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Effect of Gamma Ray, Infrared Irradiation, and Roasting on Amino Acids Profile, Ruminal Degradation Kinetics of Linseed Meal

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ABSTRACT

This study was conducted to investigate the effect of 20 and 40 kGy doses of Gamma-Ray (GR), 90 and 120-second- Infrared Irradiation (IR), as well as 15 and 30 minute-Roasting (R) at 140°C on Amino Acid (AA) profile, AA degradation, ruminal Dry Matter (DM), and Crude Protein (CP) degradation kinetics and *in vitro* digestibility of Linseed Meal (LSM). The results indicated that while the AAs contents of untreated LSM were relatively higher, the disappearances of AA were decreased by GR and IR after 16 hours of incubation in the rumen (P< 0.01). Moreover, irradiation decreased the water-soluble fraction and increased the potentially degradable fraction of DM and CP (P< 0.01). On the other hand, GR treatments decreased the Effective Ruminal Degradability (ERD) of DM and CP at ruminal outflow rates of 0.05 and 0.08 h-1 (P< 0.01). The digestible undegradable protein and the metabolizable protein of GR and IR at three outflow rates (0.02, 0.05 and 0.08 h^{-1}) were significantly higher than the roasted treatment and the control group (P< 0.01). Metabolizable Protein (MP) of IR did not have a significant difference with the control group in the outflow rate of 0.02, but there was significant increase in outflow rates of 0.05 and 0.08 h-1 (P< 0.01).

Keywords: Flaxseed, *In vitro* digestibility, Ruminant nutrition.

INTRODUCTION

Feeding lactating cows with protein is mainly carried out to increase the efficiency of using nitrogen sources for production purposes (NRC, 2001) and effective production of milk-extracted protein requires the supply of appropriate amounts and proportions of essential Amino Acids (AAs) to maximize protein production and reduce wastage (Doepel *et al*., 2016). The multivariate analysis performed on the examination of AAs passage to the small intestine showed that the concentration of each AA in the Rumen Undegradable Protein (RUP) and the relative proportion of RUP to the total passage of protein to the duodenum caused most of the changes made in the composition of the duodenal protein fits AAs (NRC, 2001). Moreover, current evaluation systems used for measuring the ruminant's protein levels are based on the information provided on the extent of AA supply for the animals. As a by-product of linseed (flaxseed) plant with an excellent balance of AAs (NRC, 2001), Linseed meal (IFN 5-30-287) is obtained by extracting the oil from the seed, being increasingly used for ruminant nutrition due to its high energizing contents (13.3 MJ digestible energy) and great level of Crude Protein (CP) (32- 37%, on DM basis). On the other hand, irradiation and heat treatments are the two physical methods used. Denaturing proteins and forming protein carbohydrate and protein cross-links (NRC 2001), heat treatment of substrates can help decrease the extent of soluble and degradable proteins and increase the amount of rumen's undegradable protein (Chrenkova *et al*., 2018). However, as a non-

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ionizing method, infrared radiation or micronization lacks sufficient energy to remove electrons from atoms, and it moves the atoms around at molecule level or causes them to vibrate instead. Infrared irradiation is a kind of dry heating that requires a short time and high temperature (Doymaz, 2014). The degradability of ruminal CP of sunflower meal (Ghanbari *et al*., 2015), cottonseed meal (Ghanbari *et al*., 2012), and canola meal was investigated by gamma irradiations (Shawrang *et al*., 2008) and barley grains by IR (Fattah *et al*., 2013). While the AAs degradability of the rumen is one of the most important factors in ruminant's protein evaluation systems (NRC, 2001), the influence of the gamma ray, infrared irradiation, and roasting on protein and AAs degradability of LSM has been under-research.

Therefore, this study was conducted to investigate the influence of 15 and 30 minutes-roasting at 140˚C, 20 and 40 kGy doses of GR, 90 and 120-second infrared irradiation on ruminal DM and CP degradation kinetics, pre and post-processing AAs compositions, and the LSM AAs degradation and *in vitro* digestibility.

MATERIALS AND METHODS

Sample Preparation and Treatments

The linseeds were obtained from the Oilseed Developing and Cultivation Company (Tehran, Iran). The seeds possible foreign materials were manually removed, and the seed meal was used after the oil was mechanically extracted from the seeds. Moreover, the collected samples were irradiated at Radiation Applications Research School, Nuclear Science and Technology Research Institute, and Iran' s Atomic Energy Organization. Before samples processed, sufficient water was added to the sample to increase the moisture content to 25%. Moreover, Gamma Irradiation (GI) was performed at 20°C in a cobalt-60 irradiator with an activity of 5244 Ci. The required dosage determined by Fricke dosimetry was 1.23 Gy/S, and based on the ASTM E1026-95 standard (Holm and Berry, 1970). Then, two polyethylene packages of LSM samples were irradiated at 20 and 40 kGy doses in a gamma cell (Co-60) in the presence of air, and device model was GC220. The irradiated samples were allowed to be equilibrated by air for 2 h, and were sealed in plastic bags. On the other hand, the feed samples were micronized for 90 and 120 seconds at a constant distance from the infrared source. Then, the LSM samples were roasted at 140˚C for 15 and 30 minutes. Finally, the processed samples were cooled to reach room temperature before being packed in zipper bags. All samples were kept at -20°C.

