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3 **Nutritional Value of Sesame Meal Treated by Gamma Ray and Microwave**
4 **irradiation**

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11
12 **ABSTRACT**

13 An extensive ruminal degradation of protein can reduce its quality indices such as amino acid
14 balance and digestibility. When the degradability of crude protein in the rumen is high, the bypass
15 protein to intestine and its digestibility is lower. Therefore, the extent of protein degradation in the
16 rumen determines the amount of nitrogen available to microorganisms in the rumen and supply of
17 amino acids in the small intestine of the animal. The aim of this study was to evaluate the effect of
18 gamma ray irradiation (GR) at doses of 20 and 40 kGy and microwaving (MW) at 800 W for 3 and
19 5 minutes on ruminal degradation kinetics and *in vitro* digestibility of sesame meal (SSM).
20 Degradability parameters of irradiated samples were measured by nylon bag technique. The
21 amount of histidine, threonine, valine, alanine, arginine, glutamine, glycine, and serine were lower,
22 but the amount of methionine and phenylalanine were higher in GR and MW treated samples
23 compared to untreated ones. **Irradiation reduced the fibre content of SSM and altered its chemical**
24 **composition compared to untreated SSM.** Ruminal degradability of DM and CP was diminished in
25 the treated SSM. Effective degradability (ED) of DM and CP was found to be lower in the GR
26 irradiated SSM compared to the MW irradiated SSM and the control. After sixteen hours of ruminal
27 incubation of the treated SSM the degradability of isoleucine, methionine, phenylalanine, and
28 threonine were lower, but those of glycine and serine were higher. The *in vitro* digestibility of DM
29 (DMD) and organic matter (OMD), as well as the organic matter in the dry matter (DOMD) were
30 lower and higher in GR and MW irradiated SSM respectively, compared to unirradiated SSM. It
31 can be concluded that irradiation of sesame meal by gamma ray irradiation was effective in
32 protecting crude protein and some amino acids including, methionine and phenylalanine from
33 ruminal degradation.

34 **Keywords:** Sesame Meal, Gamma Ray, Microwave, *In situ*, *In vitro*

35
36 **INTRODUCTION**
37 Characterized by a high crude protein content (30.56% to 52.9%) and good balance of amino acids,
38 sesame seed meal (*Sesamum indicum* L.) can be used in ruminant nutrition (Onsaard et al., 2010;
39 Kaneko et al., 2002; Mamputu and Buhr, 1995). However, while sesame seed meal protein is rich
40 in methionine, it is relatively poor in lysine (Passi et al., 2019).

41 Iran is situated in a dry region, characterised by low rainfall, poor natural pasture and low quality
42 fodder. As a result, modern dairy farming relies on hand feeding rather than grazing, which is of
43 intensive labour and costly. In this context, enhancing the animals' feed utilization is both
44 nutritionally and economically advantageous for the farmers. The modern high-producing and fast-
45 growing ruminants require greater amount of protein synthesized by the ruminal microbial
46 ecosystem. Increasing the fraction of protein of the ration not to be degraded and to bypass the
47 rumen can increase the protein supplied for the animal (Waltz and Stern, 1989). Protecting protein
48 through irradiation is known as a bypass protein technology. Radiation is classified in two forms
49 of ionizing and non-ionizing methods.

50 The ionizing radiation (such as gamma ray) generates enough energy to remove electrons from
51 atom and causes radiolysis of cell components, including water, protein, peptides, and amino acids
52 (Gaber, 2005; Garrison, 1987; Davies and Delsignore, 1987). Non-ionizing radiation (such as
53 microwave processing) lacks sufficient energy to remove electrons from atoms, but causes atom
54 movements or vibration (Sant'Ana, 2017). During the heating process, any alteration in tertiary
55 structure of peptides and proteins (such as the uncoiling plated structure and denaturation of the
56 whole protein molecule) reduces protein degradation in the rumen (Peng et al., 2014). The effect
57 of gamma radiation and microwave on ruminal degradation of certain feedstuffs has already been
58 studied, finding that different feedstuffs respond differently to the same irradiation (Ghanbari et
59 al., 2015; Sadeghi and Shawrang, 2007). The research showed that GR irradiation of cottonseed
60 meal at 10, 20, and 30 kGy doses (Bahraini et al., 2017), the GR irradiation of soybean meal
61 (Shawrang et al., 2007), canola meal (Shawrang et al., 2008), cottonseed meal (Ghanbari et al.,
62 2012), and sunflower meal (Ghanbari et al., 2015) at 25, 50 and 75 kGy doses, and the GR
63 irradiation of canola seed (Taghinejad et al., 2016) at 15, 30, and 45 kGy dose, reduce ruminal
64 degradability of CP. More studies need to be carried out to identify the effect of irradiation on
65 nutritive value of different types of feeds and their by-products. Microwave energy penetrates a
66 food or feed material and produces a volumetrically distributed heat source, due to molecular

67 friction, resulting from dipolar rotation of polar solvents and from conductive migration of
68 dissolved ions. The dipolar rotation is caused by variations of the electrical and magnetic fields in
69 the organic components (Alton, 1998). In conventional thermal processing, energy is transferred
70 to the material through convection, conduction, and radiation of heat from the surfaces of the
71 material. In contrast, microwave energy is delivered directly to materials through molecular
72 interaction with the electromagnetic field. The molecular structure affects the ability of the
73 microwaves to interact with materials and transfer energy (Chandrasekaran., 2012).

