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Changes in Anti-Nutritional Factors, Ruminal Degradability and *in vitro* Protein Digestibility of Gamma Irradiated Canola Meal

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Abstract: The aim of this study was to evaluate the effects of gamma irradiation (γ-irradiation) at doses of 15, 30 and 45 kGy on antinutritional factors, *in vitro* CP digestibility, ruminal Dry Matter (DM) and Crude Protein (CP) degradibility of Canola Meal (CM). Three ruminally fistulated Gezel rams were used to measure ruminal *in situ* Dry Matter (DM) and CP degradability. The chemical composition of raw and irradiated CM was similar. Results showed that phytic acid in γ-irradiated CM at dose of 15, 30 and 45 kGy γ-irradiated CS decreased ($p<0.01$) by 26.2, 51 and 56.7%, respectively. Total glucosinolate contents of 15, 30 and 45 kGy γ-irradiated CM was decreased ($p<0.01$) 26.3, 51 and 63.4%, respectively. From *in sacco* results, γ-irradiation decreased ($p<0.05$) the washout fractions of CP at doses of 30 and 45 kGy. Gamma irradiation also reduced ($p<0.05$) degradation rate of the b fraction of CP so at doses of 30 and 45 kGy, Effective Degradibility (ED) of CP (rumen outflow rate of 0.05 h^{-1}) were decreased by 8.2 and 21.0%, Compared to untreated sample. On the contrary digestibility of ruminally undegraded CP of irradiated CM at doses of 30 and 45 kGy was improved ($p<0.05$) by 10.6 and 15.5%.

Key words: Canola meal, gamma irradiation, protein degradibility, glucosinolate, phytic acid

INTRODUCTION

The growing demand by humans for monounsaturated vegetable oils has provided Canola Meal (CM) for use in dairy diets because it has an excellent balance of AA (Piepenbrink and Schingoethe, 1998). However, canola meal is not an effective source of AA for high-producing dairy cows because of its extensive degradation in the rumen, as indicated by previous research where, the effective ruminal degradabilities of the protein component of canola meal ranged from 44.3-74% (Wright *et al*., 2005). High-producing dairy cows require sufficient protein in the diet to optimize microbial growth and fiber digestion in the rumen and adequate amounts of essential AA to be available in the small intestine to provide for their increased metabolic and lactation demands (NRC, 2001; Cant *et al*., 2003). Then, it is essential that the diet contain slowly degraded proteins with a high potential for rumen escape. Meanwhile, the high ruminal degradability of CP has led researchers to process CM (Khorasani *et al*., 1993). Various physical and chemical treatments have been used to decrease its extent of ruminal degradation (Deacon *et al*., 1988; Khorasani *et al*., 1993; Wright *et al*., 2005). These processing methods may adversely affect the protein digestibility and lysine availability of the final product in the small intestine (Scott *et al*., 1991). On the other hand, it contains antinutritional factors such as phytic acid and glucosinolates, which are of concern. Glucosinolates are a large group of sulphur-containing secondary plant metabolites. Major glucosinolates in brassica are progoitrin, gluconapin and glucobrassicanapin (Tripathi and Mishra, 2007). They are known to reduce feed intake, induce iodine deficiency and depress fertility (Ahlin *et al*., 1994).

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Radiation processing is a process exposing a product or material to ionizing radiations such as gamma rays emitted from radioisotopes Cobalt-60 or Cesium-137, or, high energy electrons (electron beam) and X-rays produced by machine sources (Farkas, 2006). It has been recognized as a reliable and safe method for improving the nutritional value and inactivation or removal of certain antinutritional factors in foods and feeds (Siddhuraju *et al*., 2002). Shahbazi *et al*. (2008) concluded that electron beam irradiation of barley straw could improve its nutritional value for ruminants. Irradiation processing of soybean and canola meal at doses of 25, 50 and 75 kGy (Shawrang *et al*., 2007, 2008) showed, γ-irradiation at doses ≥25 kGy can be used as a cross-linking agent to improve protein properties of supplements in ruminant nutrition. Recently, Taghinejad *et al*. (2009) studied a lower range of γ-irradiation doses (15, 30 and 45 kGy) and reported that γ-irradiation at doses ≥15 kGy can reduce the CP degradibility of full-fat soybean in the rumen as well as reducing its phytic acid content and trypsin inhibitor activity.

Therefore, to elucidate some of the effects of radiation processing with gamma rays on chemical and antinutritional contents, DM and CP degradability parameters, *in vitro* CP digestibility of canola meal, we conducted this study.

