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Improving the Nutritional Value of Sunflower Meal by Electron Beam and Gamma Ray Irradiations

Research Article

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ABSTRACT

This research was performed to compare electron beam (EB) and gamma ray (GR) treatments at doses of 25, 50 and 75 kilo Gray (kGy) on ruminal degradation kinetics and *in vitro* digestibility of sunflower meal (SFM). Ionizing radiations of EB and GR had significant effects (P<0.05) on dry matter (DM), crude protein (CP) and amino acid (AA) degradability parameters of SFM. Effective ruminal degradability (ERD) of DM was lower in EB and GR irradiated SFM than in unirradiated SFM (P<0.05). GR treatment at a dose of 75 kGy decreased ERD of CP compared to control (P<0.05). ERD of CP was not affected by EB (P>0.05). Irradiation processing caused a decrease in AA degradation after 16 h of ruminal incubation significantly (P<0.05). GR irradiation was more effective than EB irradiation in lessening the ruminal degradability of AA. *In vitro* CP digestibility of EB and GR irradiated SFM was improved (P<0.05). This study, based on *in situ* and *in vitro* measures, showed that EB and GR processing can be used as an efficient method in improving nutritional value of SFM.

KEY WORDS degradation kinetics, digestibility, electron beam, gamma ray, sunflower meal.

INTRODUCTION

Sunflower meal (SFM), a by-product obtained from sunflower oil extraction, is used as a protein source in animal nutrition. However, its use in ruminant nutrition is often limited by high protein degradability in the rumen (Molina Alcaide *et al*. 2003). The high degradation of protein in the rumen leads to the loss of its quality indexes such as amino acid (AA) balance and digestibility (Yoruk *et al*. 2006; Salamatdoust-Nobar *et al*. 2009). Different chemical and physical processing methods have been used to reduce the protein degradation in the rumen (NRC, 2001; Tuncer and Sacakli, 2003). Chemical processing can cause environmental pollution and in some cases it has negative effects on animal products (MoshtaghiNia and Ingals, 1995; Forooshani, 2010). Utilization of alcohol, mineral and organic acids, tannin, formaldehyde and xylose can be mentioned as chemical methods (Tuncer and Sacakli, 2003). One of the disadvantages of these methods is the appearance of chemical materials, like formaldehyde in the milk (MoshtaghiNia and Ingals, 1995). The most popular physical processing method of protein resources is heating (NRC, 2001). Heating decreases protein degradability as it denatures proteins or forms protein-carbohydrate and protein-protein cross-links (Maillard reaction). However excessive heating causes amino acid (AA) destruction. Irra-

diation is another physical processing method that is considered in feedstuffs processing because of its effectiveness without any side effects on environment (Al-Masri, 1999). Feed irradiation includes a controlled use of energy from ionizing radiations such as gamma ray (GR) and electron beam (EB). EB and GR have been known to show a similar effect on materials, but they have differences regarding the penetration and method of their use (Choi *et al*. 2009). Feed processing by irradiation has high potential for substitution by other popular methods and in future, it will be used in greater extents (Mani and Chandra, 2003).

In addition to that, irradiation increases feed shelf life, it also causes physicochemical changes in feed that may affect feed availability (Mani and Chandra, 2003; Song *et al*. 2009). Advantages of irradiation compared to the other above mentioned methods are fewer damages to the nutrients especially proteins, non formation of indigestible products, elimination of microbial and fungal contaminations from feed and no residual effects after irradiation (Shawrang, 2006). The chemical changes that irradiation causes in biopolymers, such as proteins, include fragmentation, cross-linking, aggregation and oxidation by oxygen radicals generated in the radiolysis of water (Lacroix *et al*. 2002; Gaber, 2005; Lee *et al*. 2005). Recently, studies have been completed using GR and EB to process protein sources of ruminant feeds (Shawrang, 2006; Shawrang *et al*. 2007; Shawrang *et al*. 2008; Ebrahimi *et al*. 2009; Taghinejad-Roudbaneh *et al*. 2010; Ghanbari *et al*. 2012). However, there is lack of study on the effects of EB and GR irradiation on SFM protein degradability. The purpose of present study was to evaluate and compare the effects of EB and GR irradiations at doses of 25, 50 and 75 kilo Gray (kGy) on ruminal dry matter (DM), crude protein (CP) and AA degradation kinetics as well as *in vitro* digestibility of SFM.