74 The by-products of the oil extraction industry are generally protein-rich cakes and meals that are
75 comparable in CP content. Many of these by-products such as cottonseed, sunflower meal and etc.
76 have previously been treated by radiation to reduce their intense ruminal degradation (Bahraini et
77 al., 2017; Shawrang et al., 2007; Taghinejad et al., 2016). However, there is a lack of information
78 on the effects of GR and MV irradiation on SSM protein degradability. Therefore, the purpose of
79 present study was to evaluate and compare the effects of 20 and 40 kGy gamma ray (non-thermal
80 method) and 3-5- minute microwave irradiation (thermal method) on ruminal DM, CP, and AA
81 degradation kinetics using nylon bag (*in situ*) technique and *in vitro* digestibility of SSM. The
82 method of irradiation by other researchers and the results gave us the idea for planning present
83 research.

84

85 MATERIALS AND METHODS

86 Sample Preparation and Irradiation

87 The sesame seed used in this study was obtained from the Oilseed Developing and Cultivation
88 Company (Tehran, Iran). The oil of the seeds was extracted mechanically by hot extraction system
89 and the remaining meal was collected to be used in the study. The irradiation of SSM samples was
90 carried out in Radiation Applications Research School, affiliated with the Nuclear Science and
91 Technology Research Institute of the Iranian Atomic Energy Organization. The moisture of the
92 samples increased by roughly 25% via adding water prior to the irradiation process. The gamma-
93 ray irradiation was accomplished in a gamma cell irradiator of gc-220 calibrated according to the
94 ISO/ASTM 51026:2015. The dose rate was 1.023 G/S (61.5 Gy per minute) and the irradiations were
95 20 kg (in 4 hours, 31 minutes and 34 seconds) and 40 kg (in 9 hours, 3 minutes and 13 seconds).
96 Two polyethylene bags of SSM samples were irradiated in the gamma cell. The samples were
97 freeze-dried after finishing the irradiation and subsequently allowed to air equilibrate for 2 h before

98 being fastened in plastic bags. A second set of SSM samples were exposed to microwave irradiation
99 at 800 W power for 3 to 5 minutes. The processed samples were cooled to reach room temperature
100 and then packed in zipper bags. All samples were kept at -20°C temperature for subsequent
101 analyses.

102

103 **Chemical Analyses**

104 The SSM samples were ground using a laboratory hammer mill equipped with a 1 mm sieve. The
105 DM and CP of the SSM, the residues of SSM after rumen incubation, the EE and ash in samples
106 were determined as described in AOAC (1995). Neutral detergent fiber (NDF) and acid detergent
107 fiber (ADF) (sequentially after NDF) were determined as described by Van Soest et al. (1991).

108 The essential amino acids (His, Ile, Leu, Lys, Met, Phe, Thr, and Val) and non-essential amino
109 acids (Ala, Arg, Glu, Gly, Ser, and Tyr) of the SSM samples were determined before and after
110 rumen incubation (*in sacco*). About 15 ml of 6 M hydrochloric acid was added to 100 milligrams
111 of dried powderif SSM samples, and stored for 24 hours at 110°C to be hydrolyzed. The cooled
112 samples were filtered using a $0.45\ \mu\text{m}$ syringe filter and analyzed by high performance liquid
113 chromatography (HPLC Biochrom Plus amino acid analyzer, Pharmacia, Cambridge, UK) (Caidan
114 et al., 2014).

115

116 ***In Situ* Ruminant Degradability**

117 In this research three rumen cannulated bulls with an average body weight of $397 (\pm 5)$ kg were
118 used for bags incubation. The Animals were fed a mixed ration according to nylon bags standard
119 techniques on maintenance level. The ration was formulated on the basis of 67 percent forage
120 (alfalfa and wheat straw) and 33 percent concentrate (barley, corn, wheat bran, cottonseed meal
121 and vitamin supplement). Both SSM samples (treated and untreated) were ground separately to
122 pass a 2 mm sieve, and samples of 6 g each were retained in the polyester bags (10×20 cm; 45-50
123 μm pore size). Two bags were incubated at each incubation time (totally, 6 bags per treatment).
124 The bags were inserted in the rumen simultaneously, just before providing the bulls with their first
125 meal (i.e., 08:00 A.M). At the end of each incubation time, the bags were removed from the rumen
126 and immediately washed with tap water until the rinsing water became clear. Similarly,
127 disappearance at zero time was obtained by washing the bags not incubated. All of the washed bags
128 were dried in a forced-air oven at 65°C for 48 hours and weighed.

129
130 ***In Vitro* Digestibility of SSM**
131 The two-step digestion technique was used to determine the digestibility of SSM samples (Tilly
132 and Terry, 1963). The samples were dried at 65°C for 48 hours in an oven and milled through a 1.0
133 mm screen. About 500 mg of the milled sample was poured into each of three Erlenmeyer flasks
134 and three extra Erlenmeyer flasks without sample were considered as blanks. Rumen liquor was
135 collected before the morning meal by vacuum pump (60 mL syringes) of the fistulated bulls rumen.
136 Animals were fed a total mixed ration (TMR). The diet was formulated on the basis of 60% forage
137 and 40% concentrate. The diets (based on 67% forage and 33% of concentrate) were formulated
138 according to recommendations to provide approximately 12.7% CP on a DM basis. The liquor from
139 each animal was filtered through eight layers of gauze cloth, was purged with CO₂ and was kept in
140 a pre-warmed (39 °C) thermos flask until use (within approximately 20 min). The liquor of the
141 rumen and buffer were mixed at a 1:4 (v/v) ratio and 50 ml of the inoculum was added to each
142 Erlenmeyer flasks. The Erlenmeyer flasks then closed with a rubber cap and purged with CO₂ for
143 15 seconds and anaerobically incubated for 48 hours in an automatic shaking water bath kept at
144 39°C during the incubation. At the end of the incubation, 6 ml of hydrochloric acid (HCl, 20%)
145 and two mL of pepsin solution (pepsin, 20%) were added to each Erlenmeyer flask, and were then
146 incubated anaerobically for another 48 hours. At the end of this stage, the remaining sample were
147 filtered using a Whatman paper no. 41 that had previously being marked and numbered. The
148 residual contents and filter papers were dried in a forced air oven at 105°C for 24 h and the DM
149 was determined. To determine the ash content, all the samples were ashed at 560 °C for 4 h to
150 calculate digestible organic matter content in DM (OMD).

