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USING *POLYETHYLENE GLYCOL* (6000) FOR DEACTIVATION OF TANNINS IN GRAZING TREE LEAVES GROWN IN EASTERN LIBYA

UPOTREBA POLIETILENSKOG GLIKOLA (6000) ZA DEAKTIVIRANJE TANINA U LIŠĆU DRVEĆA ZA BRŠĆENJE U ISTOČNOJ LIBIJI

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SUMMARY

This study was conducted to investigate the effect of different levels of Polyethylene glycol (PEG) on *in vitro* gas production of some tree leaves grown in Eastern Libya. The samples used were *Ceratonia siliqua, Pistacia lentiscus,* and *Acacia cyanophylla.* They were incubated anaerobically with rumen liquor from growing male sheep equipped with a permanent cannula. Cumulative gas production was measured after 3, 6, 12, 24, 48 and 72 hours from the incubation of samples with rumen liquor. PEG was added at levels 15, 30 and 45 mg per 0.2 g dry matter. The chemical analysis showed that the crude protein (%) was 8.2, 9.5, 13.4, for *P. lentiscus*, *C. siliqua* and *A. cyanophylla,* respectively, and that of the ether extracts were nearly the same in all the studied samples. The NDF contents were ranged between 44.5% for *C. siliqua* and 37% for *A. cyanophylla* with *P. lentiscus* lie in the middle (39.8%). The percentages of condensed tannins were 25.4, 21.5 and 4.1 for *A. cyanophylla, P. lentiscus* and *C.siliqua* respectively. The average cumulative gas production (ml/0.2 g DM) after 48h of incubation was higher ($P<0.05$) for C*. siliqua* then *P. lentiscus* followed by *A. cyanophylla* (22.9, 12.6 and 8.2) respectively. Addition of Polyethylene glycol (15, 30 and 45 mg PEG/0.2 g DM) increased ($P<0.05$) the cumulative gas production compared with control (13.5, 16.4 and 20 vs. 8.4). The current study concluded that PEG can be used to alleviate the undesirable effects of anti-nutritional Polyphenols found in some grazing tree leaves.

Kew words: *In vitro* gas production, PEG, anti-nutritional compounds.

INTRODUCTION

Livestock production depends largely upon the availability of nutrients. Arid and semiarid areas in the world suffer from the shortage of feeds due to the scarcity and irregular distribution of rainfall. In Libya, the prevailing climate conditions constrained animal performance. Forest trees and shrubs are cultivated and/or native grown and serve as forages for livestock. Unfortunately, some tree leaves contain some anti-nutritional compounds, notably, tannins which limit the availability of nutrients. Tannins are secondary plant metabolites, polyphenolic compounds in nature. The important biochemical property of tannins is contributed to their ability to bind to proteins and form insoluble complexes. Tannins bind to proteins in the mouth reducing the palatability of the feed, hence potentially decrease intake (Hagerman and Bulter, 1991; Abu-Zanat et al. 2003.).

On the other hand, Barry et al., (2001.) stated that, low levels of tannins may have positive effects in protecting proteins against degradation in the rumen, thereby increasing the availability of amino acids in the small intestine. The quality and quantity of nutrients can be improved by proper strategies. Attempts to alleviate the negative effects of

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these compounds have led to improvements in feed utilization. Polyethylene glycol (PEG) reacts preferentially with condensed tannins and prevents the formation of tannin-protein complexes (Jones and Mangan, 1977.). This polymer is used to bind with tannins at a wide range of pH, leading to improvement of feed intake and nutrient utilization (Silanikove, 2000.). The addition of PEG improves the ability of microbes to ferment the foliages with high tannins contents (Alam, et al., 2007.). Polyethylene glycol does not appear to affect digestion and can be added to feed or given by oral or ruminal routes to ruminants as a mean for eliminating the negative effect of condensed tannins (Waghorn et al., 1999.). The objective of this study was to investigate supplementing tree leaves with different levels of polyethylene glycol and its effect on *in vitro* gas production.

MATERIALS AND METHODS

Feed samples:

Samples of tree leaves (*Ceratonia siliqua, Pistacia lentiscus ,* and *Acacia syanophylla*) were collected from Al-Jabal Al-Akhder regions which is located in the Eastern Libya (32° 45' N, 21° 44' E). Random samples from each species were collected from about ten shrubs. Samples were dried at 60 °C for 48 h, ground to pass 1 mm sieve and stored in airtight glass containers until analysis.

