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Influence of roasting, gamma ray irradiation and microwaving on ruminal dry matter and crude protein digestion of cottonseed

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ABSTRACT

The aim of the current study was to compare the effect of different physical processing methods including roasting at 140 °C for 15 (R15) or 30 minutes (R30), gamma ray irradiation (γ-irradiation) at doses of 15 (γ15), 30 (γ30) and 45 (γ45) kGy, and microwaving at 800 W for 2 (MW2), 4 (MW4) and 6 minutes (MW6) of whole cottonseed (WCS) on ruminal degradation. *In vitro* crude protein (CP) digestibility and gossypol contents were compared as well. *In situ* experiment was conducted on three permanent rumen-fistulated bulls. Gossypol content was decreased among treatments ($p < 0.05$). The lowest degradation rate of protein in rumen was obtained for γ45 treatment. The rate of degradation of the potentially degradation fraction was decreased for both dry matter (DM) ($p = 0.002$) and CP ($p = 0.006$) with different treatments. The lowest values for effective degradation in all passage rates were obtained with γ45. Both microwaving and γ irradiation showed difference for CP effective degradability parameter. The greatest value of *in vitro* CP digestibility ($p < 0.05$) was observed for a dose of 45 kGy gamma-irradiated cottonseed compared to untreated WCS. Based on the results, γ ray irradiation with 45 kGy was the most effective processing method in both reducing the gossypol content and escaping the protein through rumen for WCS in ruminant nutrition.

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Cottonseed; gamma ray irradiation; gossypol; microwaving; roasting

Introduction

According to the National Research Council (1989), the whole cottonseed (WCS) is a good source of energy, crude protein (CP) and dietary fiber for domestic animal nutrition as it contains about 2.22 Mcal/kg of net energy for lactation, 23% CP, 44% neutral detergent fiber (NDF), and 34% acid detergent fiber (ADF). Often WCS are fed at a maximum of 15% of the total diet to minimise the effect of unsaturated fat on ruminal fiber digestibility (Pires et al. 1997). Moreover, because of the difficulty in handling of this feed, processing of WCS to improve handling characteristics allows greater usage in ruminant rations (Bernard & Calhoun 1997). In addition, high gossypol content in un-treated WCS would cause negative effects in animal responses, such as decreasing growth and feed conversion, depression of fertility, as well as intestinal and internal organ abnormalities (Santos et al. 2003; Carruthers et al. 2007). Because of these negative effects, different methods (*e.g.*, chemical treatment with iron sulfate or calcium hydroxide) were considered to decrease this anti-nutrient effect in animal performance (Nagalakshmi et al. 2003). Beside the mentioned positive effects of WCS processing, processing has been used to increase the proportion of rumen un-degradable protein in whole oilseeds (Bernard & Calhoun 1997). Pena et al. (1986) reported that roasted WCS provided greater amounts of rumen undegradable protein than did extruded WCS. Hsu et al. (1993) reported linear decreases, as measured by a modified protein dispersion index, in protein degradability of delinted or linted WCS that were roasted at temperatures ranging from 134 to 210 °C and steeped for 30 min.

Gamma ray irradiation (γ) has also been recognised as a reliable and safe method to improve the nutritive value of feeds (Siddhuraju et al. 2002). Treatment of canola seed (Ebrahimi et al. 2009) and canola meal (Taghinejad et al. 2009) with γ irradiation was successful in reducing ruminal degradation of CP and increasing intestinal CP digestibility. Moreover, irradiation was effective in reducing phytic acid concentration in broad bean and velvet seed (Bhat et al. 2007).

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As another physical processing method applied for feedstuffs, microwave irradiation of different feeds also takes many considerations in different studies (Sadeghi & Shawrang 2007). It seems that different feedstuffs response differently to physical processing. For example Stutts et al. (1988) reported that soybean protein was more responsive to roasting than WCS protein, and Pena et al. (1986) reported roasting to be more effective than extruding at increasing escaping of WCS's protein through rumen. Most of the previous studies concluded decreased dry matter (DM) or CP degradation in rumen by applying different physical treatments from one hand (Ghanbari et al. 2012), and reducing anti-nutrients content from the other hand (Ebrahimi et al. 2009). It seems that there are limiting data for the comparison of effectiveness of these physical processing methods in oilseeds in animal nutrition. Therefore, the aim of the current study was to compare the effects of different physical processing methods (roasting, γ irradiation and microwave irradiation; at different levels) of cottonseed on DM and CP degradation in rumen. *In vitro* CP digestibility and gossypol contents were also evaluated and compared by applying these processing methods as well.