151 The residual contents and filter papers were dried in a forced air oven at 105°C for 24 hours, and
152 the DM was determined. Finally, all samples were burned at 560°C for 4 hours to determine the
153 digestible organic matter (OMD) in DM.

154 155 **Calculations and statistical Analysis**

156 Degradability of DM, CP, and AA was calculated as the difference between the weight of the
157 sample and the portion remained after incubation in the rumen. The DM and CP degradability
158 parameters of un-irradiated and irradiated SSM were estimated using the *Fit Curve* software.
159 Moreover, the Ørskov and McDonald's (1979) model was fitted based on the percentage of DM
160 and CP disappearance as follows:

161 $P=a+b(1-e^{-ct})$ (1)

162 where the P is the DM or CP degradability at time t (h), “a” stands for the washout or Soluble
163 Fraction, “b” represents Potentially Degradable Fraction, and “c” shows the degradation Rate (h⁻¹)
164 of b Fraction. Also, the effective ruminal disappearance (ED) of DM and CP were estimated at
165 outflow rates (k) of 0.02, 0.05 and 0.08 h⁻¹ (Tuncer and Sacakli, 2003). Using the following model:

166 $ED= a+((b\times c)/(c+k))$ (2)

167 The chemical composition, pre-incubation amino acids concentration, and *in vitro* digestibility
168 data of SSM were analyzed using a completely randomized design (3), and the degradability data
169 were analyzed as a randomized complete block design (4) (three animals × two bags for each
170 sample at each incubation time per bull) via the GLM SAS procedure. The significance of the
171 differences of the means were tested using the Duncan’s multiple range test (Steel and Torrie,
172 1980), at a significance level of P < 0.05. Also, orthogonal contrasts were used to detect significant
173 differences among the treatments.

174 $Y_{ij} = \mu + T_i + e_{ij}$ (3)

175 $Y_{ijk} = \mu + T_i + K_j + e_{ijk}$ (4)

176 where Y_{ijk} and Y_{ij} are dependent variable, μ is the overall mean, T_i stands for irradiation effect,
177 K_j shows the animal effect, and e_{ijk} and e_{ij} are residual error, which were assumed to have normal
178 distribution.

179

180 **RESULTS AND DISCUSSION**

181 The average of OM, NDF, and ADF content of SSM (Table 1) were within the range of values
182 reported in the literature (Maneemegalai and Prasad, 2011; Onsaard et al., 2010; Wang et al., 2016).
183 However, the CP and EE contents were slightly higher and lower, respectively, than those reported
184 by Wang et al. (2016) and Onsaard et al. (2010). GR and MW irradiation decreased the contents of
185 OM, CP, NDF, ADF, and EE compared to unirradiated SSM. In some studies, chemical
186 composition of cottonseed (Taghinejad et al., 2016), canola meal (Shawrang et al., 2008) and whole
187 flaxseed (Beheshti Moghadam et al., 2019) were not affected by gamma ray, microwave or electron
188 radiation. Yalcin et al. (2011) irradiated linseed with GR at 2.5-7 kGy doses and reported a loss in
189 protein and oil contents. While the MW irradiation of the whole soybeans for 2, 4, and 6 minutes
190 did not affect the OM, CP, and EE caused significant increase in NDF and ADF (Golshan et al.,
191 2019). Also, Bahraini et al. (2017) reported a decrease in crude fiber of cottonseed meal following
192 the GR radiation at 10-30 kGy doses. Al-Masri and Guenther (1999) have also reported that

193 irradiation can break down the lignocellulosic materials of the plant cell walls. Tang et al. (2012)
 194 reported that, the reduction in crude fiber by GR irradiation may be due to oxidation of the
 195 cellulose, and conversion of cellulose and lignin to the cell wall solution. By irradiation fibre level
 196 could be reduced due depolymerization and delignification (Sandev and Karaivanov, 1977). The
 197 discrepancy between the results of the studies concerning the chemical composition of irradiated
 198 seeds and meals could be attributed to factors such as seed type, whole seed or seed meal, the oil
 199 extraction method (hot or cold press), and the conditions of the processing method (e.g.,
 200 temperature, moisture, processing duration, dosage).

201

202 **Table 1.** Effects of irradiation processing on chemical composition² of sesame meal (g/kg).

Treatment ¹	OM	CP	NDF	ADF	EE
untreated	915.60 ^a	377.60 ^a	222.45 ^a	124.90 ^a	151.60 ^a
GR20	897.90 ^b	369.35 ^b	169.95 ^a	87.50 ^a	147.35 ^b
GR40	897.35 ^b	368.90 ^c	162.40 ^d	77.40 ^d	137.10 ^c
MW3	898.50 ^b	357.05 ^d	220.00 ^e	95.00 ^e	65.00 ^d
MW5	897.05 ^b	348.15 ^e	172.55 ^c	90.00 ^c	50.10 ^e
SEM	2.417	3.454	8.717	5.343	14.508
Orthogonal contrasts					
Unirradiated vs. irradiated	<0.01	<0.01	<0.01	<0.01	<0.01
Unirradiated vs. GR	0.67	<0.01	<0.01	<0.01	<0.01
Unirradiated vs. MV	<0.01	<0.01	<0.01	<0.01	<0.01
GR vs. MV	<0.01	<0.01	<0.01	<0.01	<0.01

203 SEM, standard error of the means.

204 ¹) Different treatments were as follow, untreated, intact sesame meal; GR20= 20 kGy gamma irradiation, GR40= 40
 205 kGy gamma irradiation, MV3= microwaving for 3 minutes, MV5= microwaving for 5 minutes.