Animals and Diets

Three 4-year-old bulls (with approximately 397±5 kg of body weight), were used, all of which were fitted with rumen fistula, housed in individual pens, and given free access to the salt lick and fresh water throughout the experiment. Moreover, the bulls were fed twice a day at 8:00 AM and 4:00 PM with 8 kg DM of a total mixed ration containing 75% alfalfa hay and 25% wheat straw and forage with a 65: 35% concentration. The concentrate contained ground barley grain, ground corn grain, linseed meal, cottonseed meal, wheat bran, dicalcium phosphate, and a vitamin-mineral premix (60, 9, 14.5, 8.5, 6 and 1% on DM basis, respectively). The diet was formulated to meet the nutrient requirements of beef cattle (NRC 2001) containing 13.75% of CP. The bulls were adapted to the diets for two weeks prior to incubations.

In situ Ruminal Degradability

About 6 g of untreated and treated feed samples were sealed in 10×20 cm polyester bags (45-50 mm pore size). The surface area of each bag' was approximately 15 mg cm-2. The bags were incubated in the rumen of

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three rumen-fistulated bulls for 0, 2, 4, 8, 16, 24, and 48 hours according to Michalet-Doreau and Ould-Bah (1992), which were placed simultaneously in the dorsal sac of each bull's reticulorumen (2 bags per each feed sample in each bull for each incubation time) after 8 hours of feeding. At the end of each incubation time, the bags were removed from the rumen and washed immediately with tap water until the rinsing water turned clear. On the other hand, the disappearance at time 0 was obtained by washing the un-incubated bags in a similar way. Then, all the washed bags were dried for 48 hours in a forced-air oven at 65°C and weighed. The sub-samples of the bags' residues were used for DM and CP to determine the LSMs' degradation kinetics. After 16 hours of ruminal incubation on AAs content, the residues left were analyzed to determine the rumen's AA degradation.

Calculations and Statistical Analysis

The ruminal degradability of DM and CP was calculated at each incubation time as the difference between the substrate and the portion remaining after incubation. The ruminal degradability of the AA was also measured after 16 hours of incubation. Moreover, the degradability parameters of treated and untreated LSM were estimated as follows, using the *Fit Curve* software according to the model proposed by Ørskov and McDonald (1979)**:**

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

On the other hand, the Effective Ruminal Disappearance (ERD) of DM and CP was estimated using the following model:

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

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**.**

ERD refers to the effective rumen degradability for response variables (%), and k represents the ruminal outflow rate (h−1). The effective degradability of DM and CP ruminal was calculated by applying rumen outflow rates (k) 0f 0.02, 0.05 and 0.08 h^{-1} (Tuncer and Sacakli, 2003).

Moreover, Quickly Degradable Protein (QDP), Slowly Degradable Protein (SDP), Effective Rumen Degradable Protein (ERDP), Rumen Degradable Protein (RDP), Rumen Undegradable Protein (RUP) and Metabolizable Protein (MP) were calculated according to the models outlined by AFRC (1993) based on the following equation.

$QDP (\%)= a\times CP$

Where,

$SDP (\%)= [(b\times c)/(c+k)]\times CP$

$RDP (\%)= QDP+SDP$

$ERDP (\%)= 0.8(QDP)+SDP$

$RUP (\%)= CP\times(1-a-(bc/c+k))$

$DUP (\%)= 0.9[(RUP)-(6.25\times AIDN)]$

$MP (\%)= 0.6375(ERDP)+DUP$

On the other hand, the LSMs' *in vitro* digestibility and the data concerning the chemical composition factors were analyzed as a completely randomized design (Model 1) and the degradability data were analyzed as a completely randomized block design (Model 2) using the GLM (General Linear Model) procedure developed by SAS (1996). The means differences were also determined by the LSD (Least Significant Difference) test at a significant level of $P< 0.05$. Orthogonal contrasts were used to identify significant differences among the treatment groups.

$Y_{ij} = \mu + T_i + e_{ij}$ (Model 1)

$Y_{ijk} = \mu + T_i + K_j + e_{ijk}$ (Model 2)

Where, Y_{ij} and Y_{ijk} are dependent variables, μ stands for the overall mean, T_i refers to the irradiation effect, K_j represents the animal effect and e_{ij} and e_{ijk} are residual errors, assumed to have a normal distribution and be independent.

RESULTS

Amino Acid Profile

The effect of treatments on the LSMs' essential and non-essential AA profile are shown in Table 1. Glutamic acid was the

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Table 1. Amino acid profile (% of DM) of untreated and treated linseed meal.[a]

