The Nutrient and Energy Contents of *Medicago* Varieties Growth in Saltaffected Soils of the Harran Plain¹

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Abstract

This study was carried out to determine the nutritive and energy value of some varieties of *Medicago sativa* in salt effected soil of Şanlıurfa-Harran Plain (İkizce) by using *in vitro* gas production kinetics. In this study, *Medicago sativa (Elci), Medicago sativa (Barrier), Medicago Sativa (13R Supreme)* were used and tested in two different locations having two salinity levels which are salty (EC: 8-16 dS/m) and non salty (EC< 4 dS/m), using a 3*2 factorial block design in three replications during the three years. Results indicated that there were no significant differences among varieties in terms of nutrient composition. However, dry matter (DM) was changed according to varieties and soil*variety interactions. *Medicago sativa* produced higher dry matter under all saline conditions. The dry matter values of *Medicago sativa barrier* were lower than those other varieties. The salt response was noted further in sub varieties than *Medicago sativa* in terms of dry matter. Relative feed values (RFV) of all varieties ranged from 144 to 170 and were quite satisfactory in salty soil conditions. The *in vitro* gas production kinetics and metabolizable energy (ME), net energy lactation (NE_L) and organic matter digestibility (OMD) were affected from the salinity levels of soils. Results showed that salt stress on plants decreased gas production parameters, energy and OMD values of all varieties.

Key words: Medicago sativa varieties, in vitro gas production, glycophyte, salt tolerant forages, relative feed value.

Harran Ovası Tuzlanma Etkisinde olan Topraklarda Yetiştirilen Yonca Varyetelerinin Besin Madde ve Enerji Değerleri

Özet

Bu çalışma *in vitro* gaz üretim tekniği ve besin madde içeriklerini kullanarak toprak tuzluluğundan etkilenmiş Şanlıurfa Harran Ovası topraklarındaki (İkizce bölgesi) bazı Yonca varyetelerinin besin değeri ile metabolik enerji (ME) ve net enerji laktasyon (NE_L) değerlerini tespit etmek amacı ile yürütülmüştür. Çalışmada *Medicago sativa (Elci), Medicago sativa (Barrier), Medicago Sativa (13R Supreme)* varyeteleri iki farklı tuz seviyesine (tuzlu: 8 dS/m > EC < 16 dS/m ve tuzsuz: EC < 4 dS/m) sahip topraklarda 3*2 faktöriyel blok desenine göre 3 yıl ekilmiştir. Çalışmanın sonucunda besin değerleri bakımından üç varyete arasında farklılık çıkmamış ancak sadece kuru madde içeriği varyetelere ve varyete tuzluluk interaksiyonuna göre değişmiştir. *Medicago sativa* tüm tuz seviyelerinde daha fazla kuru madde üretmiştir. *Medicago sativa barrier* ise en düşük kuru maddeye sahip varyete olmuştur. Kuru madde açısından tuzluluğa karşı *Medicago sativa* 'nın dışındaki varyeteler daha fazla tepki vermişlerdir. Nispi yem değeri (RFV) tüm yonca varyetelerinde 144.1-169.9 değerleri arasında olup tüm toprak tuzluluk düzeylerinde tatminkar düzeyde bulunmuştur. *In vitro* gaz üretim değerleri, ME ve NE_L ile organik madde sindirilebilirliği (OMD) toprak tuzluluğundan oldukça etkilenmiştir. Çalışmanın sonucunda toprak tuzu stresinin bitkilerde gaz üretim parametrelerini, enerji ve OMD değerlerini tüm yonca varyetelerinde gerilettiği ortaya çıkmıştır.