Materials and methods

Samples preparation and different treatments

The DM of WCS was determined by oven drying of 1 g sample in duplicate at 55 °C for 48 h (AOAC 1995). Sufficient water was then added to the sample to increase the moisture content to 250 g/kg. For roasting treatments, the samples of WCS were roasted at 140 °C for 15 (R15) and 30 (R30) minutes. The γ ray irradiation was completed in the Radiation Application Research School of Atomic Energy Organization of Iran by using a cobalt-60 irradiator at 20 °C. The dose rate determined by Fricke dosimetry (Holm & Berry 1970) was 0.36 Gy/s. Three paper packages of samples were irradiated to total doses of 15 (15 γ), (30γ) and (45γ) kGy in the presence of air. After irradiation and prior to sealing the plastic bags, samples were allowed to air equilibrate for 2 h, then frozen at −18 °C. For performing microwave treatments, three samples of 500 g were subjected to microwave irradiation (Butane microwave oven, Tehran, Iran, emitting a 2450 MHz microwave frequency) at a power of 800 W (1.33 W/g) for 2 (MW2), 4 (MW4) and 6 (MW6) minutes agitation. Three replicates were considered for each experimental treatment to perform later chemical analyses.

In situ *experiment*

The ruminal degradation of DM and CP in different treatments was compared. Three rumen-cannulated Taleshi bulls averaging body weight of 416 ± 18 kg were used in a 3 × 3 Latin square design experiment. Basal diet was consisting of 70% forage (consisted of 49% alfalfa hay and 21% wheat straw) and the rest 30% was concentrate which was consisted of 10.5% ground barley, 5.1% soybean meal, 7.5% cottonseed meal, 6% wheat bran, 0.3% calcium carbonate and 0.6% vitamin-mineral premix. The animals were kept in individual cages and have free access to water. The animals were fed two times daily at 08:00 and 16:00 h according to their nutrient requirements of National Research Council (1989). The treatment samples were ground to pass 2 mm screen size. Six grams of samples were weighed into nylon bags (10 cm × 20 cm with 45 μm pore size). The bags were labeled with a waterproof permanent marker. Duplicates were incubating for 0, 2, 4, 8, 16, 24, and 48 h, just before morning meal. After incubation, and immediately after removal from the rumen, bags were put in ice water to stop microbial fermentation, and then washed under tap water until the rinsing water became colorless. Then, bags were dried at 55 °C for 48 h in a forced air oven and then weighed. Aliquots of the bag residuals were used for DM and CP determination.

Chemical analyses

The DM content was determined in feed samples and nylon bag residues with three replicates at 55 °C for 48 h. The N content in feeds, residues after rumen and *in vitro* incubation was determined (triplicates/treatment) according to AOAC (1995; Method 984.13). Ash was determined by burning duplicate 2 g samples at 600 °C for 2 h in a muffle furnace (AOAC, 1995; Method 942.05). Neutral detergent fiber and ADF were analysed in all samples sequentially according to the method of Van Soest et al. (1991), using an automatic fiber analyser (Fibertec System M, Tecator, Hoganas, Sweden). The NDF was determined without α-amylase and sodium sulfite, and expressed with residual ash, while the ADF was also expressed inclusive of residual ash. A standard method was used to determine ether extract (AOAC, 1995; Method 920.39). The solution of propan and hexan were used to determining of free gossypol content, while di-ethyl formamid was used for determining the whole gossypol content (ISO 6866, 1985). In this method, gossypol has been turned to gossypol di-alanine in the present of alanine and then it was measured in 435 and 445 nm.