MATERIALS AND METHODS

Sample preparation

The used SFM in this study was provided from Golestan Union of Cattle and Horse Producers, Gorgan, Iran.

Irradiation processing

Irradiation of samples was done in Radiation Applications Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran.

Gamma irradiation

GR irradiation was accomplished in a cobalt-60 irradiator with 3.7 PBq (100 kCi) activity at 20 ˚C. The dose rate appointed by Fricke dosimetry was 3.7 kGy/h (Holm and Berry, 1970). Three polyethylene packages of SFM samples were irradiated in a gamma cell (Co-60). The doses contained 25, 50 and 75 kGy in the presence of air. Samples were freeze-dried after finishing the irradiation and subsequently allowed to air equilibrate for 2 h before being fastened in plastic bags.

Electron beam irradiation

The SFM samples were packed in polyethylene bags and were subjected to 10 MeV electron beam of a Rhodotron accelerator model TT-200 (IBA co., Belgium) at different doses (25, 50 and 75 kGy). All irradiations were carried out at room temperature in air, with 4 mA beam of 10 MeV electrons. Since the thickness of sample packages was slender, a single sided irradiation was used. The required doses were delivered to the samples by adjusting the conveyer speed when each of the sample batches was passed under the beam.

For measuring the doses delivered to the sample packages, cellulose three acetate (CTA) thin film dosimeters were used, which showed conformity with the relevant desired doses within 7%.

Animals and diet

Three rumen fistulated Taleshi bulls (350±10 kg) were used for *in situ* and *in vitro* trial. They were housed in individual 2 × 3 m pens in Karaj Animal Science Research Institute. Animals were fed a total mixed ration (TMR) according to the nylon bag standard techniques at the maintenance level. The diet was formulated on the basis of 60% forage and 40% concentrate. Bulls were fed twice daily in equal meals at 08:30 h and 16:30 h. Two weeks were assigned for adaptation of animals with the diet.

In situ trial

Samples were ground by laboratory hammer mill with a 2 mm screen. Then, approximately 5 g of samples were put in polyester bags (10 cm×21 cm; 45 µm pore size). Two bags were prepared for each sample at each incubation time per bull.

Ruminal incubation times of nylon bags containing the samples were 2, 4, 8, 16, 24, 48 and 72 h. All bags were inserted in the rumen at the same time just before the morning feeding.

At the end of each incubation time, bags containing residual materials were removed from rumen by fistula and then immediately washed by cold water to arrest fermentation and remove debris from outside of the bags. This was done until the rinse water was clear. Then the bags were placed in a washing machine with cold water for 30 min. Washed bags were dried in forced-air oven at 60 ˚C for 48 h and then weighed. The residues were analyzed for DM, AA and CP to determine degradation kinetics of SFM.

***In vitro* digestibility of SFM**

The two-step digestion technique (Tilly and Terry, 1963) was used to determine digestibility of SFM.

Preparing samples

All feeds were ground in a mill to pass a 1 mm pore-size screen and then each sample (15 g) was dried at 65 ˚C for 48 h in an oven. Each sample (500 mg) was weighed accurately and was placed into a 100 mL erlen. Each sample was considered in three replicates and three erlens as Blanks.

Preparing rumen liquor

Rumen liquor was collected before the morning meal from the rumen by vacuum pump (60 mL syringes). The liquor from each animal was filtered through eight layers of gauze cloth, was purged with CO_2 and was kept in a pre-warmed (39 ˚C) thermos flask until use (within approximately 20 min).