Anahtar kelimeler: Medicago sativa varyeteleri, in vitro gaz üretimi, glikofit, tuz tolerans bitkiler, nispi yem değeri

Introduction

Drought and high soil salinity have owned two major deleterious effects on plant growth and productivity in some regions of Şanliurfa-Harran Plain. In addition to, currently, in the area of 132000 ha, both salination and sodicity are becoming major problems due to geographic structure of the plain, excessive irrigation and poor drainage conditions (Aydemir et al., 2010). Moderately high soil salinity levels are often considered unsuitable for many crops, but Alfalfa (*Medicago* *sativa*) represents an important glycophyte leguminous forage crop throughout the world and it is best adapted to moderate level of salinity and medium textured soils with pH between 6 and 8 (Tilaki and Behtari, 2009). As a perennial forage crop, alfalfa (*Medicago sativa L.*) can be cultivated in marginal lands and has a high yield and good quality high-protein content (Croughan et al., 1978; Moran et al., 1994).

The *in vitro* gas production has been developed as a predictive tool of nutrient content and digestibility to assess the nutritional quality of feeds (Menke et al.,

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1979; Getachew et al., 2004). Although the mechanisms of salt injury and salt tolerance of whole plant have been studied extensively, the effects of salt stress on nutrient content, *in vitro* gas production, digestibility, ME and NE_L levels of alfalfa hay and its varieties have not been established yet. The objective of this study was to determine potential nutritive value of different varieties of alfalfa for livestock by gas production parameters, intake, and digestibility and nutrient composition.

Materials and Methods

In this study, it was aimed to test nutrient content of different glycophyte varieties of *Medicago sativa* species growing in different salt-affected soils by *in vitro* gas production. A 3*2 factorial design was employed with 3 *Medicago sp.* varieties (*Medicago sativa (Elci), Medicago sativa barrier, Medicago sativa 13R supreme*) and 2 salt levels as main effects. There are two different locations having two saline levels (Salty: 8 dS/m > EC < 16 dS/m and non salty: EC < 4 dS/m) (Table 1).

 Table 1. Concentrations of salinity and some other components from different fields cultivated Alfalfa Plants

Series	pH (1:1)	EC dS/m	ESP %	Lime (%)
Akçakale	7.6-8.2	8-13	10-28	19-23
Ikizce	7.9-8.2	0.7-1.0	1-3	23-26

EC : (Electrical Conductivity) Salinity values, ESP: Exchangeable Sodium Percentage

The experimental design was randomized block to determine the optimum variety of *Medicago sativa* after three experiment years by taking account necessary nutrient aspects of plants via *in vitro* gas production technique. All varieties were planted in the fall and they were harvested in the beginning of blooming by cutting at ground level and then were field-dried. After that the samples were ground in laboratory mill to pass through 1 mm screen for chemical analyses and for incubations by *in vitro* gas production assays. Dry matter, crude protein, ash, ADF, NDF and ether extract of feeds were analyzed according to AOAC (1998), ANKOM procedure and the method of Van Soest et al. (1991). All chemical analyses were carried out in triplicate for each replication of plants.

Ca, Na, Mg, and K Analysis in Plants

Representative 500 g moist plant samples taken from each plot were dried at 70°C until constant weight. After drying, solutions of ground plants with a mixture of percloric acid were subjected to the process of wet burning. Mineral substances from the plant solution after the burning process were determined by reading with ICP-OES (Chapman and Pratt, 1982).

Calculation of Relative Feed Value, Digestible Dry Matter and Dry Matter Intake

Relative Feed Value (RFV) index was estimated from the digestible dry matter (DDM) of the samples from ADF values, and the potential of DM intake was calculated (as a percent of body weight, BW) from NDF values (Jeranyama and Garcia, 2009).

DDM = Digestible Dry Matter = 88.9 - (0.779 x % ADF)

DMI = Dry Matter Intake (% of BW) = 120 / (% NDF)

 $RFV = (DDM \ x \ DMI) / 1.29$

In Vitro Gas Production Technique

Holstein cows aged 5 years with ruminal cannulas (average live weight 650 kg) were used in in vitro technique. Rumen fluid was obtained from the three fistulated cows fed twice daily (08.30-16.30) with a diet containing corn silage (60 %) and concentrates (40 %). Triplicates of each sample were used in two separate runs. Approximately 200 mg dry weights of samples were weighed in triplicate into 100 ml calibrated glass syringes following the procedures of Menke and Steingass (1998). The syringes were pre-warmed at 39 °C before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39°C. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation and data were fitted to the model of Ørskov and McDonald (1979) by NEWAY package program. OMD, ME (Menke et al., 1979) and NE_L (Menke and Steingass, 1979) contents of forages were estimated using equations given below:

