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EFFECTS OF POLYETHYLEN GLYCOL (PEG 6000) SUPPLEMENT ON *IN VITRO* GAS PRODUCTION OF CANOLA HYBRIDS AND CANOLA MEALS

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ABSTRACT: The aim of this study was to determine the effects of polyethylen glycol on *in vitro* gas production and *in vitro* gas production kinetics of some canola hybrids and canola meals. In this study, four canola hybrids (Bristol, Eurol, Capitol and Licrown) and canola meals purchased from market were used. Two rams (SakızxKarayaka) aged 4 with ruminal cannulas were used in gas production technique. All of the feedstuffs were incubated for 3, 6, 9, 12, 24, 48, 72 and 96 hours. All canola varieties had lover gas production levels except for canola meals. The effects of PEG supplement on in vitro gas production, a (the gas production from the immediately soluble fraction), b (the gas production from the insoluble fraction) and c (gas production rate) of all canola hybrids and canola meals for 3, 6, 9, 12 ,24 and 48 hour incubations were not significant (P>0.05). However, PEG supplementation decreased total gas production (a+b) and in vitro gas production for 72 and 96 hour incubations in Bristol hybrid (P<0.01). Besides, PEG supplemention increased *in vitro* gas production for 72 hour incubation in Capitol hybrid (P<0.01). In conclusion, PEG supplementation affected *in vitro* gas productions for 72 and 96 hours incubations and total gas production (a+b) for all the feeds used in this study (P<0.01). PEG addition could not show its effect due to the lower TMP contents in canola hybrids and canola meals used in this study. *In vivo* experiments should be carried out with feeds rich in tannin by using most appropriate PEG 6000 level.
Key Words: Canola, canola meal, *in vitro* gas production, hybrid, polyethylene glycol

POLIETILEN GLIKOL (PEG 6000) İLAVESININ BAZI KANOLA ÇEŞITLERİNİN VE KANOLA KÜSPESİNİN *İN VITRO* GAZ ÜRETIMI ÜZERINE ETKILERI

ÖZET: Bu çalışmanın amacı bazı kanola hibritlerinde ve kanola küspesinde *in vitro* gaz üretimi ve gaz üretim parametreleri üzerine polietilen glikol ilavesinin etkisini belirlemektir. Çalışmada dört farklı kanola hibriti (Bristol, Eurol, Capitol ve Licrown) ve piyasadan temin edilen kanola küspesi kullanılmıştır. *İn vitro* gaz üretim tekniğinde dört yaşında rumen kanülü takılmış, 2 baş Sakız-Karayaka melezi koç kullanılmış ve yemler 3, 6, 9, 12, 24, 48, 72 ve 96 saat süre ile inkübasyona bırakılmışlardır. Bütün kanola hibritleri, kanola küspesinden daha düşük gaz üretimine sahip olmuşlardır . Denemede kullanılan yemler arasında 3, 6, 9, 12, 24 ve 48 saatlik inkübasyon sonrasında *in vitro* gaz üretim miktarları, hemen çözünebilir fraksiyondan oluşan gaz miktarı (a), zamana bağlı oluşan gaz miktarı (b) ve gaz üretim hızı (c) üzerine PEG ilavesinin etkisi önemsiz bulunmuştur (P>0.05). Bununla beraber PEG ilavesinin Bristol için 72 ve 96 saatlik inkübasyonlar sonrasında oluşan *in vitro* gaz üretimini ve toplam gaz üretimini (a+b) düşürdüğü (P<0.01); Capitol için 72 saatlik inkübasyon sonrasında in vitro gaz üretimini artırdığı saptanmıştır (P<0.01). Sonuç olarak 72 ve 96 saatlik inkübasyon süreleri ile toplam gaz üretimi (a+b) bakımından PEG (6000) ilavesinin denemede kullanılan bütün yemler üzerine etkisi görülmüştür (P<0.01). Çalışmamızda kullanılan kanola hibritleri ve kanola küspesindeki toplam fenolik maddeleri (TFM) içeriklerinin düşük düzeylerde olması nedeniyle PEG ilavesinin etkisi saptanmamıştır. PEG etkisini görebilmek için, TFM içeriğince zengin yemlere en uygun PEG 6000 düzeyinin kullanıldığı *in vivo* denemelerin yürütülmesi gerekmektedir.
Anahtar Sözcükler: kanola, kanola küspesi, *in vitro* gaz üretimi, hibrit, polietilen glikol

1. INTRODUCTION

Canola, an oil-seed crop developed from rapeseed (*Brassica napus* and *Brassica campestris/rapa*), contains low levels of "erucic acid" and anti-nutritional compounds called "glucosinolates" in the meal fraction (Mailer et al., 2008). Canola can be grown in every location in Turkey. Canola has two physiologic phases such as wintery and summery. It is usually planted in winter in Turkiye. Canola seeds and meals can be used as a protein and/or lipid source in ruminant rations (Khorasani et al., 1992). Furthermore, oils obtained from canola varieties with high erucic acid levels are used as biofuel in industry and in electric transformers in France and Germany. Biodiesel production from canola oil has increased during recent years.

