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Effects of Gamma Irradiation on Nutrient Composition, Anti-nutritional Factors, *In vitro* Digestibility and Ruminal Degradation of Whole Cotton Seed

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ABSTRACT

Whole cotton seed (WCS) has become one of the major feed ingredients in TMR for dairy cattle in Korea, and WCS for feed use is mostly imported from abroad. Since this genetically modified oil seed is usually fed to the animal in raw state, its germination ability, if last long, often causes concerns about ecological disturbances. In the process of looking for effective conditions to remove germination ability of WCS this study had the objectives to evaluate the nutritional effects of gamma irradiation at doses of 8, 10 and 12 kGy on changes in nutrient contents, anti-nutritional factors, *in vitro* digestibility and ruminal degradability. No significant differences were found in proximate analysis of nutrients between raw WCS and gamma irradiated one. Glycine and threonine contents significantly increased when the WCS was exposed to gamma ray as compared to untreated WCS ($p<0.05$). As for fatty acid composition, no significant differences were observed with the irradiation treatment. Free gossypol in WCS was decreased ($p<0.05$) by gamma irradiation treatment. Of the 3 different levels of gamma irradiation, a dose of 12 kGy was found to be the most effective in reducing free gossypol concentration. Results obtained from *in situ* experiment indicated that gamma irradiation at a dose of 10 kGy significantly ($p<0.05$) lowered rumen degradability of both dry matter and crude protein as compared with raw WCS. However, there were no significant differences in rapidly degradable and potentially degradable fractions of crude protein due to 10 kGy gamma irradiation. Overall, this study show that gamma irradiation at a dose of 10 kGy is the optimum condition for removing germination ability of WCS, and could improve nutritive value for the ruminant with respect to the decrease in both ruminal protein degradability and gossypol content of WCS.

(**Key words** : Whole cotton seed, Gamma irradiation, Nutrient composition, Rumen degradability)

INTRODUCTION

Whole cotton seed (WCS) is popular feed for dairy cattle and is uniquely high in fiber, energy and protein (Prieto et al., 2003). In a nationwide survey to determine the feedstuffs fed to lactating dairy cows, it was reported that approximately 40% of dairy producers in the United States fed WCS (Mowrey and Spain, 1999). Recent genetic modifications have produced cotton plants more resistant to pests and tolerant to herbicides (Bertrand et al., 2005). However, since it is mostly GM plant imported from foreign countries and being fed in raw state, it is apprehended that it will disturb existing ecology of the country unless dispersion of the seed is under proper control. Even a part of the seed within the excreta used to maintain its ability of germination, which would occasionally come in sight around farmstead.

One of the potential processing methods to remove germination ability of the seed would be irradiation, a technique exposing food of large amount to ionizing radiation such as gamma rays emitted from radioisotope ^{60}Co (Diehl, 2002). Irradiation with gamma rays has been recognized as a safe and reliable method for improving nutritional value and removing or inactivating certain anti-nutritional factors in feeds (Farkas, 2006). The irradiation of seeds with high doses of gamma ray disturbs the synthesis of protein, hormone balance, leaf gas-exchange and enzyme activity (Borzouei et al., 2010). It has been reported that gamma irradiation (10 and 12 kGy) given to whole cotton seeds removed the germination ability of seeds (Kwon et al., 2012). Furthermore, Chaudhuri (2002) reported that the irradiation of wheat seeds reduced shoot and root lengths upon germination. Gamma rays belong to ionizing radiation and are the most energetic form of such electromagnetic radiation, having the energy level from 10 kilo electron volts (keV) to several hundred keV. Therefore, they are more penetrating than other types of radiation such as alpha and

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beta rays (Kovacs and Keresztes, 2002).

Plant seeds contain certain anti-nutrient substances that have an adverse effect on the nutritive properties of the seed. Phytic acid and its derivatives are known to bind essential dietary minerals, thus making them unavailable or only partially available for absorption by animals (Han, 1988). Although phytase activity has been shown to be present in the small intestine of various animals, its activity is not sufficient to utilize dietary phytic acid.

