TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2013) 37: 443-448
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doi:10.3906/vet-1202-25

The effect of electron beam irradiation on β-glucan content, X-ray diffraction of starch, protein subunit patterns, and in vivo digestibility of barley grain in cockerels

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Received: 21.02.2012 • **Accepted:** 27.09.2012 • **Published Online:** 29.07.2013 • **Printed:** 26.08.2013

Abstract: This study was carried out to evaluate the effect of electron beam radiation on the chemical composition, the β-glucan content, the water extract viscosity, the crystallinity of starch, the patterns of protein subunits, and the in vivo digestibility of barley grain. Barley samples were irradiated at doses of 10, 15, 20, 25, and 30 kGy under the electron beam. In a completely randomized design, cockerels were fed raw or irradiated samples to determine the digestibility of crude protein and gross energy. Irradiation had no effect on the total contents of protein, starch, and β-glucan. At doses of 10, 15, 20, 25, and 30 kGy, electron beam irradiation decreased the water extract viscosity of samples by 41%, 49%, 57%, 66%, and 78%; decreased the crystallinity of starch by 4%, 6%, 12%, 26%, and 27%; and increased the surface hydrophobicity of protein by 3%, 20%, 35%, 67%, and 80%, respectively. In vivo digestibility of energy and protein increased as irradiation dose increased. Therefore, electron beam irradiation of barley results in a better nutritional quality in respect to an increase in the bioavailability of its components.

Key words: Barley grain, cockerels, viscosity, ionizing irradiation, starch crystallinity

1. Introduction

Barley grain is used as an important ingredient in animal feeding. This grain contains a soluble nonstarch polysaccharide, termed β-glucan, in the cell walls, as well as starch and protein in the endosperm. The viscosity formed by these components has been recognized as an important cause of low nutrient digestibility and poor performance in pigs and broiler chicken fed diets containing barley grain (1,2). Therefore, the processing of barley grain to remove antinutritional factors and improve its nutrient bioavailability before including it in the diets of one-stomach animals is beneficial. Several methods such as milling, extruding, acid and alkaline hydrolyses, enzymatic digestion, ultrasound, microwave, and gamma irradiations have been evaluated for processing of barley grain (1–5); however, information concerning the effect of electron beam radiation on nutritional and antinutritional components of barley grain are scarce. Therefore, this study was carried out to evaluate the effects of electron beam radiation on the chemical composition, the β-glucan content, the water extract viscosity, the crystallinity of starch, the protein subunit patterns, and the in vivo digestibility of barley grain.

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2. Materials and methods

2.1. Sample irradiation

The barley samples (cultivar Fajr) were packed in nylon bags (30 cm × 40 cm × 5 cm, 0.5 mm in thickness) and exposed to electron beam irradiation (Rhodotron TT200 accelerator, IBA Co., Belgium) at the Yazd Radiation Processing Center (AEOI, Yazd Center, Iran) at doses of 10, 15, 20, 25, and 30 kGy at room temperature. The dose rate was determined using cellulose triacetate films (6).

2.2. Viscosity of water extract

The water extract viscosity measurements of raw and irradiated samples were based on the method described by Svihus et al. (5). Briefly, 1 g of milled barley grain was mixed with 5 mL of distilled water in centrifuge tubes. The tubes were incubated in a water bath at 40 °C for 30 min, followed by centrifugation at 3000 × *g* for 10 min. Viscosity of the supernatant was measured using a laboratory viscometer (Brookfield Engineering Laboratories, MA, USA).

2.3. In vivo digestibility

In vivo digestibility of crude protein and energy was measured as described in our previous study (7). Briefly, in a completely randomized design, 5 cockerels as replicates

were randomly given raw or irradiated samples at different doses. For each sample (raw or irradiated), the experiment was carried out with 3 days for an adaptation period, 2 days of starvation for depleting the digestive tract, and then 1 day of feeding followed by 2 days of starvation for complete excretion of the undigested material. The excreta of the 5 cockerels during the final 72-h period were collected separately, weighed, and frozen (–18 °C) until analyses of crude protein and gross energy could be done.