In vitro gas production method is widely used to evaluate the nutritive value of different classes of feeds (Getachew et al. 2002). The gas production technique is more efficient than the other *in vitro* techniques in determining the nutritive value of feeds containing tannins. The binding effect of tannins to macromolecules such as proteins and carbohydrates creates problems (Makkar et al., 1995). In the *in vitro* gas production method, the effects of tannins on rumen fermentation are reflected in the gas production. The technique has been used to assess actions of antinutritive factors on rumen fermentation.

Polyethylene glycol (PEG) has been used in gas-based techniques for assessing anti nutritional factors in tanniniferous plants for ruminants. A significant correlation was observed between per cent change in gas production on addition of polyethylene glycol (PEG) and the contents of phenolic matters . Addition of PEG to tannin-containing browses increased *in vitro* gas production (Getachew et al., 2002). Canbolat et al (2005) reported that polyethylene glycol (PEG)

supplementation had a significant effect on the gas production of *Quercus cerris* leaves. When incubated in the presence of PEG, the gas production from substrate fermentation increased (Makkar et al., 1995) and this has been attributed to the binding of PEG to tannins, which are released during fermentation (Khazaal et al., 1993). PEG compounds used in practice are PEG 2000, PEG 4000, PEG 6000 and PEG 8000. PEG 6000 compound was reported to be most efficient compound in eliminating negative effect of tannin (Makkar et al., 1995).

The aim of the study was to use *in vitro* gas production techniques to evaluate the tannin effect upon in vitro gas production and to determine *in vitro* gas production kinetics of some canola hybrids and canola meals using polyethylene glycol (PEG 6000) as an inhibitor of tannin effects.

2. MATERIALS AND METHODS

This study was conducted over the period from January 2006 to March 2007 at Department of Animal Science, Faculty of Agriculture in Ondokuz Mayis University in Samsun - Turkey. In this study, canola (*Brassica napus*) seeds from four different winter variety and four canola hybrids (Bristol, Eurol, Capitol, and Licrown) obtained from the Balck Sea Agricultural Research Institute in Samsun and, canola meals purchased from market were used. Canola seed grains were milled in a hammer mill to pass through 1 mm sieve for subsequent analysis.

2.1. Chemical analysis

Dry matter (DM), Ash, Ether extracts (EE) and crude protein (CP) were determined according to Association of Official Analytical Chemists (AOAC, 1990) and Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents were determined by the methods of Van Soest (1982). Total phenolic matter (TPM) was determined according to the method proposed by Gurses and Artik (1987). Volatile fatty acids and NH3-N contents in the rumen fluid were determined using Markham Steam Distillation procedure (1942). All chemical analyses were carried out in three replicate.

2.2. In vitro gas production technique

Two rams (SakızxKarayaka) aged 4 with ruminal cannulas were used in gas production technique (Menke and Steingass, 1988). Approximately 200 mg dry weight of samples were weighed in triplicate into 100 ml calibrated glass syringes following the procedures of Menke and Steingass (1988). The syringes were pre-warmed at 39 ºC prior to injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39 ºC. To determine the effects of polyethylen glycol on in vitro gas production and in vitro gas production kinetics of some canola hybrids and canola meals, 100 mg PEG (MW 6000) was added in each syringe. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Rumen fluid was obtained from the fistulated sheep fed twice daily (08.30-16.30) with a diet containing forage (%60) and concentrate (%40). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as below by NEWAY computer package programme.

$$y = a+b(1-exp-ct)$$

where; 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), t: incubation time (h), y: gas produced at time "t".