Treatments	Untreated	GR20	GR40	IR90	IR120	R15	R30	SEM	Untreated vs. treated	Untreated vs. GR	Untreated vs. IR	Untreated vs. R	GR vs. IR	GR vs. R	IR vs.R
Essential amino acid															
His	0.09^{b}	ND	0.08^{b}	0.14^{b}	0.08^{b}	0.08^{b}	0.07^{b}	0.112	NS	NS	NS	NS	NS	NS	NS
Ile	0.26^{a}	0.25^{ab}	0.24^{ab}	0.21^{c}	0.25^{ab}	0.25^{ab}	0.25^{ab}	0.01	< 0.001	< 0.001	NS	< 0.001	<0.001	<0.001	<0.001
Leu	1.42^{a}	1.16^{c}	1.36^{b}	1.42^{a}	1.33^{b}	1.34^{b}	1.33^{b}	0.015	< 0.001	< 0.001	< 0.05	< 0.001	<0.05	<0.05	<0.001
Lys	0.52^{a}	0.47^{abc}	0.5^{ab}	0.46^{abc}	0.44^{bc}	0.43^{bc}	0.41^{c}	0.03	< 0.001	< 0.001	< 0.05	< 0.001	NS	<0.001	<0.001
Met	0.3^{a}	0.29^{cd}	0.31^{ab}	0.29^{bc}	0.28^{d}	0.3^{bc}	0.31^{ab}	0.038	NS	NS	NS	NS	NS	NS	NS
Phe	0.25^{a}	0.21^{b}	0.21^{b}	0.23^{ab}	0.23^{ab}	0.23^{ab}	0.23^{ab}	0.013	< 0.001	< 0.01	< 0.05	< 0.001	NS	<0.001	<0.05
Thr	1.32^{a}	1.28^{abcde}	1.26^{bcde}	1.24^{e}	1.25^{cde}	1.30^{abcd}	1.31^{ab}	0.021	< 0.001	< 0.05	NS	< 0.001	<0.05	<0.001	<0.001
Val	0.14^{a}	0.13^{abc}	0.12^{bc}	0.13^{abc}	0.13^{abc}	0.13^{abc}	0.13^{abc}	0.006	< 0.01	< 0.001	< 0.05	NS	<0.001	<0.001	<0.001
Try	0.33^{a}	0.29^{cd}	0.31^{ab}	0.29^{bc}	0.28^{d}	0.30^{bc}	0.31^{ab}	0.008	< 0.001	< 0.001	< 0.01	< 0.001	<0.001	<0.05	<0.01
Nonessential amino acid															
Ala	1.38^{a}	1.21^{c}	1.33^{a}	1.33^{a}	1.33^{a}	1.34^{a}	1.33^{a}	0.014	< 0.01	< 0.05	NS	< 0.001	NS	<0.001	<0.001
Arg	1.33^{a}	0.5^{d}	1.29^{abc}	1.28^{abcd}	1.24^{cd}	1.26^{bcd}	1.28^{abcd}	0.023	< 0.001	< 0.001	< 0.05	< 0.01	<0.01	<0.01	<0.01
Asp	1.36^{a}	1.27^{a}	1.32^{a}	0.89^{b}	1.28^{a}	1.31^{a}	1.32^{a}	0.149	< 0.001	< 0.001	NS	< 0.01	<0.001	<0.001	<0.001
Glu	1.36^{a}	1.22^{d}	1.33^{ab}	1.26^{cd}	1.32^{ab}	1.34^{a}	1.35^{a}	0.022	< 0.01	< 0.001	NS	NS	<0.001	<0.001	<0.001
Gly	0.06^{b}	nd	0.11^{a}	0.13^{a}	0.01^{def}	0.006^{ef}	0.02^{cdef}	0.011	< 0.01	< 0.001	NS	NS	<0.001	<0.001	<0.001
Ser	nd	nd	0.08^{a}	0.03^{bc}	0.07^{a}	0.01^{cd}	nd	0.013	< 0.01	< 0.01	NS	< 0.01	NS	<0.001	<0.001
Tyr	0.24^{a}	0.17^{c}	0.19^{c}	0.23^{ab}	0.22^{ab}	0.23^{ab}	nd	0.009	< 0.01	< 0.001	NS	< 0.05	<0.01	<0.01	<0.01

[a] (a-f)**:** The means in the same column with different letters are different (P< 0.05). Treatments were: Untreated= Intact linseed meal, GR20= 20 kGy GI, GR40= 40 kGy GI, IR90= IR for 90 seconds, IR90= IR for 120 seconds, R15= Roasting for 15 min, R30= Roasting for 30 min. His= Histidine, Ile= Isoleucine, Leu= Leucine, Lys= Lysine, Met= Methionine, Phe= Phenylalanine, Thr= Threonine, Val= Valine, Ala= Alanine, Arg= Arginine, Asp= Aspargin, Glu=glutamin, Gly=glycine, Ser=serine, Tyr=tyrosine. SEM: Standard Error of the Mean, ND = Not determined, NS= Not significant.

most predominant amino acid followed by arginine and aspartic acid. Except aspartic, the use of gamma irradiation had a significantly reduction in AA content of GI-LSM at both doses (P< 0.05). In addition, a significant reduction in the LSMs AA content was observed by heat treated at both doses, except for histidine, alanine, aspartic and glutamic acid (P< 0.05).

Ruminal Degradability of DM and CP

Tables 2 and 3 show the ruminal degradability parameters of DM and CP in treated and untreated LSM, respectively. Accordingly, the maximum potential degradability (a+b) of DM and CP were 82.5 and 89.8% for untreated LSM, respectively, indicating that the LSM was highly degradable in the rumen. Compared to the control group, irradiation of LSM decreased the washout fraction and degradation rate of DMs *b* fraction (P< 0.001). However, the DMs potentially degradable fraction and maximum potential degradability were increased by irradiation processing (P< 0.001). The greatest increase in DM s ERD was obtained by GR at a ruminal outflow rate of 0.02 h^{-1} compared with the other treatments (P< 0.001). On the other hand, the lowest DM's ERD was found in gamma and infrared irradiated LSM at ruminal outflow rates of 0.05 and 0.08 h^{-1} (P< 0.001). Moreover, the effect of GR and IR on DM's degradation parameters and ERD was comparable, except for DM's degradation rate of b fraction. Also, the DM's washout fraction, potential degradability and ERD were decreased by IR at a ruminal outflow rate of 0.02 h^{-1} compared with GR (P< 0.01). Furthermore, compared to GR-irradiated and 15-minute-roasted-LSM (P< 0.001), the DM's maximum potential degradability was decreased by 90 second- Infrared-irradiated LSM.