2.4. Chemical analysis

Chemical composition, including dry matter (Method 925.09), nitrogen (Method 984.13), ether extract (Method 920.39), and ash (Method 942.05), was determined according to the methods of AOAC (8). Gross energy of the grain and excreta samples was determined by adiabatic bomb calorimeter using a Parr-4 Model 1241 calorimeter. The amounts of β-glucans were analyzed using an enzyme kit (Megazyme International, Co., Wicklow, Ireland). The assay procedure was based on the McCleary method for mixed-linkage β-glucans (9), which has been accepted by AOAC (Method 995.16). Total starch content was determined using a Megazyme kit according to the method of McCleary et al. (10), which was adopted by AOAC (Method 996.11). Surface hydrophobicity of the protein-rich fraction of the samples was determined according to the method of Hayakawa and Nakai (11).

2.5. Monitoring the pattern of protein subunits

Protein subunits were fractionated by a SDS-PAGE technique as described by Sadeghi and Shawrang (4). Briefly, 12 mg of dried raw or irradiated barley grain was placed into 750 µL of SDS-PAGE sample buffer. After 30 min of thorough mixing (vortex and inverse), samples were immersed at 90 °C for 3 min and centrifuged at 10,000 × *g* for 1 min, and 25 µL of each sample was then loaded into the sample cell of gel (12.5% acrylamide in resolving gel and 3.75% acrylamide in stacking gel).

2.6. Crystalline structure of starch

The analysis of X-ray diffraction patterns was done with a diffractometer (D-8 Advance, Siemens) operating with copper Kα radiation at 30 mA and 40 kV. The starch powder of raw and irradiated barley grains was packed in a rectangular glass cell (40 × 30 mm, 0.3 cm thickness) and scanned at room temperature at a rate of 2°/min from the diffraction angle (2θ) of 4° to 80°. The degree of crystallinity was calculated following the method of Hayakawa et al. (12).

2.7. Statistical analysis

Data were analyzed with a completely randomized design using the general linear model procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC, USA). When a significant difference was found, means were separated using Duncan's multiple range tests. Differences were considered to be significant if $P \leq 0.05$. Linear response was determined using orthogonal contrast.

3. Results

3.1. Effects on chemical composition

The chemical composition of the raw and irradiated barley grain are shown in the Table. There were no significant differences among the raw and irradiated grains at various doses for contents of dry matter, crude protein, ether extract, ash, β-glucan, and starch.

3.2. Effects on viscosity

Figure 1 shows the effect of electron beam radiation on water extract viscosity of barley grain. Decline in the viscosity increased (linear effect; $P < 0.01$) as irradiation doses increased. At doses of 10, 15, 20, 25, and 30 kGy, the electron beam irradiation decreased the viscosity by 41%, 49%, 57%, 66%, and 78%, respectively. In the low-dose range (doses of 10, 15, and 20 kGy), decline in the viscosity was greater than that under a higher dosage (doses of 25 and 30 kGy).

Table. Chemical composition of raw and electron beam irradiated barley grain (g/kg dry matter).

Treatments	Dry matter	Crude protein	Ether extract	Ash	Starch	β-glucan
Raw barley	914	101	32	25	708	49
10 kGy	927	106	31	29	715	46
15 kGy	919	103	30	27	709	42
20 kGy	934	107	33	28	716	44
25 kGy	925	106	31	27	725	41
30 kGy	931	105	32	28	714	45
SEM	4.3	7.9	5.3	4.9	18.9	8.5

Means in the same column do not significantly differ ($P > 0.05$). SEM: standard error of the mean.

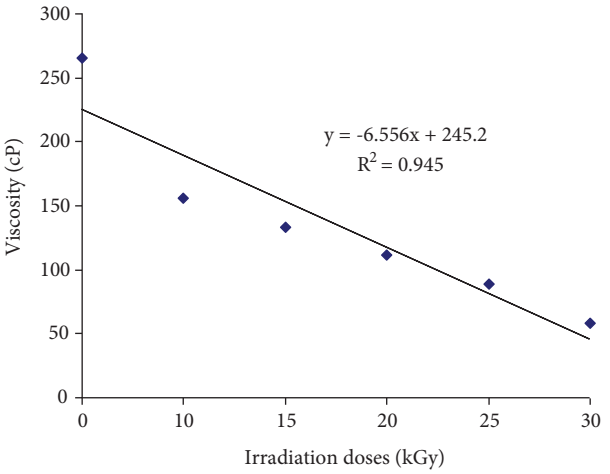

Figure 1. The water extract viscosity of raw and irradiated barley grain.

