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In situ Rumen Degradability, *in vitro* Digestibility and *in vitro* Gas Production of Full Fat Canola Seeds

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Abstract: The objective of this study was to determine the chemical composition, *in vitro* gas production, *in vitro* digestibility and *in situ* rumen degradability of canola hybrids. In the study, canola seeds of four different hybrids (Bristol, Eurol, Capitol and Licrown), which were obtained from the Institute of Karadeniz Agricultural Research in Samsun, Turkiye were used. Two rams aged 2 years with permanent ruminal fistulated were used in gas production and *in situ* nylon bag techniques. All of the feedstuffs were incubated for 3, 6, 9, 12, 24, 48, 72 and 96 h in *in vitro* incubations for gas production. Feedstuffs were incubated for 48 h in nylon bag technique. The results of the present study suggested that there were no differences among the hybrids in terms of feed value. All of the hybrids had low *in vitro* gas production values due to their high fat contents. Licrown variety had the lowest production level up to 48 h of the incubation, but there were no differences after 24 h of the incubation ($p>0.05$). There were not significant differences among the hybrids in terms of estimated parameters except for gas production rate (c). The gas production rate of Licrown was significantly ($p<0.05$) lower than that of Bristol. While, *in vitro* enzyme digestibility Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD) and Metabolisable Energy (ME)) was not different among the hybrids ($p>0.05$), rumen degradabilities Dry Matter Degradability (DMD_{48}), Organic Matter Degradability (OMD_{48}) and Crude krotein Degradability (CPD_{48}) were significantly different ($p<0.01$).

Key words: Full fat canola seed, *in vitro* gas production, degradability, digestibility, energy value

INTRODUCTION

Canola is an oil-seed crop developed from rapeseed (*Brassica napus* and *Brassica campestris/rapa*) by the Canadian plant breeders in 1970's. Uulike traditional rapeseed, canola contains low levels of erucic acid and anti-nutritional compounds called glucosinolates in the meal fraction (Mailer *et al*., 2008).

Canola (*Brasicca napus Oleifera* sp.) has two physiologic phases such as wintry and summery. Canola can be produced in every location of our country. Canola is usually planted in winter in Turkiye. Canola seed containing 38-50% Ether Extract (EE) and 16-24% Crude Protein (CP) can be used as a protein and/or lipid source in ruminant rations (Shahidi, 1990; Khorasani *et al*., 1992). Inclusion of canola seed containing high level of lipids helps to increase energy density of the ration, which is an important aspect particularly for today's high producing cows. In addition, canola oil fraction contains higher content of unsaturated fatty acids. Since, canola seed has a highly lignified seed coat, which is resistant to both ruminal and small intestinal degradation, some form of processing is necessary for effective utilization of canola seed (Khorasani *et al*., 1992; Leupp *et al*., 2006).

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Chichlowski *et al*. (2005) reported that 3.9% added fat from ground canola seed for a total of 6.4% dietary fat (DM basis) to lactating cow diets favorably altered the fatty acid profile in milk fat. The changes in fatty acid profile were not associated with reduced milk yield or composition. Adding ground canola seed to the diets of lactating dairy cows resulted in an increase in the proportions of C_{18} monounsaturated fatty acids including vaccenic acid and isomers of conjuge linoleic acid in milk fat. Ammonia and total volatile fatty acids tended to be lower in ruminal fluid from ground canola seeds cows, however, rumen pH was unchanged. Feeding canola seed to lactating dairy cows resulted in milk fat with higher proportions of healthful fatty acids without affecting milk yield or composition of milk.

The amount of whole canola seed used in diets for beef and dairy cattle and sheep depends upon the total fat level in the diet. At higher concentrations usually above 5.5 to 6% of total diet dry matter, fat interferes with fiber digestion and may reduce feed intake. However, fat at lower levels if properly formulated into the diet becomes a safe and efficient way of adding energy (Prairie and Christensen, 2004).

Whole canola seed can be used to advantage for growing and finishing animals and also for wintering beef cows. In feedlot diets, the oil content is levels up to 20% of total diet dry matter have successfully been fed providing total dietary fat on a dry matter basis is below 6%. This could be 10% of whole canola if 40% or 15% whole canola at 27% oil (Prairie and Christensen, 2004).