In vitro *CP digestibility*

Digestibility of rumen undegraded CP was estimated using the three-steps *in vitro* procedure of Calsamiglia and Stern (1995). Samples of the ruminal undegradable fraction collected at the 16 h ruminal incubation period containing 15 mg N was incubated for 1 h in 10 mL of 0.1 N HCl solution containing 1 g/L of pepsin. Following incubation, the pH was neutralised with 0.5 mL of 1N NaOH and 13.5 mL of phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin. Samples (triplicates/treatment) were incubated for 24 h at 38 °C, and then undigested protein was precipitated using trichloroacetic acid (3 mL TCA). Samples were centrifuged at 10 000 × *g* for 15 minutes at room temperature, and then supernatant was analysed for soluble N (AOAC, 1995; Method 984.13). *In vitro* digestibility of CP was calculated as soluble N divided by the amount of initial sample N content (i.e. nylon bag residues).

Calculations and statistical analyses

Digestion kinetics of DM or CP were determined according to the equation of Ørskov and McDonaled (1979) as $P = a + b(1 - e^{-ct})$ where P is the amount degraded at a time, 'a' the washout fraction, 'b' the potentially degradable fraction, 'c' the constant rate of disappearance of b, and 't' the time of incubation (h). The effective degradability (ED) in the rumen was calculated as: $ED = a + [(b \times c)/(c + k)]$, using NEWAY software; where 'a' is the water-soluble fraction or washout fraction, 'b' the potentially degradable fraction, 'c' the rate of degradation of 'b', and 'k' the passage rate of the digesta out of the rumen which estimated at ruminal outflow rates of 0.02, 0.05 and 0.08/h.

The data were analysed to evaluate differences among the eight treatments (R15, R30, γ15, γ30, γ45, MW2, MW4, MW6) with the untreated (control group) according to the GLM procedure of SAS (1996).

Degradability data were analysed as a completely randomised block design according to the GLM procedure of SAS (1996) with the statistical model $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$.

Chemical composition and gossypol data were analysed as a completely randomised design according to the GLM procedure of SAS (1996) with the statistical model of $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$, where Y_{ijk} is dependent variable, μ is overall mean, T_i is different processing methods (roasting, γ ray irradiation and microwave irradiation), B_j is animal effect, and e_{ijk} is residual error, assumed normally and independently distributed. The results were presented as mean value ± standard error and differences were considered to indicate a trend toward significance at $0.05 < p < 0.10$.

Results

No effect was observed ($p > 0.05$) on the chemical composition of WCS due to the physical treatments. However, gossypol contents were decreased ($p < 0.05$) with all physical treatments compared to untreated WCS and the lowest value ($p < 0.05$) was achieved for 45 kGy dose irradiated method (Table 1).

The wash out fraction 'a' of DM was decreased by different treatments whereas the lowest value ($p < 0.05$) was observed for R30 treatment with no differences ($p > 0.05$) between R15, γ15, γ30, γ45, MW2 and MW4 than control. Potentially degradable fraction 'b' of DM did not differ among treatments ($p > 0.05$); however, the degradation rate of potentially degradable fraction for DM 'c' decreased with different treatments compared to untreated one with the lowest value ($p < 0.05$) for MW6 treatment with no differences between R15, R30, γ15, γ30, MW2 and MW4 than untreated one. Effective degradation for DM was differed among treatments for 0.08, 0.05 but not for 0.02 h^{-1} (Table 2). All the parameters 'a', 'b' and 'c' of CP were

Table 1. Chemical composition[1] and gossypol content (g/kg, mean ± standard error) of untreated and physically treated cottonseed.

