



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

**THE EFFECT OF ELECTRON BEAM IRRADIATION ON IN VIVO, IN VITRO AND
IN SACCO DIGESTION OF WHEAT STRAW**

HAIPOUR M¹, NIKKHAH A^{1*}, SHAWRANG P², SADEGHI AA¹, CHAMANI M¹

1: Department of Animal Science, Science and Research Branch, Islamic Azad University,
Tehran, Iran.

2: Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute,
Atomic Energy Organization of Iran, Karaj, Iran.

***Corresponding Author: E-mail: aasdghi@gmail.com**

ABSTRACT

The effect of electron beam radiation on dry matter, organic matter and fiber digestibility in sheep was studied. Samples of untreated or electron beam irradiated wheat straw were subjected to the rumen of three fistulated rams and also *in vitro* and *in vivo* conditions to determine the digestibility and ruminal degradation parameters of dry matter (DM) and neutral detergent fiber (NDF). The water soluble fraction, degradable fraction, degradation rate and ruminal effective degradability of DM and NDF increased as electron beam irradiation dosage increased ($P < 0.05$). *In vivo* and *in vitro* digestibility of dry matter had the same trend. The data suggest that electron beam irradiation, up to 500 kGy, could improve ruminal fiber degradability and total tract fiber digestibility of wheat straw.

**Keywords: Electron Beam Irradiation, Neutral Detergent Fiber, Digestibility, Wheat
Straw**

INTRODUCTION

Lignocellulosic material such as cereal straw is one of the renewable energy sources [1]. However, a large amount of cereal straw is burned or discarded every year. This is both a waste of resources and a source of pollution [2, 3]. The treated form of straw is a major source of biomass that can be fed to

ruminants. The biggest challenge is changing the structure of lignocellulose to increase its enzymatic digestibility. Physical and chemical treatments (grinding, steaming, mineral acids, sodium hydroxide and ammonia) have been used to breakdown lignocelluloses structure and to improve its

industrial properties [4] and nutritional value [5]. These pretreatments have had a number of issues, including high transportation and storage costs in mass production, pollution due to low recovery of acids and bases used in the pretreatment, high cellulase usage, and low hydrolysis efficiency [6]. There is an urgent need for an efficient, non-polluting, cost-effective pretreatment method. Ionizing irradiation, a process in which material exposed to gamma rays or electron beam, has been recognized as a reliable and safe method to improve the nutritive value of foods [7] and feeds [8,9,10]. Electron beam irradiation has been shown to generate free radicals and reduce the degree of polymerization of cellulose, resulting in increased reactivity [11, 12]. Recently, it was reported that electron beam irradiation could enhance cellulose hydrolysis in wood and paper industry [13]. However, Information about the effect of electron beam irradiation on ruminal fiber degradability of roughages is limited. Therefore, objective of this study was to determine the dry matter and fiber degradation characteristics of wheat straw following electron beam irradiation.

MATERIALS AND METHODS

Sample preparation and irradiation treatments

Wheat straw was obtained from the experimental farm in Alborz province of

Iran and dried at 40°C for 7 d. After thorough hand-mixing, straw was divided into two batches to form duplicate sources for electron beam irradiation. Samples were packed in nylon bags (30 × 40 × 5 cm dimensions, 0.5 mm thickness) and exposed to electron beam irradiation (Rhodotron accelerator model TT200, IBA co., Belgium) at the Yazd radiation processing center (AEOI, Yazd center, Iran) to doses of 250 and 500 kGy at room temperature. Each sample was placed in a metal tray and passed through the accelerator at a rate of 77 cm per min. With setting of 10 MeV and a current flux of 2.0 mA, one passage results in an exposure of 28.5 kGy. Multiple passages (9 and 18 passages) were used to obtain 250 and 500 kGy. The dose rate was determined using cellulose triacetate films [14]. Uncertainty and Dmax/Dmin were about 3% and 1.2, respectively. Similarly packed samples without irradiation served as control.

