



THE EFFECT OF SUPPLEMENTATION OF *ATRIPLEX HALIMUS* WITH EITHER *SALVIA OFFICINALIS* OR *ROSMARINUS OFFICINALIS* ON ITS *IN VITRO* DRY MATTER DIGESTIBILITY

DJELOVANJE DODAVANJA *ATRIPLEX HALIMUSA* SA *SALVIOM OFFICINALIS* ILI *ROSMARINUSOM OFFICINALIS* NA *IN VITRO* PROBAVLJIVOST SUHE TVARI

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ABSTRACT

A study was conducted to investigate the effect of supplementation of *Atriplex halimus* with dried powders of aromatic plants on its *in vitro* dry matter digestibility. 400 ± 5 mg of dried *Atriplex* samples were incubated for 12 or 24 hours with buffered rumen liquor from sheep and supplemented with 100 mg of either *Salvia officinalis* or *Rosmarinus officinalis*. The result of this study demonstrated that the *in vitro* dry matter digestibility of *A. halimus* was 48.4% and 58.9% after the incubation for 12 or 24 hr respectively. Supplementation of this stuff with *S. officinalis* resulted in a significant reduction ($P < 0.05$) *in vitro* DMD (44.7 and 51.6%). The same trend was observed with the addition of *R. officinalis* to the *A. halimus* samples (44.8 and 53.2%). It would therefore appear that, the supplements used in the current study may have a negative effect on the *in vitro* dry matter digestibility of *A. halimus*.

Key words: *Atriplex halimus*, aromatic plants, *in vitro* dry matter digestibility

INTRODUCTION

Due to the scarcity of rainfall in Libya, it is difficult to grow forage crops especially those of high quality and productivity because of their high water requirements. Ruminants depend on poor pastures and/or crop residues for the most of the year. It is estimated that the total grazing area in Libya is about 3.2 million hectare i.e 8% of the total land area of the country, the majority lies between the rainfalls of 50-200 ml/year (Final Report, 2005). *Atriplex halimus* is one of forage plants which exhibit high resistance to drought and salinity and its cultivation can be one of the challenges for recovering the vegetation and face the desertification thereby increasing land productivity (Le-Houerou, 1992). In Eastern part of Libya, *Atriplex* grow in rocky soils which tend to be calcareous and saline and also in shallow wades (Final Report, 2005). *Atriplex* can be used as a sole ration for sheep and goats without any supplements and this can only meets the maintenance requirements (Valderrában et al, 1996). In

the same context, Al-Owaimer et al, (2008) reported that *Atriplex* could replace hay in feeding growing lamb.

Chemical analyses showed that *Atriplex* leaves contained 18% crude protein and young stems contained as little as 6% crude protein, whereas the neutral-detergent fiber content was 31-32% and 69-84% for leaves and young stem respectively (Andueza et al (2005). It is known that high neutral-detergent fiber and acid-detergent fiber lead to a reduction in digestibility of dry matter (Kamalak et al, 2005), and that is the reason for reduced animals efficiency and productivity.

Many antibiotics have been tried as feed additives may improve cellulose digestion (Russell and Strobel, 1989). However, the concerns about growing resistance of microorganisms against some kinds of antibiotics have led the European Union to decide to withdraw antibiotics from the feed additives programs (E. C., 2003). The research activities were mainly directed towards other al-

ternative means to improve digestion in the rumen. One of these alternatives was the use of plant products or their extracts (Hart et al, 2008). It was demonstrated that extracts of aromatic plants resulted in an improvement in fiber digestion (Broudiscou et al, 2002). The type and the active ingredient content in the aromatic plant vary according to the species of the plant, and in this context, Gachkar et al (2007) reported that *Rosmarinus officinalis* contained α -pinene, 1,8-cineole and linalool as major active ingredients. On the other hand, the main active ingredients of *Salvia officinalis* were 1,8- cineole and α -thujone whereas β -thujone was present in a small amount (Fellah et al, 2006).

Chemical treatments of low quality forages may be associated with some application difficulties in addition to the fear from any toxic residue so their application may be limited (Akraim et al, 2009; Rode et al, 1997). The objective of this study was to investigate the effect of adding dried powder of either *Rosmarinus officinalis* or *Salvia officinalis* on the *in vitro* dry matter digestibility of *Atriplex halimus*.

