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ENZYMATIC DIGESTIBILITY OF ELECTRON BEAM IRRADIATED MILLET AND TRITICALE SEEDS

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ABSTRACT

Millet and triticale seeds were exposed to electron beam irradiation and chemical properties and subsequent effects on digestibility in broilers were investigated. Seed samples were packed in 30 × 40 × 5 cm nylon bags (0.5 mm thickness) and exposed to electron beam irradiation to various doses of 10, 20 and 30 kGy at room temperature by a Rhodotron accelerator model TT200. Ileal apparent digestibility of dry matter, crude protein, true protein and gross energy were measured in broilers. Total protein efficiency of raw and irradiated seeds was also measured. Based on the results, irradiation had no effect ($p > 0.05$) on chemical compositions. Irradiation improved ($p < 0.05$) *in vivo* digestibilities of dry matter, crude protein, true protein and gross energy. Electron beam irradiation increased digestibility of starch significantly. It can be concluded that electron beam irradiation has an important effect on the properties of starches. Clearly the reaction pattern seems complex and cannot be related to degradation of starch molecules alone. Electron beam irradiation increased digestibility of starch significantly. Unlike chemical treatments, which are time consuming, irradiation can be a quick and efficient method for modifying the properties of starch. Total protein efficiency of processed seeds at the doses applied was increased, as compared with the value for raw seeds.

Keywords: Electron Beam Irradiation, Starch, Millet, Triticale and Digestibility

INTRODUCTION

Millet grain (*Setaria italica*) is a tropical plant processing the C_4 photosynthetic pathway which out-yields other cereal grains under conditions of infertile soil, intense heat, limited rainfall, and short growing seasons. Relative to corn, millet contains greater concentrations of crude protein (CP) but less starch. Millet grain contains about 5% oil, resulting in greater GE than corn or sorghum grains **[1]**. Millet grain may be utilized as a primary feed grain in poultry and swine diets. Triticale (*Triticosecale rimpaui*) is a hybrid that is a cross between wheat and rye and is used in grain production or more commonly as forage. Triticale is used as feed for swine, cows and poultry and is generally higher in protein and amino acids than wheat or barley. Despite rich carbohydrate content of millet and triticale which is about 65% for millet and 67% for triticale **[2]** their potentials are still not fully exploited like other cereals such as wheat, rice, barley and maize.

Among various processes for starch degradation, radiation-chemical degradation occupies an important place and the radiolysis of starch has been described in a variety of original articles.

Electron-beam modification of starch has been studied to a lesser extent, despite the environmental and economic benefits of electron-beam treatment as compared to X-ray and γ-irradiation. Electron beam irradiation has been shown to reduce or inactivate some of the limitation factors in wild cereals seeds or meals, thereby enhancing their edibility **[3].** But information about irradiated millet and triticale starches is not available. Millet and triticale starches have good potential and it is reasonable to explore all the potentials of their nutrients to bring them to full industrial utilization since origin of starches have pronounced effect on their physicochemical properties and each of them is unique in its characteristics. Therefore, in this study, it was aimed to evaluate effects of electron beam irradiation on chemical properties and subsequent effects on *in vivo* digestibility of millet and triticale starches in broilers.

MATERIALS AND METHODS

The experiment was performed at the experimental farm of Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran, Karaj, Iran. All bird protocols were approved by the relevant Ethical Review Committee and all experimental conditions followed official guidelines for the care and management of birds.

Collection of the Seed Samples

The seeds were collected from the Agricultural and Natural Resources Research Center, Sari, Mazandaran, Iran. Soon after collection, after removing immature and damaged seeds, the mature seeds were dried in direct sunlight for 2 days and stored in plastic containers at room temperature (25 °C) until further use.

Sample Irradiation

Seed samples were packed in 30 × 40 × 5 cm nylon bags (0.5 mm thickness) and exposed to electron beam irradiation at the Yazd radiation processing centre (AEOI, Yazd centre, Iran) to various doses (10, 20 and 30 kGy) at room temperature by a Rhodotron accelerator model TT200 (IBA Co., Belgium). All samples were irradiated at fixed beam energy of 10 MeV and the required irradiation doses were obtained by adjusting the electron beam parameters (electron beam current, Conveyor speed, etc.). Double side irradiation (exposure to both sides) was performed for uniform dose delivery. The dose was determined with cellulose triacetate films. Similarly packed seed samples without irradiation served as control. Irradiated samples evenly placed in a sifter drying in a constant temperature oven with air velocity at 0.5 ± 0.1 m/s and temperature at 40ºC. The samples were dried until it reached a final moisture content of 14.5 ± 0.02% (dry base), which represented the safe moisture value for grain storage.