OMD, % = 14.88+ 0.889 *GP* + 0.45 *CP* + 0.651 *A*

ME, (MJ/kg DM) = 2.20+0.136 GP + 0.0574 CP

 NE_L , (MJ/kg DM) = 0.101 GP + 0.051 CP + 0.112 EE;

where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), A: Ash content (%), EE: Ether extracts (%).

Statistical Analysis

Completely Randomized Block Design was used to compare nutrients, gas production parameters, energy values using GLM procedure of SPSS (2006) package programs. All parameters from 2 salinity levels with 3

	Medicago sativa(Elçi)		Medicago sativa barrier		Medicago sativa 13R suppreme		_			
Minerals	Non-Salty	Salty	Non-Salty	Salty	Non-Salty	Salty	SEM	V	S	V*S
Mg, ppm	15.74 ^{c*}	20.96 ^a	15.29 ^c	18.58 ^b	11.73 ^d	18.71 ^b	0.48	0.01	0.01	0.01
Na, ppm	140.04 ^c	168.09 ^b	133.38 ^c	183.26 ^a	107.05 ^d	159.92 ^b	4.31	0.01	0.01	0.05
K, ppm	9.02 ^{bc}	19.49 ^a	8.49 ^c	12.92 ^b	9.29 ^{bc}	11.17 ^{bc}	1.20	0.01	0.01	0.01
Ca, ppm	115.93 ^a	93.12 ^b	102.57 ^b	103.09 ^b	97.21 ^b	99.13 ^b	3.04		0.05	0.01

Table 2. Some mineral components of Alfalfa Plants cultivated from different salt-affected fields

*: Significance between individual means was identified using the Duncan's multiple comparative tests, SEM: Standard error of means, V: Varieties, S: Salinity

alfalfa varieties were analyzed by 2*3 factorial patterns. Significance between individual means was identified using the Duncan's comparative tests.

Results

Nutrient Contents of Medicago Plants Grown in Soils with Different Salt Levels

Chemical compositions of all forages were presented in Table 2 and 3.

Mineral concentrations were significantly affected by variety, salinity and variety*salinity interaction. There was no difference between varieties in terms of the accumulation of Ca. Soil salinity caused a significant accumulation of Mg, Na and K. The level of Ca decreased in response to increasing salt stress, while that in *Medicago sativa (Elçi)* was lower than in others. Salinity did not cause virtually any effect on the nutrients (Table 3).

Each Alfalfa variety dry matter content showed no significant change with the salt levels, but varieties (between each others) significantly affected by salinity. *Medicago sativa elçi* hay used current study showed most dry matter content than other forages and *Medicago sativa barrier* and *Medicago sativa 13R supreme* also showed grater response to salinity in terms of dry matter than *Medicago sativa elçi*. As for dry matter, *Medicago sativa barrier* showed the lowest value and a positive reaction. However, *Medicago sativa 13R suppreme* gave a negative response. DDM, DMI and RFV values of varieties were not affected significantly by salinity.

Table 3. Chemical compositions, dry matter intake and relative feed value of samples used in the study

	Medicago sativa (Elçi)		Medicago sativa barrier		Medicago sativa 13R suppreme					
Nutrients	Non-Salty	Salty	Non-Salty	Salty	Non-Salty	Salty	SEM	V	S	V*S
DM, %	94.6 ^{bcd} *	95.2 ^{cd}	93.3 ^a	94.5 ^{bc}	95.3 ^d	94.2 ^{ab}	0.1	0.01	-	0.01
CP, %	17.6	18.0	17.4	19.0	17.3	17.8	1.2	-	-	-
EE, %	2.3	2.5	2.1	2.3	1.9	1.9	0.1	-	-	-
Ash, %	11.7	12.9	12.4	11.9	10.9	11.8	0.3	-	-	-
ADF, %	32.1	30.3	27.4	31.0	32.6	31.5	1.0	-	-	-
NDF, %	37.5	32.4	37.1	37.7	41.8	37.9	1.1	-	-	-
Feed evaluation parameters										
DDM	63.9	65.3	67.6	64.7	63.5	64.4	0.8	-	-	-
DMI	3.3	3.7	3.2	3.3	2.9	3.2	0.1	-	-	-
RFV	162.7	187.6	169.9	166.3	144.1	159.4	5.8	-	-	-

