period, soil and climate. ME and OMD were not significantly affected by soybean varieties (P>0.05).

There are variation among soybean varieties in terms of *in vitro* gas production and estimated parameters, such as OMD and ME contents of soybean.

Table 1 Nutrient analysis of soybean varieties

Nutrient	Soybean varieties				
	Yeşilsoy	Yemsoy	Türksoy	Adasoy	Nazlıcan
DM (%)	90.2	90.5	90.7	90.3	91.0
Ash (% DM)	5.59	5.46	5.49	5.42	5.54
CP (% DM)	34.43[b]	34.02[b]	36.91[a]	35.38[ab]	37.13[a]
EE (% DM)	19.93	20.33	19.17	21.00	18.72
NDF (% DM)	25.0[a]	21.2[b]	20.0[b]	18.5[b]	16.4[c]
ADF (% DM)	21.6[a]	19.6[a]	18.2[b]	16.7[b]	13.0[c]
CF (% DM)	7.66	7.37	7.06	7.60	6.87

DM: dry matter %; CP: crude protein; EE: ether extract; NDF: neutral detergent fiber; ADF: acid detergent fiber and CF: crude fiber.

Table 2 Means of gas production by different soybean varieties (mL) at different times of incubation

Varieties	Incubation time (h)							
	3.00	6.00	9.00	12.00	24.00	48.00	72.00	96.00
Yeşilsoy	4.29	14.62	22.39[abc]	27.31	33.31	35.02	36.46	37.45
Yemsoy	3.74	13.35	21.11[c]	26.93	34.56	37.90	39.70	40.87
Türksoy	3.75	14.45	21.86[bc]	27.13	35.31	36.48	37.39	37.84
Adasoy	3.89	14.32	24.57[a]	27.79	33.02	35.44	36.88	37.68
Nazlıcan	4.81	14.90	23.52[ab]	30.20	35.09	36.62	38.05	39.04
SE	0.40	0.63	0.71	1.31	1.15	1.04	0.91	0.97
P-value	0.35	0.52	0.04	0.43	0.54	0.38	0.18	0.15

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standart error.

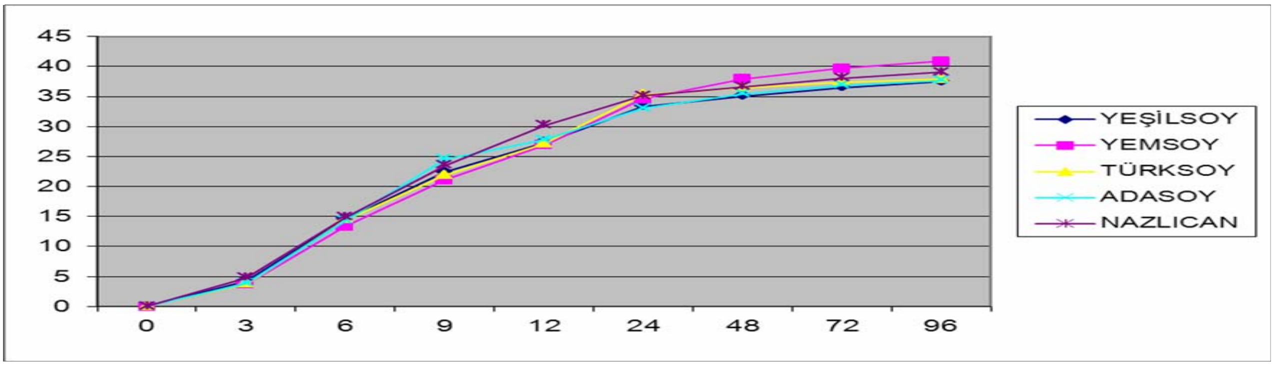

Figure 1 The effect of different varieties of soybean on gas production

Table 3 The effect of different varieties of soybean on the gas production kinetics, metabolisable energy (ME, MJ/kg DM) and organic matter digestibility (OMD, %)

Item	pH	a	b	c	OMD	ME	NE_L
Yeşilsoy	6.820	-11.887	48.050	0.137[a]	72.470	7.070	8.397
Yemsoy	6.818	-9.907	49.323	0.11[b]	72.843	7.267	8.517
Türksoy	6.855	-12.580	49.910	0.13[ab]	75.320	7.383	8.537
Adasoy	6.822	-14.943	51.220	0.15[a]	71.953	7.053	8.500
Nazlıcan	6.838	-13.687	51.503	0.143[a]	73.253	7.333	8.477
SE	0.011	1.445	1.597	0.008	0.917	0.179	0.086
P-value	0.155	0.220	0.560	0.023	0.172	0.600	0.804

a: gas production (mL) from quickly soluble fraction; b: gas production (mL) from the insoluble fraction; c: gas production rate (%) and NE_L: net energy for lactation.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SE: standart error.

The variation in gas production and estimated parameters among soybean varieties can be attributed to compositional differences of soybean varities.

Gas production (mL) from quickly soluble fraction (a) was not affected by soybean varieties (P>0.05). The study of gas production from soybean fast degradable parts (a) showed a negative effect due to delayed fermentation substrate and delay in gas production.

Gas production from slowly fermentable fraction (b) for soybean varieties was not significant (P>0.05). The potential gas production (b) of soybean variety changed from 48.050 to 51.503 mL. The potential gas production of soybean variety from Nazlıcan and Adasoy were higher than the others. Mostly, potential gas produciton is concerned with the type of carbohydrates and whether microorganisms have enough time to break them (Qheshlagh *et al.* 2015).

Canbolat and Bayram (2007) conducted a study in soybean seeds and they estimated that the potential of gas production was 72.2 mL. Moghaddam *et al.* (2013) reported that gas production was not influenced by any factor other than the chemical and physical properties of feed but changes in the microbial activity of rumen fluid may affect the rate of fermentation.

The rate of gas production is associated with the total gas. Gas production rate (c) was affected by soybean varieties (P<0.05). Gas production rate ranged from 0.110% to 0.150%. The gas production rate of soybean varities from Adasoy and Nazlıcan were significantly higher than the others. In a study conducted on soybean gas production rate was 7.5 ml per hour (Canbolat and Bayram, 2007).

Kökten *et al.* (2013) reported increased gas production with an increasing amount of NDF. This result is contrary to the findings of Karabulut *et al.* (2007). Kökten *et al.* (2014) found that the varieties: Türksoy, Erensoy, Cinsoy, Blaze, Ataem-7 and Nova were considered as primary varieties to be used in animal feed and agricultural activities of the region with regard to hay yield and quality.

CONCLUSION

There are considerable differences in the CP, NDF and ADF between different soybean varieties. Türksoy and Nazlıcan varieties are recommended for use in ration because of higher protein and digestibility.

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