Preparing artificial saliva

To provide artificial saliva, mineral salts as Na_2CO_3, 9.8 g/L, $Na_2H_2PO_4$, 1.0 g/L, KCl, 0.57 g/L; NaCl, 0.47 g/L and 0.12 g/L of $MgSO_4$ were combined and then diluted to 40-mL volume with distilled water.

Fermentation process

Thirty min before doing fermentation process, KCl solution (1 mL of 4% solution v/w) was added to artificial saliva and continued with purging CO_2 to the buffer (10-15 min) to reduce pH lower than 7. The incubation inoculums were prepared by diluting the rumen liquor with the buffer in a 1:4 (v/v) ratio and were put in a water bath (39 ˚C) and were purged with CO_2 for 4-5 min.

Two mL distilled water was added to each erlen containing the samples, then 50 mL of prepared solution of mixed rumen liquor and buffer was added to either blank and other erlen was purged with CO_2 for 15 sec and finally all erlens tips were closed. All samples were kept in warm bath water (39 ˚C) for 48 h and hand swirled 3-4 times during incubation.

HCl pepsin digestion

At the end of 48 h incubation period, erlen contents were centrifuged at 2500 g for 15 min and the supernatant was discarded.

The residual content was acidified by adding 6 mL of hydrochloric acid (HCl, 20%) during 3 steps. After all, 2 mL of pepsin solution was added to each erlen. Then all samples were kept in water bath (39 ˚C) for 46 h and were swirled infrequently like previous step. At the end of this stage, all samples were filtered through standard filter paper (Watman no. 41) that had previously being marked and numbered. The residual contents and filter papers were dried in forced air oven at 105 ˚C for 24 h and the DM was determined. To determine the ash content, all samples were burned at 560 ˚C for 4 h to calculate digestible organic matter (OMD) in DM.

Chemical analysis

The SFM samples were ground in a laboratory hammer mill equipped with a 1 mm sieve. Samples were assayed in duplicate according to AOAC (1995) for DM (method 930.15), CP (method, 984.13) contents. Total AAs (peptide bound and free) concentrations of samples were determined at MTT Agrifood Research, Finland with Mass Trak UPLC according to some studies.

Calculations and statistical analysis

Degradability amount of DM, CP and AA at incubation times in the rumen was calculated as the difference between the feed and the portion remained after incubation in the rumen.

The DM and CP degradability parameters of unirradiated and irradiated SFM were estimated using Fit Curve software. The exponential model of Orskov and McDonald (1979) was used for fitting DM and CP degradability data:

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

Where:
P: DM or CP degradability at time t (h).
a: washout (soluble) fraction.
b: potentially degradable fraction.
c: degradation rate (h^{-1}) of b fraction.

By using the fractional outflow rate from the rumen, k, the effective ruminal degradability (ERD) of DM and CP was calculated as:

$$ERD= a + ((a\times c)/(c+k))$$

Where:
Estimated solid outflow rates (k) of 0.02, 0.05 and 0.08 h^{-1} were used (Tuncer and Sacakli, 2003).

In situ and *in vitro* data were analyzed using General Linear Model (GLM) procedure of SAS (2003). Differences among the means were tested using Duncan's multiple range test, at a significant level of $P < 0.05$.

The analysis of simple linear regression was used to define the relationship between the dosage of ionizing radiations and effective ruminal degradability of dry matter and crude protein of sunflower meal.

RESULTS AND DISCUSSION

ERD and degradation kinetics of DM, CP and AA in unirradiated (control) and irradiated SFM