*: Significance between individual means was identified using the Duncan's multiple comparative tests, DM: Dry matter, CP: Crude protein, EE: Ether extracts, NDF: Neutral detergent fiber, ADF: Acid detergent fiber RFV: Relative feed value, DDM: Digestible dry matter (% of body weight), DMI: Dry matter intake (% of body weight), SEM: Standard error of means, V: Varieties, S: Salinity

	Medicago (Elç.	o sativa i)	Medicago sativa barrier		Medicago sativa suppreme					
Inc. times	Non-Salty	Salty	Non-Salty	Salty	Non-Salty	Salty	SEM	V	S	V*S
3	5.9 ^b	1.9 ^a	5.7 ^b	2.1 ^a	5.8 ^b	1.9 ^a	0.2	-	0.01	-
6	9.03 ^b	5.1 ^a	8.8 ^b	5.4 ^a	8.8 ^b	5.2 ^a	0.05	-	0.01	-
9	14.1 ^c	9.8 ^a	13.9 ^c	10.7 ^b	13.8 ^c	9.9 ^a	0.09	-	0.01	-
12	18.9 ^c	14.5 ^a	18.6 ^c	15.6 ^b	18.5 ^c	14.5 ^a	0.1	-	0.01	-
24	31.5 ^c	26.3 ^a	30.9 ^c	28.2 ^b	30.7 ^c	26.3 ^a	0.2	-	0.01	-
48	40.8 ^b	36.5 ^a	40.1 ^b	39.1 ^b	39.5 ^b	36.5 ^a	0.4	-	0.01	-
72	44.7 ^b	39.5 ^a	44.0 ^b	42.3 ^b	43.5 ^b	39.4 ^a	0.3	-	0.01	-
96	46.4 ^b	41.1 ^a	45.7 ^b	44.1 ^b	45.1 ^b	40.8 ^a	0.3	-	0.01	-
pН	6.5 ^{ab}	6.9 ^b	6.9 ^b	6.9 ^b	6.3 ^a	6.8 ^b	0.1	-	0.03	-
а	-1.3 ^b	-4.94 ^a	-1.26 ^b	-5.24 ^a	-1.09 ^b	-5.02 ^a	0.2	-	0.01	-
b	48.12	46.65	47.33	49.94	46.64	46.5	0.5	-	0.01	-
c	0.04	0.05	0.04	0.05	0.04	0.05	0.001	-	0.01	-
OMD, %	53.8 ^c	49.6 ^a	53.2 ^c	51.2 ^b	53.0 ^c	49.5 ^a	0.2	-	0.01	-
ME, MJ/kg DM	7.8 ^c	7.1 ^a	7.7 ^c	7.4 ^b	7.7 ^c	7.1 ^a	0.03	-	0.01	-
NE ₁ .MJ/kg DM	4.5°	4.1^{a}	4.5°	4.3 ^b	$4.5^{\rm c}$	4.1^{a}	0.02	-	0.01	-

Table 4. In vitro gas productions (ml) parameters and ME, NE_L and OMD values of Medicago varieties

*: Significance between individual means was identified, OMD: Organic matter digestibility, a: the gas production from the immediately soluble fraction (ml), b: the gas production from the insoluble fraction (ml), c: the gas production rate constant for the insoluble fraction (ml/h), SEM: Standard error of means; V: Varieties, S: Salinity