Gossypol, a yellow polyphenolic compound found primarily in the pigment glands of the cotton plant, exists in both the free and bound forms. In the intact whole seed, gossypol is mostly found in the free form (Mena et al., 2004). A primary concern associated with feeding large amounts of cotton seed is the possibility of gossypol toxicity (Arieli, 1998). Feeding recommendations for cotton seed products have been based on nutrient content, but little information is available on safe feeding levels of free and total gossypol in diets for lactating dairy cows (Mena et al., 2001). Gharaghani et al. (2008) reported that gamma irradiation seems to be a good procedure to improve the nutritional quality of canola meal for broiler chickens.

Therefore, we conducted this study in order to elucidate the effects of different doses of gamma irradiation on nutritional content, *in vitro* digestibility, degradation in the rumen and anti-nutritional contents of whole cotton seed.

MATERIALS AND METHODS

1. Sample preparation and irradiation treatment

Linted whole cotton seed (WCS), imported from USA, was purchased from Korea Silo Ltd. (Inchon, Korea). It was exposed to gamma rays of ^{60}Co with doses of 8, 10, and 12 kGy, which were proved to be optimal strength in previous germination tests (Kwon et al., 2012). Irradiation treatment was carried out at Korea Atomic Energy Research Institute (KAERI). To get representative analytical samples, WCS was ground in 2 steps, firstly using Disc mill (BM-D-100, McCoy Corporation, TX, USA) and then laboratory cyclone mill (SMBT 3000, Shinmyung BT, Seoul, Korea).

2. Nutrients analysis

WCS samples were analyzed for DM, CP and lipid by AOAC procedures (AOAC, 1995). NDF and ADF were determined using an ANKOM200 Fiber Analyzer (ANKOM Technology, NY, USA). Fatty acid composition was measured by gas chromatography with flame ionization detection (GC-FID) using a HP-6890 GC instrument (Hewlett-Packard, CA, USA) with a FID and a HP-FFAP column (30 m × 0.32 mm × 0.25 μm). The oven temperature conditions were as follows: the initial temperature of the column, 100°C, was held for 2 min, after which the temperature was ramped a 4°C / min to 240°C, where it was held for 20 min. The flow rate of the carrier gas (helium) was 1.5 mL/min. Fatty acids were identified by comparing the relative retention times of FAME peaks with those of standards. Total amino acid composition was determined with an amino acid analyzer (Biochrom 20, Pharmacia Biotech, Buckinghamshire, England). Samples were hydrolyzed in 6 N HCl in evacuated sealed tubes at 110°C for 24 h. The hydrolysates were evaporated to dryness in a vacuum evaporator and then diluted with sodium citrate buffer for analyzing amino acids.

3. Gossypol analysis of WCS

The AOCS procedure was used for extraction and determination of free gossypol in the samples of WCS (AOCS, 1987). Briefly, gossypol extraction was completed in the presence of hexane for the determination of free gossypol. Then, gossypol was converted to gossypol-dianiline using aniline. Measurement of the absorbance was conducted at the wavelength of maximum absorbance, i.e. 440 nm. The purity of standard gossypol acetic acid was checked and its absorption spectrum in cyclohexane was recorded in the UV region. The absorption was measured at 358 nm. Different concentrations of this standard were used to draw the standard graph. Thus, an average factor was obtained to calculate the gossypol content of the samples.

4. Phytic acid analysis of WCS

The extraction and estimation of phytic acid in the WCS were done according to De Boland et al. (1975). Briefly, samples were extracted with a solution containing 12 mL/L HCl and 100 g/L Na_2SO_4 and centrifuged at 1,430 × g for 15 min at room temperature. Then, 10 ml of extract was diluted with 10 ml of water and treated with 5 ml solution of 4 g/L $FeCl_3$ in 6 mL/L HCl containing 50 g/L Na_2SO_4. After boiling for 15 min, the samples were centrifuged at 3,220 × g for 15 min at room temperature. Analysis of phosphorus

content of the insoluble ferric salt was determined colorimetrically at 660 nm after digestion with 3 ml of sulfuric acid and 5 ml nitric acid. Phytic acid was calculated on the assumption that it contains 282 g/kg of phosphorus.

5. *In vitro* digestibility

Fresh rumen contents from two ruminal-fistulated Holstein cows were collected 3 h after the morning feeding for use in the cultures. Approximately, 1.5 L rumen fluid was collected by straining digesta through four layers of cheese cloth into a flask flushed previously with CO_2. In this trial, 0.5 g of each sample was placed in to 50 mL tubes, the incubation inoculum was prepared by diluting the digesta inoculum with the McDougall's buffer and fresh rumen liquor in a 1:4 (vol/vol) ratio and stirring in a water bath at 39°C with purging CO_2 until its use (10~15 min later). The inoculated test tubes were allocated in shaking incubator at 39°C for 48 h.