206 ²) OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; EE, ether extract.

207

208 Table 2 shows the results of irradiation effect on amino acids profile before ruminal incubation
 209 of un-irradiated and irradiated SSM. Irradiation of SSM was found to have significant effects on
 210 amino acids quantity except for the isoleucine, leucine, lysine, and tyrosine (P<0.01), leading to an
 211 increase in SSM methionine and phenylalanine content (P<0.01). Concentrations of certain
 212 essential and non-essential amino acids were higher or lower in irradiated SSM compared to the
 213 control. However, while the concentration of methionine and phenylalanine increased when treated
 214 by gamma rays and microwave, the concentration of histidine, threonine, valine, alanine, arginine,
 215 glutamine, glycine, and serine decreased under the same treatment. The results of Erkan and Ozden
 216 (2007) study showed a significant difference in the contents of the sea bream amino acids following
 217 the gamma irradiation at 2.5 and 5 kGy doses. Also, Xiang et al. (2020) reported that irradiation of
 218 gluten with 1000 W microwave for 5 minutes resulted in a cross-linkage between amino acids,
 219 reducing their total amount. Moreover, Bamidele and Akanbi (2015) showed a decrease in essential

220 and nonessential amino acids of pigeon pea flour when they were irradiated by gamma irradiation
221 at 5-20 kGy doses. Xiang et al. (2020) suggested that the impact of irradiation on amino acids
222 depended on the irradiation dosage and the structure of amino acids. Accordingly, the reduction
223 in the radiated amino acids could be attributed to the deamination and dehydrogenation-induced
224 formation of amino acid radicals, the breakdown of amino acids induced by the loss of groups of
225 NH_3 , H_2O , and COOH , the breakdown of covalent and peptide bonds, and the water-based
226 chemical reactions (Cheftel et al., 1985; Cho and Song, 2000). Reacting with water molecules,
227 ionizing rays destroy the structure of amino acids by forming free radicals. In current study it was
228 found that the amount of crude protein (Table 1) and amino acids (Table 2) decreased with an
229 increase in the moisture (Table 1) of irradiated sesame meal. As found by previous studies, sulfur-
230 containing and aromatic amino acids are extremely sensitive to radiation. However, the current
231 study found that whereas radiation increased the concentration of methionine (a sulfur-containing
232 amino acid) and phenylalanine (an aromatic amino acid), it reduced the concentration of histidine.
233 In this regard, Orias et al. (2002) argued that those amino acids protected in the protein complex
234 structure were not affected by irradiation, and that the same might held true for isoleucine (a
235 nonpolar amino acid) which remained unaltered when irradiated.

236 **Irradiation** may result in physical, chemical and biological changes in some feed materials (Afify
237 et al., 2011). Studies on the effect of gamma radiation on the protein profile and functional
238 properties of sesame seeds are scarce. It is known that the physiochemical properties of proteins
239 are modified by irradiation as reported by Hafez et al. (1985). Afify and Shousha (1988)
240 demonstrated that γ -irradiation caused molecular changes resulting in condensation or
241 polymerization, degradation, hydrogen-bonding distribution and cleavages of intermolecular
242 disulphide bonds (Casarett, 1968). Therefore, it is assumed that such molecular rearrangements
243 **may change the** secondary structure and distribution of the native conformation of proteins,
244 especially at higher dose levels or **probably** increase bioavailability of amino acid for animal.
245 Different types of amino acids have different variations in the microwave heating process. **The**
246 **concentration of sulfur-containing and hydrophobic amino acids increases under the influence of**

247 heat and Maillard reaction (Some feed sources contain cystine, which is formed from two cysteine
 248 amino acids, Cystine may break under the influence of radiation and produce two cysteines or even
 249 be converted to methionine) while the concentration of histidine decreases. Of course, the variation
 250 trends of the same amino acid in different feed are different, which could be connected to feed type
 251 and microwave and Gama irradiation processing settings.
 252
 253
 254

255 **Table 2.** Effects of irradiation processing on amino acid² profile (%) of sesame meal prior
 256 incubation in rumen.

Treatment ¹	Unirradiated	Irradiated				SEM	P-values			
		GR20	GR40	MW3	MW5		Unirradiated vs. irradiated	Unirradiated vs. GR	Unirradiated vs. MV	GR vs. MV
Essential										
His	0.48 ^a	0.35 ^b	0.24 ^c	0.37 ^b	0.35 ^b	0.022	<0.01	<0.01	<0.01	0.03
Ile	0.71 ^{ab}	0.54 ^b	0.92 ^a	0.43 ^b	0.61 ^{ab}	0.057	0.45	0.88	0.14	0.06
Leu	1.69 ^{ab}	1.25 ^c	1.96 ^a	1.43 ^{bc}	1.53 ^{bc}	0.081	0.35	0.62	0.22	0.36
Lys	0.05 ^{ab}	0.06 ^{ab}	0.07 ^a	0.02 ^b	0.02 ^b	0.006	0.48	0.54	0.08	<0.01
Met	0.05 ^c	0.23 ^{ab}	0.26 ^a	0.10 ^c	0.19 ^b	0.022	<0.01	<0.01	<0.01	<0.01
Phe	0.05 ^c	0.45 ^b	0.55 ^{ab}	0.46 ^b	0.73 ^a	0.063	<0.01	<0.01	<0.01	0.14
Thr	0.84 ^a	0.35 ^c	0.30 ^c	0.34 ^c	0.50 ^b	0.055	<0.01	<0.01	<0.01	0.03
Val	1.04 ^a	0.66 ^{bc}	0.47 ^c	0.60 ^c	0.87 ^{ab}	0.060	<0.01	<0.01	<0.01	0.03
Non-essential										
Ala	0.85 ^a	0.53 ^{cd}	0.35 ^d	0.58 ^{bc}	0.73 ^{ab}	0.050	<0.01	<0.01	0.03	<0.01
Arg	1.71 ^a	1.16 ^b	1.02 ^b	1.32 ^{ab}	1.20 ^b	0.083	<0.01	<0.01	0.03	0.28
Glu	3.77 ^a	3.30 ^b	2.45 ^c	2.61 ^c	3.43 ^{ab}	0.142	<0.01	<0.01	<0.01	0.27
Gly	1.08 ^a	0.80 ^{bc}	0.67 ^c	0.085 ^d	0.85 ^b	0.090	<0.01	<0.01	<0.01	<0.01
Ser	0.82 ^a	0.48 ^c	0.50 ^{bc}	0.51 ^{bc}	0.68 ^{ab}	0.042	<0.01	<0.01	0.01	0.127
Tyr	0.62	0.53	0.58	0.57	0.46	0.028	0.26	0.43	0.21	0.53