Gas Production Parameters

As a result of this study, significant changes in all gas parameters occurred due to salinity levels (Table 4). First of all, all Medicago varieties growing in the saline fields (EC = 8-13 dS/m) showed less gas production in all incubation times. Especially due to increased salinity, the total gas production in 24-hour period fell from 31 0 to 26.9, even 96-hour period fell from 45.7 to 42.0. Depending on the level of salt in the soil, significant changes were observed with respect to the immediately soluble fraction (a), insoluble fraction (b), and the rate of gas production constant from the insoluble fraction (c). It was seen significant decline in the value of soluble fraction (a) and the rate of gas production (c), but there was no change in b-value in general using the Duncan's multiple comparative tests. As for the pH values, significant increases were observed in conjunction with the soil salinity. Depending on the level of soil salinity, pH level increased and ranged between 6.3 and 6.9.

Digestibility of organic matter in all varieties significantly deteriorated with increasing salinity (OMD; from 53.3 to 50.1 %). In addition, metabolic and net energy lactation levels were decreased significantly in all varieties (ME from 7.8 to 7.3 MJ/kg DM and NE_L from 4.5 to 4.2 MJ/kg DM). In terms of energy values and relative feed values only *Medicago sativa elçi* variety showed a significant difference. For gas production values, organic matter digestibility and all energy values, a significant interaction effect of salinity and *Medicago* varieties were not observed.

Discussion

The nutrient components of all varieties were relatively similar in two salinity levels. *Medicago sativa elçi* gained more concentrations of dry matter compared to others. The higher content may attribute to its characteristic structure. As results of this study, the experimental varieties in salty and non-salty soils were nutritious for ruminants. In all components, especially CP is enough to cover their nutrient requirements. According to El Shaer (2010), most of salt tolerant fodders attain reasonable CP but they have high ash content. Furthermore such plants grown in saline soils are usually fairly poor in energy and high in CP (Le Houérou et al., 1982). This may be generally attributed that alfalfa is a plant of moderately resistant to salinity. These plants are already known as a glycophyte plant (Ungar, 1967). Therefore all three varieties are among the potential salt tolerant grass species as good quality fodders for livestock in Sanliurfa-Harran region of South East Turkey. Absence of significant changes in nutrient content have shown that these Medicago varieties growing on saline soils can produce high to moderate consumable biomass for livestock feeding at 8-13 dS/m in Akçakale Soil Series.

Based on the data in Table 2, at the end of the study, salinity caused a significant accumulation of some minerals in all varieties. Data on mineral content revealed that Na, K, Mg and Ca retention were significantly affected by the levels of salinity in soils. Jia et al. (2008) suggested that high level of salts imposes both ionic and osmotic stresses on plants, resulting in an excessive accumulation of sodium (Na) in plant tissues. Because mentioned minerals in Akçakale region was higher than the Harran region (Ikizce soil series), some of the mineral contents of plants such as Mg, Na, K and Ca were higher in this area. Suyama et al. (2007) reported more Na accumulation for 'Salado' alfalfa grown under irrigation with drainage water causing soil salinity of 5.2-6.4 dS/m. It is well known that at a whole plant level, salinity stress induces an increase in Na, as well as a decrease K and Ca concentrations (Lutts, 1996). Salinity also causes to decrease the rate of photosynthesis and leads to decrease nutrient uptake and Ca⁺⁺ transport (Taban and Katkat, 2000). Although concentrations of Mg, Na and K were higher than the level of non-salty soils, the concentrations are still within the maximum tolerance levels for sheep and beef cattle specified by NRC (1984, 1985). As stated by Masters et al. (2001), sheep can tolerate a sodium chloride intake in feed of about 100-150g/day provided that they have access to a non saline drinking water. The high Na and K content may limit feed intake but we don't know whether these forages growing salty conditions are consumed less in current conditions because this trial was not in-vivo conditions. But DMI calculated from data seems unaffected by salinity. Various studies on this subject should be more in *in vivo* conditions on farm animals to investigate the effect of feeding Medicago growing salty soils.