MATERIALS AND METHODS

Sample preparation and irradiation treatments: The CM sample, which was grown in Iran, was obtained from Oilseed Developing and Cultivation Company (Tehran, Iran). Two kg CM was divided into 4 equal portions and placed in paper packages. Three paper packages of samples were irradiated in a gamma cell for total doses of 15, 30 and 45 kGy in the presence of air. One package (control) was left at a room temperature similar to the others. After completing the 45 kGy irradiation (final sample) and prior to sealing samples in plastic bags, all samples were spread in trays and allowed to air-equilibrate for 2 h. Gamma irradiation was carried out in the Radiation Applications Research School, Atomic Energy Organization of Iran by a Gammacell 220 research irradiator at room temperature. At the time of irradiation, the activity of the irradiator was 1460 Ci (5.4×1013 Bq) with the dose rate of 0.325 Gy s^{-1}. The samples were irradiated with >10% deviation relative to the mean dose.

Animals and diet: Three ruminally fistulated Gezel rams (48.3±1.7 kg) were used. The rams were fed with 1.5 kg of DM; a total mixed ration containing 600 g kg^{-1} of DM forage (80% alfalfa hay and 20% wheat straw) and 300 g kg^{-1} of DM concentrate. The concentrate consisted of ground barley, canola meal, dicalcium phosphate and a vitamin-mineral premix (750, 230, 10 and 20 g kg^{-1} DM, respectively). Water and salt lick were available *ad libitum*. Diet was formulated according to NRC (2001) and fed twice daily at 07:00 and 15:00 h.

***In situ* ruminal degradability:** Nylon bags (8×15 cm; 45 μm pore size) were filled with approximately 3 g sample (size: bag surface area of 12.5 mg cm^{-2}). The samples were ground to pass a 2 mm screen according to Nocek (1988). Bags were filled with untreated or irradiated CM and incubated in the rumen for 0, 2, 4, 8, 16, 24 and 48 h. All bags were simultaneously placed in the rumen, just before the rams were offered their second meals in the morning (i.e., 15:00 h). After retrieval from the rumen, bags were thoroughly washed with tap water until the rising water was clear. The same procedure was applied to 2 bags to obtain the 0 h value. The residues were dried and analyzed for DM and CP to determine degradation kinetics of CM.

***In vitro* crude protein digestibility:** Digestibility of rumen undegraded CP was estimated using the 3-steps *in vitro* procedure of Calsamiglia and Stern (1995). Samples of the ruminal undegradable fraction collected at the 16 h ruminal incubation period containing 15 mg Nitrogen (N) were incubated for 1 h in 10 mL of 0.1 N HCl solution containing 1 g L^{-1} of pepsin. Following incubation, pH was neutralized with 0.5 mL of 1 N NaOH and 13.5 mL of phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin were added. Samples were incubated for 24 h at 38°C and then undigested protein was precipitated using trichloroacetic acid (3 mL TCA). Afterward supernatant of centrifuged samples were collected and analyzed for soluble N. *In vitro* digestibility of protein was calculated as soluble N divided by amount of initial sample N (i.e. Nylon bag residues).

Chemical analyses: The DM content was determined in feed samples and nylon bag residues at 60°C for 48 h. The nitrogen in feeds and residues after rumen and *in vitro* incubation was determined according to AOAC (1995) (Method 984.13). Ash was determined by burning duplicate 2 g samples at 600°C, for 2 h in muffle furnace (Method 942.05; AOAC, 1995). Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed according to the method of Van Soest *et al*. (1991), using an automatic fiber analyzer (Fibertec System M, Tecator, Sweden). Standard methods were also used to determine ether extract (AOAC, 1995). Phytic acid was determined

by the methods of De Boland *et al.* (1975). Total glucosinolate, was determined by the methods of Wetter and Youngs (1976).

Statistical analyses: Digestion kinetics of DM or CP was determined according to the equation as:

$$P = a + b (1 - e^{-ct})$$

where:

P = The amount degraded at a time
a = Washout fraction
b = Potentially degradable fraction
c = The constant rate of disappearance of b
t = The time of incubation (h)

Effective Degradability (ED) was calculated using ED = a + bc/(c+k) at estimated outflow rates (k) of 0.02, 0.05 and 0.08 h^{-1}.

Degradability data were analyzed as completely randomized block design according to the general linear models procedure of SAS (1996) with the following statistical model of $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$. Chemical composition and antinutritional factors data were analyzed as a completely randomized design according to the general linear models procedure of SAS (1996) with the following statistical model of

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

where:

Y_{ijk} = Dependent variable
μ = Overall mean
T_i = γ-irradiation effect
B_j = Animal effect and
e_{ijk} = Residual error

Assumed normally and independently distributed. Means were compared by least squares means.

RESULTS AND DISCUSSION

Chemical composition and antinutritional contents of γ-irradiated CM: The chemical composition and antinutritional factors of CM are listed in Table 1. γ-irradiation had no effect on chemical composition of CM but phytic acid and total glucosinolate contents of CM were decreased ($p<0.01$) compared to untreated CM. The present findings are in agreement with the data previously obtained (Shawrang *et al.*, 2008; Taghinejad *et al.*, 2009).