The *a* fraction of CPs washout was decreased by irradiating and roasting the LSM for 15 minutes in comparison with untreated LSM (P< 0.001) while increasing its potentially degradable fraction (*b*) and reducing its ERD. Moreover, compared to other treatments, roasting the LSM for 15 minutes increased the CP s degradation rate of b fraction (P< 0.05). On the other hand, while the effect of GR and IR on the CP s potentially degradable fraction and degradation rate of *b* fraction was not comparable, the lowest amount of the CP s washout fraction degradation content was observed by infrared–irradiated LSM (P< 0.001). Moreover, the lowest amount of the CPs ERD was found in the 90-second-infrared–irradiated LSM at a rumen outflow rate of 0.05 h^{-1} (P<0.001).

Metabolizable Protein

All values of QDP, SDP, RDP, DUP, ERDP, RUP and MP differed (P< 0.05) among the treatments (Tables 4, 5, 6). Both types of irradiation decreased the QDP and increased the ERDP values of LSM at ruminal outflow rates of 0.05 and 0.08 h^{-1} (P< 0.001). Furthermore, the highest RDP value was estimated in untreated 30-minute-roasted LSM at ruminal outflow rates of 0.02, 0.05 and 0.08 h^{-1} (P< 0.001). On the other hand, gamma and infrared irradiations increased the RUP value at ruminal outflow rates of 0.02, 0.05, and 0.08 h^{-1} compared to the untreated LSM (P< 0.001). However, GI and IR increased LSM's MP values at LSM at ruminal outflow rates of 0.05 and 0.08 h^{-1} (P< 0.05). It should be noted that compared to IR, GI exerted a greater influence on LSMs MP (P< 0.05).

Amino Acid Degradability

The results of essential and non-essential AA degradation after 16 h of ruminal incubation are presented at Table 7. The individual AA of all treatments disappeared in the rumen s different extensions. Moreover, there was a significant difference between treatments performed on the

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Table 2. Rumen degradation parameters of dry matter of untreated and treated linseed meal.[a]

Treatments [a]	Degradation parameters				Effective degradability at outflow rate (%)		
	a (%)	b (%)	a+b (%)	C (h^{-1})	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
Untreated	45.5^{b}	36.9^{b}	82.5^{d}	0.092^{a}	75.5^{b}	69.1^{a}	64.9^{a}
GR20	39.6^{d}	57.3^{a}	96.9^{ab}	0.036^{b}	78.6^{a}	66.1^{bc}	59.6^{b}
GR40	41.3^{cd}	57.4^{a}	98.8^{a}	0.043^{b}	79.9^{a}	67.2^{bc}	60.8^{b}
IR90	37.1^{e}	52.5^{a}	89.6^{c}	0.060^{b}	75.6^{b}	64.6^{c}	58.5^{b}
IR120	36.1^{e}	56.0^{a}	92.1^{bc}	0.053^{b}	76.4^{b}	64.6^{c}	58.2^{b}
R15	43.8^{bc}	38.1^{c}	82.5^{d}	0.090^{a}	75.4^{b}	68.7^{b}	64.9^{a}
R30	51.7^{a}	35.6^{b}	87.3^{cd}	0.072^{ab}	79.1^{a}	72.2^{a}	68.1^{a}
SEM	0.82	1.74	1.67	0.0104	0.613	0.925	1.08
P value	0.001	0.001	0.001	0.05	0.001	0.001	0.001
Orthogonal contrasts							
Untreated *vs.* treated	< 0.001	< 0.001	< 0.001	< 0.05	< 0.05	NS	< 0.05
Untreated *vs.* GR	< 0.001	<0.001	< 0.001	< 0.01	< 0.001	< 0.05	< 0.01
Untreated *vs.* IR	< 0.001	< 0.001	< 0.001	< 0.05	NS	< 0.01	< 0.001
Untreated *vs.* Roasting	NS	NS	NS	NS	< 0.05	NS	NS
GR vs.IR	< 0.001	NS	< 0.001	NS	< 0.001	< 0.05	NS

[a] (a-d): The means in the same column with different letters are different (P< 0.05). Some reatments are defined under Table 1. a= Wash out fraction degradation, b= Potentially degradable fraction, a+b= Maximum potential degradability, c= Degradation rate of b fraction, SEM: Standard Error of the Mean, NS= Not Significant.