Ruminal degradation kinetics of DM was affected (P<0.05) by ionizing radiations (Table 1). Irradiation of SFM decreased washout fraction and increased potentially degradable fraction of DM (P<0.05). However, degradation rate of b fraction was unaffected by irradiation processing (P>0.05). GR irradiation at doses of 25, 50 and 75 kGy decreased washout fraction of DM by 28.29%, 37.93% and 38.78% respectively, compared to unirradiated SFM. The EB treatment at the same doses decreased this trait by 15.89%, 19.23% and 26.69% compared to untreated SFM. Ionizing radiations of GR and EB at doses of 25, 50 and 75 kGy increased potentially degradable fraction of DM compared to control by 15.64%, 6.13%; 22.17%, 16.09%; and 24.56%, 9.22% respectively. Ruminal degradability parameters of CP are presented in Table 2. Compared to the control, irradiation of SFM caused a reduction in washout fraction and increased potentially degradable fraction of CP (P<0.05). Nonetheless, potential degradability (a+b) and degradation rate of b fraction were unaffected by irradiation (P>0.05). The ERD of CP in irradiated SFM at rumen outflow rates of 0.02, 0.05 and 0.08 h^{-1} was decreased in comparison to control group (P<0.05). Effects of GR and EB on washout and potentially degradable fractions of CP were similar. However, Compared to EB, GR decreased ERD of CP (P<0.05). Washout (soluble) fraction of CP in GR-irradiated SFM at doses of 25 and 75 kGy decreased by 16.08% and 37% respectively, compared to unirradiated SFM. It was decreased by18.32% and 20.92% at doses of 50 and 75 kGy in EB-irradiated SFM, compared to untreated SFM. Overally, maximum reduction in washout fraction of CP was observed in GR treatment at a dose of 75 kGy. Irradiation of SFM with GR and EB at a dose of 75 kGy, increased potentially degradable fraction of CP compared to control by 13.93% and 10.57% respectively. Treatment of SFM by GR at a dose of 75 kGy caused a reduction in degradation rate of b fraction of CP by 21% compared to untreated SFM. Decrease in washout (soluble) fraction is because of reduction in protein availability for rumen microbes and it could be due to change in protein structure caused by irradiation (Shawrang, 2006).

Ionizing radiation, through the production of free radicals, can affect proteins by promoting reactions such as protein–protein association, deamination, and cleavage of peptide and disulfide bonds (Abu *et al*. 2006). Oxygen radicals that are generated during iradiation processing can cleave disulfide bonds and other cross-links involved in protein secondary and tertiary structures, leading to denaturation and fragmentation of proteins (Taghinejad-Roudbaneh *et al*. 2010). High doses of irradiation may result in cross-linking, aggregation and formation of high molecular weight proteins (Cies´la *et al*. 2000; Lee *et al*. 2005). It can also decrease protein solubility due to denaturation, cross-linking of chains and protein aggregation (Lacroix *et al*. 2002).

Processing of SFM with GR at doses of 25, 50 and 75 kGy significantly decreased ERD of DM at rumen outflow rate of 0.05 h^{-1} by 5.11%, 6.61% and 15.79% respectively, compared to control. Whereas, EB irradiation at doses of 25 and 75 kGy decreased the trait mentioned above by 4.17% and 7.51% respectively, compared to unirradiated SFM (Table 1).

Irradiation of SFM with GR at the dose of 75 kGy decreased significantly ERD of CP at rumen outflow rate of 0.02, 0.05 and 0.08 h^{-1} by 12.68%, 21% and 26.10% respectively, compared to unirradiated SFM. However, ERD of CP was not affected by EB (Table 2).

The relationships between ERD of DM and CP of SFM and irradiation dose respectively are illustrated in Figures 1 and 2. Regression analysis demonstrated that there is not any relationship between ERD of DM and CP of SFM and EB dose (P>0.05). However by increasing irradiation dose of GR, the ERD of DM and CP decreased linearly (P<0.01). Accordingly, increase for each kGy of irradiation dose of GR, resulted to 11.7% and 19.9% decrease in ERD of DM and CP.

