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The effect of physical and chemical treatments of canola seed varieties on crude protein fractions using CNCPS and in vitro gas production

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Abstract The effect of physical and chemical treatments on crude protein fractions, using CNCPS method, of five different canola seed varieties (Brassica napus) were studied. *In vitro* gas production of the varieties was also evaluated. Treating with formaldehyde as chemical treatment and toasting and autoclaving as physical treatments were used. *In vitro* protein solubility of treated canola varieties were evaluated to determine the different fraction of protein, including non-protein nitrogen (A), rapidly degradable protein (B_1), intermediately degradable protein (B_2), slowly degradable protein (B_3), unavailable protein (C) and neutral detergent insoluble protein (NDIP). *In vitro* gas production was recorded at 2, 4, 6, 9, 12, 24, 48, 72 and 96 h incubation. This experiment was carried out as a completely randomized design and the data were analyzed using factorial method and the mixed model procedure. Significant difference ($P<0.01$) was observed in crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF) content of varieties and ranged from 214.2 to 237.1, 288.6 to 466.6, 232 to 264.3 and 147.7 to 200.6 g/kg DM, respectively. Treated varieties had greater B_2 and B_3, and lower A and B_1 fractions compared with untreated varieties. Formaldehyde and heat treatment increased neutral detergent-insoluble protein. Acid detergent-insoluble protein was not affected or slightly affected by heat or formaldehyde treatments, respectively. Results of the *In vitro* gas production techniqueshowed that regardless oftreatment methods, treating resulted in decrease and increase in both potential gas production (*A*) and lag times compared with untreated varieties, respectively. The results emphasized that treating canola seeds especially with formaldehyde can effectively increase the RUP in ruminant diets.

Keywords: canola, formaldehyde, heat, *in vitro* gas production, protein solubility

Introduction

Among oil seeds canola is considered as poor sources of ruminal undegraded protein owing to their high ruminal degradability. Canola seed is utilized as an energy and protein source in ruminant diets. It contains

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approximately 210 g/kg crude protein (CP) and 430 g/kg oil and has an amino acid composition well suited for ruminants (Wang *et al*., 1999) but the protein of canola seed is highly degraded by rumen microbes (Madsen and Hvesplund, 1985; Wang *et al*., 1999). Especially, high-producing dairy cows and rapidly growing ruminants cannot satisfy their CP requirements from microbial protein alone (NRC, 2001) making it essential that the diet contain slowly degraded proteins with a high potential for rumen escape. Several workers have found that moist heat treatment of protein meals such as seeds of pea, lupin, field bean, vetch, bitter vetch and mustard meal reduces ruminal protein degradability (Mustafa, 1999; Aguilera, 1992). The effect of moist heat treatment on ruminal degradation of protein of feedstuffs has been intensively studied, mostly in situ. Various approaches are available to assess the ruminal degradability of protein in feedstuffs, which include *in vivo*, *in sacco*, and *in vitro* methods (Elwakeel*et al*., 2007). The Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen*et al*., 1992) is one of the schemes developed for the fractionation of protein in feeds. The CNCPS is a mathematical model to evaluate cattle ration and animal performance based on principles of rumen fermentation, feed digestion, feed passage and physiological status of the animal (Fox *et al*., 2004). *In vitro* gas production is a method that detects small differences in nutritional characteristics between feedstuffs, allows for more frequent sampling than with *in vitro* digestibility, and is rapid and precise (DePeters*et al*., 2003). There is a lack of information regarding evaluation of the response of canola seed to different treatment methods using different varieties. This study was carried out to investigate not only the variation in the protein fractions of different canola seed varieties but also to investigate the effects of physical and chemical treatments on kinetics of in vitro gas production of different varieties of canola seed.

Material and methods

Whole canola seeds varieties (Licord, Zarfan, Slm-046, Talaye, Rgs 003) obtained from the Gene Bank of seed and plant breeding Institute, Karaj, Iran. Standard methods as described in AOAC (1990) were used for determination of dry matter (DM), ash crude protein (CP) and ether extract (EE). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991).

Varieties of seeds were treated as follows: Toasting at 150°C for 15 min, autoclaving with a steam pressure of 117 KPa at 125°C for 30 min. For preparing the treated canola seed with formaldehyde the varieties (20 g) was mixed with 0.7 g formalin 37% and sealed for 5 days. Then, the treated seeds

were poured on to a plastic sheet to remove formaldehyde for 24h. The untreated oilseeds were considered as control group.

