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Effects of Gamma Irradiation on Ruminal DM and NDF Degradation Kinetics of Alfalfa Hay

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Abstract: The effects of gamma irradiation on ruminal dry matter, Neutral Detergent Fiber (NDF) degradation of alfalfa hay were investigated. Alfalfa hay samples were irradiated by gamma irradiator at doses of 50, 100 and 150 kGy under identical conditions of temperature and humidity. Nylon bags of untreated or irradiated samples were suspended in the rumen of three Taleshi bulls for up to 96 h and resulting data were fitted to non-linear degradation model to calculate degradation parameters. Results indicated that the washout fractions of dry matter and NDF increased linearly ($p<0.001$) with increasing irradiation dose. The b fraction and the degradation rate of the b fraction (c) of DM and NDF were the highest at 50 kGy dose. Effective degradability of DM and NDF increased linearly with increasing irradiation dose. Gamma irradiation at doses of 50, 100 and 150 kGy increased the effective NDF degradability of alfalfa hay at rumen outflow rate of 0.05 h^{-1} by about 8, 11 and 12%, respectively. Gamma irradiation affects on the hydrogenic bonds and with theirs breakdowning causes the wander-valls power weaken, that results in the degradation of cellulose and increasing of DM and NDF degradability.

Key words: Gamma irradiation, dry matter, NDF, degradability, nylon bags

INTRODUCTION

Feeds such as alfalfa hay, are commonly fed to the animals, especially the ruminants since the cellulose can be digested to glucose and then fermented to volatile fatty acids by bacteria in the rumen. The cellulose in this hay is associated with lignin matrix; therefore, the enzymatic hydrolysis of such cellulose during the ruminant digestion process may not be efficient. Many studies have been done to increase the degradation of the cellulosic components of this feed by physical, chemical and biological processes. Chemical treatment by ammonia, sodium hydroxide and urea was commonly used to break down the lignocellulose materials of roughages or to solubilize the hemicellulose (Ballet *et al*., 1997). However, these methods are harmful for animal health and cause environment pollution.

Gamma radiation was commonly used to decrease cell wall constituents or depolymerize and delignify the fiber of agricultural by-products (Al-Masri and Zarkawi, 1994). As far as we know, little information is available concerning effects of gamma irradiation on ruminal degradation kinetics of lignocellulosic materials used in ruminant nutrition. Therefore, present purpose of this study was to investigate the effect of gamma irradiation on dry matter and NDF disappearance characteristics and degradability parameters of alfalfa hay.

MATERIALS AND METHODS

Sample preparation and irradiation treatments: This experiment was conducted from December 2006 to June 2007. The alfalfa hay samples were collected from five farms in Kerman-Shah province of Iran. Samples were mixed and air dried for 48 h and stored in seal plastic bags. The dry matter of alfalfa hay was determined before of the irradiation treatment. Samples irradiated by cobalt-60 irradiator (Gamma cell) at 20°C. The dose rate determined by Fricke dosimetry was 0.37 kGy h^{-1} (Holm and Berry, 1970). The irradiation was accomplished in Agricultural,

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Medical and Industrial Research School, Nuclear Science and Technology Research Institute. Three polyethylene packages of samples were gamma irradiated at doses of 50, 100 and 150 kGy in the presence of air.

Animals and diets: Three Taleshi bulls with an average live weight of 400 kg fitted with rumen fistulas were placed in individual pens (3.4×4.9 m) with concrete floors that were cleaned regularly. Bulls were fed 8 kg dry matter; a total mixed ration containing 700 g kg^{-1} of dry matter of high quality alfalfa hay and 300 g kg^{-1} of dry matter concentrate. The concentrate consisted of ground barley, soybean meal, cottonseed meal, wheat bran, salt, dicalcium phosphate and vitamin + mineral premix (500, 160, 100, 210, 10, 10 and 10 g kg^{-1} dry matter, respectively). Diet was fed twice daily at 08:00 and 15:00 h.