Ruminal degradability

Three castrated Shal rams with an average live weight of 65 kg fitted with rumen fistulas were used for in situ experiments. Nylon bags (7 cm × 14 cm) with a pore size of 45 µm were filled with approximately 3 g of the samples ground to pass a 3 mm screen according to Ørskov and McDonald [15]. Duplicate bags filled with untreated or irradiated wheat straw were incubated in the

rumen for periods of 0, 4, 8, 12, 16, 24, 48 and 72 h. Two series of incubations (96 samples included of four replicates \times eight incubation periods \times three sheep) were completed for each feed and sheep. In each series, all bags were simultaneously placed in the rumen, just before the animals were offered their first meal in the morning at 07:30 h. After retrieval 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. The residues were dried and analyzed for dry matter (DM) and neutral detergent fiber (NDF) to establish degradation kinetics of wheat straw.

***In vitro* digestibility trial**

In a 50-ml flask, 0.5 gram sample of raw or irradiated wheat straw powder was added to 35 ml of artificial saliva and rumen fluid (4:1 ratio). The mixture was incubated in a water bath with shaker at 39°C and 150 rpm for 72 h. Then pellet was obtained via centrifugation. The pellet was subjected to 35 ml of hydrochloric acid and pepsin. After digestion completed, dry matter and ash contents of pellet were determined and DM and OM digestibility were calculated.

***In vivo* digestibility trial**

Samples of wheat straw were taken and composited on an equal weight basis. Non-

irradiated or irradiated wheat straw was fed to three Shal rams for 7 days. Fecal grab samples were taken completely from three rams per treatment. Feces were collected between 07:30 and 09:00h, weighed and stored at 2°C . Samples were dried in a forced-air oven at 60°C for 24h and ground to pass a 1-mm screen. Food and fecal samples were analyzed for DM, CP, neutral-detergent fibre (NDF). Then total tract dry matter digestibility was calculated.

Chemical analyses

Moisture content was determined from the mass of samples before and after they were stored overnight in an oven at 105°C [16]. Neutral detergent fiber and acid detergent fiber (ADF) were analyzed according to a validated method [17], using an automatic fiber analyzer (Velp Scientifica, Milan, Italy). Sodium sulfite was omitted from the neutral detergent solution.

Statistical analyses

Disappearances (P) of DM or NDF (including 0 h values) were fitted for each sheep to the exponential model [15] as:

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

In this model, the constants "a" and "b" represent, respectively, the soluble 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.

The statistical normality of all data were tested in MINITAB[®] software (confidence level=95%). Then treatments analyzed by ANOVA procedure using the GLM procedure of SAS[®] software as follows:

$$Y_{ijk} = \mu + T_i + B_j + e_{ijk}$$

Where Y_{ijk} is dependent variable, μ is overall mean, T_i is irradiation effect, B_j is animal effect, and e_{ijk} is residual error. When significant differences among means were found, means were separated using Duncan's Multiple Comparison test ($\alpha=5\%$) for post hoc multiple comparisons.

RESULTS AND DISCUSSION

Effects on NDF degradability

Ruminal degradation characteristics of NDF of untreated and irradiated wheat straw are in **Table 1**. Increasing electron beam irradiation dose increased the soluble fraction (a), degradable fraction (b), degradation rate (c) and the effective degradability of NDF. Irradiation resulted in increase of effective degradation by cellulose and hemicellulose decomposition and their cross-linking. There is limited information about electron beam irradiation effects on NDF degradability of wheat straw, but there is some works on lignocellulosic decomposition. It has been shown that, when irradiated free radicals are formed within lignocellulose molecules

[18]. There are both cleavage and cross-linking reactions in cellulose. These radicals could also cause degradation of lignocellulose via certain reactions. At high irradiation dosages, the cleavage reactions dominate, resulting in increased breakage of glycosidic bonds and decreased cross-linkage to lignocellulose. This results in direct decomposition of lignocellulosic materials [19,20]. Similar ruminal degradabilities of DM and NDF were obtained by Gralak et al. [21] in wheat and triticale straws. In their study, irradiation raised the potential rumen degradability and effective degradability of the DM of both straws. Also, it was showed that DM digestibility of wheat, oat, barley and rye straws can increase up to 80% by treatment with gamma rays or accelerated electron [22].

Effects on *in vitro* degradability

Combining cellulase and irradiation treatments yielded better digestion than the treatments alone did, suggesting synergy between the treatments (**Table 2**). The highest *in vitro* digestibility of DM and OM was for 500 kGy and the lowest for non-irradiated sample. Takács *et al.* [8] found that under beam irradiation, cell wall constituents undergo degradation which is due to the breaking off the glucosidal bond and modification in their structures. Modification may be due to several factors

that finally lead to the opening of the anhydroglucose ring. Moreover, The improvement in *in vitro* digestibility of fiber with irradiation observed is likely to be due to a combination of the decreased particle size, increasing the surface area exposed for microbial attachment, a possible increase in solubility, the alteration in the chemical composition (especially the reduction in NDF content and corresponding increase in sugar content), random depolymerisation and decomposition of cellulose and hemicellulose and seriously weakens the cellulosic fiber [23,24].