MATERIALS AND METHODS

Feed samples:

Atriplex halimus samples were collected from locations of grazing area in the south east Benghazi (32° 11` N and 20 ° 03` E). Samples of *Salvia officinalis* and *Rosmarinus officinalis* were collected from Al-Jabal Al-Akhdar mountain area (Final Report, 2005). Randomization and representative sampling were applied throughout the collection of samples. Samples were then dried at 60 °C for 48 hours, milled through a 1.0 mm sieve and stored at room temperature in airtight glass containers until chemical analyses. All feed samples (*A. halimus*, *S. officinalis*, and *R. officinalis*), that were studied in the course of the current study were analyzed for dry matter, ether extract, and crude protein. The dry moisture content was determined by drying at 105 °C for 24 h. The ash content was determined by ignition in a muffle furnace at 550 °C overnight, the ether extract was determined by refluxing the sample against diethyl ether and the total nitrogen was determined using a Kjeldahl technique and the crude protein was calculated by multiplying N content by 6.25 according to the method of (A.O.A.C, 1980). The neutral-detergent fiber and acid-deter-

gent fiber were determined by the methods of Goering and Van Soest (1970). All the above chemical analyses were done in replicate.

In vitro dry matter digestibility (IVDMD):

Sample preparation: The dried ground samples were transferred to a digestion tubes (containing 500 mg of *Atriplex halimus*, or 400 mg *Atriplex halimus* plus either 100 mg *Rosmarinus officinalis* or 100 mg of *Salvia officinalis*, a set of blank containing no sample but only the incubation media were also included. All runs were done in two replicates.

Source of rumen liquor: Rumen liquor was collected from at least four sheep that were slaughtered in the abattoir, selected from animals that had eaten mainly roughages, and filtered through three layers of cheesecloth into a warmed thermos flask. On arrival to the laboratory the liquor was flushed with CO₂ then kept in an incubator maintained at 39 °C until used for the inoculation of samples as soon as possible.

Preparation of buffered rumen liquor: The buffer solution was prepared according to the method described by Enjalbert et al (2003). The buffer was maintained at 39 °C in a water bath, rumen liquor was then added so that the ratio of buffer to rumen liquor was 4:1 v/v. Forty ml of buffered rumen liquor were added to each tube. The tubes were then stopped with a rubber septum connected to a tiny plastic tube immersed in the water bath for the evacuation of the gases which were produced during the fermentation. The tube contents were then maintained in the water bath at 39 °C and thoroughly mixed two to three times a day. At the end of the fermentation period, whether after 12 h or 24 h, the tubes were transferred to an icy bath to terminate the fermentation. Tubes were then centrifuged and the supernatant discarded carefully and the residue dried at 105 °C for 24 h and weighed and this will represent the indigestible part of the sample (Tilley and Terry, 1963).

Dry matter digestibility was calculated as follows:

$$\% \text{ IVDMD} = 100 * \{(\text{Sample DM} - (\text{Residual DM} - \text{Residual DM of blank})) / \text{Sample DM}\}$$

Data analyses:

Data were analyzed using Statistical Packages for the Social Sciences (SPSS, 1998), and the differences between mean were tested according to Turkey's test. The model used in this study was as follow:

$$Y = \mu + t_i + E_{ij}$$

where:

Y: the *in vitro* dry matter digestibility,

μ : the general mean,

t: the treatment effect, and

E: the experimental error

RESULTS AND DISCUSSION

The chemical analyses of samples used in the current study are presented in Table 1. It was observed that crude protein and neutral detergent fiber content of *Atriplex halimus* was in agreement with figures reported by Andueza et al (2005) provided that the content were in leaves. *Rosmarinus officinalis* contained more fat compared with the other feeds used in this study. *A. halimus* was the highest in ash contents followed by *S. officinalis* and then lastly the *R. officinalis*. NDF and ADF were intermediate. Crude protein contents in supplements were almost the same.

Table 2 shows the effects of adding dried aromatic plants on the *in vitro* dry matter digestibility of *Atriplex halimus*. It was clearly shown that nearly 50% of the dry matter of *Atriplex halimus* is digested after 12h, and its further incubation to 24h resulted

in additional digestion which reached about 60%. This figure was lower than the results reported by Laudadio et al (2009) who stated that the *in vitro* dry matter digestibility reached 72.6%, and Al-Owaimer et al (2008) who reported that *Atriplex* was efficiently digested (74.7%) when fed as a sole feed to growing lambs.

This difference might be attributed to the time of incubation since the increased time from 12 h to 24 h resulted in a 10% increase in digestion. On the other hand, extending the fermentation time up to 48 hr may give better results because it can coincide with the resident time of the fibrous feeds in the rumen. Based in this observation, if the incubation time was extending to 48 h, this might agree with the results reported by the above mentioned works. On the other hand, Andueza et al (2004) observed that there was a substantial difference in the *in vitro* dry matter digestibility (46 - 90%), when they tested 142 samples of *Atriplex*. On the other hand, addition of aromatic plants used in this study resulted in a significant reduction ($P < 0.05$) in the digestibility of *Atriplex halimus* after 12h (48.4 vs 44.7 and 4.8). The corresponding results of 24 h incubation were (59 vs 51.6 and 53.2). It can be noted that the addition of either *Rosmarinus officinalis* or *Salvia officinalis* did not differ significantly ($P > 0.05$). It can be concluded that this dietary manipulation has a negative effect on *in vitro* dry matter digestibility of *Atriplex halimus*.

