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THE EFFECT OF *ROSMARINUS OFFICINALIS* OR *SALVIA OFFICINALIS* DRIED LEAVES ON THE *IN VITRO* DRY MATTER DIGESTIBILITY OF BARLEY STRAW

DJELOVANJE SUHIH LISTOVA *ROSMARINUS OFFICINALIS* I *SALVIA OFFICINALIS* NA *IN VITRO* PROBAVLJIVOST SUHE TVARI SLAME JEČMA

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ABSTRACT

The study was conducted to evaluate the effect of the addition of *Rosmarinus officinalis* or *Salvia officinalis* dried leaves on the *in vitro* dry matter digestibility of barley straw. Barley straw samples (0.5 g control) or (0.4 g of barley straw + 0.1 g of *R. officinalis* or *S. officinalis* dried leaves) were incubated with sheep rumen liquid for 12 hours and 24 hours. Dry matter digestibility of barley straw was 21.7% after 12 hours and 43.62% after 24 hours of incubation without additives. The addition of the aromatic plants did not affect the dry matter digestibility of barley straw after 12 hours of incubation ($P>0.05$). Dry matter digestibility of barley straw was reduced with the addition of the aromatic plants after 24 hours of incubation; the inhibition was significant with *R. officinalis* ($P>0.05$). The addition of *R. officinalis* or *S. officinalis* dried leaves inhibits the dry matter digestibility of barley straw *in vitro*.

Key words: straw, *Rosmarinus officinalis*, *Salvia officinalis*, *in vitro* dry matter digestibility

INTRODUCTION

Libya suffers from acute shortage of feed resources because of the fluctuation in rainfall rates. In Libya, straw (such as barley and wheat straw) represents the main source of roughage in ruminant nutrition. Cultivated area of barley and wheat was 204.08 and 132 thousand hectares, respectively (AOAD, 2008). Dry matter digestibility of straw is low because of high levels of NDF and ADF in cell wall (Kamalak et al., 2005). Chemical treatments of straw (i,e., urea, ammonium sulphate, anhydrous ammonia) increase dry matter digestibility but the difficulty of handling the treatments and the problems associated with safety limit their use (Akraim et al., 2009; Rode et al., 1997).

Use of antibiotics like Monensin, Lasalocide, salinomycin, Lysocilin, Narasin etc. as feed additives may enhance cellulose digestibility through decreasing the production of lactic acid and increasing pH number in rumen (Russell and Strobel, 1989). The inclusion of monensin to cows before parturition increased the apparent digestibility of NDF and ADF (Plaizier et al., 2000). Concerns about microbial antibiotic resistance led the European Union to prohibit the use of antibiotics as feed additives (EC, 2003). Interest was increased to investigate alternatives to antibiotics as growth promoter and rumen modifiers. One of these possible alternatives are natural plant products (Hart et al., 2008). The digestibility of NDF and ADF has been increased with the addition of some plant extracts and decreased with others (Broudiscou et al., 2002), and this could be related to active compounds in different plants, Gachkar et al. (2007) reported that *R. officinalis* active compounds were α-pinene, 1,8-cineole and linalool. Active compounds exist in great quantity in leaves, stems and flowers of *S. officinalis* where 1,8-cineole and α-thujone, β-thujone exist in a small quantity (Fellah et al., 2006). This study was conducted to evaluate the effect of addition of *R. officinalis* or *S. officinalis* dried leaves on dry matter digestibility of barley straw.

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MATERIALS AND METHODS

Salvia officinalis and *Rosmarinus officinalis* plants were obtained from Al-Jabal Al-Akhdar Mountain area (Final report, 2005). Leaves were isolated from plants and dried at 60 °C for 48 h, then ground through 0.5 mm.

Barley straw was obtained through local feed market. Samples were obtained in several bales and mixed together and a representative sample was then ground through 0.5 mm. Plants and barley straw samples were analyzed for crude protein, ether extracts, ash, neutral detergent fiber and acid detergent fiber as described by Galyean (2010).