Protein solubility of oilseeds

The protein fractionation in CNCPS is based on solubility in buffer and detergent solutions. Buffer-soluble protein, non-protein nitrogen (NPN), and acid and neutral detergent-insoluble protein were determined following the procedures of Licitra*et al*. (1996). The equations of Sniffen*et al*. (1992) were used to fractionate the true protein fraction of unheated and heated canola seed varieties protein into rapidly (B_1), intermediately (B_2) and slowly (B_3) degradable fractions.

In vitro gas production trial

The method used for gas production measurements was as described by Theodorou*et al*. (1994). All samples were ground to pass a 1mm screen. About 125 mg of each sample was weighed into tubes kept at approximately 39 ºC and flushed with CO_2 before use. Each sample was incubated in three replicates. Fifteen ml of buffered rumen fluid (20% rumen fluid + 80% buffer solution) prepared and were anaerobically dispensed in each tube at 39 ºC. All the tubes were crimped, placed in an incubator at 39 ºC, and shaken at regular times. The pressure of gas produced in each tube was recorded using a pressure transducer (Manometer Digital testo 512) at 2, 4, 6, 9, 12, 18, 24, 48, 72 and 96 h after the start of the incubation. To estimate the kinetics of gas production, data on cumulative gas volume produced were fitted using the generalized Mitscherlich model proposed by France *et al*. (1993):

$$G = A\,(1-e^{-c\,(t-L)-d\,(\sqrt{t}\,-\sqrt{L}\,)})$$

Where, *G* (ml) denotes cumulative gas production at time *t*, *A*(ml) is asymptotic gas production, *c* (h^{-1}) and *d* ($h^{-1/2}$) are rate constants and *L* (h) is lag time. The half-life ($t_{1/2}$, h) of the degradable fraction of each substrate was calculated as the time taken for gas accumulation to reach 50% of its asymptotic value. All gas volumes were adjusted to a common sample weight of 200 mg DM (Lopez *et al*., 2007).

Result and discussions

Chemical composition

Chemical compositions of untreated canola seed varieties are shown in Table 1. There were significant differences between varieties in terms of CP, EE, NDF and ADF concentrations. The CP levels ranged from 214/2 to237/1 g/kg DM. The CP levels of the canola varieties used in this experiment were consistent with those of canola varieties reported by Kulich and Garipoglu (2009) who showed that the CP levels of four canola varieties ranged from 211.1 to 234.8 g/kg DM. The EE content ranged from 288.6 to 466.6 g/kg DM with Licord having the lowest EE level. The mean value of EE (352.4 g/kg DM) was consistent with those reported by Kulich and Garipoglu (2009). Although different amounts of EE and CP content of canola seed has been reported in other studies. This might have been due to differences between varieties and growing conditions.

Table 1. Chemical composition (g/kg) of untreated canola and safflower varieties

Varieties	DM	OM	OM	ASH	CP	EE	NDF	ADF
Licord	971.9^{b}	935.2^{bc}	935.2^{bc}	36.7^{c}	237.1^{a}	288.6^{d}	253.4^{b}	200.6^{a}
Zarfan	971.3^{b}	926.0^{c}	926.0^{c}	45.3^{a}	214.2^{d}	354.2^{b}	241.6^{c}	175.2^{b}
SLM-046	985.0^{a}	942.1^{ab}	942.1^{ab}	42.9^{ab}	227.9^{b}	320.4^{c}	264.3^{a}	147.6^{d}
Talaye	979.6^{ab}	954.4^{a}	954.4^{a}	25.2^{d}	227.9^{b}	332.5^{c}	232.0^{d}	171.0^{b}
RGS003	983.3^{a}	947.6^{b}	947.6^{b}	38.7^{bc}	226.6^{c}	466.6^{a}	237.9^{cd}	164.4^{c}
Avg.	978.8	941.0	941.0	37.76	226.7	352.4	245.8	171.7
P.value	**	**	**	**	**	**	**	**
SEM	2.11	2.98	2.98	1.93	1.95	16.42	3.26	4.64

Means within the same column with differing superscripts are significantly different.
DM= dry matter. CP= crude protein. EE= ether extract. NDF= neutral detergent fiber. ADF= acid detergent fiber
**P < 0.01. SEM= Standard Error Mean, Significantly.