2.3. Statistical analysis

The experiment was planned as completely randomized factorial experimental design and conducted with three replications.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where; Y_{ijk}: observation value (in vitro gas production, gas production kinetics, organic matter digestibility and energy values), μ: Population mean, α_i: Effect of i^{th} canola hybrids and canola meal (1 = Eurol, 2= Capitol, 3= Brtistol, 4= Licrown, 5= Canola meal), β_j: Effect of j^{th} PEG treatment (1= without PEG, 2 = with PEG), $(\alpha\beta)_{ij}$: The effect of interaction between feeds and PEG, e_{ijk}: Residual error. DUNCAN Multiple Range Test was used to determine these differences (SPSS, 10.0). There is no comparison test (posthoc test) taht can be used to determine which interactions are significant for 72, 96 and a+b parameters in which interactions are found to be significant. For this reason, one way variance analysis was applied by combining factors.

3. RESULTS AND DISCUSSION

Chemical composition and total phenolic matter (TPM) content of whole fat canola seeds and canola meals were given in Table 1. Nutrient contents and cell wall components of feeds used in the present study were generally in consistence with those in the other studies. However, there can be some variations among results due to the differences in climate, soil structure, raising techniques, fertilising, variety, harvesting time and storage conditions (Kutlu and Baykal Celik, 2005).

Rumen pH, ammonia N (NH_3-N) and total volatile fatty acid (VFA) contents determined in rumen liquid using *in vitro* gas production technique were; 5.97 (5.73-6.34), 319 mg/l (247-502 mg/l) and 121 mmol/l

(84-141 mmol/l), respectively. pH, TVFA and NH_3-N values related to the rumen liquids used in *in vitro* gas production technique might be affected by composition of ration, rumen sampling time, species, feed processing methods (Ørskov, 1994; Karsli and Tasal, 2003).

The *in vitro* gas production and gas production kinetics were given in Table 2 and Table 3. All varieties had low gas production levels except for canola meals.

The effects of PEG supplement on *in vitro* gas production, a (the gas production from the immediately soluble fraction), b (the gas production from the insoluble fraction) and gas production rate (c) of all canola hybrids and canola meals for 3, 6, 9, 12 ,24 and 48 hour incubations were not significant (P>0.05).

Table 1. Chemical compositions of canola seeds and canola meal, g/kg DM

%	Eurol	Capitol	Bristol	Licrown	Canola Meal
CP	21.93	22.78	21.11	23.48	37.40
Ash	4.41	3.91	4.61	4.84	8.47
EE	47.59	46.97	46.52	44.57	1.72
NDF	37.43	33.75	39.02	40.12	29.14
ADF	34.51	31.74	36.12	32.90	24.17
ADL	8.68	9.60	7.57	9.44	10.27
TPM	1.84	1.81	1.77	2.03	1.76

DM: dry matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; ADL: Acid detergent lignin, TPM: total phenolic matters,

Table 2. The effects of PEG on in vitro gas production and gas production kinetics of canola seeds and canola meal, ml/200 mg DM

	Epeg	**Econt**	**Cpeg**	**Ccont**	**Bpeg**	**Bcont**	**Lpeg**	**Lcont**	**CMpeg**	**CMcont**	**Sig**
72	21,86±	22,02±	22,29±	20,96±	19,92±	21,86±	21,45±	21,97±	36,09±	35,73±	0,000
	0,21**bc**	0,52**bc**	0,44**b**	0,91**c**	0,04**d**	0,10**bc**	0,16**bc**	0,11**bc**	0,13**a**	0,05**a**	
96	22,53±	22,42±	22,46±	22,01±	20,06±	23,25±	22,59±	22,06±	37,77±	35,85±	0,000
	0,17**c**	0,51**c**	0,49**c**	0,02**c**	0,14**d**	0,28**c**	0,64**c**	0,08**c**	0,46**a**	0,10**b**	
a+b	22,28±	21,69±	21,91±	21,31±	19,71±	22,42±	21,91±	21,95±	37,15±	35,66±	0,000
	0,26**c**	0,60**c**	0,41**c**	0,76**c**	0,36**d**	0,39**c**	0,01**c**	0,42**c**	0,45**a**	0,16**b**	

Means in the same row with different letters indicate significance. Epeg: Eurol with PEG, Econt: Eurol control, Cpeg: Capitol with PEG, Ccont: Capitol control, Bpeg: Bristol with PEG, Bcont: Bristol control, Lpeg: Licrown with PEG, Lcont: Licrown control, CMpeg: canola meal with PEG, CMcont: canola meal control. a,b..:P<0.01.