257 SEM, standard error of the means.

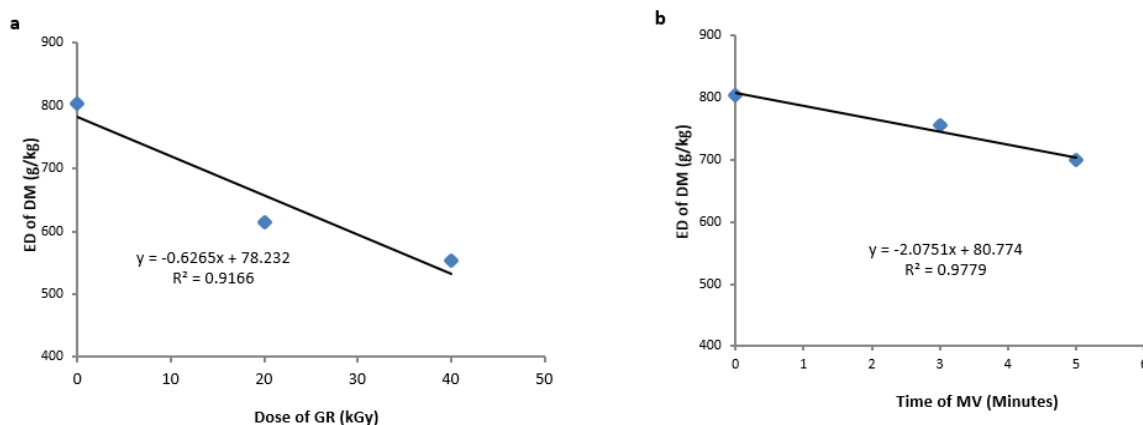
258 ¹⁾ Different treatments were as follow, untreated, intact sesame meal; GR20= 20 kGy gamma irradiation,
 259 GR40= 40 kGy gamma irradiation, MV3= microwaving for 3 minutes, MV5= microwaving for 5 minutes.

260 ²⁾ His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val,
 261 valine; Ala, alanine; Arg, arginine; Glu, glutamin; Gly, glycine; Ser, serine; Tyr, tyrosine.
 262

263 Table 3 shows the ruminal degradability parameters of DM and CP in treated SSM. The
 264 irradiation of SSM by GR and MW decreased the rapidly degradable fraction (a) and effective
 265 degradability (ED) of DM and CP at ruminal outflow rates of 0.02, 0.05, and 0.08h⁻¹ and increase
 266 of (P<0.01) the slowly degradable fraction (b) without any influence on potential degradability
 267 compared to the control (P<0.01). Furthermore, GR irradiation at 40 kGy had the greatest effect on

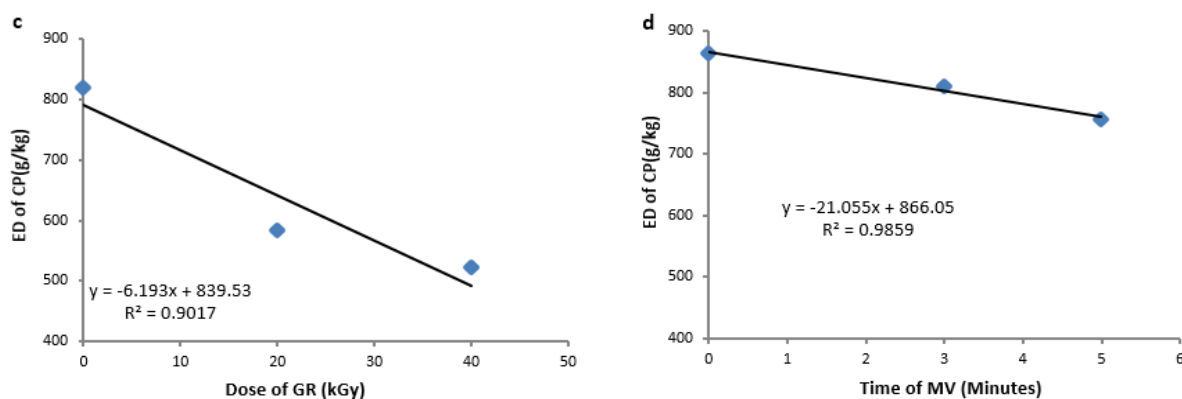
268 reducing the ED of DM and CP, which could be attributed to a low soluble fraction, high insoluble
269 fraction, and low degradation rate of DM and CP in GR-irradiated SSM. In this regard, the results
270 of previous studies have shown some changes in rapidly degradable fraction and potentially
271 degradable fraction of DM and CP when irradiated by GR at 25 - 75 kGy doses (Shawrang et al.,
272 2007) and 2-6-min MW (Sadeghi et al., 2005) in soybean meal, which our results are consistent
273 with their reports.