Statistical analysis

In finally total data was statistically analyzed in complete random designs (CRD) in factorial arrangement 4*5. Data on gas production and CNCPS from different canola seed varieties were analyzed using the General linear model (GLM) procedure of SAS institute Inc (SAS, 2002). Significance between individual means was identified using Duncan multiple-range test (Duncan, 1955).

Nitrogen solubility and protein fractionation

The result of protein fractionation (Table 2) showed that there were significant differences between proteinfraction of untreated canola different varieties (P<0.01). Fractionation of total CP showed that raw canola seed had high soluble protein (58.6 to 62.9 %CP) and low neutral (12.70 to 17.07 % CP) and acid (7.86 to 6.86 %CP) detergent-insoluble protein levels (Table 2). These values were all higher than those reported by Mustafa *et al.* (2000). The rapidly degradable protein (B_1) was the highest portion in all varieties while the unavailable protein (C) was the lowest fraction.

Table 2. Protein fractions based on Cornell Net Carbohydrate and Protein System for untreated different canola seed varieties (%CP)

Varieties	Protein Fraction (%CP)							
	CP	SCP	A	B1	B2	B3	C	NDIP
Licord	23.71 ^{a}	62.64 ^{a}	15.54^{bc}	47.10 ^{a}	21.32^{c}	8.18^{b}	7.86^{a}	16.04^{b}
Zarfan	21.43 ^{d}	62.87^{a}	15.74 ^{b}	47.13 ^{a}	24.43^{b}	4.87^{d}	7.83^{ab}	12.70^{d}
SLM-046	22.82 ^{b}	60.35^{b}	14.54 ^{d}	45.82 ^{b}	25.70^{a}	6.31^{c}	7.64^{c}	13.95^{c}
Talaye	22.80 ^{b}	58.63^{d}	16.76 ^{a}	41.87^{d}	24.30^{b}	9.41^{a}	7.66^{cb}	17.07^{a}
RGS003	22.66 ^{c}	59.37^{c}	15.1 ^{c}	44.27^{c}	24.02^{b}	9.75^{a}	6.86^{d}	16.61^{ab}
Avg.	22.68	60.77	15.53	45.24	23.51	7.70	7.57	15.27
P.value	**	**	**	**	**	**	**	**
SEM	0.195	0.104	0.205	0.532	0.401	0.508	0.099	0.459

Regardless oftreatment methods all kinds oftreatments decreased (P<0.01) soluble crude protein soluble crude protein (SCP) fraction considerably and increased the intermediately degradable protein (B_2) and slowly degradable protein (B_3) fraction (Table 3). The increase in B_2 fraction upon treating would account for the majority of the loss in the B_1 fraction, hence it would be expected that the overall degradation rate would decrease because of treating as a result of the shift from the rapidly ruminally degraded, B_1 fraction, to the slowly ruminally degraded, B_2 fraction. Decreased CP solubility of protein supplements as a result of heat treatment is well documented (MoshtaghiNia and Ingalls, 1992; McAllister *et al*., 1993). Similar effects of moist heat treatment on protein fractions have alsobeen reported for mustard meal (Mustafa *et al*., 1999) feed gradechickpeas (Mustafa *et al*., 2000) and sunflower seed (Mustafa *et al*., 2003). Heat facilitates the Millard or non enzymatic browning reaction between sugar aldehyde groups and free amino acid groups of protein to yield an amino-sugar complex (Lin and Kung., 1999). This complex is more resistant than normal peptides to enzymatic hydrolysis and reversibility of this reaction is dependent on temperature and duration of heat exposure (Lin and Kung, 1999).

Table 3. Protein fractions based on Cornell Net Carbohydrate and Protein System for raw (control) and different treated canola seed (%CP)