Table 3. Rumen degradation parameters of crude protein of untreated and treated linseed meal.[a]

Treatments [a]	Degradation parameters				Effective degradability at outflow rate (%)		
	a (%)	b (%)	a+b (%)	c (h^{-1})	0.02 h^{-1}	0.05 h^{-1}	**0.08 h^{-1}**
Untreated	68.7^{a}	21.1^{b}	89.8	0.320^{b}	86.7^{a}	83.9^{ab}	82.1^{a}
GR20	54.4^{bc}	37.5^{a}	91.9	0.26^{b}	79.8^{bc}	73.0^{c}	69.8^{b}
GR40	51.1^{c}	40.4^{a}	91.5	0.29^{b}	79.2^{bc}	71.9^{cd}	68.4^{b}
IR90	45.5^{d}	39.2^{a}	84.8	0.27^{b}	75.1^{c}	68.5^{e}	64.9^{c}
IR120	43.7^{d}	39.6^{a}	83.3	0.25^{b}	76.6^{c}	70.6^{d}	67.0^{bc}
R15	57.6^{b}	26.7^{b}	84.2	0.77^{a}	83.6^{ab}	82.6^{b}	81.7^{a}
R30	68.4^{a}	20.7^{b}	89.1	0.31^{b}	87.1^{a}	84.8^{a}	83.2^{a}
SEM	1.72	3.38	4.49	0.133	1.65	0.442	0.910
P value	0.001	0.01	NS	0.05	0.001	0.001	0.001
Orthogonal contrasts							
Untreated *vs.* treated	< 0.001	< 0.01	NS	NS	< 0.01	< 0.001	< 0.001
Untreated *vs.* GR	< 0.001	< 0.001	NS	NS	< 0.01	< 0.001	< 0.001
Untreated *vs.* IR	< 0.001	< 0.001	NS	NS	< 0.001	< 0.001	< 0.001
Untreated *vs.* Roasting	< 0.05	NS	NS	NS	NS	NS	NS
GR *vs.* IR	< 0.001	NS	NS	NS	< 0.05	< 0.001	< 0.01

[a] (a-d): The means in the same column with different letters are different (P< 0.05). Some treatments are defined under Table 1. a= Wash out fraction degradation, b= Potentially degradable fraction, a+b= Maximum potential degradability, c= Degradation rate of b fraction, SEM: Standard Error of the Mean, NS= Not Significant.

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Table 4. The effect of different treatments on Quickly Degradable Protein (QDP), Slowly Degradable Protein (SDP) and Effective Rumen Degradable protein (ERDP) of linseed meal.

Treatments [a]	QDP (%)	SDP (%)			ERDP (%)		
		0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
Untreated	19.4^{a}	5.1^{d}	4.3^{d}	3.8^{c}	20.7^{a}	19.9^{a}	19.3^{a}
GR20	15.4^{bc}	7.2^{c}	5.3^{cd}	4.4^{bc}	19.5^{abc}	17.6^{b}	16.7^{b}
GR40	14.5^{c}	8.0^{bc}	5.9^{bcd}	4.9^{abc}	19.5^{abc}	17.5^{b}	16.5^{b}
IR90	12.9^{d}	8.4^{ab}	6.5^{abc}	5.5^{abc}	18.7^{c}	16.8^{c}	15.8^{b}
IR120	12.4^{d}	9.3^{a}	7.6^{a}	6.6^{ab}	19.2^{bc}	17.5^{b}	16.5^{b}
R15	16.3^{b}	7.4^{c}	7.1^{ab}	6.8^{a}	20.4^{ab}	20.1^{a}	19.9^{a}
R30	19.4^{a}	5.3^{d}	4.6^{d}	4.2^{c}	20.8^{a}	20.1^{a}	19.7^{a}
SEM	0.487	0.307	0.501	0.680	0.396	0.158	0.328
P value	0.001	0.001	0.05	0.05	0.05	0.001	0.001
Orthogonal contrasts							
Untreated vs. treated	< 0.001	< 0.001	< 0.01	< 0.05	< 0.05	< 0.001	< 0.001
Untreated vs. GR	< 0.001	< 0.001	0.058	0.366	< 0.05	< 0.001	< 0.001
Untreated vs. IR	< 0.001	< 0.001	< 0.001	< 0.05	< 0.01	< 0.001	< 0.001
Untreated vs. Roasting	< 0.05	< 0.01	< 0.05	0.008	0.674	0.257	0.285
GR vs.IR	< 0.001	< 0.01	< 0.05	0.061	0.195	< 0.05	0.222

[a] (a-d): The means in the same column with different letters are different (P< 0.05). Some treatments are defined under Table 1.

Table 5. The effect of different treatments on Rumen Degradable Protein (RDP), Rumen Undegradable Protein (RUP) and Metabolizable Protein (MP) of linseed meal.[a]

Treatments[a]	RDP (%)			RUP (%)		
	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
Untreated	24.5^{a}	23.7^{ab}	23.2^{a}	3.8^{c}	4.6^{de}	5.1^{c}
GR20	22.6^{bc}	20.7^{c}	19.8^{b}	5.7^{ab}	7.6^{c}	8.5^{b}
GR40	22.4^{bc}	20.4^{cd}	19.4^{b}	5.9^{ab}	7.9^{bc}	8.9^{b}
IR90	21.3^{c}	19.4^{e}	18.4^{c}	7.0^{a}	8.9^{a}	9.9^{a}
IR120	21.7^{c}	20.0^{d}	19.0^{bc}	6.6^{a}	8.3^{b}	9.3^{ab}
R15	23.7^{ab}	23.4^{b}	23.1^{a}	4.6^{bc}	4.9^{d}	5.2^{c}
R30	24.6^{a}	24.0^{a}	23.4^{a}	3.7^{c}	4.3^{e}	4.8^{c}
SEM	0.468	0.125	0.258	0.468	0.125	0.258
P value	0.001	0.001	0.001	0.001	0.001	0.001
Orthogonal contrasts						
Untreated vs. treated	< 0.01	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
Untreated vs. GR	< 0.01	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
Untreated vs. IR	< 0.01	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
Untreated vs. Roasting	0.227	0.089	0.825	0.227	0.089	0.802
GR vs.IR	< 0.05	< 0.01	< 0.01	< 0.05	< 0.001	< 0.01

[a] (a-e): The means in the same column with different letters are different (P< 0.05). Some treatments are defined under Table 1.