***In sacco* ruminal degradability:** Nylon bags (9×21 cm) with a pore size of 46 μm were filled with approximately 4.5 g of untreated or irradiated alfalfa samples ground to pass 3 mm screen. All bags were simultaneously placed in the rumen, just before the animals were offered their first meal in the morning (i.e., 08:00 h). Bags were incubated in the rumen for periods of 0, 6, 12, 24, 48, 72 and 96 h. After retrieved from the rumen, bags were washed with tap water and stored at -20°C. After thawing, bags were washed three times for 5 min in a turbine washing machine. The same procedure was applied to two series of two bags to obtain the 0 h value. All residues were oven dried (65°C for 48 h) and dry matter determined (Hvelplund and Weisbjerg, 2000).

Statistical analysis: Disappearances of dry matter were fitted for each bull to the exponential model of Orskov and McDonald (1979) as: $p = a+b(1-e^{-ct})$. In this model, the constant a and b represent, respectively, the washout fraction and the non-soluble but degradable component, which disappears at a constant fractional rate c per unit time. The Effective Degradability (ED) was calculated using ED = a+ bc/(c+k), estimated outflow rates (k) of 0.02, 0.05 and 0.08 h (Agricultural and Food Research Council, 1993). Data were analyzed using the general linear models procedure of SAS (1996) with the following statistical model of $Y_{ijk} = \mu + T_i + B_j + e_{ijk}$, where Y in the dependent variable, μ the overall mean, T_i the gamma effect, B_j the animal effect and e_{ijk}, is the residual error, assumed normally and independently distributed. Differences among treatments were separated using polynomial orthogonal contrasts to determine linear, quadratic and cubic responses. The means comparative of treatments for various ruminal incubation periods was accomplished with using of Duncan's Multiple Range Tests (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Untreated alfalfa hay showed increase of disappearance of DM and NDF with increasing incubation time to 96 h ($p<0.001$). The disappearance of DM and NDF were greater for treated samples than for untreated alfalfa at almost all incubation periods ($p<0.001$). Significant differences between treatments for the disappearance of DM and NDF during almost all incubation periods were also observed ($p<0.001$) (Table 1, 3).

Table 1: Dry matter disappearance (%) of alfalfa hay at different incubation periods

	Ruminal incubation periods						
Irradiation dose (kGy)	0	6	12	24	48	72	96
Untreated	34.30^{b}	56.20^{e}	65.10^{d}	72.70^{d}	75.40^{d}	75.80^{d}	76.60^{d}
50	35.90^{b}	58.00^{bc}	68.40^{c}	74.00^{c}	77.70^{c}	78.90^{c}	79.40^{c}
100	44.20^{a}	60.10^{b}	71.40^{b}	76.40^{b}	80.40^{b}	82.40^{b}	85.90^{b}
150	45.60^{a}	65.80^{a}	76.60^{a}	82.50^{a}	84.90^{a}	89.30^{a}	90.00^{a}
SEM	1.07	1.89	1.23	1.11	0.92	0.74	0.53

Means with different superscripts within column are differ ($p<0.05$), Each value is a mean of six samples

Table 2: Rumen degradation parameters of dry matter of untreated and gamma irradiated alfalfa hay

		Gamma irradiated alfalfa hay (kGy)				Contrasts		
Parameters	Untreated alfalfa hay	50	100	150	SEM	L	Q	C
a	34.73	35.99	44.49	46.02	1.316	***	NS	***
b	40.94	42.55	38.52	41.95	1.585	NS	NS	*
a + b	75.67	78.54	83.01	87.97	2.901	***	NS	NS
c (/h)	0.127	0.111	0.088	0.103	0.0097	***	**	NS
Effective rumen degradation (h^{-1})								
0.02	70.03	72.04	75.86	81.14	0.599	***	**	NS
0.05	64.00	65.30	69.04	74.24	0.722	***	**	NS
0.08	59.74	60.69	64.66	69.62	0.786	***	**	NS

SEM: Standard Error of the Means; L: Linear contrast; Q: Quadratic contrast; C: Cubic contrast, Significance: NS: Not Significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$; a: The washout fraction, b: The potentially degradable fraction, c: The rate of degradation

Table 3: NDF disappearance (%) of alfalfa hay at different incubation periods

Irradiation dose (kGy)	Ruminal incubation periods						
	0	6	12	24	48	72	96
Untreated	3.45^{d}	6.83^{d}	8.41^{c}	9.04^{d}	28.80^{d}	29.28^{c}	30.74^{d}
50	8.39^{c}	11.48^{c}	13.77^{b}	23.41^{c}	27.36^{c}	29.05^{c}	29.54^{c}
100	11.43^{b}	13.61^{b}	16.47^{a}	24.73^{b}	25.72^{b}	26.48^{b}	28.54^{b}
150	13.08^{a}	14.07^{a}	16.68^{a}	26.77^{a}	11.79^{a}	12.28^{a}	13.86^{a}
SEM	0.424	0.205	0.236	0.270	0.203	0.292	0.227