Protected canola seeds decreased dry matter intake. Feeding canola seeds reduced the content of C_8 to C_{16} fatty acids in milk and increased the content of oleic acid ($C_{18:1c9}$). Canola seeds had no significant effects on insulin, triglycerides, or cholesterol present in serum, but increased the concentration of nonesterified fatty acids; a greater increase was obtained with protected canola seeds (Delbecchi *et al*., 2001).

High production dairy diets may use some added fat in the diet to provide additional energy in a form other than starch. Similar rules apply to dairy as for beef cattle with whole canola seed. Added dietary oil levels of up to 400 g per cow per day can be used. Because interference with fiber digestibility, high levels of fat are not well tolerated without lowering butterfat levels or reducing feed intake (Prairie and Christensen, 2004).

Furthermore, oil obtained from canola varieties with high erucic acid levels are used as biofuel in industry and in electric transformers of countries such as France and Germany. Biodiesel production from canola oil has increased during recent years.

The objective of the study was to determine the chemical composition, *in vitro* gas production, *in vitro* enzyme technique and *in situ* rumen degradability of four canola hybrids.

MATERIALS AND METHODS

This study was conducted over the period from January 2006 to March 2007 at University of Ondokuz Mayis, Faculty of Agriculture, Department of Animal Science in Samsun Province of the Republic of Turkiye.

Canola Seeds

In this study, canola (*Brassica napus*) seeds from 4 different winter variety hybrids (Bristol, Eurol, Capitol and Licrown) obtained from the Institute of Karadeniz Agricultural Research in Samsun, Turkiye were used. Canola seed grains were milled in a hammer mill to pass through a 1 mm sieve for subsequent analysis.

Chemical Analysis

Dry Matter (DM) was determined by drying samples at 105°C overnight. Organic Matter (OM) content was determined by ashing in a muffle furnace at 550°C for 8 h. Nitrogen (N) content was determined using Kjeldahl method (AOAC, 1990). Crude protein was calculated as N×6.25. Crude Fiber (CF) and EE were determined by the methods described by AOAC (1990) and Nitrogen Free

Extract (NFE) was determined by difference [100 - (CP + EE + CF +ash)]. Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid ketergent Lignin (ADL) contents were determined by the methods of Van Soest (1991). Total phenolic matter was determined according to the method proposed by Gurses and Artik (1987). Volatile fatty acids and NH_3-N contents in the rumen fluid were determined using Markham Steam Distillation procedure (Markham, 1942). All chemical analyses were carried out in triplicate.

***In vitro* Gas Production**

Approximately, 200 mg dry weight of samples was weighted in triplicate into 100 mL calibrated glass syringes following the procedures of Menke and Steingass (1988). The syringes were pre-warmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture consisting of 10 mL strained rumen fluid and 20 mL buffer solution into each syringe followed by an incubation in a water bath at 39°C. Rumen fluid from three fistulated Sakýz x Karayaka rams was collected before the morning feeding and strained through two layers of muslin. Sheep were fed twice daily (08.30-16.30) with a diet of grass hay (60%) and concentrate (40%). Gas volume was recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Total gas volumes were corrected for blank incubations. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) by NEWAY computer package program.

$$y = a + b\left(1 - \exp^{-CT}\right)$$

where, a is the gas production from the immediately soluble fraction (mL), b is the gas production from the insoluble fraction (mL), c is the gas production rate constant for the insoluble fraction (mL h^{-1}), a+b is potential gas production (mL), t is incubation time (h), y is gas produced at time t.

Organic matter digestibility (Menke *et al*., 1979) and ME (Close and Menke, 1986) contents of canola seeds were estimated using equations given below:

$$OMD(\%) = 14.88 + 0.889GP + 0.45CP + 0.065Ash$$

$$ME\left(MJ\,kg^{-1}\,MD\right) = 1.06 + 0.157GP + 0.00884CP + 0.022EE - 0.0081Ash$$

where, GP is 24 h net Gas Production (mL/200mg DM), CP is crude protein (%), EE is Ether Extract (%)

Cellulase Method (*In vitro* Enzyme Technique)

In vitro digestibility of DM and OM were determined according to Alcicek and Wagener (1995) as follows: ONUZUKA cellulase enzyme was used in this study.

$$DMD(\%) = \left(S_1\left(T_1 - T_0\right)/S_1\right) \times 100$$

$$OMD(\%) = 1 - \left(\left(T_1 - T_2\right)/\left(S_1 - S_2\right)\right) \times 100$$

where, S_1 is sample amount as (DM), T_0 is weight of crucible (105°C, 48 h), T_1 is dry sample (105°C, 24 h)+T_0, T_2 is ashed sample (550°C, 4 h)+T_0, A_1 is crude ash amount of sample, (g).