After incubation, the tubes were kept in ice and centrifuged at 10,000 rpm for 15 min and the supernatant was discarded. Samples were subjected to rumen fluid and pepsin-HCl digestion as described by Tilley and Terry (1963). The residue remaining after drying was used to calculate the *in vitro* dry matter digestibility.

6. *In situ* ruminal degradability

Two ruminally cannulated Holstein heifers were used to determine *in situ* dry matter and protein degradability of both WCSs. The animals were maintained on a standard diet having forage to concentrate ratio of 7 to 3. WCS samples (3.5 g) were weighed in to 5 × 10 cm nitrogen-free polyester bags (ANKOM Technology, NY, USA) with a 50 μm mean pore size. Before placement in the rumen, the bags (n = 24) were soaked briefly in water, and then introduced serially into the rumen and suspended for 2, 4, 8, 16, 24 and 48 h in four replicates for each incubation time. Solubility at 0 h was evaluated by immersing the bags in warm water (39°C). At the end of each incubation time, the bags were removed from the rumen and rinsed thoroughly with cold tab water until rinsing water became colorless. Bags then were placed in a forced air oven set at 60℃ for 48 h. The residue was analyzed for dry matter by drying oven (60°C / 48 h), and nitrogen content by micro-Kjeldahl method.

7. Statistical analysis

All analyses were replicated a minimum of 3 times. Data were analyzed by one-way analysis of variance using the SAS software package (version 9.2; SAS Institute, Cary, NC, USA). All values were presented as means ± SD. Group means were considered to significantly differ at $p<0.05$, as determined by Duncan's multiple range analysis or Student's t-test.

RESULTS AND DISCUSSION

When WCS was subjected to gamma irradiation treatment at 8, 10 and 12 kGy, the germination percentage was significantly decreased (Kwon et al., 2012). However, no studies have yet reported about chemical composition and degradability of gamma irradiated WCS.

No significant differences were found in proximate analysis of nutrient contents between WCS and gamma irradiated one ($p>0.05$) (Table 1). Similar results were obtained by Taghinejad

Table 1. Chemical composition of untreated and irradiated whole cottonseed

(%, DM basis)

Parameters	Untreated WCS	Gamma-irradiated WCS		
		8 kGy	10 kGy	12 kGy
DM	95.00±0.11[1)]	98.27±0.13	98.70±0.13	98.83±0.02
Ash	4.14±0.04	4.06±0.07	4.03±0.05	4.12±0.07
CP	22.21±0.07	21.81±0.09	21.92±0.10	22.10±0.12
EE	17.76±0.23	17.39±0.20	18.18±0.12	17.55±0.34
NDF	52.12±0.26	51.72±0.58	52.50±0.86	51.93±0.40
ADF	39.01±0.19	40.77±0.47	39.87±0.74	40.52±0.58
NFC[2)]	3.77±0.15	5.02±0.10	3.37±0.13	4.30±0.14

[1)] Values are mean ± standard deviation (n = 3).
[2)] NFC = 100 − (Ash + CP + EE + NDF).

(2009), who reported gamma irradiation had no effect on chemical composition of soybean. Moreover, extensive research showed that the contents of macronutrients (carbohydrates, proteins and lipids) were relatively stable against irradiation doses up to 10 kGy (Ahn and Lee, 2003). However, gamma irradiation has a slight effect on the amino acid profile at recommended doses to foods (WHO, 1999). The contents of compositional amino acids of WCS and gamma irradiated WCS are showed in Table 2. Changes in some amino acid content were observed with gamma irradiation treatment. Lower levels of arginine, proline, tyrosine, methionine and isoleucine were obtained in WCS as compared to gamma irradiated WCS ($p<0.05$). Glycine and threonine levels were significantly higher in gamma irradiated WCS than untreated one ($p<0.05$). Authors concluded that simple amino acids increased upon irradiation such as glycine, which undergo reductive deamination and decarboxylation (Piri et al., 2011). Wang and von Sonntage (1991) reported that sulfur containing as well as aromatic amino acids are, in general, the most sensitive to irradiation. Other amino acids existed in unchanged contents. The differences, however, seemed partly to include some variation originated from sampling spot and also some that might come from different environmental factors affecting the plant quality and seed morphology during the vegetation period (Van Soest, 1994). As for fatty acid composition, no significant differences were observed by the gamma irradiation treatment (Table 3). Linoleic acid ($C_{18:2}$) was consistently present in the highest quantity with averaged 56% of the total fatty acid. The fatty acid existing in second highest quantity was palmitic acid ($C_{16:0}$) showing 23% on the average, followed by oleic acid ($C_{18:1}$) averaged 16%. Those three major fatty acids existing in WCS were reported by Bertrand et al. (2005) and Berberich et al. (1996). Kwon et al. (1988) found in a Korean garlic cultivar that immediately after gamma irradiation with 100 kGy there were no differences in levels of linoleic, palmitic, oleic and linolenic acids, the predominant fatty acids of bulbs. The present findings are in agreement with the nutrient contents data previously obtained with gamma irradiation treatment.