Ileal Apparent Digestibility

All three irradiated millet and triticale samples together with a raw unprocessed sample were incorporated into diets at a rate of inclusion of 108 g/kg. Other dietary components were shown in Table 1.

Each of the four experimental diets was evaluated with four replicates of a cage of two Ross-type male broilers (initial allocation to replicate was at day 13 with the live weight of each bird in a replicate not differing by more than 10 g) of initial age of 19 days. On day 4 following the introduction of the experimental diets, birds were starved for 1 h and then fed for 2 h to ensure sufficient gut fill for digesta sample collection. The two birds were then euthanized by being placed in an airtight box with rising CO_2 concentration.

Digesta samples were taken from the lower ileum, which was taken to be the length between Meckel's Diverticulum and the ileal–caecal-colonic junction. The lower ileum was used as it was assumed that all possible dietary nutrients digestion and absorption would be complete before the digesta reached this portion of the intestine and thus only indigestible and endogenous nutrients would be present. After excising these portions of the gut, gentle digital pressure was used to remove the digesta samples, taking care not to disturb the mucosal lining of the intestine or to contaminate the sample with blood.

The digesta sample was collected in a pot, mixed and frozen at −20ºC. Samples from both birds in a cage were pooled in order to provide sufficient digesta for analysis. All digesta samples were freeze dried in an oven drier to a constant mass. The samples were then ground as finely as possible using a pestle and mortar. They were then stored in plastic sealed pots in a cool room. Analyses of dry matter, crude protein, true protein and gross energy of untreated and irradiated samples were conducted and calculations were carried out.

Chemical Compositions

Moisture content was determined from the mass of samples before and after they were stored overnight in an oven at 105ºC **[5]**. Nitrogen was determined by using a Dosimat-776 Metrohm apparatus (Metrohm Co., Switzerland) according to AOAC **[5]**. The instrument was calibrated each time with ammonium sulphate as a nitrogen standard. Starch contents were determined on a spectrophotometer at 510 nm after extraction with boiling water. Fat content was determined with a Solvent Extractor (Behr Labour-Technik, Düsseldorf, Germany) equipped with six Soxhlet posts. Fat content was determined with a Solvent Extractor (Behr Labour-Technik, Düsseldorf, Germany) equipped with six Soxhlet posts **[5].** Ash was determined by burning duplicate 2 g samples at 540 °C, for 3 h in a muffle furnace **[5].** Crude fiber was determined by treating an oil-free sample by sulphuric acid (0.26 N) and potassium hydroxide (0.23 N) solution using an automatic fiber analyzer (Velp Scientifica, Milan, Italy), followed by oven drying and muffle furnace incineration **[5]**. Gross energy of grain and excreta samples were determined by adiabatic bomb calorimeter using Parr-4 Model 1241 Calorimeter. The True protein of the samples was quantitatively estimated following the method of **[6]**. The protein contents of the samples were calculated using a calibration curve obtained for bovine serum albumin standards (0–1.5 mg) treated in the same way. Two extractions were carried out per sub-sample and each sample was analyzed in duplicate.

Total Protein Efficiency

TPE was determined following the method of **[4]**. The preliminary feeding period was 5 days. On the sixth day, Chicks were banded, individually weighed to the nearest gram and grouped by weight. A group of 100 chicks of uniform size was divided randomly into four treatments with five replicates of five chicks.

The experimental diets were given to their respective for 10 days. Body weight and feed consumption data were recorded at the end of the experimental period (10 days). The TPE was calculated as weight gain of chicks divided by the total protein consumed.

At all times, feed and water were provided on an *ad libitum* basis. During the trial period, temperature was maintained at 21ºC and the birds were kept under artificial light for 23 h per day, with one hour of dark. The air in the metabolism room was continuously circulated and humidity monitored.

Statistical Analysis

Treatments were analyzed as a completely randomized design under the general model:

$Yij = \mu + Ti + eij$

Where *Yij* is the dependent variable; μ is the general mean; *Ti* is the treatmenti 1, 2, 3; and *eij* is the experimental error, calculated using the GLM procedure of the SAS software **[7]**. The broilers were the experimental units for all analyses. Treatment means were compared using the Duncan method, an α-value of 0.05 was used to assess significance.