Table 3 shows the results of AA degradation after 16 h of ruminal incubation. EB, as well as GR treatments decreased AA degradability in the rumen. However the effectiveness of GR to decrease ruminal degradability of AA was greater than EB. The greatest reduction was seen for AAs which were irradiated by GR in 75 kGy dose. No reference was found in the literature dealing with the effects of ionizing radiations on ruminal degradation kinetics of SFM. However, decreased ruminal degradability of some protein supplements has been reported (Shawrang, 2006; Shawrang *et al*. 2007; Shawrang *et al*. 2008; Ebrahimi *et al*. 2009; Taghinejad-Roudbaneh *et al*. 2010; Ghanbari *et al*. 2012). Irradiation causes the unfolding of the protein structures and denaturation, thus increasing surface hydrophobicity of proteins by exposing non-polar groups (Gaber, 2005). It is possible that, under these conditions, the unfolded proteins formed cross links and / or aggregates that were less susceptible to enzyme hydrolysis, since most bacteria involved in degradation of protein in the rumen have proteases that are associated with the cell surface and absorption of soluble proteins to bacteria is essential for protein degradation (Taghinejad-Roudbaneh *et al*. 2010). Gaber (2005) found that hydroxyl and superoxide anion radicals that are generated by radiation could modify the molecular properties by cross linking and aggregation of polypepdie chains.

Table 1 Effects of irradiation processing of sunflower meal on ruminal degradation characteristics of dry matter

	Parameters				ERD of DM		
	a (%)	b (%)	a + b (%)	c (h^{-1})	0.02 (h^{-1})	0.05 (h^{-1})	0.08 (h^{-1})
Unirradiated	32.03^{a}	37.22^{d}	69.25^{a}	0.161^{b}	64.67^{b}	59.93^{b}	56.37^{b}
GR-irradiated (25 kGy)	22.97^{d}	43.04^{b}	66.01^{b}	0.181^{b}	61.90^{cd}	56.87^{cd}	52.97^{cd}
GR-irradiated (50 kGy)	19.88^{e}	45.47^{a}	65.35^{bc}	0.195^{b}	61.03^{de}	55.97^{de}	52.03^{d}
GR-irradiated (75 kGy)	19.61^{e}	46.36^{a}	65.97^{b}	0.100^{c}	58.17^{f}	50.47^{f}	45.30^{e}
EB-irradiated (25 kGy)	26.94^{b}	39.50^{c}	66.44^{b}	0.170^{b}	62.27^{c}	57.43^{c}	53.77^{c}
EB-irradiated (50 kGy)	25.87^{c}	43.21^{b}	69.08^{a}	0.245^{a}	65.93^{a}	61.83^{a}	58.50^{a}
EB-irradiated (75 kGy)	23.48^{d}	40.65^{c}	64.13^{c}	0.185^{b}	60.17^{e}	55.43^{e}	51.80^{d}
SEM	0.341	0.506	0.441	0.010	0.329	0.365	0.408
Orthogonal contrasts							
Unirradiated *vs.* irradiated	< 0.0001	< 0.0001	< 0.0001	0.1613	< 0.0001	< 0.0001	< 0.0001
Unirradiated *vs.* EB	< 0.0001	< 0.0001	0.0002	0.0119	0.0004	0.0035	0.0112
Unirradiated *vs.* GR	< 0.0001	< 0.0001	< 0.0001	0.9046	< 0.0001	< 0.0001	< 0.0001
EB *vs.* GR	< 0.0001	< 0.0001	0.0597	0.0008	< 0.0001	< 0.0001	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
ERD: effective ruminal degradability; DM: dry matter; GR: gamma ray and EB: electron beam.
SEM: standard error of mean.

Table 2 Effects of irradiation processing of sunflower meal on ruminal degradation characteristics of crude protein