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Table 6. The effect of different treatments on Digestible Undegradable Protein (DUP) and Metabolizable Protein (MP) of linseed meal.[a]

Treatments[a]	DUP			MP		
	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
Untreated	0.185	0.90^{b}	1.4^{b}	13.4	13.6^{b}	13.7^{b}
GR20	1.18	2.9^{a}	3.7^{a}	13.6	14.2^{a}	14.4^{a}
GR40	1.13	3.0^{a}	3.9^{a}	13.6	14.2^{a}	14.4^{a}
IR90	1.46	3.2^{a}	4.1^{a}	13.4	13.9^{ab}	14.2^{ab}
IR120	1.15	2.7^{a}	3.6^{a}	13.4	13.9^{ab}	14.1^{ab}
R15	0.55	0.79^{b}	1.0^{b}	13.6	13.7^{b}	13.7^{c}
R30	0.411	0.98^{b}	1.4^{b}	13.7	13.9^{ab}	14.0^{bc}
SEM	0.415	0.158	0.276	0.175	0.010	0.104
P value	NS	0.001	0.001	NS	0.05	< 0.01
Orthogonal contrasts						
Untreated vs. treated	0.002	< 0.001	< 0.001	0.001	< 0.01	< 0.01
Untreated vs. GR	0.005	< 0.001	< 0.001	0.005	< 0.001	< 0.001
Untreated vs. IR	< 0.05	< 0.001	< 0.001	0.012	< 0.05	< 0.01
Untreated vs. Roasting	0.078	0.628	0.394	0.035	0.685	0.982
GR vs.IR	0.227	0.839	0.925	0.345	< 0.05	< 0.05

[a] (a-c): The means in the same column with different letters are different (P< 0.05). Some treatments are defined under Table 1.

ruminal disappearance of AA (P< 0.05). On the other hand, GR irradiation at 20 and 40 KGY, decreased the rumen degradability of essential and non-essential AA (P< 0.05). Also, the IR for 90 and 120 seconds decreased the ruminal degradability of the LSMs EAA (Essential Amino acid) (P< 0.01). The study also found that the AA Glycin in GI- LSM at 40 KGY dosage, EI-LSM at 40 KGY dosage and 90-second-infrared irradiated LSM were more resistant to rumen degradation compared to the untreated LSM (P< 0.01).

DISCUSSION

The ruminants' ingestion of highly degradable protein supplements would lead to the loss of the supplements' quality indexes such as AA balance and digestibility reducing the animals' performance, therefore, increasing the ruminal escape protein in the ruminants' nutrition regime seems critically important. According to our results, all the EAA (Essential Amino acid) (% on DM basis) values were found to beat lower levels than those reported by NRC (2001), except for methionine, leucine, and threonine. The results are also in line with the findings of Bamidele *et al*. (2015) who reported a decrease in some AA content of pigeon pea (*Cajanus Cajan*) flour. However, Hamza *et al*. (2013) reported an increase in some AA content of soy flour. The irradiation-induced reduction in the LSM's AA content may result from the reaction of GI with water in the feed, releasing of electrons and the formation with high reactivity free radicals. Both heating and radiation will lead to the denaturation of protein, which in turn decreases the solubility and degradation of the rumen, getting more escape proteins reach the duodenum as a result (Abu *et al*., 2006). As found in this study, the washout fraction and the potential degradable fraction value of DM and CP were higher than those found for flaxseed by Lashkari *et al*. (2015). The lower degradability of flaxseed could be explained by the hard pericarp surrounding the seed. On the other hand, irradiating LSM with GR and IR decreased the degradability of DM and CP. In this regard, Fattah *et al*. (2013) suggested that infrared irradiation reduced the rapidly degradable fraction of barley grain's DM and increased its slowly degradable fraction. Such a decrease in DM degradability could be attributed to the formation of complexes between protein and non-protein compounds such as starch, which consequently reduced the

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Table 7. Amino acid[b] degradation (%) of untreated and treated linseed meal.