Chemical analyses

Samples were analyzed for dry matter, ether extract, crude protein and ash according to the methods described by A.O.A.C. (1980.). Neutral detergent fiber and acid detergent fiber contents were determined using the method described by Goering and Van Soest (1970.). All chemical analyses were done in duplicate. Total condensed tannins and total bound tannins were also determined using butanol-HCl method as described by Makkar, 1995.

In vitro gas production

Samples were incubated anaerobically with strained rumen fluid obtained from two growing male sheep fitted with a permanent cannula. A 200 mg of dried tree leaves samples of either *Ceratonia siliqua, Pistacia lentiscus,* or *Acacia syanophylla* were weighted in plastic syringes (100 ml). A 30 ml of buffer rumen solution (2:1) was added to the samples which were then incubated in a water bath at 39° C. Polyethylene glycol was added at levels 15, 30, or 45 mg. All was done in triplicate accompanied with three syringes containing only buffer rumen solution but no samples to serve as a blank. The syringes were gently shaken every two hours for the first 24 hours. Gas production was recorder at specific time intervals (3, 6, 12, 24, 48, and 72 h) for the determination of cumulative gas production (ml/gm dry matter) as mentioned by Menke and Steingass (1989.).

Statistical analysis

Data of cumulative gas production were subjected to analysis of variance as a completely randomized design. The comparison among means was analyzed by the least significant difference using LSD procedure (SPSS programme, version 10, 1999.).

RESULTS AND DISCUSSIONS

Chemical analyses of tree leaves used in the current study are shown in Table 1. It can be demonstrated that crude protein contents (%) were 9.5 and 8.2 for *C. siliqua* and *P. lentiscus* respectively. These results were obtained early by Silanikove et al, (1996.). Higher crude protein content was found in *A. syanophylla* leaves (13.4%), this finding was in agreement with that reported by Moujahed et al (2005.). In respect of neutral detergent fiber it was 44.7%, 37%, and 40% for *C. siliqua*, *A. syanophylla* and *P. lentiscus* respectively. These findings were in agreement with that of Silanikove et al, (1996.) for *C. siliqua* and *P. lentiscus* while for *A. syanophylla* it was nearly the same finding of Ben-Salem et al (2005). Ether extract contents were about 6% for all samples studied, and it may have adverse effect on rumen microorganism.

The concentration of condensed tannins in *C. siliqua* leaves was 4.1%, which is similar to Silanikove et al. (1994) who reported that carob leaves contained 5% of condensed tannins. On the other hand, *P. lentiscus* and *A. syanophylla* contained high concentrations of total condensed tannins, 21.5 and

Table 1 The chemical composition (%) of tree leaves used in this study
Tablica 1. Kemijski sastav (%) lišća drveća upotrijebljenog u ovom istraživanju

	C. siliqua	*A. syanophylla*	*P. lentiscus*
Dry matter – Suha tvar	91.0	84.5	80.0
Ash - Pepeo	3.7	6.0	4.2
Crude protein – Sirove bjelančevine	9.5	13.4	8.2
Ether extract – Ekstrakt etera	6.4	5.6	6.8
NDF	44.7	37.0	39.8
ADF	36.6	31.2	28.5
Total condensed tannins – Ukupni kondenzirani tanini	4.1	25.4	21.5
Total bound tannins – Ukupni vezani tanini	3.7	5.1	4.8
Soluble tannins – Topivi tanini	0.4	20.3	16.7

NDF - neutral detergent fibre
ADF - acid detergent fibre

Table 2 Means ± standard error of cumulative gas production (ml/ 0.2 gm DM) of tree leaves after anaerobicall incubation for 48 h with sheep rumen liquor

Tablica 2. Srednja standardna greška nakupljenog plina (ml/ 0.2 gm DM) lišća nakon anaerobne inkubacije kroz 48 h s tekućinom iz buraga ovce

Type of substrate – Vrsta supstrata	Cumulative gas production – Nakupljeni plin (ml/0.2 gm DM)
C. siliqua	$22.9^{a} \pm 0.923$
P. lentiscus	$12.6^{b} \pm 0.923$
A. syanophylla	$8.2^{c} \pm 0.923$

$^{a, b, c}$ Means with different subscriptions are significantly different ($P < 0.05$).