Metabolisable Energy (ME) was estimated using equations given below (Jarrige, 1989; Malossini *et al*., 1993). Calculated values were converted to MJ kg^{-1} DM:

$$ME\left(kcal\,kg^{-1}\,DM\right) = \left[\left(86.82 - 0.0099CF - 0.0196CP\right)DE\right]/100$$

In situ Nylon Bag Technique

The *in situ* degradability characteristics of samples were measured using the nylon bag technique of Orskov and McDonald (1979). Two rumen fistulated Sakýz×Karayaka rams were used in *in situ* study. Three bags for each feed in each of the rams were incubated for 48 h. Triplicate bags containing about 5 g DM were placed into the rumen and incubated for 48 h. After incubation, bags were rinsed in running tap water to remove adhering digesta and then washed twice in a pool of water (30°C) for 5 min to remove rumen fluid. They were dried at 65°C for 72 h. In a forced-drought oven, allowed to air equilibrate and weighed. After incubation, DM, OM and CP degradability (DMD, OMD and CPD) for each bag, for each incubation period and for each ram were calculated separately with formulas suggested by Susmel *et al*. (1990). Metabolizable Energy (ME) contents of canola seeds were estimated using equations given below (Bhargava and Orskov, 1987):

$$ME,\left(MJ\,kg^{-1}\right) = 2.27563 + 0.1073 \times DMD$$

where, DMD is rumen dry matter degradability for 48 h.

Statistical Analysis

One-way Analysis of Variance (ANOVA) was carried out to compare the chemical composition, gas production kinetics, ME, NE_L and OMD values using General Linear Model (GLM) of SPSS 10.0 package programs. Significance between individual means was identified using the Duncan's multiple range tests.

RESULTS

Chemical composition and Total Phenolic Matter (TPM) content of whole fat canola seeds were given in Table 1. Rumen pH, ammonia N (NH_3-N) and total Volatile Fatty Acid (VFA) contents determined for rumen liquid using *in vitro* gas production technique were; 6.18 (5.88-6.45), 321 mg L^{-1} (293-438 mg L^{-1}) and 112 mmol L^{-1} (93-128 mmol L^{-1}), respectively.

All varieties had low gas production levels. Licrown variety had the lowest production level up to 48 h of the incubation, but there were no differences after 24 h of the incubation ($p>0.05$). There were not significant differences among the hybrids in terms of estimated parameters except for gas production rate (c). The gas production rate of Licrown was significantly ($p<0.05$) lower than that of Bristol (Table 2).

There were no differences among the hybrids in terms of DMD, OMD and ME values ($p>0.05$). (Table 3). There were significant differences among the hybrids in terms of DMD_{48}, OMD_{48}, ME ($P<0.001$) and CPD_{48} ($p<0.01$) (Table 4). The DMD_{48} and ME values of Eurol and Licrown are higher than those of Capitol and Bristol ($p<0.001$). OMD_{48} ($p<0.001$) and CPD_{48} ($p<0.01$)values were found different between Eurol and Bristol.

Table 1: Chemical compositions and TPM contents of whole fat canola seeds, %DM

	Hybrids					
Contents (%)	Eurol	Capitol	Bristol	Licrown	SEM	p-value
CP	21.93ab	22.78ab	21.11b	23.48a	0.432	**
Ash	4.41	3.91	4.61	4.84	0.336	NS
EE	47.59a	46.97a	46.52ab	44.57b	0.470	**
NDF	37.43	33.75	39.02	40.12	1.532	NS
ADF	34.51ab	31.74b	36.12a	32.90ab	0.787	**
ADL	8.68	9.60	7.57	9.44	0.765	NS
TPM	1.84b	1.81b	1.77b	2.03a	0.034	**

DM: Dry matter, CP: Crude protein; EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, TPM: Total phenolic matter, SEM: Standard error of mean, NS: Non significant. Means in the same row with different letter(s) indicate significance. $p<0.01$

Table 2: *In vitro* gas productions, gas production kinetics and estimated parameters of whole fat canola seeds