Varieties	CP	Protein Fraction (%CP)						
		SCP	A	B1	B2	B3	C	NDIP
Licord	23.93[a]	53.56[a]	8.89[b]	44.67[a]	24.79[e]	13.38[b]	7.52[a]	21.65[a]
Zarfan	22.49[e]	53.64[a]	9.65[a]	43.99[b]	27.80[d]	11.36[d]	7.20[c]	18.56[c]
SLM-046	23.79[b]	46.44[d]	7.1[d]	39.34[d]	33.87[a]	11.78[c]	7.04[d]	18.82[c]
Talaye	23.63[c]	48.02[c]	8.79[b]	39.23[d]	31.88[b]	13.48[ab]	7.37[b]	20.1[b]
RGS003	22.69[d]	48.62[b]	7.66[c]	40.96[c]	31.47[c]	13.69[a]	6.97[d]	19.91[b]
SEM	0.011	0.034	0.051	0.048	0.092	0.091	0.029	0.094
Processing								
Control	22.68[d]	60.77[a]	15.53[a]	45.24[a]	23.95[d]	7.70[d]	7.47[a]	15.27[c]
Toasting	23.52[b]	53.39[b]	10.51[b]	42.86[b]	31.18[b]	9.22[c]	7.43[a]	15.45[c]
Autoclaving	23.67[a]	45.87[c]	6.29[c]	39.58[c]	30.94[c]	15.70[b]	7.49[a]	23.19[b]
Formaldehyde	23.37[c]	40.22[d]	1.34[d]	38.88[d]	33.78[a]	18.33[a]	6.38[b]	25.31[a]
Avg.	23.31	50.06	8.42	41.64	29.96	12.74	7.22	19.81
SEM	0.009	0.03	0.045	0.043	0.082	0.081	0.026	0.084
Varieties effect	**	**	**	**	**	**	**	**
Processing effect	**	**	**	**	**	**	**	**
Processing×Varieties	**	**	**	**	**	**	**	**

Different letters within column indicates differences (P < 0.001). SEM= Standard Error Mean. **P < 0.001.CP= crude protein. SCP= soluble crude protein. A= Non-protein nitrogen. B1= rapidly degradable protein. B2= Intermediately degradable protein. B3= Slowly degradable protein. C= unavailable protein. NDIP= neutral detergent insoluble protein.

Autoclaving and formaldehyde treatment increased (P<0.01)neutral detergent-insoluble protein from 15.27 in control to 23.19 and 25.31, respectively but toasting had no effect on this fraction. Increase in NDIP fraction is also in accordance with the neutral detergent insoluble protein (NDIP) results for micronized flaxseed reported in Gonthier*et al*. (2004), as well as with results for autoclaved sunflower seed in study of Mustafa *et al*. (2003). However, no effect on acid detergent-insoluble protein was noted as a result of heating. These results indicate that possibly heat inputs used in the present study were not severe enough to generate unavailable protein via the Millard reaction. Other researchers have reported similar findings when modest heat treatments were applied to soybean (Demjanec *et al*., 1995) and canola (MoshtaghiNia and Ingalls, 1992) meal. Van Soest (1989) suggests that an optimum heat treatment will minimize soluble CP and maximize NDIP without a substantial increase in ADIP. As shown in Table 4 treating with formaldehyde resulted in the lowest SCP and highest B_2 and B_3 among treatments in all varieties. Nitschmann*et al*. (1943) found that the structure of proteins with formaldehyde forms strong complexes that resist against the proteolysis

enzymes in rumen pH, but the pH of the abomasum broken connections and a lot of protein is removed.

Table 4. Protein fractions based on Cornell net carbohydrate and protein system for raw (control) and different treated canola seeds (%CP)