The Phytic acid content of 15, 30 and 45 kGy γ-irradiated CS decreased by 26.2, 51 and 56.7%, respectively. In agreement with our findings, Al-Kaisey *et al.* (2003) reported that irradiation of broad bean at doses of 2.5, 5, 7.5 and 10 kGy could reduce the phytic acid content. Moreover, Taghinejad *et al.* (2009) reported that phytic acid of soybean seed was completely eliminated on exposure to doses of 15 and 30 kGy. Numerous studies have led to the conclusion that phytic acid can bind essential dietary minerals, thus making them unavailable or only partially available for absorption. It seems that irradiation at doses between 15-30 kGy is more effective in reducing the phytic acid content (Bhat *et al.*, 2007; Taghinejad *et al.*, 2009). Phytate chelate with certain metal ions such as calcium, magnesium, zinc, copper and iron, to form insoluble complexes that are not readily broken down and may pass through the digestive tract unchanged (Al-Kaiesy *et al.*, 2003). This reduction in phytic acid content is probably due to chemical degradation of phytate to the lower inositol phosphates and inositol by the action of free radicals produced by the radiation or cleavage of the phytate ring itself (Siddhuraju *et al.*, 2002).

Glucosinolate content of 15, 30 and 45 kGy γ-irradiated CS decreased by 26.3, 51 and 63.4%, respectively. Up to 30 kGy, γ-irradiation reduced the content of glucosinolate, but further irradiation dose had no effect. Similar to our results, Gharaghani *et al.* (2008) reported that γ-irradiation at doses of 10, 20 and 30 kGy deceased the gluosinolates content of rapeseed meal by 40, 70 and 89%, respectively, compared to the untreated sample. Although, the mechanism of glucosinolate reduction in CM by γ-irradiation has not been demonstrated, it seems that γ-irradiation causes glucosinolates destruction. Major harmful effects of glucosinolates ingestion in animals are: reduced palatability, decreased growth and production. Inclusion of low glucosinolate rapeseed meal at higher levels in dairy cows diet could depress fertility and induce thyroid disturbances (Ahlin *et al.*, 1994). Dietary glucosinolate level of 11 μmol g^{-1} should be safe, in dairy cattle feeding (Tripathi and Mishra, 2007). Howover, in a study by Mandiki *et al.* (2002) consuming diets containing 1.2-2.2 μmol g^{-1} decreased body weight in sheep. Results observed in this study suggest that γ-irradiated CM might be fed to ruminants in higher levels without glucosinolate harmful effects.

Ruminal degradation and *in vitro* digestibility of γ-irradiated CM: Ruminal degradability parameters of DM and CP and *in vitro* CP digestibility of untreated and γ-irradiated CM are shown in Table 2. Maximum potential degradability (a+b) of DM and CP were 873 and 975 g kg^{-1} for untreated CM, respectively, indicating CM to be highly degradable in the rumen. Gamma irradiation

Table 1: Chemical composition and anti-nutritional factors of untreated and irradiated canola meal (n = 3)

		γ-irradiated canola meal			
Parameters	Untreated canola meal	15 kGy	30 kGy	45 kGy	SEM
Dry matter (g kg^{-1})*	930.0	924.0	927.0	929.0	3.5
Crude protein (g kg^{-1})	348.0	352.0	350.0	347.0	7.2
Ash (g kg^{-1})	67.0	67.0	69.0	68.0	2.6
Neutral detergent fiber (g kg^{-1})	269.0	271.0	276.0	265.0	9.7
Acid detergent fiber (g kg^{-1})	176.0	178.0	173.0	181.0	7.2
Ether extract (g kg^{-1})	36.0	37.0	34.0	33.0	3.4
Phytic acid (mg 100 g^{-1})	41.6^{a}	30.7^{b}	20.4^{c}	18.0^{d}	1.1
Glucosinolate (μmol g^{-1})	18.6^{a}	13.7^{b}	10.3^{c}	7.8^{c}	0.9

*On dry matter basis; $^{a, b, c, d}$ Means in the same row with different letters differ ($p<0.01$)

Table 2: Rumen degradation parameters of dry matter and crude protein and *in vitro* crude protein digestibility of undegraded protein of untreated and irradiated soybean