	Hybrids					
Incubation times	Eurol	Capitol	Bristol	Licrown	SEM	p-value
Gas volume (mL)						
3	4.69bc	5.65ab	7.17a	3.41c	0.427	**
6	8.43bc	10.08ab	11.98a	6.53c	0.644	**
9	11.49bc	13.70ab	15.60a	9.21c	0.769	**
12	14.00bc	16.65ab	18.34a	11.52c	0.838	**
24	20.30ab	24.04a	24.31a	18.13b	0.880	*
48	24.63	29.01	27.45	24.53	0.792	NS
72	25.65	30.12	27.97	27.17	0.753	NS
96	25.90	30.38	28.07	28.35	0.744	NS
Gas production kinetics and estimated parameters						
a (mL)	0.12	-0.22	0.86	-0.24	0.354	NS
b (mL)	25.87	30.24	27.40	29.58	1.557	NS
c (mL h^{-1})	0.07ab	0.07ab	0.11a	0.04b	0.013	*
ME (MJ kg^{-1} DM)	5.45	6.03	5.86	5.05	1.511	NS
OMD (%)	43.07	46.75	45.24	41.87	0.246	NS

DM: Dry matter, a: Gas production from the immediately soluble fraction (mL), b: Gas production from the insoluble fraction (mL), c: Gas production rate constant for the insoluble fraction (mL h^{-1}), ME: Metabolisable energy, OMD: Organic matter digestibility, SEM: Standard error of mean, NS: Non significant; Means in the same row with different letters indicate significance. *p<0.05, **p<0.01

Table 3: DMD, OMD and ME contents of whole fat canola seeds

	Hybrids					
Estimated parameters	Eurol	Capitol	Bristol	Licrown	SEM	p-value
DMD	40.98	40.84	41.87	41.07	0.825	NS
OMD	38.78	37.76	40.16	39.57	0.636	NS
ME	8.87	8.83	9.52	9.43	0.149	NS

DMD: Dry matter digestibility, OMD: Organic matter digestibility, ME: Metabolisable energy, SEM: Standard error of mean, NS: Non significant

Table 4: *In situ* DMD48, OMD48, CPD48 and ME values of canola seeds

	Hybrids					
Estimated parameters	Eurol	Capitol	Bristol	Licrown	SEM	p-value
DMD48	72.09a	63.25b	65.12b	71.90a	1.542	***
OMD48	74.28a	66.75ab	65.39b	71.96ab	1.817	***
CPD48	75.46a	71.69ab	66.94b	76.06a	2.013	**
ME	9.96a	9.01b	9.21b	9.94a	0.165	***

DMD48: Dry matter degradability, OMD48: Organic matter degradability, CPD48: dry matter degradability, ME: Metabolisable energy, SEM: Standard error of mean, NS: Non significan. Means in the same row with different letters indicate significance. **p<0.01, ***p<0.001

DISCUSSION

In vitro gas production, gas production kinetics, estimated parameter values are largely influenced by the differences in the chemical cosmpositions of feedstuffs. The increase in ash contents of the feedstuffs leads to a decrease in the amount of gas produced (Menke and Steingass, 1988). The similarity of varieties in terms of ash content can be one of the reasons for similar total gas production levels.

Feedstuffs with high CP result in low gas production (Chenost *et al*., 2001). Lower gas production level observed for Licrown variety might be attributed to its higher CP content. 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 can lead to less gas production. The hybrids used in the present study did not affect microbial activity

significantly. Khorasani *et al*. (1992) reported that supplementation of ruminally protected canola seed, at levels>3% of the diet, decreased concentrations of total ruminal VFA and molar proportions of acetate, propionate and butyrate.

Licrown had numerically the lowest gas volumes up to 48 h of the incubation. This can be attributed to higher TPM content in Licrown compared to other hybrids (Kilic and Sarıcicek, 2006). Furthermore, gas production rate differs with relation to the amount and availability of rumen microorganisms (Mauricio *et al*., 2001). Low gas production rate in Licrown might be caused by higher TPM content which had influence on amount of rumen microorganisms. However, there were no differences among the varieties in terms of gas production levels. 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).