Rumen liquid was obtained from 3 adult female sheep consuming barley straw as main roughage in their diets. Rumen liquid was immediately transformed to thermos after passing through sieve. The thermos was equipped with a valve permitting the delivery of the liquid without contact with the air. Time of transport to the laboratory was 10 minutes. Artificial saliva (Enjalbert et al., 2003) was placed to a water bath of 39 °C before the arrival of rumen liquid. Three samples in duplicates were incubated in each incubation time: the control contained only 0.5 g of straw, R. treatment contained 0.4 g of straw and 0,1 g of *R. officinalis* and S. treatment contained 0.4 g of straw and 0.1 g of *S. officinalis*. Eight ml of rumen liquid and 32 ml of artificial saliva were added to each tube; CO_2 was injected and then the tubes were closed firmly. In addition two tubes containing only rumen liquid and artificial saliva were incubated. Incubation times were 12 and 24 h. The tubes were equipped with needles placed in a plastic tube and immersed in water, so fermentation gases could escape without inflow of air. Tubes were shaken manually each 4 h. At the end of incubation time, the tubes were transferred to iced water. Tubes contents were centrifuged for 10 min., the residual which was undigested dry mater was dried at 100 °C over the night.

Statistical analysis:

Comparison between treatments was carried out by Systat (Statistical Packages for the Social Sciences, 1998) according to the following model:

Mean of dry matter digestibility
= general mean + effect of incubation time
+ effect of additive + experimental error.

A Tukey pairwise comparison test was used to compare the different additives and the differences were declared significant at $P<0.05$.

RESULTS AND DISCUSSION

Chemical composition of barley straw, *S. officinalis* and *R. officinalis* is presented in Table 1. Dry matter digestibility of barley straw is presented in Table 2. and Table 3.

The added aromatic plants were in the form of dried leaves. This represented the dry matter that could be digested, so, 0.5 g of straw was added to control tubes and 0.1 g of tested additives and 0.4 g of straw were added to treatment tubes.

Dry mater digestibility of barley straw in this study was 21.7% after 12 h of incubation and in-

Table 1. Chemical composition of barley straw, *S. officinalis* **and** *R. officinalis*[1,2]

Tablica 1. Kemijski sastav slame ječma, *S. officinalis* **i** *R. officinalis*[1,2]

Item - Stavka	CP	EE	NDF	ADF	Ash - Pepeo
	% (air dry basis – na osnovu suhe tvari)				
Barley straw – Slama ječma	3.6	1.0	71.2	43.6	7.5
R. officinalis	8.3	17.5	28.8	24.6	12.6
S. officinalis	7.8	9.7	27.8	24.2	14.6

[1,2]- Mean of two samples – srednja vrijednost dva uzorka, (CP) Crude proteins – Sirove bjelančevine, (EE) Ether extract – Ekstrakt etera, (ADF) Acid detergent fiber – kisela deterdžent vlakna, (NDF) neutral detergent fiber – neutralna deterdžent vlakna

Table 2. Dry matter digestibility of barley straw after 12 h of incubation

Tablica 2. Probavljivost suhe tvari slame ječma nakon 12 sati inkubiranja

Item - Stavka	Digestibility coefficient Koeficijent probavljivosti (%)	S.E
Control - Kontrola	21.67	0.99
Straw + *R. Officinalis* – Slama + *R. Officinalis*	21.23	0.99
Straw + *S. Officinalis* – Slama + *S. Officinalis*	21.98	0.99

Table 3. Dry matter digestibility of barley straw after 24 h of incubation

Tablica 3. Probavljivost suhe tvari slame ječma nakon 24 sata inkubiranja

Item - Stavka	Digestibility coefficient Koeficijent probavljivosti (%)	S.E
Control - Kontrola	43.62 [a]	3.89
Straw + *R. Officinalis* – Slama + *R. Officinalis*	27.63 [b]	3.89
Straw + *S. Officinalis* – Slama + *S. Officinalis*	31.93 [ab]	3.89

Means in the same column with different superscripts differ (P<0.05)
Srednje vrijednosti u istim kolonama sa različitim slovima značajno se razlikuju (P<0.05)

creased to 43.62% after 24 h of incubation. In general, dry matter digestibility of barley straw was low in comparison with other highly digestible roughages and there was difference between digestibility coefficients obtained in different studies due apparently to variation in experimental conditions. *In vitro* dry matter digestibility of barley straw was 61.7% (Hervás et al., 2004), of organic matter was 45.68% (Arisoy, 1998) and 34.8% (Mir et al., 1986). Incubation time in the previous studies was 48 h. Kamalak et al., (2005) reported a dry matter digestibility of barley straw in sheep of 41.53% which was comparable to our findings after 24 h of incubation.