RESULTS AND DISCUSSION

Effects on Chemical Compositions

There were no significant differences in chemical composition between the irradiated and non-irradiated grains except for starch and crude fiber (Table 2). Starch contents for millet were reduced by 6.9%, 10.65% and 15.77% and starch contents for triticale reduced by 7.16%, 13.13% and 19.62% at 10, 20 and 30 kGy doses, respectively. Also, crude fiber contents for millet were reduced by 11.9%, 16.6% and 45.23% and Crude fiber contents for triticale reduced by 11.53%, 30.7% and 46.15% at 10, 20 and 30 kGy doses, respectively.

Since the irradiation treatment at any dose would not increase the temperature of seeds, no changes were observed in moisture content. Ash content was not affected with electron beam irradiation doses, a finding that agrees with those of many researchers **[8]**. The quantity of ash in any seed sample assumes importance, as it determines the nutritionally important minerals. Although the crude protein, crude fiber and starch of seeds decreased on irradiation, it was only significant for crude fiber and starch content ($p < 0.05$), A finding that was previously reported by many investigators **[8]**. It has been suggested that decrease of starch content was induced by structural degradations in granules and molecules of starch **[9]**. It would be interesting to determine total, soluble and insoluble dietary fiber fractions in raw and electron beam irradiated seeds, to gain a better insight into the fiber contents. Fiber levels reduced in direct proportion to the level of irradiation that may be due to depolymerisation of fiber. It appears that radiation resulted in random depolymerisation and decomposition of cellulose and seriously weakens the cellulosic fiber.

Effects on *in vivo* Digestibility

The results of *in vivo* digestibility of untreated and irradiated millet and triticale grains are shown in Table 3. With increase in doses, digestibility of starch, dry matter, crude protein, true protein and gross energy increased significantly compared to control. There were 9.4%, 18.29%, 10.29%, 19.9% and 22% increase in digestibility of millet starch, dry matter, crude protein, true protein and gross energy by irradiation doses of 0 and 30 kGy, respectively. Increase in digestibility of triticale starch, dry matter, crude protein, true protein and gross energy by irradiation doses of 0 and 30 kGy were 7.96%, 14.98%, 9.55%, 20.48% and 15.49%, respectively.

The reason for increasing in protein digestibility is modification in the three dimensional structure of millet proteins due to irradiation. Studies of **[10]** illustrated that protein denaturation occur by irradiation that lead to improvement in intestinal protein digestion.

Despite rich carbohydrate content of millet and triticale which is about 60-70% **[11]**, existence of non-starch polysaccharides (NSPs) and high level of crude fiber in these grains causes that their potentials is not fully exploited like other cereals such as wheat, rice, barley and maize. It appears that radiation results in random depolymerisation and decomposition of cellulose and seriously weakens the cellulosic fiber. Hence, irradiation treatment has the potential to increase the nutritive value of grains containing fiber.

Mahalati and Devegowda **[11]** showed that millet grain contains about 6.6% NSPs, and triticale grain contains almost 7.7% NSPs. These carbohydrate polymers cause the intestinal contents to become viscous and interfere with nutrient assimilation and the general well-being of the animals. Classen *et al*., **[12]** showed that irradiation of grains containing NSPs, fed to chicks improves the apparent absorption of fat, amino acids and starch. It seems that improvement noted for mentioned grains is thought to be due to depolymerisation of NSPs.

Yoon *et al*., **[9]** revealed an increase in starch digestibility by gamma irradiation. It seems that this increase was induced by structural degradations in granules and molecules of starch which allowed easy access of the digestive enzymes to starch.

Effects on the Total Protein Efficiency (TPE)

The TPE value was significantly ($p<0.05$) increased for each dose of irradiation by 17%, 26.58% and 41.77% for millet, and by 14.5%, 25.23% and 37.23% for triticale, respectively (Table 4).

The data suggest a beneficial effect of irradiation on the nutritional value of millet and triticale. These results are in good agreement with those obtained by Rady *et al*., **[13]** who reported that irradiation of wheat at dose levels of 0.4 and 5 kGy, increased the net protein utilization in chicks fed the irradiated wheat.

CONCLUSION

It can be concluded that electron beam irradiation has an important effect on the properties of starches. Clearly the reaction pattern seems complex and cannot be related to degradation of starch molecules alone. Electron beam irradiation increased digestibility of starch significantly. Unlike chemical treatments, which are time consuming, irradiation can be a quick and efficient method for modifying the properties of starch.