Treatments[a]	Untreated	GR20	GR40	IR90	IR120	R15	R30	SEM	Untreated vs. treated	Untreated vs. GR	Untreated vs. IR	Untreated vs. R	GR vs. IR	GR vs. R	IR vs. R
Essential amino acids															
His	89.97^{abc}	85.55^{d}	86.62^{bcd}	90.75^{ab}	86.44^{cd}	90.42^{abc}	91.43^{a}	1.40	< 0.0001	< 0.0001	< 0.0001	0.0177	0.0067	< 0.0001	0.0002
Ile	91.01^{ab}	87.50^{c}	87.88^{c}	89.86^{b}	87.94^{c}	91.40^{ab}	92.53^{a}	0.83	0.0003	< 0.0001	0.2303	0.9848	< 0.0001	< 0.0001	0.2227
Leu	79.45^{a}	75.16^{b}	58.48^{c}	71.82^{b}	72.03^{b}	80.42^{a}	83.14^{a}	1.93	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Lys	92.11^{bcd}	89.02^{f}	89.95^{ef}	90.33^{def}	89.15^{f}	93.49^{ab}	94.48^{a}	0.88	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.7968	< 0.0001	< 0.0001
Met	83.50^{abc}	79.89^{cd}	79.50^{cd}	77.12^{d}	81.33^{bcd}	84.27^{abc}	86.59^{a}	2.19	0.0141	0.0397	0.0065	0.1751	0.2219	0.5131	0.1122
Phe	97.99^{ab}	97.52^{cd}	97.75^{bc}	97.35^{cd}	97.27^{d}	98.12^{ab}	98.31^{a}	0.2	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Thr	86.99^{a}	81.53^{b}	82.83^{b}	82.48^{b}	82.14^{b}	87.25^{a}	88.58^{a}	1.15	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Val	88.23^{abc}	83.35^{d}	85.67^{bcd}	84.54^{cd}	83.70^{d}	88.80^{ab}	90.04^{a}	1.73	< 0.0001	< 0.0001	< 0.0001	0.0010	0.0150	0.0001	0.0049
Try	82.83^{a}	78.13^{a}	50.39^{b}	76.40^{a}	79.10^{a}	84.08^{a}	84.73^{a}	0.02	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Nonessential amino acids															
Ala	85.69^{a}	81.72^{a}	80.34^{b}	80.58^{b}	80.07^{b}	86.25^{a}	87.73^{a}	1.44	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Arg	87.97^{b}	87.30^{a}	72.64^{c}	66.70^{d}	69.25^{cd}	79.97^{b}	82.04^{b}	2.12	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Asp	89.97^{abc}	85.55^{d}	86.62^{bcd}	90.75^{ab}	86.44^{cd}	90.42^{abc}	91.43^{a}	1.78	< 0.0001	< 0.0001	0.0796	0.0202	< 0.0001	< 0.0001	0.0004
Glu	89.78^{a}	86.74^{b}	86.02^{b}	86.81^{b}	85.69^{b}	89.94^{a}	91.04^{a}	0.97	< 0.0001	< 0.0001	< 0.0001	0.0826	< 0.0001	< 0.0001	< 0.0001
Gly	91.29^{b}	100^{a}	74.76^{c}	71.45^{c}	97.23^{a}	99.09^{a}	97.19^{a}	1.92	0.0015	0.0037	0.0002	0.6581	0.0092	0.0061	0.0002
Ser	100^{a}	100^{a}	78.85^{c}	89.95^{b}	77.06^{c}	98.16^{a}	ND	3.58	-	-	-	-	0.6001	0.4739	0.2012
Tyr	90.14^{a}	83.84^{b}	66.59^{c}	86.60^{bc}	84.70^{b}	88.35^{ab}	ND	1.40	0.0175	0.0023	0.1260	0.3091	0.0148	0.0178	0.6748

[a] (a-f): The means in the same column with different letters are different (P< 0.05). Some treatments are defined under Table 1.
[b] His= Histidine, Ile= Isoleucine, Leu= Leucine, Lys= Lysine, Met= Methionine, Phe= Phenylalanine, Thr= Threonine, Val= Valine, Ala= Alanine, Arg= Arginine, Asp= Aspargin, Glu= Glutamin, Gly= Glycine, Ser= Serine, Tyr= Tyrosine.

accessibility of the rumen's micro-organisms. For instance, Shawrang *et al*. (2018) found that irradiation can reduce DM's degradability by gelatinizing starch and protein, thus limiting their degradability. In contrast to the results of the current study, GI at 15, 30, and 45 KGY doses had no influence on the cottonseed DM's washout fraction and a slowly degradable fraction (Taghinejad-Roudbaneh *et al*., 2016). However, Wahyono *et al*. (2017) found that GI at 50 and 100 KGY doses increased the sweet sorghum's DM degradation of the rumen. Gamma irradiation at 40 kGy dosage decreased the effective protein degradation value of the ruminal outflow at 0.08 h-1 from 82.1% to 68.4%. The lower effective CP degradability of the un-irradiated-LSM could be attributed to the shift of N disappearance from the rumen to the small intestine. In this regard, GI of cottonseed at 30 and 45 kGY doses (Taghinejad-Roudbaneh *et al*., 2016) and EBI (Electron Beam Irradiation)of the cottonseed meal at 25-75 kGY doses (Ghanbari *et al*., 2012) significantly decreased the rapidly degradable fraction (*a*) with increasing the slowly degradable fraction (*b*) of CP. As found in this study, irradiating the LSM with gamma decreased the washout fraction of CP by 23.23% compared with the untreated-LSM. In consistent with the result of this study, Ghanbari *et al*. (2012) found 38.80% reduction in the washout fraction of the cottonseed meal's CP after the meal was processed by B-irradiation. In this regard, it could be argued that the increase in the slowly degradable fraction in gamma or infrared-radiated meal might be derived from the increase in hydrophobicity of protein molecule surface caused by the separation of hydrogen bonds and other weakly non-covalent bonds and the changes made in AA position. The results of the current study revealed that infrared radiation had a greater effect on decreasing the ERD of CP compared to GI and roasting performed at all ruminal outflow rates. Such greater influence may be due to the disruption or modification that occurred in the protein, changing the functional properties such as water absorption capacity, swelling capacity, and solubility (Semwal and Manchanahally, 2021). Radiation causes the formation of gels by making structural changes, oxidation of amino acids, breaking covalent bonds, and forming of free radicals, thus reducing the availability of chemical groups for the action of microbial proteolytic enzymes and decreasing the rate of protein degradation in the rumen. According to the findings of this study, the significant reduction in QDP, and thus the ERDP of LSM, caused by irradiation processing led to an increase in the by-pass of protein from LSM, increasing the amounts of MP in the small intestine as a result. In this regard, previous studies have also reported that irradiation processing could reduce the washout fraction and increase the slowly degradable fraction, which in turn increases the RUP and MP of the feed (Ghanbari *et al*. 2015; Taghinejad-Roudbaneh *et al*., 2016). The results of the current study also showed that irradiation was effective in changing the place of digestion from the rumen to the small intestine, thus altering the amounts of RUP and MP in the small intestine. On the other hand, different processes including decarboxylation, disulfide bonds reduction, sulfhydryl groups oxidation, peptide-chains cleavage, polypeptide cross-linking, denaturation and protein aggregation may occur after the irradiation, all of which are responsible for altering the place of protein digestion from rumen to the small intestine (Tang *et al*., 2012). The differences between CP and AA and the AA in terms of the rate and extent of degradation indicated that the amount of CP degradation does not represent the AA degradation and that the AA are not degraded in the same manner. As found in the current study, gamma and infrared irradiations had the greatest influence on reducing the rumen's AA degradability, which is consistent with the results found by Ghanbari *et al*. (2012, 2015), who reported that GI at 75 kGY dosage decreased the ruminal degradation of