The addition of aromatic plants did not affect dry matter digestibility of barley straw after 12 h of incubation (P>0.05). Dry matter digestibility was negatively affected with the addition of aromatic plants after 24 h of incubation; this effect was particularly significant with the addition of *R*. *officinalis* to the incubation media. *R*, *officinalis* tended to exert more adverse effect on dry matter digestibility. The effect of inhibition was apparently more pronounced with increased time of incubation.

O'Grady et al., (2006) also reported that *R*. *officinalis* inhibited the fermentation of barley grain and this effect began in the last phase of incubation period which lasted for 72 h. In contrast to our findings, Demirtas et al., (2011) reported that *R*. *officinalis* and *S*. *officinalis* extracts did not affect dry matter digestibility of barley straw *in vitro*. *R. officinalis* and *S. officinalis* oils had no effect on VFA concentration *in vitro* in (10:90) forage concentration diet (Castillejos et al., 2008).

Aromatic plants contain mixture of complex chemical compounds. Broudiscou et al., (2002) reported a decrease in digestibility of cell wall components with some plant extracts and increase with others and presented three hypotheses that could be acted alone or in combination: (1) the inhibitory or stimulatory action of flavonoids on some rumen microorganisms; (2) the effect of the degradation products of flavonoids; and (3) the direct action of other secondary metabolites.

However, the inclusion of *S. officinalis* plants in lamb diets enhanced daily gain and feed conversion rate (Badias and Yaniz, 2004). These results may be

in contrast with our findings because the increase in daily gain normally related to improvement in feed dry matter digestibility. There is a discrepancy between the results obtained *in vitro* and *in vivo* concerning the effect of aromatic plant extracts (Villalba and Provenza, 2010). *R. officinalis* and *S. officinalis* contain phenolic compounds (Curvelier et al., 1996). Phenolics exhibited antimicrobial activity as exemplified by reduced diet fermentability and a shift in VFA profile from less propionate towards more butyrate (Benchaar et al., 2007). However, different results reported *in vivo* concerning the effect of *R. officinalis*: while the inclusion of rosemary leaves into the Murciano-Granadina goat diet modified neither animal productivity (milk yield) nor milk quality (Jordan et al., 2010), dietary rosemary extract supplementation in dairy ewes improved milk production and quality (Chiofalo et al., 2011). Amer and Amazik (2012) found that chick performance was decreased with increasing rosemary in broiler chick diets. Additional *in vivo* studies are needed before confirming our in vitro findings on the effects of those plants on dry matter digestibility.

CONCLUSION

The addition of *R. officinalis* and *S. officinalis* dried leaves did not enhance the *in vitro* dry matter digestibility of barley straw. Contrarily, the addition of those two plants to the incubation media adversely affects the digestibility of barley straw after 24 h of incubation. *In vivo* study and different doses are needed to determine the potential use of these plants in ruminant nutrition.

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SAŽETAK

Istraživanje je provedeno radi procjene djelovanja dodatka suhih listova biljaka *Rusmarinus officinalis* ili *Salvia officinalis* na in vitro probavljivost suhe tvari slame ječma. Uzorci slame ječma (0,5 g kontrola) ili (0,4 g slame ječma + 0,1 g suhih listova *R. officinalis* ili *S. officinalis*) inkubirani su s tekućinom iz buraga ovce 12 i 24 sata. Probavljivost suhe tvari slame ječma bila je 21,7% nakon 12 sati i 43,62% nakon 24 sata inkubacije bez dodataka. Dodatak aromatičnih biljaka nije djelovao na probavljivost suhe tvari slame ječma nakon 12 sati inkubacije ($p>0,05$). Probavljivost suhe tvari slame ječma smanjena je dodatkom aromatičnog bilja nakon 24 sata inkubacije; smanjenje je bilo značajno s *R.officinalis* (0,005). Dodatak suhih listova *R.officinalis* ili *S.officinalis* koči in vitro probavljivost suhe tvari slame ječma.

Ključne riječi: slama, *R.officinalis*, *S.officinalis*, *in vitro* probavljivost suhe tvari