There were generally significant declines among groups with salinity levels in terms of the gas production from the immediately soluble fraction (a), the gas production from the insoluble fraction (b) and the rate of gas production constant from the insoluble fraction (c, p < 0.05). This can be attributed to the lower gas production in salinity conditions. It is well documented that the nutrient content of feeds effects their potential production of gas quantity and level of gas produced tends to decrease or increase with changing chemical content of feeds (Abdulrazak et al., 2000; Doane et al., 1997). In this study, both the salinity and differences of varieties did not cause a significant change in chemical contents of plants. It was not easy to explain why the low levels of gas production are seen in all varieties growing on soils with high salinity. All varieties had lower gas production parameters and due to low gas production values, all varieties had a low energy values and low digestibility for fields having salty soil. Poor energy of plants in saline soils is frequently seen¹⁵. Significant declines in energy content were observed in current study (p=0.01) compared to plants grown in nosaline soils. All varieties had ME above 7.1 MJ/kg DM. In general, an ME value below 7MJ/kg DM is considered to be unacceptable for beef cattle and goats (NRC, 1981; 1984). Varieties with an ME between 7 and 9 MJ/kg DM are suitable for low production or maintenance level of beef cattle but not acceptable for dairy cattle and rapidly growing calves.

In this study, the nutrient content of all varieties was sufficient and similar level for microbial fermentation, but probably another factor was effective in reducing gas production. This was probably associated with reduction of ruminal microbial activity in medium with plants having high salt and mineral content. It is known that bacterial growth is most severely inhibited in soils containing high levels of sodium due to effects of osmotic stress on microbial activity (Mavi et al., 2010; Polonenko et al., 1981). In this study, *in vitro* microbial fermentation with a similar mechanism was likely to be affected negatively. Similar findings were reported by Hamilton and Webster (1987); the high Na and K content may reduce digestibility by shortening rumen turnover times.

When examining the Table 3, the calculated values of DMI and RFV did not show a negative trend in current salinity conditions. Therefore the lower levels of energy and digestibility in salinity conditions have not level of adversely affect on calculated animal performance but

cultivated land in current study should be noted that salinity has a moderate level. In animal production based on forage, more attention should be given soils having higher salt concentrations if there is no even enough drinking salt free water. RFV is used generally to compare similar forages for two important qualities; "how well it will be consumed and how well it will be digested". RFV has no units; instead, it is used to rank similar forages for potential dry matter intake (Horrocks and Vallentine, 1999). RFV, dry matter intake (DMI) and digestible dry matter (DDM) were presented in Table 3. The Hay Marketing Task Force of the American Forage and Grassland Council (AFGC) endorses the use of RFV as a measure of forage quality (Linn and Martin, 2008). According to the Quality Grading Standard assigned by the Hay Market Task Force of AFGC, the RFV values were founded as "Premium" 2 or "Prime" for all Medicago varieties in current study and they were quite satisfactory levels in salty soil conditions (all Medicago varieties ranged from 144.1 to 169.9; Table 3).

Ruminal gas production has been highly affected by pH and it is well documented that gas production decreases clearly at pH values below 6.2. Because, it was mentioned that microbial activity due to the absence of buffer matter in medium had decreased in the low pH in previous some studies (Beuvink and Spoelstra, 1992; Ørskov, 1994). Observed pH values after the incubations of 96 h (Table 4) for rumen liquid using *in vitro* gas production technique did not fall below this level and tended to increase with salinity in all varieties. The high pH is likely to be related to the increase in mineral matter in some *Medicago* varieties (especially *Medicago sativa elçi* and *M sativa 13 R supreme*) planted in saline soils.

In conclusion, the study shows that *Medicago sativa* can be used as salt tolerant forage in the ruminant diets. Glycophyte *Medicago* varieties yield low to high edible biomass in saline lands where non-salt tolerant species cannot grow. This study shows that the energy values and digestibility of *Medicago* varieties were adversely affected from soil salinity. Salinity is not a significant impact on nutritional value of alfalfa varieties in moderate salinity conditions, but it was observed that the salinity had a significant potential in reducing the use of these some nutrients. Provided sufficient water consumption, accumulation of mineral elements in these *Medicago* and its varieties does not limit the consumption of dry matter.

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