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essential and non-EAA of cottonseed and sunflower meal. However, while limited information is available concerning the AA changes made in protein supplements following the irradiation, both gamma and infrared irradiation will lead to the denaturation of protein, decreasing the CP's solubility and degradation in the rumen, and thus helping more escape proteins and more AAs reach the duodenum (Abu *et al*. 2006). In heat radiation, depending on the intensity and duration of the heating, a biochemical reaction occurs among the proteins, resulting in cross-linkages between polypeptide chains that are resistant to protease enzymes (Shawrang *et al*. 2018). These reactions occur mainly between the lysine and the amide group of the Asp, Ala, and Glu, while the Lys, Arg, Met and Cys are more sensitive to heat than other AA (Shawrang *et al*. 2018). In our study, different AA responded differently to various processing methods, with the ruminal degradation of AA decreasing via irradiation. On the other hand, irradiation decreased the ruminal degradation of AA and increased the undegradable protein in the small intestine, assisting in adjusting the diet of high-producing animals as an important strategy (Borucki-Castro *et al*., 2008). Irradiation helps unfold protein structures, making a cleavage in the peptide and disulfide bonds, which can expose hydrophobic AAs (as positions) to active sites of pepsin and trypsin, thus increasing the digestibility (Murray *et al*., 2003). Furthermore, changes in the secondary and tertiary structure of the protein caused by GI, will expose more peptide bonds to the proteolytic enzyme (Fombag *et al*., 2005).

CONCLUSIONS

Physical processing methods such as GI (non-heat method), IR (micronizing) and roasting (heat methods) can be used to increase protein quality. On the other hand, all physical processing methods applied to LSM, including gamma and infrared irradiations, could decrease the ruminal degradation of AA and RDP while increasing RUP, DUP, and MP. However, gamma irradiation significantly increases the LSM's RUP and MP. It can be concluded that GI at either 20 or 40 kGY doses is an effective strategy for enhancing the nutritional value of LSM to be used in a ruminant diet.

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تأثیر اشعه گاما، تابش مادون قرمز و برشته کردن بر مشخصات اسیدهای آمینه، سینتیک تخریب شکمبه کنجاله بزرک

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چکیده

این مطالعه به منظور بررسی اثر دوزهای ۲۰ و ۴۰ کیلوگری پرتو گاما(GR) ، تابش مادون قرمز ۹۰ و ۱۲۰ ثانیه (IR)و برشته کردن ۱۵ و ۳۰ دقیقه (R) در دمای ۱۴۰ درجه سانتی گراد بر روی پروفایل اسید آمینه(AA) ، تجزیهAA ، ماده خشک شکمبه (DM)، و سینتیک تخریب پروتئین خام (CP) و قابلیت هضم در شرایط آزمایشگاهی کنجاله بزرک (LSM) انجام شد. نتایج نشان داد که در حالی که محتوای اسیدهای آمینه AA در LSMتیمار نشده نسبتاً بالاتر بود، ناپدید شدن AA توسط GR و IR پس از ۱۶ ساعت انکوباسیون در شکمبه کاهش یافت ($P< 0.01$).علاوه بر این، تابش قسمت محلول در آب را کاهش داد و قسمت تجزیه پذیر بالقوه DMو CP را افزایش داد ($P< 0.01$). از سوی دیگر، تیمارهای GR تجزیه پذیری موثر شکمبه DM (ERD) و CP را در نرخ خروجی شکمبه ۰.۰۵ و ۰.۰۸ در ساعت کاهش دادند ($P< 0.01$) . پروتئین تجزیه ناپذیر قابل هضم و پروتئین قابل متابولیسم GR و IR در سه سرعت خروجی (۰.۰۲، ۰.۰۵ و ۰.۰۸ در ساعت) به طور معنی داری بیشتر از تیمار برشته شده و گروه کنترل بود ($P<0.01$). MP از IR تفاوت معنی داری با گروه کنترل در نرخ خروج ۰.۰۲ نداشت، اما افزایش معنی داری در نرخ خروجی ۰.۰۵ و ۰.۰۸ در ساعت مشاهده شد ($P<0.01$) .

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