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Table 1: Experimental Diets Composition, Amount (g/kg diet)

Component	Trail one	Trail two
Millet	108	-
Triticale	-	108
Wheat	700	700
maize	60	60
Soya	110	110
Calcium Phosphate	3.3	3.3
Vitamin and Mineral Premix [a]	12.5	12.5
Lysine	2.5	2.5
L Threonine	1.2	1.2
Salt	2.5	2.5
GE (MJ/kg)	17.86	17.86

[a] Content per g of premix: 0.1 g phosphorus, 0.017 g magnesium, 0.152 g calcium, 0.030 g sodium, 150 IU vitamin A, 30 IU vitamin D3, 0.2 IU vitamin E (as α-tocopherol acetate), 0.012mg copper (as copper sulphate), 3.2μg selenium (as selenium BCP)

Table 2: Chemical Composition of Irradiated Millet and Triticale Grains (as g/100g Dry Matter)

	Irradiation Dose (kGy)	Starch	Moisture	Ash	Crude protein	Ether extract	Crude fiber
millet	Control	50.7^{a}	10.3	3.6	14.5	2.4	4.2^{a}
	10	47.2^{b}	10.5	3.8	14.1	2.4	3.7^{ab}
	20	45.3^{c}	10.5	3.5	14.2	2.3	3.5^{b}
	30	42.7^{d}	10.3	3.9	14	2.5	2.3^{c}
	SEM	0.8	0.4	0.5	0.53	0.61	1.3
triticale	Control	58.6^{a}	10.86	2.1	14.75	1.9	2.6^{a}
	10	54.4^{b}	10.68	2.1	14.2	2.0	2.3^{a}
	20	50.9^{c}	10.72	1.8	14.1	1.95	1.8^{b}
	30	47.1^{d}	10.81	2.2	13.95	2.1	1.4^{c}
	SEM	1.1	0.2	0.4	0.35	0.7	0.9

^{a,b,c} Values followed by the different superscripts letter within a column differ significantly ($P < 0.05$) from each other ;SEM: standard error of the means

Table 3: Effects of Electron Beam Irradiation on Millet and Triticale Apparent *in vivo* Digestibility

	Irradiation dose (kGy)	*In vivo* digestibility (%)				
		starch	Dry matter	Gross energy	Crude protein	True protein
millet	Control	65.8	49.2^{c}	58.3^{b}	41.7^{c}	50^{c}
	10	67.1	53.4^{b}	58.8^{b}	43.5^{bc}	52^{c}
	20	69.2	55.6^{ab}	61.6^{ab}	45.9^{b}	55.5^{b}
	30	72	58.2^{a}	64.3^{a}	50^{a}	61^{a}
	SEM	0.2	0.63	0.57	0.54	0.5
triticale	Control	60.3	45.39^{c}	54.1^{c}	34.52^{c}	40.85^{c}
	10	60.9	47.86^{b}	55.12^{bc}	35.19^{c}	41.28^{c}
	20	62.2	50.26^{a}	56.72^{ab}	38.17^{b}	43.87^{b}
	30	65.1	52.19^{a}	59.27^{a}	41.59^{a}	47.18^{a}
	SEM	0.3	0.33	0.39	0.32	0.15

^{a,b,c} Values followed by the different superscripts letter within a column differ significantly ($P < 0.05$) from each other; SEM: standard error of the means

Table 4: Effects of Radiation Processing on Total Protein Efficiency (TPE) of Millet and Triticale

	Irradiation dose (KGy)	Group starting weight (g)	Group finishing weight (g)	Weight gain (g)	Feed intake (g)	Crude protein intake (g)	TPE
millet	Control	139	500	361	485	114	3.16^{d}
	10	139	609	470	552	127	3.7^{c}
	20	138	686	548	595	137	4^{b}
	30	140	727	587	569	131	4.48 ^{a}
	SEM	4.46	3.67	2.58	2.07	0.06	0.02
triticale	Control	139	460	321	439	101	3.17^{d}
	10	138	566	428	513	118	3.63^{c}
	20	140	650	505	552	127	3.97^{b}
	30	139	680	536	535	123	4.35^{a}
	SEM	3.1	1.23	2.04	1.6	0.03	0.04

^{a,b,c} Values followed by the different superscripts letter within a column differ significantly ($P < 0.05$) from each other; SEM: standard error of the means