274 For instance, Maity et al. (2009) reported dose-dependent reduction in total and soluble protein
275 content of rice seeds when irradiated at 6 kGy by GR. Moreover, Sadeghi and Shawrang (2007)
276 argued that the post irradiation decreases in DM degradability could be attributed to the decrease
277 in degradability of starch and/or protein due to the formation of complex bonds between protein
278 and non-protein compounds, which consequently reduced their accessibility to rumen
279 microorganisms. Depressive effect of GR on protein solubility could also be related to changes in
280 the physicochemical properties of the protein. The oxygen radicals generated in the radiolysis of
281 water can change protein conformation in the forms of fragmentation (breakdown of the
282 polypeptide chain and formation of low-molecular weight molecules), aggregation (development
283 of inter-protein cross-linkage, disulfide bonds, and formation of high-molecular-weight
284 molecules), and oxidation of amino acids (Lee and Song, 2002; Cho and Song, 2000) all of which
285 are dependent upon the protein concentration and the irradiation dosage (Lee and Song, 2002).
286 Heat processing methods such as microwave radiation can alter the structure of proteins by
287 uncoiling the β -pleated sheets or denaturizing the whole protein molecule. Some factors such as
288 racemization, cross-linkages among the uncoiled peptides and amino acids, and Maillard reaction
289 can decrease the CP solubility, and thus, increase the resistance of protein molecules to microbial
290 degradation (Yan et al., 2014). Significant negative relationship was observed between the ED of
291 DM, and irradiation dosage ($R^2=0.91$), as well as MV time ($R^2=0.97$) (Fig 1 a and b). The
292 percentage of effective ruminal degradability of DM was decreased linearly as the irradiation
293 dosage of GR and time of the MW increased. The DM- ED decreased 6% with every 1 kGy increase
294 of GR and 2% for every 1 minute increase in MW time, respectively.



295
 296 **Fig. 1.** Relation of effective ruminal degradability (ED) (g/kg) of dry matter (DM) with GR
 297 irradiation dose and MW time.

298
 299 A negative linear relationship was also found between CP effective ruminal degradability of SSM
 300 and GR irradiation dose ($R^2=0.90$), and MW time ($R^2=0.98$) (Fig 2 c and d). **The effective ruminal**
 301 **degradability of CP decreased by 6% for each 1 kGy increase in GR dose and by 21% for each 1**
 302 **min increase in MW time.**



303
 304 **Fig. 2.** Relation of effective ruminal degradability (ED) (g/kg) of crude protein (CP) with GR
 305 irradiation dose and MW time.

306
 307 **Table 3.** Effects of irradiation processing of sesame meal on ruminal degradation characteristics
 308 of dry matter and crude protein².

Treatments ¹	Unirradiated	Irradiated				SEM	P-values			
		GR20	GR40	MW3	MW5		Unirradiated vs. irradiated	Unirradiated vs. GR	Unirradiated vs. MV	GR vs. MV
Dry matter										
a(g/kg)	476.53 ^a	173.83 ^d	120.67 ^e	389.59 ^b	299.60 ^c	35.426	<0.01	<0.01	<0.01	<0.01
b(g/kg)	500.07 ^e	825.53 ^b	879.23 ^a	606.23 ^d	673.33 ^c	37.615	<0.01	<0.01	<0.01	<0.01

a+b(g/kg)	976.60	999.37	999.90	995.82	972.93	4.262	0.13	0.05	0.46	0.10
c(h ⁻¹)	0.095 ^a	0.058 ^{bc}	0.049 ^c	0.080 ^{ab}	0.076 ^{abc}	0.0061	0.03	<0.01	0.19	0.03
Effective degradability at outflow rate (g/kg)										
0.02 h ⁻¹	889.57 ^a	783.54 ^c	742.38 ^d	867.13 ^a	826.24 ^b	15.683	<0.01	<0.01	0.02	<0.01
0.05 h ⁻¹	804.14 ^a	613.39 ^c	553.55 ^d	754.51 ^{ab}	698.58 ^b	25.932	<0.01	<0.01	<0.01	<0.01
0.08 h ⁻¹	748.02 ^a	517.94 ^d	452.93 ^e	686.00 ^b	621.43 ^c	30.168	<0.01	<0.01	<0.01	<0.01
Crude protein										
a(g/kg)	588.60 ^a	268.73 ^d	195.90 ^e	476.13 ^b	343.67 ^c	38.075	<0.01	<0.01	<0.01	<0.01
b(g/kg)	402.80 ^e	726.56 ^b	803.83 ^a	517.90 ^d	649.70 ^c	38.719	<0.01	<0.01	<0.01	<0.01
a+b(g/kg)	991.40	995.29	999.73	994.03	993.36	1.595	0.36	0.23	0.64	0.35
c(h ⁻¹)	0.107 ^a	0.062 ^{bc}	0.054 ^c	0.095 ^a	0.090 ^{ab}	0.0072	0.02	<0.01	0.28	<0.01
Effective degradability at outflow rate (g/kg)										
0.02 h ⁻¹	927.91 ^a	815.06 ^c	784.08 ^d	899.37 ^b	871.09 ^b	14.803	<0.01	<0.01	<0.01	<0.01
0.05 h ⁻¹	863.14 ^a	668.44 ^d	615.42 ^e	810.15 ^b	756.42 ^c	25.054	<0.01	<0.01	<0.01	<0.01
0.08 h ⁻¹	819.19 ^a	584.37 ^d	521.97 ^e	752.85 ^b	683.26 ^c	29.728	<0.01	<0.01	<0.01	<0.01

309 SEM, standard error of the means.

310 ¹)Different treatments were as follow, untreated, intact sesame meal; GR20= 20 kGy gamma irradiation, GR40= 40
311 kGy gamma irradiation, MV3= microwaving for 3 minutes, MV5= microwaving for 5 minutes.

312 ²) ERD, effective ruminal degradability; a, wash out fraction degradation; b, potentially degradable fraction; a+b,
313 potential degradability; c, rate constant of degradation of b fraction.