Treatments[2]	DM	CP	TP	NDF	ADF	EE	Ash	Gossypol
Untreated	918.4 ± 17.1	226.4 ± 11.7	210.4 ± 7.1	511.9 ± 13.4	409.3 ± 11.4	240.5 ± 10.3	42.1 ± 0.8	0.47 ± 0.01^{a}
R15	927.5 ± 21.8	227.6 ± 10.5	206.9 ± 9.0	516.8 ± 14.2	410.7 ± 11.2	238.7 ± 11.5	42.3 ± 0.9	0.40 ± 0.008^{bc}
R30	935.0 ± 22.7	232.9 ± 8.6	215.1 ± 10.4	508.3 ± 11.7	415.6 ± 9.6	237.7 ± 8.5	40.8 ± 0.7	0.37 ± 0.01^{cd}
γ15	910.9 ± 23.6	219.1 ± 9.4	208.9 ± 8.5	515.6 ± 12.9	405.7 ± 10.1	235.2 ± 9.2	44.3 ± 0.7	0.42 ± 0.01^{b}
γ30	921.8 ± 17.1	227.1 ± 10.6	210.7 ± 8.9	517.9 ± 12.2	411.8 ± 9.3	238.8 ± 10.3	45.2 ± 0.6	0.38 ± 0.01^{c}
γ45	918.3 ± 24.2	225.6 ± 8.9	212.2 ± 9.2	510.7 ± 13.1	413.6 ± 10.0	237.8 ± 12.1	42.7 ± 1.1	0.34 ± 0.008^{d}
MW2	922.4 ± 26.9	230.8 ± 8.3	211.3 ± 8.4	517.8 ± 11.6	408.5 ± 9.7	234.7 ± 8.1	41.5 ± 0.6	0.46 ± 0.01^{ab}
MW4	931.6 ± 25.1	223.5 ± 9.6	209.5 ± 7.4	515.8 ± 12.0	407.1 ± 10.4	234.5 ± 8.6	41.1 ± 0.9	0.43 ± 0.01^{b}
MW6	938.3 ± 20.8	228.7 ± 10.1	217.3 ± 8.6	512.5 ± 11.9	412.7 ± 9.8	229.8 ± 9.5	41.4 ± 1.2	0.41 ± 0.009^{bc}

Mean in the same column with different letters differ ($p < 0.05$).
[1]DM = dry matter; CP = crude protein; TP = true protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract.
[2]Different treatments were as follow: untreated = intact whole cottonseed, R15 = roasting for 15 minutes, R30 = roasting for 30 minutes, γ15 = 15 kGy gamma irradiation, γ30 = 30 kGy gamma irradiation, γ45 = 45 kGy gamma irradiation, MW2 = microwave irradiation for 2 minutes, MW4 = microwave irradiation for 4 minutes, MW6 = microwave irradiation for 6 minutes.

Table 2. Rumen degradation parameters of dry matter of untreated and physically treated cottonseed.

	Degradation parameters[2]			Effective degradability at outflow rate (g/kg)		
Treatments[1]	*a* (g/kg)	*b* (g/kg)	*c* (h^{-1})	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
Untreated	31.7 ± 2.5^{a}	23.2 ± 2.8	0.152 ± 0.01^{a}	52.2 ± 3.0	49.2 ± 2.7^{a}	46.9 ± 2.5^{a}
R15	29.9 ± 2.4^{ab}	25.1 ± 3.5	0.13 ± 0.038^{ab}	51.6 ± 5.4	48.0 ± 4.8^{ab}	49.4 ± 4.5^{a}
R30	24.6 ± 2.7^{c}	26.0 ± 3.6	0.110 ± 0.02^{ab}	46.9 ± 3.3	42.6 ± 3.0^{b}	39.7 ± 2.9^{b}
γ15	31.9 ± 4.1^{a}	22.8 ± 3.4	0.130 ± 0.06^{ab}	50.4 ± 5.5	47.1 ± 5.3^{ab}	44.9 ± 4.9^{ab}
γ30	30.6 ± 3.4^{ab}	23.6 ± 2.8	0.116 ± 0.04^{ab}	49.0 ± 4.2	46.0 ± 4.2^{ab}	44.0 ± 3.0^{ab}
γ45	28.5 ± 1.9^{ab}	24.2 ± 3.0	0.105 ± 0.01^{b}	50.4 ± 2.1	45.0 ± 1.7^{ab}	42.4 ± 1.4^{ab}
MW2	31.0 ± 1.8^{ab}	22.1 ± 2.5	0.122 ± 0.04^{ab}	49.8 ± 3.2	46.4 ± 2.9^{ab}	44.0 ± 2.9^{ab}
MW4	29.7 ± 1.2^{ab}	24.1 ± 2.8	0.011 ± 0.02^{ab}	50.0 ± 2.6	46.1 ± 2.2^{ab}	43.5 ± 2.1^{ab}
MW6	26.0 ± 3.6^{bc}	26.1 ± 2.7	0.094 ± 0.02^{b}	47.5 ± 5.1	43.0 ± 5.8^{b}	40.5 ± 5.6^{b}