Varieties	Processing	Protein fractions						
		SCP	A	B1	B2	B3	C	NDIP
Licord	Control	62.64^{a}	15.54^{a}	47.10^{a}	21.32^{d}	8.18^{c}	7.86^{a}	16.04^{c}
	Toasting	56.46^{b}	10.89^{b}	45.57^{b}	27.51^{a}	8.17^{c}	7.86^{a}	16.3^{c}
	Autoclaving	52.22^{c}	7.60^{c}	44.62^{c}	23.95^{c}	16.48^{b}	7.35^{b}	23.83^{b}
	Formaldehyde	42.91^{d}	1.52^{d}	41.40^{d}	26.38^{b}	20.69^{a}	7.02^{c}	30.71^{a}
Zarfan	Control	62.87^{a}	15.74^{a}	47.13^{a}	24.43^{c}	4.87^{d}	7.83^{a}	12.70^{c}
	Toasting	55.65^{b}	13.52^{b}	42.12^{c}	28.03^{b}	8.88^{c}	7.44^{b}	16.32^{b}
	Autoclaving	50.02^{c}	7.73^{c}	42.29^{c}	27.62^{b}	14.68^{b}	7.67^{a}	22.36^{a}
	Formaldehyde	46.04^{d}	1.61^{d}	44.43^{b}	31.10^{a}	17.00^{a}	5.86^{c}	22.86^{a}
SLM-046	Control	60.35^{a}	14.53^{a}	45.82^{a}	25.70^{d}	6.31^{c}	7.64^{a}	13.95^{d}
	Toasting	51.16^{b}	8.12^{b}	43.03^{b}	33.68^{c}	8.002^{b}	7.16^{b}	15.16^{c}
	Autoclaving	40.09^{c}	4.72^{c}	35.37^{c}	36.01^{b}	16.36^{a}	7.54^{a}	23.90^{a}
	Formaldehyde	34.17^{d}	1.02^{d}	33.15^{d}	40.10^{a}	16.45^{a}	5.82^{c}	22.27^{b}
Talaye	Control	58.63^{a}	16.76^{a}	41.87^{a}	24.30^{d}	9.41^{d}	7.66^{b}	17.07^{c}
	Toasting	51.61^{b}	10.98^{b}	40.63^{b}	32.68^{c}	10.85^{c}	7.86^{a}	15.71^{d}
	Autoclaving	42.46^{c}	6.11^{c}	36.35^{d}	34.76^{b}	14.95^{b}	7.83^{a}	22.78^{b}
	Formaldehyde	39.36^{d}	1.30^{d}	38.06^{c}	35.79^{a}	18.74^{a}	6.11^{c}	24.84^{a}
RGS003	Control	59.37^{a}	15.10^{a}	44.27^{a}	24.02^{d}	9.75^{d}	6.86^{b}	16.61^{c}
	Toasting	51.98^{b}	9.02^{b}	42.96^{b}	34.00^{b}	10.22^{c}	6.80^{b}	14.02^{d}
	Autoclaving	44.56^{c}	5.29^{c}	39.27^{c}	32.34^{c}	16.02^{b}	7.08^{a}	23.10^{b}
	Formaldehyde	38.59^{d}	1.24^{d}	37.35^{d}	35.52^{a}	18.77^{a}	7.13^{a}	25.89^{a}
SEM		0.094	0.101	0.096	0.183	0.181	0.058	0.188

Different letters within row indicates differences (P<0.01). SEM= Standard Error Mean.
CP= crude protein. SCP= soluble crude protein. A= non protein nitrogen. B1= rapidly degradable protein. B2= intermediately degradable protein. B3= slowly degradable protein. C= unavailable protein. NDIP= neutral detergent insoluble protein.

In vitro gas production from different varieties

Gas production kinetic parameters of untreated canola seed varieties are presented in Table 5. There were significant differences between potential gas production (*A*) and the rate constants (*c*) of canola seed varieties (P<0.01). Significant difference was observed in potential gas production (*A*) and constant rate (*c*) among different varieties. The *A* values ranged from 25.1 to 29.1 (ml 200 mg^{-1}DM) and the highest total gas production and constant rate (*c*) was observed in Licord variety. Kinetics parameters of varieties with different treatments are presented in Table 6 and 7. Effect of treatments on kinetic parameters was variable among different varieties (Table 7). Generally, toasting, autoclaving and formaldehyde treatment decreased (P<0.01) asymptotic gas production (*A)* (24.48, 23.33 and 22.34 ml/200 mg DM, respectively) compare to untreated seed (26.69 ml/200 mg DM).

In all varieties formaldehyde considerably increased lag time compare to other methods. The reduction in total gas production and constant rate as aresult of toasting and autoclaving was consistent with those reported by El-Wazir*yet al.* (2005) and Canbolat *et al*. (2005) respectively.

Table 5. Cumulative gas production and kinetic parameters[1] estimated of untreated canola seeds varieties

Varieties	Gas parameters				
	A (ml/200mgDM)	C (h^{-1})	Lag time (h)	Half Life (h)	ME(MJ kg^{1}DM)
Licord	29.11^{a}	0.08^{a}	1.34^{c}	8.86^{cd}	5.75^{a}
Zarfan	25.10^{d}	0.03^{c}	1.25^{cd}	10.55^{a}	4.89^{d}
SLM-046	26.61^{c}	0.08^{a}	1.10^{d}	8.73^{d}	5.52^{b}
Talaye	27.47^{b}	0.07^{b}	1.58^{b}	8.93^{c}	5.53^{b}
RGS003	25.13^{d}	0.07^{b}	2.07^{a}	10.27^{b}	5.45^{c}
Avg.	26.69	0.07	1.47	9.47	5.43
P.value	**	**	**	**	**
SEM	0.405	0.005	0.093	0.208	0.076