A concern associated with feeding large amounts of WCS is the possibility of gossypol toxicity (Calhoun et al., 1995). Although ruminants have a well-developed rumen microbial population that is able to detoxify by converting gossypol from free form to bound one within the rumen and impeding its absorption into the blood, it is possible that feeding excessive amounts of the toxin in free form may overpass the protective mechanism and impair animal performance (Mena et al., 2001). Therefore, several solvent

Table 2. Contents of compositional amino acids of untreated and irradiated whole cotton seed

(mg/100g)

Components	Untreated WCS	Gamma-irradiated WCS		
		8 kGy	10 kGy	12 kGy
Aspartic acid	1,959.2±11.9$^{b1)}$	1,964.2± 6.5^{b}	1,933.7±10.1^{c}	2,020.1±29.0^{a}
Serine	888.3± 3.5^{b}	919.9± 5.2^{a}	892.0± 6.8^{b}	918.0±30.0^{a}
Glutamic acid	4,587.4± 4.3^{a}	4,490.7±15.7^{b}	4,424.0±28.5^{c}	4,620.3±60.7^{a}
Glycine	861.3± 5.7^{c}	877.0± 3.5^{b}	865.9± 6.0^{bc}	904.6±15.3^{a}
Histidine	589.0± 2.8^{a}	543.8±10.2^{c}	546.5± 5.3^{c}	572.7± 5.7^{b}
Threonine	635.7± 4.5^{d}	671.1± 5.3^{b}	659.6± 3.9^{c}	687.0±13.3^{a}
Arginine	2,430.6±10.7^{a}	2,198.1± 3.1^{b}	2,166.7±74.9^{b}	2,217.8±63.5^{b}
Alanine	866.9± 7.5^{a}	828.5± 9.1^{b}	818.4± 5.6^{b}	856.6± 8.6^{a}
Proline	779.2± 7.8^{a}	669.0± 2.6^{b}	669.6±15.0^{b}	679.5± 0.34^{b}
Tyrosine	494.9± 3.3^{a}	370.0±18.2^{b}	339.4±16.2^{c}	323.0±14.9^{c}
Valine	960.4±29.5^{a}	885.8± 0.7^{b}	888.5± 8.4^{b}	948.3± 4.9^{a}
Methionine	255.8± 2.2^{a}	202.2±17.4^{c}	175.3± 4.6^{d}	226.6± 2.6^{b}
Lysine	969.7± 4.9^{b}	965.0±17.6^{b}	969.4±34.3^{b}	992.4±34.7^{a}
Isoleucine	706.1± 8.1^{a}	620.4± 3.4^{d}	632.0± 3.4^{c}	676.9± 0.3^{b}
Leucine	1,331.0±23.4^{c}	1,383.0± 5.7^{b}	1,369.3±13.7^{b}	1,437.4±11.5^{a}
Phenylalanine	1,196.0±12.1^{a}	1,160.2±14.6^{b}	1,151.6± 5.3^{b}	1,195.7±23.6^{a}

[1)] Values are Mean±standard deviation (n=3).

Values with different superscripts are significantly different by Duncan multiple range test ($p<0.05$).