		γ-irradiated canola meal			
Parameters	Untreated canola meal	15 kGy	30 kGy	45 kGy	SEM
Dry matter					
a (g kg^{-1})	274^{a}	265^{ab}	251^{ab}	224^{b}	14.0000
b (g kg^{-1})	597	613	630	659	20.3000
a+b (g kg^{-1})	873	878	881	883	13.4000
c (h)$^{-1}$	0.078^{a}	0.075^{a}	0.069^{ab}	0.058^{b}	0.0041
Effective rumen degradation (g kg^{-1})					
0.02 h^{-1}	753	748	740	714	12.3000
0.05 h^{-1}	641^{a}	632^{ab}	616^{ab}	578^{b}	13.0000
0.08 h^{-1}	572^{a}	561^{a}	543^{a}	501^{b}	11.8000
Crude protein					
a (g kg^{-1})	264^{a}	252^{a}	221^{b}	174^{c}	7.5000
b (g kg^{-1})	771^{c}	720^{bc}	755^{b}	804^{a}	12.2000
a+b (g kg^{-1})	975	981	976	970	9.2000
c (h)$^{-1}$	0.088^{a}	0.083^{a}	0.069^{b}	0.048^{c}	0.0035
Effective rumen degradation (g kg^{-1})					
0.02 h^{-1}	843^{a}	831^{a}	806^{a}	741^{b}	11.6000
0.05 h^{-1}	718^{a}	670^{ab}	659^{c}	567^{d}	12.5000
0.08 h^{-1}	637^{a}	617^{a}	571^{b}	475^{c}	5.4000
In vitro crude protein digestibility (g kg^{-1})	643^{c}	660^{bc}	711^{ab}	744^{a}	16.5000

$^{a, b, c, d}$Means in the same row with different letters differ ($p<0.05$); a: the washout fraction (g kg^{-1}); b: the potentially degradable fraction (g kg^{-1}); c: the rate of degradation

at dose of 30 and 45 kGy decreased ($p<0.05$) the washout fractions of CP (by 10.2 and 34.1%) of CM. It also increased potentially degradable fraction (b) of CP at dose of 45 kGy ($p<0.05$). Decrease in protein solubility of protein observed in the current study is in agreement with the result of Abu *et al.* (2006), who demonstrated that γ-irradiation decreases protein solubility due to denaturation, occurring cross-linking of chains and proteins aggregation. Gamma irradiation decreased degradation rate of the b fraction of DM (at dose of 45 kGy) and CP (at dose of 30 and 45 kGy) compared to untreated CM ($p<0.05$). At irradiation doses of 30 and 45 kGy, Effective Degradibility (ED) of CP at rumen outflow rate of 0.05 h^{-1} were decreased by 8.2 and 21.0%, Compared to untreated sample. Irradiation decreased the effective protein degradability of CM, which is in agreement with the report of Shawrang *et al.* (2008), which γ-irradiation of canola meal at doses of 25, 50 and 75 kGy, decreased the effective protein degradability of canola meal at ruminal outflow rate of 0.05 h^{-1} by 19, 27 and 32%, respectively. Decreasing protein degradability as a result of irradiation is due to the occurrence of cross-linking of chains, denaturation and proteins aggregation (Gaber, 2005; Abu *et al.*, 2006). Irradiation induces the unfolding of the protein structures and denaturation, thus, increase surface hydrophobicity of proteins by exposing non polar groups (Gaber, 2005). Since most bacteria, which involved in degradation of protein in the rumen have proteases that are associated with the cell surface and adsorption of soluble proteins to bacteria is essential for protein degradation (Kopecny and Wallace, 1982). Consequently γ-irradiation may have reduced the protein degradability of soybean protein by reducing its solubility.

Gamma irradiation increased *in vitro* digestibility of CP at doses of 30 and 45 by 10.6 and 15.5%, ($p<0.05$). The CP digestibility value of untreated CM in this study (643 g kg^{-1}) was lower than value of 680 g kg^{-1} that was reported by Shawrang *et al.* (2008) for intestinal CP digestibility of canola meal that γ-irradiation at doses of 25, 50 and 75 kGy, increased intestinal CP digestibility of canola meal by 4, 13 and 20%, respectively. The

Discrepancies in reported CP digestibility values may be due to differences in methods of estimating the CP digestibility and variety of seed. Gamma irradiation may be induced, the unfolding of the protein and denaturation, exposing hydrophobic amino acids (especially aromatics) that are position groups for active site of pepsin and trypsin enzymes (Murray *et al*., 2006; Abu *et al*., 2006). Moreover, with modification in secondary and tertiary structures of protein by γ-irradiation, more peptide bonds are exposed to proteolytic enzymes (Fombang *et al*., 2005).

CONCLUSION

The results suggest that γ-irradiation of CM at doses ≥30 kGy can decreas its ruminal protein degradability and increasing of intestinal CP digestibility. In addition, results indicate that γ-irradiation decreased the phytic acid and glucosinolate content of CM at doses higher than 15 kGy. Further investigation is needed to elucidate the economical benefits of this processing.

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