Aromatic plants may contain mixtures of complex chemical substances, and so it is too difficult to ascertain the exact active ingredient which is responsible for this adverse effect on digestibility. Broudiscou et al (2002) observed an inhibitor effect

Table 1. Concentration (g/kg dry matter, DM), neutral and acid-detergent fibre (NDF and ADF), crude protein (CP), Ether extract EE) and Ash of the feeds used in this study.

Tablica 1. Koncentracija (g/kg suhe tvari, DM), neutralno kiselo-deterdžentno vlakno (NDF i ADF), sirove bjelančevine (CP), ekstrakt etera (EE) i pepeo u hrani upotrijebljenoj u ovom istraživanju

Fees sample Uzorak hrane	CP	EE	NDF	ADF	Ash
<i>Atriplex halimus</i> *	165	95	324	138	256
<i>Rosmarinus officinalis</i>	83	175	282	246	126
<i>Salvia officinalis</i>	78	97	278	242	146

*(Data from: Azooz, 2009)

Table 2. Mean \pm E.E of *in vitro* dry matter digestibility of *Atriplex halimus* with or without either *Rosmarinus officinalis* or *Salvia officinalis* after incubation with sheep rumen liquor for 12 and 24 h.

Tablica 2. Prosjek \pm E.E. *in vitro* probavljivosti suhe tvari *Atriplex halimusa* sa ili bez *Rosmarinus officinalis* ili *Salvia officinalis* nakon inkubiranja s tekućinom iz buraga ovce 12 ili 24 sata

Treatment / Tretman	In vitro dry matter digestibility In vitro probavljivost suhe tvari		E.E.
	12 h	24 h	
Atriplex halimus	48.4 ^a	59.0 ^a	0.76
Atriplex halimus + Rosmarinus officinalis	44.7 ^b	51.6 ^b	0.76
Atriplex halimus + Salvia officinalis	44.8 ^b	53.2 ^b	0.76

of aromatic plants extracts on the digestion of cell wall component, whereas an improvement with other extracts. They attributed this effect to the inhibitory or stimulation effect of flavinoids or its other metabolites and/or from direct effect of the secondary products of metabolism on the activity of rumen microorganism. In contrast, Baidias and Yaniz (2004) reported that *Salvia officinalis* fed to lambs resulted in an improvement in daily gain and feed conversion rate. These findings disagree with the results of this study since the daily gain resulted from efficient digestion. Although Benchaar et al (2003) did not find any effect on the population of cellulolytic bacteria when they used a mixture of essential oils because those oils derived from plants different from the aromatic plants used in the current study. O`Grady et al (2006) noted that extracts from *Rosmarinus officinalis* had a negative effect on barley fermentation as indicated by the amount of gas and changes in the proportion of volatile fatty acids produced. It was observed that aromatic plants extracts altered the manner of rumen fermentation and the total volatile fatty acids (Busquet et al, 2006). Using aromatic plants may have adverse effect on rumen fermentation, which was confirmed by Faixova and Faix (2008) and Delamare et al (2007) for *Rosmarinus officinalis* and *Salvia officinalis* respectively because these plants contain phenolic compounds. These compounds may cause an inhibitory effect on the activity of rumen microorganisms, and that change may be in the form of changing the percentage of volatile fatty acids with decreasing the proportion of propionate and increasing butyrate (Cuvelier et al, 1996; Benchaar et al, 2007). It can be concluded that, the addition of *Rosmarinus officinalis* or *Salvia officinalis* to *Atriplex halimus* resulted in a negative

effect on its *in vitro* dry matter digestibility. More investigation is needed to ascertain this effect *in vivo*.

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SAŽETAK

Istraživanje je provedeno radi proučavanja djelovanja dodavanja *Atriplex halimusa* sa suhim prahom aromatičnog bilja na *in vitro* probavljivost suhe tvari. 400+- 5 mg uzoraka suhog *Atriplexa* inkubirano je 12 ili 24 sata s puferiranom tekućinom iz buraga ovce i nadopunjeno sa 100 mg *Salviae officinalis* ili *Rosmarinus officinalis*. Rezultati ovog istraživanja pokazali su da je *in vitro* probavljivost suhe tvari *A. halimus* bila 48,4% odnosno 58,9% nakon inkubiranja 12 odnosno 24 sata. Rezultat dodavanja *S. officinalis* bilo je znatno smanjenje ($P < 0.05$) *in vitro* DMD-a (44,7 i 51,6%) Isti je trend primijećen dodavanjem *R. officinalis* uzorcima *A. halimusa* (44,8 i 53,2 %). Prema tome, izgleda da dodaci upotrijebljeni u ovom istraživanju mogu negativno djelovati na *in vitro* probavljivost suhe tvari *A. halimusa*.

Ključne riječi: *Atriplex halimus*, aromatično bilje, *in vitro* probavljivost suhe tvari