Table 3. Fatty acid composition of untreated and irradiated whole cottonseed

Fatty acid (% of total fat)	Untreated WCS	Gamma-irradiated WCS		
		8 kGy	10 kGy	12 kGy
Myristic acid ($C_{14:0}$)	0.7	0.7	0.7	0.7
Palmitic acid ($C_{16:0}$)	23.0	23.0	23.0	23.0
Palmitoleic acid ($C_{16:1}$)	0.5	0.5	0.5	0.5
Stearic acid ($C_{18:0}$)	2.6	2.7	2.7	2.6
Oleic acid ($C_{18:1}$)	16.0	16.2	16.2	16.2
Linoleic acid ($C_{18:2}$)	56.5	56.2	56.2	56.1
Linolenic acid ($C_{18:3}$)	0.2	0.2	0.2	0.2
Arachidic acid ($C_{20:0}$)	0.4	0.3	0.3	0.4
Behenic acid ($C_{22:0}$)	0.1	0.2	0.2	0.3
Total	100.0	100.0	100.0	100.0

extraction methods have been used to reduce the gossypol content of cotton seed (Cherry and Gray, 1981; Rahma and Narasinga Rao, 1984). Although solvent extraction reduces the gossypol content, the flavor of the meal was objectionable (Alyevand et al., 1967). In contrast, gamma irradiation approach requires fewer steps and does not affect the flavor of the meal compared to solvent extraction methods. The effect of gamma irradiation on free gossypol is given in Table 4. Free gossypol in WCS decreased ($p<0.05$) by the treatment. Of the 3 different doses of gamma irradiation, the 12 kGy dose was found to be the most effective in reducing free gossypol level. The result is similar to that reported by Abu-Tarboush (1998), who observed a lowered free gossypol concentration of cotton seed by gamma irradiation at a dose level of 10 kGy, although this dose was not effective in removing gossypol completely. Shawrang et al. (2011) reported that gamma irradiation at doses over 25 kGy could completely remove free gossypol of cotton seed meal. In comparison with other physical modification methods, such as microwave, UV and hydrothermal treatment, irradiation treatment is rapid, convenient and more extensive due to the ionizing energy. Table 4 shows the effect of gamma irradiation on phytic acid of WCS. The doses of 8, 10 and 12 kGy gamma irradiation had no effect on phytic acid of WCS. Taghinejad et al. (2009) reported that gamma irradiation decreased the phytic acid content of soybean and completely eliminated when doses of 30 and 45 kGy were adopted. One possible reason that our results differed from those reported by Taghinejad et al. (2009) might be the doses of gamma irradiation. Bhat et al. (2007) also reported that phytic acid of velvet seed was completely eliminated with an exposure to doses of 15 and 30 kGy.

Table 4. Gossypol and phytic acid contents of untreated and irradiated whole cottonseed

	Free gossypol (%)	Phytic acid (%)
Untreated WCS	0.17[a]	1.26
Gamma-irradiated WCS		
8 kGy	0.13[b]	1.28
10 kGy	0.14[b]	1.30
12 kGy	0.11[c]	1.26

Values with different superscripts are significantly different by Duncan multiple range test ($p<0.05$).

The results of present study showed that gamma irradiation of WCS effectively increase *in vitro* digestibility ($p<0.05$) (Table 5). Irradiation may induce unfolding of the protein and its denaturation, exposing hydrophobic amino acids that are positional groups for the active site of pepsin enzyme (Siddhuraju et al., 2002). Based on the nutritional content and *in vitro* study results, we examined the effect of 10

Table 5. *In vitro* digestibility of untreated and irradiated whole cottonseed

	IVDMD (%)[1)]	IVOMD (%)[2)]
Untreated WCS	51.42±1.81[b]	51.49±1.01[b]
Gamma-irradiated WCS		
8 kGy	56.89±2.97[a]	57.13±2.77[a]
10 kGy	57.19±0.76[a]	58.53±1.14[a]
12 kGy	57.28±1.36[a]	59.05±2.18[a]

[1)] Dry matter digestibility by *in vitro* procedure (Tilly and Terry, 1963)
[2)] Organic matter digestibility value by *in vitro* procedure (Tilly and Terry, 1963)
Values are mean ± standard deviation. n = 6.
Values with different superscripts are significantly different by Duncan multiple range test ($p<0.05$).