Means with the different superscripts within column are differ ($p<0.05$), Each value is a mean of six samples

Table 4: Ruminal NDF degradation parameters of untreated and gamma irradiated alfalfa hay

Parameters	Untreated alfalfa hay	Gamma irradiated alfalfa hay (kGy)				Contrasts		
		50	100	150	SEM	L	Q	C
a	4.16	7.45	10.52	11.25	0.34	***	NS	NS
b	9.19	21.18	19.65	19.95	0.37	**	*	NS
a + b	13.35	28.63	30.17	31.20	0.71	***	NS	NS
c (/h)	0.039	0.042	0.041	0.041	0.0016	***	NS	NS
Effective rumen degradation (h^{-1})								
0.02	10.27	21.81	23.73	24.66	0.115	***	NS	NS
0.05	8.23	17.13	19.38	20.24	0.108	***	NS	NS
0.08	7.21	14.75	17.18	18.01	0.109	***	NS	NS

SEM: Standard Error of the Means; L: Linear contrast; Q: Quadratic contrast; C: Cubic contrast, Significance: NS: Not Significant; *$p<0.05$; **$p<0.01$; ***$p<0.001$; a: The washout fraction, b: The potentially degradable fraction, c: The rate of degradation

Increasing gamma irradiation dose, increased the washout fraction (α) of DM and NDF of alfalfa hay linearly ($p<0.001$). The potentially degradable fraction (b) and the degradation rate of the b fraction (c) of DM and NDF were the highest at 50 kGy irradiation dose ($p<0.001$). Effective degradability of DM and NDF linearly increased ($p<0.001$) as irradiation dose increased (Table 2, 4).

This study examined the effects of gamma irradiation treatments on rumen degradation *in sacco* in cow. The organic matter digestibility as a result of gamma irradiation increased for wheat straw with a clear decrease in the crude fiber and NDF content (Yu *et al*., 1975; Bear *et al*., 1980). In a feeding experiment on lambs fed with barley straw by a gamma ray, disappearance of NDF decreased and of dry matter increased (Al-Masri, 1997), significantly. In addition, Pritchard and Pigden (1962) reported that the solubility and digestibility of wheat straw increased by gamma irradiation. Han *et al.* (1981) reported that the dry matter solubility of sugarcane baggasse irradiated with gamma ray increased significantly. In other experiments, the digestibility of organic matter and dry matter degradability increased in wheat straw, cotton wood, olive cake and apple pruning products after treatment with γ-irradiation (Al-Masri and Guenther, 1993). Shawrang *et al.* (2007) reported that gamma irradiation at doses higher than 50 kGy could increase ruminal dry matter degradability of feedstuffs. McManus and Manta (1972) have indicated that an effect of irradiation on poor-quality roughages (Lucerne straw and rice straw) given to sheep.

Each glucose residue of cellulose has inter and intra molecular two hydrogenic bonds. These bonds stabilize the long and parallel chains of cellulose (Krassig, 1993). Gamma irradiation affects these bonds and causes the wander-valls power weakens, that results in the degradation of cellulose and increasing of degradability of cell wall constituents (Iller *et al*., 2002; Muto *et al*., 1995). With the breaking of hydrogenic bonds, free radicals are produced. The concentration of free radicals and also, the number of separated chains from cellulose, increases with the increasing of irradiation dose (Muto *et al*., 1995; Seaman *et al*., 1952). The irradiation causes the formation of carbonyl groups of cellulose at the presence of oxygen that helps cellulose breakdown (Muto *et al*., 1995; Seaman *et al*., 1952). Gamma rays leads to the hydrolysis of the glycoside bonds.

CONCLUSION

In fact, irradiation results in the breaking of the hydrogenic bonds between cellulose and hemicellulose as well as the degradation of the inter-linkages in lignin structure. Based upon these results, the best dose of gamma irradiation for processing of Alfalfa hay was 100 kGy.

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