Effects on *in vivo* dry matter digestibility

The results of *in vivo* digestibility of dry matter are shown in **Table 3**. *In vivo* dry matter digestibility increase as irradiation dosages increased. Reducing in crystallinity of cellulose [25, 26] is a reason of increasing *in vivo* digestibility of irradiated wheat straw. In the study of Alberti *et al.* [27], the reduction in crystallinity of cellulose was most evident at doses above 100 kGy. They found that crystallinity index of microcrystalline cellulose, flax, cotton and viscose reduced up to 12% with a dose of 200 kGy. In addition, the link of lignin with other compounds in cell wall is broken by irradiation [28]. Lignin is linked to both hemicellulose and cellulose forming a physical seal around the latter two compounds that is impenetrable barrier

preventing penetration of solutions and enzymes [29]. Hence, irradiation treatment has the potential to increase the nutritive value of wheat straw for ruminants.

REFERENCES

- [1] Alvira P, Tomas-Pejo E, Balesteros M, and Negro MJ, Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review, *Biores Technol*, 101, 2010, 4851-4861.
- [2] Cara C, Ruiz E, Ballesteros I, Negro MJ and Castro E, Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification, *Process Biochem*, 41, 2006, 423-429.
- [3] Cazetta ML, Celligoi MAPC, Buzato JB and Scarmino, IS, Fermentation of molasses by *Zymomonas mobilis*: Effects of temperature and sugar concentration on ethanol production, *Biores Technol*, 98, 2007, 2824-2828.
- [4] Silva ASA, Inoue H, Endo T, Yano S, and Bon EPS, Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. *Biores Technol*, 101, 2010, 7402-7409.

- [5] Deschamps FC, Ramos LP, and Fontana JD, Pre-treatment of sugarcane bagasse for enhanced ruminal digestion. *Appl Biochem Biotech*, 57, 1996, 177–182.
- [6] Jin S, and Chen, H. Superfine grinding of steam-exploded rice straw and its enzymatic hydrolysis, *Biochem Eng*, 30, 2006, 225-230.
- [7] Siddhuraju P, Makkar HPS and Becker K, The effect of ionising radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food. *Food Chem*, 78, 2002; 187–205.
- [8] Takács E, Wojnárovits L, Borsa J, Földvári CS, Hargittai P and Zöld O, Effect of gamma irradiation on cotton cellulose. *Radiat Phys Chem*, 55, 1999, 663–666.
- [9] Shawrang P, Sadeghi AA, Behgar M, Zareshahi H, and Shahhoseini G, Study of chemical compositions, anti-nutritional contents and digestibility of electron beam irradiated sorghum grains. *Food Chem*, 125, 2011, 376-379.
- [10] Shawrang P, Mansouri MH, Sadeghi AA and Ziaie F, Evaluation and comparison of gamma- and electron beam irradiation effects on total and free gossypol of cottonseed meal. *Radiat Phys Chem*, 80, 2011, 761-762.
- [11] Kumakura M and Kaetsu I, Radiation-induced decomposition and enzymatic hydrolysis of cellulose, *Biotechnol Bioeng* 20, 1978, 1309-1315.
- [12] Liu RQ, Xie LD and Sheng KL, ESR signals from silk fabrics irradiated by UV-rays,” *Nuclear Sci Technol*, 18(5), 2007, 268-271.
- [13] Bouchard J, Methot M and Jordan B, The effects of ionizing radiation on the cellulose of wood free paper. *Cellulose*, 13, 2006, 601–610.
- [14] ISO/ASTM: Practice for Use of a Cellulose Triacetate Dosimetry System. f. West Conshohocken, PA: ASTM International. ISO/ASTM 51650, 2005.
- [15] Ørskov ER and McDonald I, The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J Agric Sci (Camb.)*, 92, 1979, 499–503.
- [16] AOAC, Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA, 1995.
- [17] Van Soest PJ, Robertson JB, and Lewis BA, Methods for dietary fiber, neutral detergent fiber and

- nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci*, 74, 1991, 3583–3597.
- [18] Von Sonntag C, Free radical reactions of carbohydrates as studied by radiation techniques. *Carbohydrate Chem Biochem*, 37, 1980. 1-7.
- [19] Toth T, Borsaa J and Takacs E, Effect of preswelling on radiation degradation of cotton cellulose. *Radiat Phys Chem*, 67, 2003, 513-515.
- [20] Shabaka AA, El-Agramy AM and Nada AMA, Infrared spectroscopic study of g irradiated cotton linters. *Isotopenpraxis*, 27, 1991, 248-252
- [21] Galak MA, Mahmood S and Barej W, Rumen degradability of dry matter and crude fiber of irradiated and sodium hydroxide treated straws. *Arch Anim Nutr*, 47, 1994, 63–74.
- [22] Leonhardt JW, Henning A, Nehring K, Baer M, Flachowsky G and Wolf I, Proceeding for nuclear techniques for assessing and improving ruminant feeds. Vienna, Austria, FAO/IAEA, 1983.
- [23] Yang C, Zhiqiang S, Guoce Y and Jianlong W, Effect and aftereffect of gamma radiation pretreatment on enzymatic hydrolysis of wheat straw. *Biores Technol*, 99, 2008, 6240–6245.
- [24] Driscoll M, Stipanovic A, Winter W, Cheng K, Manning M, Spiese J, Galloway RA and Cleland MR, Electron beam irradiation of cellulose. *Radiat. Phys. Chem.*, 2009; 78: 539–542.
- [25] Iller E, Kukeielka A, Stupinska H and Mikolajczyk W, Electron-beam stimulation of the reactivity of cellulose pulps for production of derivatives. *Radiat Phys Chem*, 63, 2002, 253–257.
- [26] Kasprzyk H, Wichlacz K and Borysiak S, The effect of gamma radiation on the supramolecular structure of pine wood cellulose in situ revealed by X-ray diffraction. *Electron. J Pol Agric Univ*, 7, 2004, 234-242.
- [27] Alberti A, Bertini S, Gastaldi G, Iannaccone N, Macciantelli D, Torri G and Vismara E, Electron beam-irradiated textile cellulose fibers. *Eur Polym J*, 41, 2005, 1787–1797.
- [28] Wasikiewicz JM, Yoshii F, Nagasawa N, Wach RA and Mitomo H, Degradation of chitosan and sodium alginate by gamma radiation, sonochemical and ultraviolet methods. *Radiat Phys Chem*, 73, 2005, 287–295.

- [29] Arora DS, Chander M and Gill PK, the degradation and selective involvement of lignin peroxidase, lignolysis of wheat straw. *Int biodet manganese peroxidase and laccase in Biodeg*, 50, 2002, 115–120.

Table 1: Neutral detergent fiber degradation parameters of untreated and irradiated wheat straw

	Degradation traits			ERD (g/kg DM) at outflow rate (/h)		
	a (g/kg DM)	b (g/kg DM)	c (%/h)	0.02	0.05	0.08
Untreated	142 ^c	212 ^c	3.1 ^c	143 ^c	95 ^c	73 ^c
250 kGy	48 ^b	256 ^b	3.9 ^b	217 ^b	160 ^b	132 ^b
500 kGy	50 ^a	294 ^a	4.5 ^a	254 ^a	190 ^a	156 ^a
SEM	13.4	25.6	0.21	12.5	17.4	16.5

^{a,b,c} Means in the same column with different superscripts differ ($P<0.05$)

Table 2: *In vitro* digestibility of dry matter and organic matter of wheat straw

Irradiation dosages	DM digestibility (g/kg DM)	OM digestibility (g/kg DM)
Non irradiated	252 ^c	312 ^c
250 kGy	325 ^b	367 ^b
500 kGy	453 ^a	399 ^a
SEM	21.8	20.1

^{a,b,c} Means in the same column with different superscripts differ ($P<0.05$)

Table 3: *In vivo* digestibility of dry matter and organic matter of wheat straw

Irradiation dosages	DM digestibility (g/kg DM)
Non irradiated	385 ^c
250 kGy	419 ^b
500 kGy	487 ^a
SEM	28.1

^{a,b,c} Means in the column with different superscripts differ ($P<0.05$)