	Parameters				ERD of CP		
	a (%)	b (%)	a + b (%)	c (h^{-1})	0.02 (h^{-1})	0.05 (h^{-1})	0.08 (h^{-1})
Unirradiated	31.16^{a}	58.67^{c}	89.83^{a}	0.207^{a}	84.40^{a}	78.10^{a}	73.17^{a}
GR-irradiated (25 kGy)	26.15^{b}	62.53^{abc}	88.68^{a}	0.243^{a}	83.90^{a}	77.97^{a}	73.13^{a}
GR-irradiated (50 kGy)	28.05^{ab}	60.71^{bc}	88.75^{a}	0.223^{a}	83.33^{a}	77.20^{a}	72.27^{a}
GR-irradiated (75 kGy)	19.63^{c}	66.84^{a}	86.46^{b}	0.085^{b}	73.70^{b}	61.70^{b}	54.07^{b}
EB-irradiated (25 kGy)	28.84^{ab}	59.54^{c}	88.37^{a}	0.208^{a}	83.13^{a}	76.80^{a}	71.77^{a}
EB-irradiated (50 kGy)	25.45^{b}	63.21^{abc}	88.66^{a}	0.235^{a}	83.63^{a}	77.47^{a}	72.50^{a}
EB-irradiated (75 kGy)	24.64^{b}	64.87^{ab}	89.51^{a}	0.219^{a}	83.93^{a}	77.33^{a}	72.00^{a}
SEM	1.360	1.477	0.572	0.012	0.438	0.422	0.463
Orthogonal contrasts							
Unirradiated *vs.* irradiated	0.0056	0.0397	0.0519	0.7325	< 0.0002	< 0.0001	< 0.0001
Unirradiated *vs.* EB	0.0211	0.0758	0.1924	0.3464	0.1414	0.0947	0.0664
Unirradiated *vs.* GR	0.0035	0.0357	0.0208	0.1261	< 0.0001	< 0.0001	< 0.0001
EB *vs.* GR	0.2175	0.5743	0.1026	0.0025	< 0.0001	< 0.0001	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
ERD: effective ruminal degradability; CP: crude protein; GR: gamma ray and EB: electron beam.
SEM: standard error of mean.

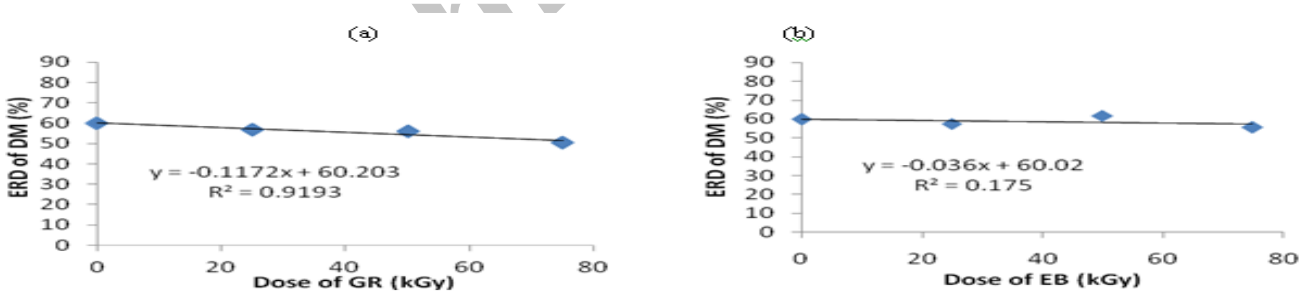

Figure 1 Relationship and linear regression equation between ionizing radiations dose and effective ruminal degradability (ERD) of dry matter (DM) (a): gamma ray (GR) and (b): electron beam (EB)

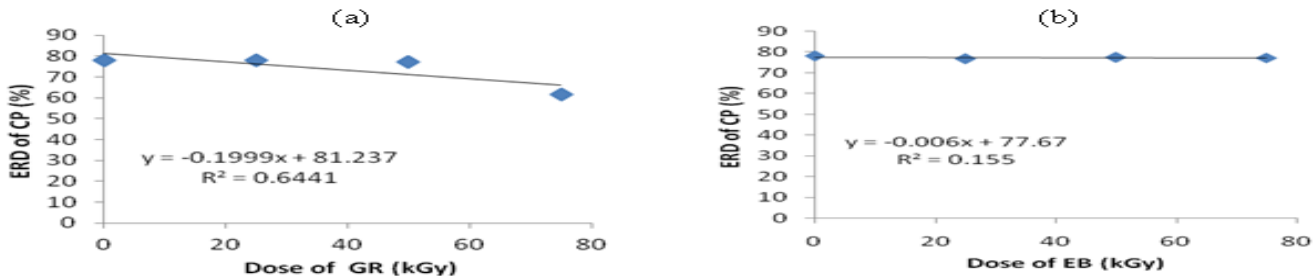

Figure 2 Relationship and linear regression equation between ionizing radiations dose and effective ruminal degradability (ERD) of crude protein (CP) (a): gamma ray (GR) and (b): electron beam (EB)