Mean in the same column with different letters differ ($p < 0.05$).
[1]Different treatments were as follow: untreated = intact whole cottonseed, R15 = roasting for 15 minutes, R30 = roasting for 30 minutes, γ15 = 15 kGy gamma irradiation, γ30 = 30 kGy gamma irradiation, γ45 = 45 kGy gamma irradiation, MW2 = microwave irradiation for 2 minutes, MW4 = microwave irradiation for 4 minutes, MW6 = microwave irradiation for 6 minutes.
[2]*a* = wash out fraction degradation, *b* = potentially degradable fraction, *c* = rate constant of degradation of *b* fraction.

Table 3. Rumen degradation parameters of crude protein of untreated and physically treatedf cottonseed.

	Degradation parameters[2]			Effective degradability at outflow rate (g/kg)		
Treatments[1]	*a* (g/kg)	*b* (g/kg)	*c* (h^{-1})	0.02 h^{-1}	0.05 h^{-1}	0.08 h^{-1}
Untreated	46.6 ± 2.5^{a}	44.6 ± 3.7^{a}	0.132 ± 0.01^{a}	85.3 ± 2.8^{a}	78.9 ± 2.9^{a}	74.3 ± 3.1^{a}
R15	45.4 ± 2.5^{bc}	45.3 ± 3.2^{d}	0.109 ± 0.02^{b}	83.5 ± 2.9^{ab}	76.2 ± 3.0^{ab}	71.3 ± 2.2^{ab}
R30	39.1 ± 2.2^{cd}	50.3 ± 2.6^{bc}	0.091 ± 0.01^{bc}	80.3 ± 4.9^{bc}	71.5 ± 4.8^{bc}	65.8 ± 4.9^{cd}
γ15	43.9 ± 1.2^{ab}	47.3 ± 4.6 ^{cd}	0.114 ± 0.01^{ab}	84.1 ± 3.4^{ab}	76.7 ± 2.5^{ab}	71.6 ± 2.9^{c}
γ30	39.6 ± 4.1^{cd}	49.6 ± 4.8^{bc}	0.090 ± 0.01^{bc}	80.1 ± 3.7^{bc}	71.4 ± 3.8^{bc}	65.7 ± 2.0^{cd}
γ45	35.1 ± 3.3^{de}	53.9 ± 3.4^{ab}	0.072 ± 0.01^{c}	77.2 ± 2.8 ^{d}	66.8 ± 2.6^{cd}	60.6 ± 3.7^{d}
MW2	42.1 ± 2.6^{c}	48.0 ± 4.0^{bc}	0.114 ± 0.01^{ab}	82.9 ± 1.0^{bc}	75.4 ± 1.0^{ab}	70.2 ± 2.4^{bc}
MW4	38.3 ± 4.2^{de}	52.1 ± 1.6^{ab}	0.098 ± 0.01^{bc}	81.5 ± 4.3^{bc}	72.7 ± 4.2^{bc}	66.9 ± 1.3^{cd}
MW6	33.1 ± 3.9^{d}	56.7 ± 3.1^{a}	0.078 ± 0.01^{c}	78.0 ± 3.1 ^{cd}	67.4 ± 2.5^{d}	60.8 ± 4.2^{d}

Mean in the same column with different letters differ ($p < 0.05$).
[1]Different treatments were as follow: untreated = intact whole cottonseed, R15 = roasting for 15 minutes, R30 = roasting for 30 minutes, γ15 = 15 kGy gamma irradiation, γ30 = 30 kGy gamma irradiation, γ45 = 45 kGy gamma irradiation, MW2 = microwave irradiation for 2 minutes, MW4 = microwave irradiation for 4 minutes, MW6 = microwave irradiation for 6 minutes.
[2]*a* = wash out fraction degradation, *b* = potentially degradable fraction, *c* = rate constant of degradation of *b* fraction.

differed among different treatments. The '*a*' fraction decreased from 46.6% for untreated WCS to 33.1% for MW6. The parameter '*c*' which shows the degradation rate of fraction '*b*' was decreased from 0.132 to 0.072 h^{-1} in γ45 treatment. At all considered passage rates, the lowest ED value was observed for a dose of 45 kGy irradiated WCS, whereas the untreated WCS had the highest value (Table 3).