Table 3 Means ± standard error of cumulative gas production (ml/ 0.2 gm DM) of tree leaves after anaerobicall incubation for 48 h with sheep rumen liquor as affected by different levels of PEG

Tablica 3. Srednja standardna greška ukupne proizvodnje plina (ml/0,2 gm DM) nakon djelovanja raznih razina PEG-a

PEG levels – Razina PEG-a, mg	Cumulative gas production – Nakupljeni plin (ml/0.2 gm DM)
0	$8.4^{d} \pm 1.006$
15	$13.5^{c} \pm 1.006$
30	$16.4^{b} \pm 1.006$
45	$20.0^{a} \pm 1.006$

$^{a, b, c, d}$ Means with different subscriptions are significantly different ($P < 0.05$)
PEG - polyethylene glycol

25.4 respectively. This finding was in agreement with the results of Alam et al. (2007.) who reported that Acacia contained the highest level of total phenolics (20.1%) It was slightly more than that of Kamalak et al (2005.) for condensed tannins in *P lentiscus* (16.3%). Gregorio et al. (2005) stated that total condensed tannins in *A. syanophylla* varied from 2.3 to 33.5% which may be due to differences between species and environmental conditions.

Data of cumulative gas produced as a result of incubating tree leave with rumen liquor for 48 h are shown in Table 2. It was revealed that *C. siliqua* produced higher gas as compared with ether *P*. *len-*

tiscus or *A. syanophylla* ($P < 0.05$), with the latter producing lowest amount of gas ($P < 0.05$). This may be attributed to the concentration of total condensed tannins (Table 1), although *C. siliqua* contained more fiber than the other two samples, but on the other hand it contained less total condensed tannins.

The effects of supplementing tree leave with different levels of polyethylene glycol of in vitro gas production are given in Table 3. The cumulative gas produced (ml/0.2 gm DM) increased significantly ($P < 0.05$) as the level of PEG increased. The increase in gas production in response to increased PEG inclusion was linear for the three species used in this study. This finding was previously stated by Kamalak et al. (2005.) on *P. lentiscus***.** This may indicate that this compound has a positive effect on the fermentation of tree leaves which contain different concentration of tannins. The same trend was reported by Canbolat et al. (2005.) on *Quercus cerris* leaves.

Addition of Polyethylene glycol (15, 30 and 45 mg PEG/0.2 g DM) increased ($P<0.05$) the cumulative gas production compared with the control (13.5, 16.4 and 20 vs. 8.4). It seems that PEG had a positive effect on fermentation. It was documented by Priolo et al (2000) Inclusion of 40 g of PEG/kg diet eliminated the effects of condensed tannins so that lamb performance and meat quality were similar to lambs given a maize based diet. From the results of the current study, it can be concluded that PEG can be used to alleviate the undesirable effects of anti-nutritional Polyphenols which may be found in some trees for grazing. More studies are needed to emphasize the levels of PEG inclusion with species from arid and semi-arid regions.

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SAŽETAK

Ovo je istraživanje provedeno radi ispitivanja djelovanja raznih razina polietilenskog glikola (PEG) na in vitro proizvodnju plina lišća nekog drveća što raste u Istočnoj Libiji. Upotrijebljeni uzorci bili su *Ceratonia siliqua, Pistacia lentiscus i Acacia cyanophylla*. Uzorci su inkubirani anaerobno s tekućinom iz buraga muških ovaca u porastu s trajno ugrađenom kanilom. Nakupljeni plin mjeren je nakon 3, 6, 12, 24, 48 i 72 sata od inkubacije uzoraka s tekućinom iz buraga. PEG je dodan u razinama od 15,30 i 45 mg na 0,2 g suhe tvari. Kemijska analiza je pokazala da su vrijednosti sirovih bjelančevina iznosile 8,2; 9,5 i 13,4 za *P. lentiscus, C.siliqua* odnosno *A.cyanophylla*, a bjelančevine drugih ekstrakata bile su gotovo iste u svim ispitivanim uzorcima. Sadržaj NDF iznosio je 44,5% za *C.siliqua* i 37% za *A.cyanophylla* dok je *P. lentiscus* bio u sredini (39,8%). Ostatci kondenziranih tanina bili su 25,4; 21,5 i 4,1 za *A.cyanophylla, P. lentiscus* odnosno *C.siliqua*. Prosječni nakupljeni plin (ml/0,2 g DM) nakon inkubacije od 48h bio je viši($P<0,05$) za *C.siliqua* zatim slijede *P. lentiscus* i *A.cyanophylla* (22,9,12,6 i 8,2).Dodavanje polietilenskog glikola (15,30 i 45 mg PEG/0.2 g DM) povisilo je ($P<0,05$) ukupnu proizvodnju plina u usporedbi s kontrolom (13,5;16,4 i 20 vs. 8,4). Prema ovom istraživaanju PRG se može upotrijebiti za ublažavanje nepoželjnog djelovanja antinutritivnih polifenola što se nalaze u lišću drveća za bršćenje

Ključne riječi: *In vitr*o proizvodnja plina, PEG, antinutritivni spojevi