[1] *A*= potential gas production. (*c*)= rate constants. *L*= lag time. ME= metabolizable energy. Values in the same columns without common letters are significantly different (P < 0.01). SEM= standard error of the means. **P < 0.01

Table 6. Cumulative gas production and kinetic parameters[1] estimated of treated canola seeds varieties

Varieties	Gas parameters			
	A(ml 200 mg^{-1})	*c* (h^{-1})	Lag time (h)	Half life (h)
Licord	26.51^{a}	0.07^{a}	1.58^{c}	9.14^{c}
Zarfan	22.30^{e}	0.05^{d}	1.34^{d}	10.15^{b}
SLM-046	24.21^{c}	0.06^{b}	1.40^{d}	8.77^{d}
Talaye	25.14^{b}	0.058^{c}	1.71^{b}	9.27^{c}
RGS003	22.90^{d}	0.058^{c}	2.24^{a}	11.06^{a}
SEM	0.051	0.001	0.021	0.062
Processing				
Control	26.69^{a}	0.07^{a}	1.47^{c}	9.47^{b}
Toasting	24.48^{b}	0.07^{a}	1.61^{b}	9.27^{c}
Autoclaving	23.33^{c}	0.05^{b}	1.61^{b}	9.02^{d}
Formaldehyde	22.34^{d}	0.04^{c}	1.93^{a}	10.95^{a}
Avg.	24.21	0.06	1.65	9.68
SEM	0.046	0.001	0.019	0.055
Varieties effect	**	**	**	**
Processing effect	**	**	**	**
Processing × Varieties	**	**	**	**

[1]*A*= potential gas production. (*c*)= rate constants. *L*= lag time. SEM= standard error of the means. Values in the same columns without common letters are significantly different (P < 0.01). **P<0.01.

Table 7. Gas production kinetics in untreated and different treated canola seed varieties

Varieties	Gas production kinetics (ml 200 mg^{-1})				
	processing	*A*(ml 200 mg^{-1})	c(h^{-1})	Lag time(h)	Half laife(h^{-1})
Licord	Control	29.11^{a}	0.09^{a}	1.35^{c}	8.86^{b}
	Toasting	26.37^{b}	0.07^{b}	1.36^{c}	8.91^{b}
	Autoclaving	26.21^{b}	0.07^{b}	1.65^{b}	9.20^{b}
	Formaldehyde	24.35^{c}	0.05^{c}	1.98^{a}	9.59^{a}
Zarfan	Control	25.10^{a}	0.03^{d}	1.25^{b}	10.55^{b}
	Toasting	23.02^{b}	0.07^{a}	1.36^{ab}	9.23^{c}
	Autoclaving	21.50^{c}	0.05^{b}	1.37^{ab}	8.42^{d}
	Formaldehyde	19.59^{d}	0.04^{c}	1.39^{a}	12.38^{a}
SLM-046	Control	26.61^{a}	0.08^{a}	1.10^{c}	8.73^{b}
	Toasting	24.80^{b}	0.07^{b}	1.39^{b}	7.54^{d}
	Autoclaving	23.27^{c}	0.06^{c}	1.40^{b}	8.24^{c}
	Formaldehyde	22.15^{d}	0.04^{d}	1.72^{a}	10.56^{a}
Talaye	Control	27.47^{a}	0.07^{a}	1.58^{b}	8.93^{b}
	Toasting	25.10^{b}	0.06^{b}	1.65^{b}	9.15^{b}
	Autoclaving	23.53^{d}	0.04^{c}	1.59^{b}	8.47^{c}
	Formaldehyde	24.45^{c}	0.06^{b}	2.00^{a}	10.55^{a}
RGS003	Control	25.14^{a}	0.07^{a}	2.08^{c}	10.27^{c}
	Toasting	23.09^{b}	0.06^{b}	2.28^{b}	11.54^{a}
	Autoclaving	22.14^{c}	0.05^{c}	2.02^{c}	10.77^{b}
	Formaldehyde	21.18^{d}	0.04^{d}	2.59^{a}	11.65^{a}
SEM		0.103	0.002	0.043	0.124

A= potential gas production. (*c*)= rate constants. *L*= lag time.
Different letters within row indicates differences ($P < 0.01$). SEM= Standard Error Mean.

Conclusion

The result of the present study showed that different treatments of canola seed decreased solubility of protein, decreased A+ B_1 and increased B_2 fraction. Therefore, it can be concluded that treating canola seeds especially with formaldehyde can effectively increase RUP in ruminant diets.

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