314

315 The results of ruminal disappearance of amino acids in unirradiated and irradiated SSM after 16
316 h of ruminal incubation are shown in Table 4. Ruminal disappearance of methionine, phenylalanine
317 and threonine were decreased and that of glycine and serine were increased by GR irradiations
318 (P<0.01). For GR-irradiated SSM, ruminal disappearance of threonine decreased as the irradiation
319 dose increased from 20 to 40 kGy (P<0.01). The ruminal disappearance of isoleucine, methionine,
320 phenylalanine, and threonine decreased and that of the serine increased with the increase of MW
321 irradiation time (P<0.01). In general, the effectiveness of MV to decrease ruminal degradability of
322 AA was greater than GR. Reducing the extent of protein breakdown in the rumen and increasing
323 the amount of the protein passage from the rumen to the intestine are among the significant goals
324 in setting the diet of high-producing animals seeking to optimize the absorbable amino acids
325 (Borucki Castro et al., 2007). In this regard, the current study found that processing sesame meal
326 increased the resistance of isoleucine, methionine, phenylalanine and threonine, but decreased the
327 resistance of glycine and serine to microbial degradation in the rumen, which could be related to
328 their structure type (Matloubi et al., 2004). Little evidence is available concerning the effects of
329 seed meals irradiation on the level of amino acids resistance to ruminal degradation. In a study,
330 Fathi Nasri et al (2008) found significant decrease in ruminal degradation of amino acids when
331 roasted (thermal method) and steep-roasted at 140-145 °C, suggesting that the influence of
332 processing on the degradability of amino acids in the rumen depends on the amino acid profile of
333 the feed sample before its processing. In other studies, the treatment made at 25, 50, and 75 kGy

334 doses of electron and gamma rays decreased ruminal disappearance of amino acids in sunflower
 335 meal (Ghanbari et al. 2015) and cotton seed meal (Ghanbari et al. 2012). In this research, the
 336 irradiation of SSM increased the amount of methionine and phenylalanine but exerted no influence
 337 on the amount of isoleucine (Table 2) and decreased the ruminal degradability of these amino acids
 338 (methionine, phenylalanine and isoleucine) (Table 4). Therefore, irradiation processing by GR and
 339 MV can be effective in increasing bypass of methionine, phenylalanine and isoleucine amino acids
 340 from the rumen

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 344

345 **Table 4.** Amino acid² ruminal degradation (%) of unirradiated and irradiated sesame meal.

Treatment ¹	Unirradiated	Irradiated				SEM	P-values			
		GR20	GR40	MW3	MW5		Unirradiated vs. irradiated	Unirradiated vs. GR	Unirradiated vs. MW	GR vs. MW
Essential										
His	20.54 ^b	25.29 ^{ab}	37.24 ^a	21.43 ^{ab}	14.66 ^b	2.646	0.48	0.12	0.69	0.03
Ile	67.01 ^a	71.81 ^a	28.84 ^b	22.02 ^b	12.83 ^b	7.084	<0.01	0.15	<0.01	<0.01
Leu	63.03 ^{ab}	93.80 ^a	9.25 ^c	64.58 ^{ab}	23.35 ^{bc}	9.644	0.31	0.485	0.25	0.57
Lys	31.10	59.72	48.02	53.33	58.89	4.945	0.11	0.15	0.12	0.85
Met	75.17 ^a	18.88 ^b	27.54 ^b	59.25 ^a	29.43 ^b	6.268	<0.01	<0.01	<0.01	<0.01
Phe	91.55 ^a	33.70 ^{bc}	50.98 ^b	18.89 ^c	13.26 ^c	8.010	<0.01	<0.01	<0.01	0.01
Thr	87.14 ^a	42.81 ^b	12.67 ^c	45.09 ^b	31.89 ^{bc}	6.862	<0.01	<0.01	<0.01	0.11
Val	24.51 ^{ab}	27.55 ^{ab}	24.81 ^{ab}	51.48 ^a	16.66 ^b	4.816	0.601	0.89	0.43	0.43
Non-Essential										
Ala	39.15 ^{ab}	41.67 ^a	9.71 ^b	26.87 ^{ab}	12.53 ^{ab}	5.157	0.16	0.28	0.13	0.55
Arg	40.65 ^a	33.65 ^{ab}	37.84 ^a	13.10 ^b	25.11 ^{ab}	3.781	0.15	0.60	0.05	0.06
Glu	40.61 ^{ab}	15.16 ^b	43.77 ^a	17.12 ^b	18.51 ^{ab}	4.331	0.09	0.28	0.05	0.18
Gly	18.79 ^c	62.61 ^{ab}	78.41 ^a	53.33 ^b	14.28 ^c	7.346	<0.01	<0.01	0.1301	<0.01
Ser	18.22 ^c	31.38 ^b	32.22 ^b	31.43 ^b	51.47 ^a	4.083	<0.01	<0.01	0.0033	0.90
Tyr	23.15 ^{ab}	21.21 ^b	53.44 ^a	37.34 ^{ab}	46.47 ^{ab}	5.034	0.16	0.26	0.15	0.65

346 SEM, standard error of the means.

347 1) Different treatments were as follow, untreated, intact sesame meal; GR20= 20 kGy gamma irradiation,
 348 GR40= 40 kGy gamma irradiation, MV3= microwaving for 3 minutes, MV5= microwaving for 5 minutes.

349 2) His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val,
 350 valine; Ala, alanine; Arg, arginine; Glu, glutamine; Gly, glycine; Ser, serine; Tyr, tyrosine.