There is a strong relationship between the OMD of feedstuffs and the rate of gas production (Chenost *et al*., 2001). In the present study, Licrown variety with lowest c value had numerically the lowest OMD value. This finding is consistent with the results of Chenost *et al*. (2001). However, Kilic and Sarıcicek (2006) suggested that feeds with lowest c value do not always have lowest OMD value.

Lower c value of Licrown compared to Bristol explains why the Licrown had lower gas production level up to 48 h of the incubation. As expected, the feeds with lower c values had lower gas productions at the beginning of the incubation. Thus, the lack of difference with the progression of incubation explains this case.

Data from cattle fed with whole canola suggest that the seeds are relatively resistant to digestion in the rumen and intestine unless processed (Khorasani *et al*., 1992; Leupp *et al*., 2006). Gralak *et al*. (1997) reported 71.90 and 74.98% values for effective DM degradability and CP degradability of whole canola seeds. This finding is consistent with present findings. Micronized whole canola seeds had higher gas productions and lower DM and CP degradability compared to unprocessed seeds (Wang *et al*., 1997). DM and CP degradability found in this study are similar to present findings.

At 5%/h flow rate, effective CP degradability of WCS was 86.7.2%. Extrusion did not affect WCS and rumen CP degradability. Without some form of protection whole canola seed CP is obviously, highly degradable (Deacon *et al*., 1986, 1988). These results are in agreement with our findings. The highest degradability values were found for CP.

If whole canola seed makes up more than 12-14% of the ration it may lead to depressed rumen function reduced feed intake and digestibility of nutrients. Conversely, fed dairy cows whole canola seeds, raw or extruded, at 14% of the diet without effecting Crude Fibre digestibility (Ellwood, 2004). Murphy *et al*. (1987) reported reduction in rumen digestibilities of DM, NDF and cellulose, however hindgut fermentation compensated for the reduction at 1 kg/day Whole Canola Seed supplementation, but not at 2 kg day^{-1}.

Although, there were no differences between the canola hybrids in terms of dry matter digestibility, organic matter digestibility and *in vitro* ME values in enzyme technique, Bristol, which had the lowest CP value, also had the lowest CPD_{48} value in *in vitro* gas production technique. Variability in feeds and also their production locations is one of the most important factors affecting the results of nylon bag technique (Kilic and Sarıcicek, 2004). Yilmaz (1997a, b) found wide variations for SunFlower Meal (SFM) samples obtained from 12 different locations and for Alfalfa Hay (AH) samples obtained from 15 different locations in terms of degradability characteristics. The author reported a value, from which DMD was calculated, as 3.90 -60-72% for SFM and 0.00- 41.75% for AH. This explains why the large variations were observed among the canola varieties used in present study.

ME values found in *in vitro* gas production technique and in *in vitro* enzyme technique were not different, but there were differences among the hybrids in *in situ* bag technique. This can be attributed to the fact that the varieties have significant effects on the results of nylon bag technique (Yilmaz,

1997a, b; Kilic and Sarıcicek, 2004). ME values found in *in vitro* gas production technique were lower than those found in *in vitro* enzyme technique and *in situ* nylon bag technique due to the lower gas productions of the canola seeds. This difference might be due to the fact that high oil levels found in all the canola varieties prevent gas production.

Leupp *et al*. (2006) reported that supplementation with canola seed at 8% of dietary DM did not affect intake or fiber digestion in low-quality forage diets. Canola supplementation increased apparent and true ruminal CP degradation but decreased small intestinal CP digestion. Canola supplementation decreased ruminal pH and the molar proportion of acetate. Decrease in acetate content explains the lower gas productions in canola seeds. The researchers explanained that their study results suggest that ground canola included at 8% of the diet can alter ruminal VFA concentrations and increase *in situ* degradation of canola seed when offered as a supplement for cattle fed low-quality forage diets.

CONCLUSION

The results of the present study indicate that feed value of different canola seed varieties is similar. Furthermore, they have lower *in vitro* gas production values due to their higher oil content. While there were no differences among the hybrids in *in vitro* enzyme technique, there were significant differences in *in situ* technique ($p<0.01$). If whole canola seed makes up more than 12-14% of the ration it may lead to a depressed rumen function, reduced feed intake and digestibility of the nutrients. However, canola seeds should be used up to 20% of the total ration dry matter or the oil supplied from canola should not exceed the 6% of the total oil content of the ration. It can be said that canola seeds incorporated into the ration can decrease the feed energy waste in rumen due to lower gas production levels.

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