Lotus Newsletter (2006) Volume 36 (1), 7-10.

The effect of PEG on *in vitro* organic matter digestibility and metabolizable energy of *Lotus corniculatus* L

ALI KARABULUT[1], ONDER CANBOLAT[1] and ADEM KAMALAK[*2]

[1]*Bursa Uludag University, Faculty of Agriculture, Department of Animal Nutrition, Bursa, Turkey*

[2]*Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Animal Nutrition, Kahramanmaras, Turkey. Fax: 00 90 344 2230048*

**Corresponding author*

Abstract

The aim of this experiment was to determine the effect of polyethylene glycol (PEG) on the *in vitro* organic matter digestibility and metabolizable energy of *Lotus corniculatus* using Menke gas production technique. The PEG supplementation had a significant effect on the gas production, organic matter digestibility (OMD) and metabolizable energy (ME). PEG inclusion increased the gas production and estimated parameters such as OMD and ME of *Lotus corniculatus*. The improvement in gas production, OMD and ME with PEG emphasizes the negative effect of tannins on digestibility.

Key words: *Lotus corniculatus*, Digestibility, Metabolizable energy, Condensed Tannin, PEG

Introduction

Lotus corniculatus is a temperate plant containing condensed tannin which is well known to improve the efficiency of protein digestion in ruminants (Waghorn *et al*., 1987). However the effect of condensed tannin in *Lotus corniculatus* on organic matter digestibility and metabolizable energy has not been tested in details. The PEG is widely used to determine the effect of condensed tannin in Menke gas production technique.

The aim of this experiment was to determine the effect of PEG on the *in vitro* organic matter digestibility and metabolizable energy of *Lotus corniculatus* using Menke gas production technique.

Materials and Methods

Lotus corniculatus were hand harvested at flowering stage and oven dried at 60 ^{o}C at 48 h. Dry matter (DM) was determined by drying the samples at 105 ^{0}C overnight and ash by igniting the samples in muffle furnace at 525 ^{o}C for 8 h. Nitrogen (N) content was measured

by the Kjeldahl method (AOAC, 1990). Crude protein was calculated as N X 6.25. Condensed tannin was determined by butanol-HCl method as described by Makkar *et al*. (1995). All chemical analyses were carried out in triplicate.

Rumen fluid was obtained from two fistulated sheep fed twice daily with a diet containing alfalfa hay (60%) and concentrate (40%). Samples were incubated *in vitro* rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). 0.200 g dry weight of the sample was weighed into calibrated glass syringes of 100 ml in the absence and presence of 1 g PEG. The syringes were pre-warmed at 39^{o}C before the injection of 30 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39^{o}C. Readings of gas production recorded at 24 h incubation time.

The metabolizable energy (MJ/kg DM) of sample was calculated using equations of Menke *et al*. (1979) as follows:

$$ME\ (MJ/kg\ DM) = 2.20 + 0.136\ GP + 0.057\ CP + 0.0029CP^2$$

Where,
GP is 24 h net gas production (ml/200 mg),
CP = Crude protein (%)

The OMD of sample was calculated using equations of Menke *et al*. (1979) as follows:

$$OMD\ (\%) = 14.88 + 0.889\ GP + 0.45\ CP + 0.0651XA$$

Where,
GP is 24 h net gas production (ml / 200 mg),
CP = Crude protein (%)

The t-test was used to determine the effect of PEG supplementation on in vitro gas production, organic matter digestibility and metabolizable energy of *Lotus corniculatus* L using Statistica for Windows (1993).

Results and Discussion

The chemical composition of *Lotus corniculatus* harvested at flowering stage is given in Table 1. The effect of PEG on the gas production, organic matter digestibility and ME content of *Lotus corniculatus* harvested at flowering stage is given in Table 2. The PEG supplementation significantly ($P<0.001$) increased the gas production, OMD and ME content of *Lotus corniculatus* harvested at flowering stage.