351

352 The results of *In vitro* digestibility of the SSM dry matter (DMD), organic matter (OMD), and its
 353 digestible organic matter in dry matter (DOMD) were affected by treatment, differed among the
 354 treatments, the highest and lowest values for these variables which were observed in 5-min MW
 355 and 20 kGy-dose GR treatments, respectively (Table 5). The results of the current study regarding
 356 the *in vitro* trial are consistent with the results found by Zarei et al. (2015) who reported a decreased
 357 *in vitro* DMD and OMD of pomegranate seeds when irradiated by gamma ray and electron beam
 358 at 5-20 kGy doses. Similarly, Shishir et al. (2020) reported that the MW- treatment for 20–80
 359 seconds could increase the digestibility of some hays. However, contrary to what found in the
 360 current study, GR irradiation at 25-75 kGy (Ghanbari et al., 2015) and 8-12 kGy (Hahm et al.,
 361 2013) increased the *in vitro* digestibility of the DM and OM of sunflower meal and the whole
 362 cottonseed, respectively. Some reports indicate cross-linkages between protein molecules and
 363 trapping the protein within the cellulose fibers cell wall can have a large effect on the reduction of
 364 DMD and OMD (Tien et al., 2000). Moreover, GR-induced cross linkages between protein
 365 molecules increase the protein molecular weight, causing changes in the protein structure and
 366 making it insensitive to degradation by proteolytic enzymes (Englard and Seifter, 1990). The MW
 367 irradiation may affect the unfolding of the protein and its denaturation, helping the digestion of the
 368 proteins by exposing the hydrophobic amino acids (especially aromatics) that are well-suited for
 369 the activity of pepsin and trypsin enzymes (Bhat et al., 2021).

370 **Table 5.** *In vitro* digestibility² of unirradiated, and irradiated sesame meal (%).

Treatment ¹	Unirradiated	Irradiated				SEM	P-values			
		GR20	GR40	MW3	MW5		Unirradiated vs. irradiated	Unirradiated vs. GR	Unirradiated vs. MV	GR vs.MV
DMD	74.37 ^b	70.37 ^c	69.50 ^c	74.87 ^{ab}	75.62 ^a	0.839	<0.01	<0.01	0.04	<0.01
OMD	72.82 ^b	68.54 ^c	67.17 ^c	73.70 ^{ab}	74.46 ^a	0.983	<0.01	<0.01	<0.01	<0.01
DOMD	65.32 ^b	61.55 ^c	60.12 ^c	66.22 ^b	68.17 ^a	1.004	0.01	<0.01	0.05	<0.01

372 SEM, standard error of the means.

373 ¹⁾ Different treatments were as follow, untreated, intact sesame meal; GR20= 20 kGy gamma irradiation, GR40= 40
 374 kGy gamma irradiation, MV3= microwaving for 3 minutes, MV5= microwaving for 5 minutes.

375 ²⁾ DMD, digestible dry matter; OMD, digestible organic matter; DOMD, digestible organic matter in dry matter.

376 CONCLUSIONS

378 The results of this experiment showed that irradiation increased the *in-vitro* digestibility of sesame
379 meal, while the ruminal degradability of some amino acids, including methionine, phenylalanine
380 and isoleucine decreased. Overall, irradiation of SSM appears to increase its protein content
381 bypassing rumen degradation, thereby increasing the amount of digestible protein entering the
382 small intestine.

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538
539 ارزش غذایی کنجاله کنجد تیمار شده با اشعه گاما و مایکروویو

540
541 تجزیه گسترده پروتئین در شکمبه می تواند شاخص های کیفی آن مانند تعادل اسید آمینه و قابلیت هضم را کاهش دهد. هنگامی
542 که تجزیه پذیری پروتئین خام در شکمبه بالا باشد، پروتئین بای پس به روده و قابلیت هضم آن کمتر می شود. بنابراین، میزان
543 تخریب پروتئین در شکمبه میزان نیتروژن موجود برای میکروارگانیسم ها در شکمبه و تامین اسیدهای آمینه در روده کوچک
544 حیوان را تعیین می کند. هدف از این مطالعه بررسی اثر تابش پرتو گاما (GR) در دوزهای 20 و 40 کیلوگری و مایکروویو
545 (MW) در 800 وات به مدت 3 و 5 دقیقه بر سینتیک تخریب شکمبه و قابلیت هضم در شرایط آزمایشگاهی کنجاله کنجد
546 (SSM) بود. پارامترهای تجزیه پذیری نمونه های پرتو دهی شده با روش کیسه ناپلونی اندازه گیری شد. مقدار هیستیدین،
547 ترئونین، والین، آلانین، آرژنین، گلوتامین، گلیسین و سرین کمتر بود، اما میزان متیونین و فنیل آلانین در نمونه های تیمار شده
548 با GR و MW بیشتر از نمونه های تیمار نشده بود. تابش محتوای فیبر SSM را کاهش داد و ترکیب شیمیایی آن را در مقایسه
549 با SSM تیمار نشده تغییر داد. تجزیه پذیری شکمبه DM و CP در SSM تحت درمان کاهش یافت. تجزیه پذیری موثر
550 DM (ED) و CP در SSM تابیده شده با GR در مقایسه با SSM تابش شده با MW و کنترل کمتر بود. پس از شانزده
551 ساعت انکوباسیون شکمبه ای SSM تیمار شده، تجزیه پذیری ایزولوسین، متیونین، فنیل آلانین و ترئونین کمتر بود، اما
552 گلیسین و سرین بیشتر بود. قابلیت هضم در شرایط آزمایشگاهی DM (DMD) و ماده آلی (OMD)، و همچنین ماده آلی در
553 ماده خشک (DOMD) به ترتیب در SSM تابیده شده با GR و MW در مقایسه با SSM پرتو دهی نشده کمتر و بالاتر بود.
554 می توان نتیجه گرفت که تابش کنجاله کنجد با پرتو گاما در محافظت از پروتئین خام و برخی اسیدهای آمینه از جمله متیونین
555 و فنیل آلانین در برابر تخریب شکمبه موثر است.