Table 3. The effects of PEG on in vitro gas production (ml) and gas production kinetics of canola seeds and canola meal

	E	**C**	**B**	**L**	**CM**	**Sig**	**PEG +**	**PEG -**	**Sig**
3	4,46±	4,10±	3,96±	3,37±	9,76±	0,000	5,37±	5,73±	0,767
	0,13b	0,16b	0,17bc	0,35c	0,14 a		0,81	0,84	
6	9,30±	8,85±	7,84±	7,40±	15,60±	0,000	10,07±	10,59±	0,734
	0,25b	0,48bc	0,61cd	0,42d	0,30a		1,03	1,10	
9	12,13±	11,81±	10,73±	10,30±	19,73±	0,000	13,22±	13,90±	0,708
	0,19b	0,74b	0,86b	0,76b	0,54a		1,17	1,36	
12	14,33±	14,69±	12,89±	12,18±	22,40±	0,000	15,52±	16,37±	0,666
	0,25b	0,67b	1,12b	1,17b	0,59a		1,27	1,43	
24	20,19±	19,28±	18,14±	18,83±	29,21±	0,000	21,41±	22,31±	0,673
	0,71b	0,64b	0,54b	0,70b	0,78a		1,38	1,59	
48	21,00±	20,72±	20,07±	20,57±	34,00±	0,000	23,86±	24,63±	0,780
	0,48b	0,64b	0,72b	0,35b	0,82a		1,87	1,99	
a, ml	-1,28±	-1,86±	-1,17±	-1,56±	4,75±	0,000	0,47±	-0,02±	0,719
	0,53b	0,44b	0,29b	0,71b	0,75a		1,12	0,74	
b, ml	23,26±	23,47±	22,23±	23,49±	31,66±	0,000	25,26±	25,63±	0,840
	0,73b	0,82b	0,72b	0,70b	0,48a		1,24	1,30	
c, ml/h	0,10±	0,10±	0,09±	0,08±	0,07±	0,044	0,08±	0,09±	0,397
	0,00a	0,01a	0,01ab	0,01ab	0,01b		0,01	0,00	

Means in the same row with different letters indicate significance. E: Eurol,C: Capitol, B: Bristol, L: Licrown, CM: canola meal, PEG+: with PEG, PEG-: without PEG, a.b..:P<0.01, Sig: Significant

However, PEG supplementation decreased total gas production (a+b) and in vitro gas production for 72 and 96 hour incubations in Bristol hybrid ($P<0.01$), whereas it increased *in vitro* gas production for 72 hour incubation in Capitol hybrid ($P<0.01$).

There were no differences among the varieties in terms of gas production amount. In vitro gas production and gas production kinetics are largely influenced by the differences in the chemical compositions of feedstuffs (Menke and Steingass, 1988). Gas production levels are known to be decreased due to the increases in ash contents of feeds (Menke and steingass, 1988), but in our study canola meal (CM) had higher gas production levels compared to canola hybrids although it had high ash content. This indicates that the gas production level might be higher even in feeds with higher ash content.

Feedstuffs with high CP result in low gas production (Chenost et al., 2001). Feedstuffs should contain at least 10% CP for optimum microbial activity in the rumen (Norton, 1998). Feedstuffs with below 10% CP can cause a reduction in the microbial activity in the rumen, thus it can lead to less gas production. The samples in the present study did not affect microbial activity significantly. Lower gas production amount observed for canola hybrids compared to CM which had higher CP and ash content. This discrepancy might be attributed to lower EE content of CM compared to the canola hybrids. Canola seeds with higher EE content had low gas production level. This difference might be due to the fact that high oil levels found in all the canola varieties prevent gas production. Low gas production levels in canola varieties can be attributed to their higher crude fat contents due to the fact that oils decrease VFA concentration and hence gas production in the rumen (Wettstein et al., 2000).

In vitro gas production and total gas production (a+b) were increased by PEG supplementation for 72 hours incubation in Capitol hybrid and for 96 hours incubation in Canola meal ($P<0.01$). In conclusion, PEG supplementation affected in vitro gas productions for 72 and 96 hours incubations and total gas production (a+b) for all the feeds used in this study ($P<0.01$). The effect of PEG addition might be determined more easily in feeds with high tannin contents (Getachew et al., 2002; Kamalak et al., 2005). PEG addition could not show its effect due to low TMP contents in canola hybrids and canola meals used in this study. *In vivo* experiments should be carried out with feeds rich in tannin (Kamalak, 2007) by using the most appropriate PEG level (PEG 6000) (Makkar et al., 1995).

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