In vitro protein digestibility differed among treatments ($p < 0.05$). The greatest value was observed with the treatment γ45 compared to untreated WCS (Table 4).

Discussion

Chemical composition and gossypol content

Chemical composition of WCS was not differed by different physical processing. This result was consistent with the previous studies of Shawrang et al. (2007) and Taghinejad et al. (2009), who did not found any effect of γ ray irradiation on the chemical composition of soybean and canola meal.

Feeding large amounts of WCS is the possibility of gossypol toxicity; however, ruminants have a well-developed rumen microbes which enable them to detoxify gossypol at some levels. Researchers studied to detoxify gossypol and proposed a number of methods, such as, solvent extraction by liquid cyclone and/or acetone (Gardner et al. 1976); chemical treatment with iron sulfate (Barraza et al. 1991) or calcium hydroxide (Nagalakshmi et al. 2003). Unfortunately, these methods affect the protein nutritive quality and are not in commercial use now. Moreover, such methods affect the flavor of the feed (Alyevand et al. 1967) compared to irradiation methods which do not affect the flavor of the feed. The results of the present study showed that among physical processing methods, γ ray irradiation could apply to reduce gossypol content that probably would improve performance of animal fed this feedstuff. The lowest content of gossypol was observed in γ45 treatment and roasting for 30 minutes. However roasting decreased gossypol content, it is not a preferred method. Roasted cottonseed always had higher available gossypol than unprocessed

Table 4. *In vitro* protein digestibility of untreated and physically treated cottonseed.

Treatments[1]	Protein digestibility *in vitro* (g/kg)
Untreated	55.6 ± 1.8^{c}
R15	57.0 ± 2.3^{b}
R30	58.9 ± 2.5^{ab}
γ15	56.4 ± 1.4^{bc}
γ30	58.7 ± 1.5^{ab}
γ45	59.8 ± 1.8^{a}
MW2	56.3 ± 2.8^{bc}
MW4	56.1 ± 2.0^{bc}
MW6	58.8 ± 2.1^{ab}

Mean in the same column with different letters differ ($p < 0.05$).
[1]Different treatments were as follow: untreated = intact whole cottonseed, R15 = roasting for 15 minutes, R30 = roasting for 30 minutes, γ15 = 15 kGy gamma irradiation, γ30 = 30 kGy gamma irradiation, γ45 = 45 kGy gamma irradiation, MW2 = microwave irradiation for 2 minutes, MW4 = microwave irradiation for 4 minutes, MW6 = microwave irradiation for 6 minutes.

cottonseed suggesting that cottonseed that has gone through a heat process is likely to be more toxic (Bernard & Calhoun 1997). Therefore, only the effect of gamma-radiation will be discussed. Decreasing amount of gossypol with gamma-radiation was relatively small than those report by Jaddou et al. (1983), who reported that 25 KGy of gamma-radiation is the best level for decreasing gossypol content in cottonseeds meal. The differences between our results and those of Jaddou et al. (1983) may be related with different methods of gossypol determination, different cotton seeds variety, different maturity stage, different field practices and many other factors. Decreased ratio of gossypol may not enough as 'detoxification' even after 45 kGy gamma-irradiation (Gadelha et al. 2014). The mechanism in which gamma radiation can decrease gossypol content is unknown; but oxidation of phenol rings might be involved. Moreover, it has been illustrated that gossypol molecule aggregation, gossypol cross-linking with other molecules, and gossypol molecule fragmentation or breakdown may cause gossypol destruction (Mahmoudabad & Taghinejad 2011).