Table 3 Amino acid degradation (%) of unirradiated and irradiated sunflower meal after 16 h of ruminal incubation

	EB-irradiated (kGy)				GR-irradiated (kGy)				Orthogonal contrasts			
	Unirradiated	25	50	75	25	50	75	SEM	Unirradiated *vs.* irradiated	Unirradiated *vs.* EB	Unirradiated *vs.* GR	EB *vs.* GR
Essential amino acid												
His	92.49^{a}	90.41^{a}	90.80^{a}	84.39^{b}	89.11^{a}	91.10^{a}	73.41^{c}	1.25	0.0031	0.0287	0.0009	0.0057
Ile	89.10^{a}	88.94^{a}	88.02^{a}	81.33^{b}	87.17^{a}	87.32^{a}	71.77^{c}	0.72	0.0003	0.0084	< 0.0001	0.0002
Leu	89.22^{a}	88.91^{a}	86.98^{a}	80.86^{b}	86.82^{a}	86.61^{a}	71.23^{c}	1.12	0.0023	0.0263	0.0006	0.0032
Lys	88.24^{a}	88.25^{a}	87.53^{a}	80.33^{b}	85.98^{a}	86.45^{a}	75.24^{c}	0.97	0.0045	0.0365	0.0014	0.0091
Met	88.24^{a}	87.57^{a}	87.14^{a}	87.45^{b}	85.00^{a}	86.19^{a}	70.35^{c}	1.00	0.0010	0.0124	0.0003	0.0021
Phe	89.78^{a}	88.95^{a}	85.80^{ab}	80.97^{b}	86.98^{ab}	85.16^{ab}	70.91^{c}	2.08	0.0211	0.1008	0.0082	0.0417
Thr	87.25^{a}	86.01^{ab}	86.34^{ab}	77.97^{c}	83.81^{b}	85.24^{ab}	68.67^{d}	0.91	0.0005	0.0084	0.0001	0.0008
Val	88.77^{a}	87.84^{a}	86.64^{a}	79.46^{b}	86.37^{a}	86.26^{a}	70.44^{c}	1.13	0.0018	0.0158	0.0006	0.0056
Ala	87.94^{a}	86.76^{a}	86.73^{a}	76.32^{b}	85.61^{a}	84.87^{a}	72.31^{b}	1.91	0.0253	0.0718	0.0155	0.1771
Nonessential amino acid												
Arg	95.22^{a}	93.74^{a}	92.98^{a}	85.61^{bc}	93.56^{a}	92.22^{ab}	83.45^{c}	2.05	0.0604	0.1031	0.0542	0.5567
Asn	93.82^{a}	87.21^{ab}	85.62^{ab}	84.18^{b}	85.39^{ab}	83.83^{b}	72.49^{c}	2.48	0.0052	0.0249	0.0024	0.0400
Gly	85.26^{a}	82.68^{a}	83.57^{a}	77.40^{b}	81.14^{ab}	82.32^{ab}	71.35^{c}	1.44	0.0109	0.0611	0.0040	0.0271
Pro	89.89^{a}	87.44^{ab}	86.43^{ab}	81.18^{b}	85.62^{ab}	85.10^{ab}	72.16^{c}	2.29	0.0271	0.1087	0.0119	0.0669
Ser	88.58^{a}	84.44^{a}	87.01^{a}	76.93^{b}	84.09^{a}	86.38^{a}	70.20^{c}	1.35	0.0019	0.0075	0.0010	0.0523
Tyr	87.91^{a}	86.59^{a}	85.06^{a}	80.06^{b}	84.77^{a}	85.02^{a}	68.80^{c}	1.28	0.0029	0.0301	0.0008	0.0041
Glu	94.30^{a}	93.40^{a}	93.13^{a}	85.86^{b}	92.26^{a}	90.37^{ab}	78.57^{c}	1.70	0.0223	0.1179	0.0078	0.0312
Orn	90.25^{a}	87.00^{a}	85.90^{a}	73.50^{b}	86.30^{a}	85.86^{a}	61.31^{c}	1.64	0.0007	0.0037	0.0003	0.0148

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
EB: electron beam and GR: gamma ray.
SEM: standard error of mean.