kGy gamma irradiation on ruminal degradation of WCS by using cannulated Holstein heifers. Gamma irradiationat a dose 10 kGy caused a significantly ($p<0.05$) lowered rumen degradability both in DM and CP as compared with raw WCS (Fig. 1, 2). However, there were no differences in the rapidly degradable and potentially degradable fractions of CP by 10 kGy gamma irradiation (Table 6). Results in the present work are consistent with those of Taghinejad et al. (2009), who demonstrated that gamma irradiation at a dose of 15 kGy did not significantly decrease the washout and potentially degradable fractions of CP but at doses of 30 and 45 kGy it reduced the washout fraction of DM and CP. As seen in Table 6, relatively higher fraction A obtained from the *in situ* incubation to other ones reported (NRC, 2001) might be due to the 2 step fine grinding of WCS for sample preparation. However, decrease in the solubility of protein observed in current study is in agreement with the results of Abu et al. (2006) and Lacroixa et al. (2002) who demonstrated gamma irradiation decreased protein solubility due to denaturation occurring through cross-linking of chains and protein aggregation. Moreover, treatment of canola meal with gamma irradiation was successful in reducing degradation of protein by rumen microorganisms and increasing intestinal digestibility of feed protein (Shawrang et al., 2008).

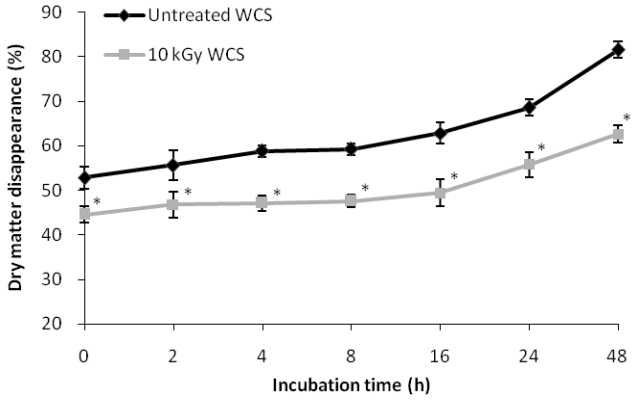

Fig. 1. *In situ* dry matter degradability of untreated and irradiated whole cottonseed.
* Mean values at each time point significantly differ at $p<0.05$.

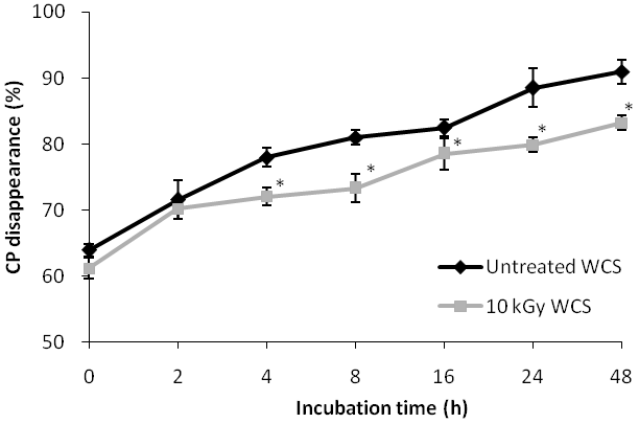

Fig. 2. *In situ* protein degradabilities for untreated and irradiated whole cottonseed.
* Mean values at each time were significantly different at $p<0.05$.

Table 6. Fractions and degradation of protein *in situ* from untreated and irradiated whole cottonseed

Item	Untreated WCS	10 kGy WCS
Fraction A (washout), % of N	63.9	61.1
Fraction B, % of N	33.9	33.8
Fraction C, % of N remaining at 48 h	2.2	5.1
K_d, /h	0.055	0.060
K_p, /h	0.05	0.05
RUP, % of CP	18.3	20.5
RDP, % of CP	81.7	79.5

Fraction A (washout) = N leaving *in situ* bag after immersing the bags in warm water (39℃), fraction B = 100% minus fraction A minus fraction C, K_d = rate of digestion, and K_p = rate of passage (National Research Council, 1985).

In conclusion, gamma irradiation of WCS seems to have some lowering effect on gossypol level and also ruminal degradability of DM and CP although the differences in major nutrients were not obtained by gamma irradiation. This study indicates that gamma irradiation at a dose 10 kGy, the optimum condition for removing germination ability of WCS, could improve nutritive value for the ruminant. Further study is needed to evaluate the extent of improvement in nutritive value and economic benefits of gamma irradiation at doses higher than 12 kGy.

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