DM and CP ruminal degradation

Different physical processing method decreased wash out fraction of DM and CP in WCS. Analysing the acquired data emphasis that from the processing method which applied on WCS, as the more powerful processing was microwave irradiation for 6 minutes (i.e., MW6), with more reduction in '*a*' parameter of DM and CP. Moreover, gamma-radiation and roasting had good results. Previous studies had been conducted to evaluate the effect of different physical methods on animal feedstuffs. Sadeghi and Shawrang (2006a) showed that microwave treatment reduced the rumen degradable starch fraction of corn grain. Moreover, Sadeghi and Shawrang (2006b, 2007) obtained decreased CP degradation after treating canola meal and cottonseed meal with microwave compared with untreated samples. Ghanbari et al. (2012) reported that γ ray irradiation of cottonseed meal had significant effect on decreasing CP and amino acid degradation in rumen but this effect was less than that of electron beam treatment. Taghinejad et al. (2009) stated that gamma irradiation at dose of 30 and 45 kGy decreased the washout fractions of CP of canola meal with increasing the potentially degradable fraction (*b*) of CP at dose of 45 kGy. Moreover, Aldrich et al. (1995) by roasting of soybean with different temperatures concluded that roasting processing could increase escape of CP through rumen with more amino acids concentrations in the intestines of roasted soybean fed steers. Most of these studies which applied different physical treatments on feedstuffs reported reduction in CP degradation in rumen and hence resulted in CP escaping to small intestine through rumen. Different mechanisms were presented to justify these findings; Broderick and Craig (1980) suggested that heat treatment of feedstuffs can decrease degradation of DM and CP by blocking reactive sites for microbial proteolytic enzymes. Heat treatment increased the supply of dietary CP to the duodenum in a study conducted by Tagari et al. (1986). Moreover, Van Soest (1982) declared the increase in CP escape through heating as a result of CP denaturation and reduction in solubility and degradation rate which favors more rumen escape of intact undegraded proteins to the lower gastrointestinal tract. Applying irradiations on feedstuffs decreased CP degradability, and this might be due to the occurrence of cross-linking of polypeptide chains, denaturation and protein aggregation (Gaber 2005; Abu et al. 2006). Irradiation induces unfolding of protein structures and their denaturation, thereby increasing surface hydrophobicity of proteins by exposing non-polar groups (Woods & Pichaev 1994). Gaber (2005) showed that if secondary and tertiary structures of a protein are unfolded, proteins may be converted to high molecular weight aggregates due to generation of inter-protein cross-linkages, hydrophobic and electrostatic interactions, and formation of disulfide bonds (Davies & Delsignore 1987; Lee Maire et al. 1990). Moreover, hydrophobic interactions lead to aggregation, followed by coagulation and precipitation (Englard & Seifter 1990), probably reducing ruminal CP degradability (Ebrahimi et al. 2009). Abu et al. (2006) demonstrated that γ-irradiation decreases protein solubility due to denaturation. Insoluble nitrogen is very important in ruminant nutrition as solubility renders the nitrogen more available for microbial activity and

metabolism (Van Soest 1994). Microwave presented the same as other physical processing methods caused to decrease protein degradation in rumen (Sadeghi & Shawrang 2006b, 2007). Our results suggest that among all different treatments, irradiation of γ with 45 kGy dose caused to the lowest protein degradation in rumen. Maximum potential degradability ($a+b$) of CP for untreated WCS indicating WCS protein to be relatively high degradable in the rumen. This value showed the greatest reduction in γ45 treatment (89%) compared to other treatments. Moreover, this finding showed that applying γ radiation in a dose of 45 kGy could be recommendable for escaping protein through rumen degradation and increase its digestibility in small intestine of ruminant animals.

Conclusions

The results suggested that all physical processing methods (roasting, γ ray irradiation and microwaving) decreased gossypol content of WCS. Moreover, protein degradation in rumen was reduced and its digestibility was improved in both within and between treatments. Gamma irradiation at a dose of 45 kGy (γ45) could be recommendable as more useful physical processing method to reduce the gossypol content, to increase escaping of protein through rumen and to increase digestibility and absorption of protein of WCS in the small intestine.

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Disclosure statements

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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