Cross linking results in formation of chemical bonds between two adjacent protein molecules. Protein-protein interaction increases because the electrostatic forces of the molecules are minimized and less water interacts with the protein. This is favorable condition for protein molecules to approach each other and possibly precipitate (Ebrahimi *et al*. 2009).

In vitro digestibility of unirradiated and irradiated SFM

In vitro digestibility of SFM was affected (P<0.05) by ionizing radiations (Table 4).

Table 4 *In vitro* digestibility of unirradiated and irradiated sunflower meal

	DMD (%)	OMD (%)	DOMD (%)
Unirradiated	50.92^{b}	47.95^{b}	44.96^{b}
GR-irradiated (25 kGy)	57.96^{a}	54.95^{a}	50.88^{a}
GR-irradiated (50 kGy)	57.34^{a}	54.80^{a}	51.38^{a}
GR-irradiated (75 kGy)	58.71^{a}	56.52^{a}	53.13^{a}
EB-irradiated (25 kGy)	55.71^{a}	52.99^{a}	49.85^{a}
EB-irradiated (50 kGy)	57.88^{a}	55.70^{a}	52.43^{a}
EB-irradiated (75 kGy)	58.13^{a}	55.74^{a}	52.45^{a}
SEM	1.443	1.492	1.402

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
DMD: digestible dry matter; OMD: digestible organic matter; DOMD: digestible organic matter in dry matter; GR: gamma ray and EB: electron beam.
SEM: standard error of mean.

Compared to control group, irradiation of SFM with EB and GR at doses of 25, 50 and 75 kGy increased *in vitro* values of digestible dry matter (DMD), digestible organic matter (OMD) and digestible organic matter in dry matter (DOMD). The highest values of the mentioned traits were observed at a dose of 75 kGy of EB and GR (58.13% and 58.71%; 55.74% and 56.52%; 52.45% and 53.13% respectively).

There is a lack of evidence concerning the effects of ionizing irradiation on digestibility of SFM. In some cases, increased susceptibility of irradiated protein supplements to enzyme hydrolysis has been observed (Fombang *et al*. 2005). Increased *in vitro* digestibility of CP after ionizing irradiation of legumes, cereals and oilseeds meal has been reported (Mostafa, 1987; Shawrang, 2006; Shawrang *et al*. 2007; Bhat *et al*. 2008; Shawrang *et al*. 2008; Taghinejad-Roudbaneh, 2008; Ebrahimi *et al*. 2009; Forooshani, 2010; Taghinejad-Roudbaneh *et al*. 2010; Ghanbari *et al*. 2012). Some authors demonstrated that ionizing irradiation might cause unfolding of proteins, and its denaturation, thereby exposing hydrophobic AAs (especially aromatics) that are position groups for active sites of pepsin and trypsin enzymes (Murray *et al*. 2003; Abu *et al*. 2006). In addition, the secondary and tertiary structures of protein will be modified via irradiation, which causes more peptide bonds be exposed to proteolytic enzymes (Fombang *et al*. 2005).

CONCLUSION

The results of the present study indicated that irradiation of SFM by EB and GR successfully decreased ruminal degradation of SFM. Treatment of SFM by GR had a greater potential than EB treatment to decrease ruminal degradability of DM, CP and AA.

Ionizing radiations of EB and GR increased *in vitro* CP digestibility of SFM. Accordingly, irradiation processing can be used as an efficient method in improving nutritional value of SFM. Subsequent *in vivo* studies are required to determine the effects of ionizing radiations, used for processing feedstuff, on fattening and lactation performances of the animals.

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