Table 1. The chemical composition of *Lotus corniculatus* harvested at flowering stage

Chemical constituents	**% (of DM)**
Crude protein	17.2
Ash	7.3
Condensed tannin	2.2

This result is in agreement finding of Kamalak *et al*. (2005), Getachew *et al*. (2001), Getachew *et al*. (2002) and Seresinhe and Iben (2003) who found that PEG supplementation increased the gas production and volatile fatty acid production. The increase in the gas production in the presence of PEG is possibly due to an increase in the available nutrients to rumen micro-organisms, especially the available nitrogen.

Table 2. The effect of PEG on the gas production, organic matter digestibility and ME content of *Lotus corniculatus* harvested at flowering stage

Parameters	**O PEG**	**1 g PEG**	**SEM**	**Sig.**
24 h GP	54.7[a]	63.5 [b]	0.443	***
OMD	71.7 [a]	79.5 [b]	0.404	***
ME	10.6 [a]	11.8 [b]	0.047	***

[a b] Means within the same row with differing superscripts are significantly different GP: Gas production (ml) at 24 h incubation, OMD: Organic matter digestibility (%), ME: Metabolisable energy (MJ/kg DM), SEM: standard error of mean, Sig: Significance level,

This result is also in agreement with findings of Rubanza *et al*. (2005) who found that PEG supplementation resulted in the increase in OMD of leaves from Acacia species. Rubanza *et al*. (2003) also found that PEG supplementation resulted in the increase in ME of leaves from browse fodders.

Conclusion

PEG inclusion increased the gas production and estimated parameters such as OMD and ME of *Lotus corniculatus*. The improvement in gas production, OMD and ME with PEG emphasizes the negative effect of tannins on digestibility.

References

AOAC 1990. Official Method of Analysis. (15th.ed.) Association of Official Analytical Chemist, Washington, DC. USA.

GETACHEW G., MAKERS H.P.S. and BECKER K. 2002. Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. *Journal of Agricultural Science*. **139**, 341-352.

GETACHEW G., MAKKAR H.P.S. and BECKER K. 2001. Method of polyethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. *Animal Feed Science and Technology*, **92**, 51-57.

KAMALAK A., CANBOLAT O., SAHIN M., GURBUZ Y, OZKOSE, E and OZKAN C.O. 2005. The effect of polyethylene glycol (PEG 8000) supplementation on in vitro gas production kinetics of leaves from tannin containing trees. *South African Journal of Animal Science*, **35**(4), 229-236.

MAKKAR H.P.S., BLUMMEL M. and BECKER K. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and their implication in gas production and true digestibility *in vitro* techniques. *British Journal of Nutrition*, **73**, 897-913.

MENKE K.H., RAAB L., SALEWSKI A., STEINGASS H., FRITZ D. and SCHNEIDER W. 1979. The estimation of digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they incubated with rumen liquor *in vitro*. *Journal of Agricultural Science* (Camb.), **92,** 217-222.

MENKE H.H. and STEINGASS H. 1988**.** Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research Development*, **28**, 7-55.

RUBANZA C.D.K., SHEM M.N., OTSYINA R., BAKANGESA S.S., ICHINOHE T. and FUJIHARA T. 2005. Polyphenolics and tannins effect on *in vitro* digestibility of selected Acacia species leaves. *Animal Feed Science and Technology*, **119**, 129-142.

RUBANZA C.D.K., SHEM M.N., OTSYINA R., ICHINOHE T. and FUJIHARA T. 2003. Nutritive evaluation of some browse tree legume foliages native to semi arid areas in western Tanzania. *Asian-Australian* Journal *of Animal Science*, **16**(10), 1429-1437.

SERESINHE T. and IBEN C. 2003. *In vitro* quality assessment of two tropical shrub legumes in relation to their extractable tannins content. *Journal of Animal Physiology and Animal Nutrition*, **87**, 109-115.

STATISTICA. 1993. Statistica for windows release 4.3, StatSoft, Inc. Tulsa, OK

WAGHORN G.C., ULYATT M.J., JOHN A. and FISHER M.T. 1987. The effect of condensed tannins on site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *British Journal of Nutrition*, **57**, 115-126.