3.3. Effects on the crystallinity of starch

The X-ray diffractograms of the raw and irradiated barley starches at 10, 20, and 30 kGy are presented in Figure 2. The raw starch showed the diffraction peak at 18.4°, 22.8°, and 23.9°, with A-type crystallinity patterns. A comparison among diffraction patterns of the raw and irradiated barley starch showed that intensity of the reflections markedly declined by electron beam irradiation. The crystallinity of the raw barley starch and those irradiated at 10, 15, 20, 25, and 30 kGy was 26.8%, 25.7%, 25.1%, 23.6%, 19.8%, and 19.5%, respectively. A low decrease rate in the crystallinity of starch at low doses (doses of 10, 15, and 20 kGy) and a high decrease rate at high doses of irradiation (doses of 25 and 30 kGy) was observed.

3.4. Effects on protein hydrophobicity

The surface hydrophobicity of protein-rich fractions related to the raw and irradiated barley grain is shown in Figure 3. Electron beam radiation at a dose of 10 kGy had no significant effect on protein hydrophobicity compared to the raw sample. At doses of 15 kGy and above, a significant difference for protein hydrophobicity among the raw and irradiated samples was observed. Irradiation at doses of 15, 20, 25, and 30 kGy increased the surface hydrophobicity by 21%, 35%, 68%, and 80%, respectively, as compared with that in the raw sample.

3.5. Effects on pattern of protein subunits

The effect of electron beam irradiation on the barley protein subunits monitored by electrophoresis technique is shown in Figure 4. On top of the running gel, accumulated proteins as bands were observed, which were narrow in the raw sample.

3.6. Effects on the energy and protein digestibility

As shown in Figure 5, irradiation at doses lower than 15 kGy and 20 kGy had no significant effect ($P > 0.05$) on the energy and protein digestibility of barley grain, respectively, as compared with the raw sample. There were no significant differences among the energy digestibility of irradiated barley at doses of 15 kGy and higher, or for the protein digestibility at doses of 25 and 30 kGy. Irradiation at doses of 10, 15, 20, 25, and 30 kGy increased the energy digestibility of barley by 5%, 9%, 19%, 24%, and 30% and the protein digestibility by 4%, 7%, 14%, 31%, and 35%, respectively, as compared with the raw sample.

4. Discussion

No change in the chemical composition of irradiated barley grain is in agreement with previous works (13,14), in which no effect of electron beam irradiation on chemical composition of cereals and legume seeds was found.

The viscosity of irradiated barley grain decreased due to the depolymerization of the β-glucan by radiolysis, which might result in a decrease in its molecular weight and an increase in the solubility (15). An interesting study showed that gamma irradiation at doses lower than 50 kGy could change β-glucan, purified from black yeast, with high solubility and low viscosity (16). The depolymerization of starch (17) has also been considered to be responsible for the decrease of the viscosity caused by ionizing irradiation. Some studies revealed that ionizing radiation could change molecular structures of wheat starch (12), rice (17), and corn starch (18). Yu and Wang (19) reported that gamma irradiation could generate free radicals on molecules of starch, which finally alter molecular size and structures. Generated free radicals can decrease the molecular weight of starches, altering their structure and creating additional chemical functionalities on the ionized chains (20). The development of this process takes part in an increase in the water solubility of polysaccharides (21) and a decrease in the viscosity of cereals (17–19,22,23).

The starch crystallinity of irradiated samples decreased, as compared with the nonirradiated sample. This could be attributed to the formation of substances known as free radicals during irradiation. These substances attack biological molecules (starch), and they disrupt the interaction between amylopectin and amylase and the interaction between the amylopectin chains as a result of cleavage of the glycosyl bonds. Thus, the crystallinity of starch molecules declines (20).

Increase in the surface hydrophobicity of protein by irradiation occurred due to breakdown of hydrogen bonds, which results in denaturation of protein (24) and changes in the barley protein conformation to expose more hydrophobic sites. In the inherent structure of globular proteins, many hydrophobic amino acids are inside the protein molecule. Ionizing irradiation could induce the unfolding of the protein and denaturation, thus exposing nonpolar groups that were previously blocked (25). Hence,

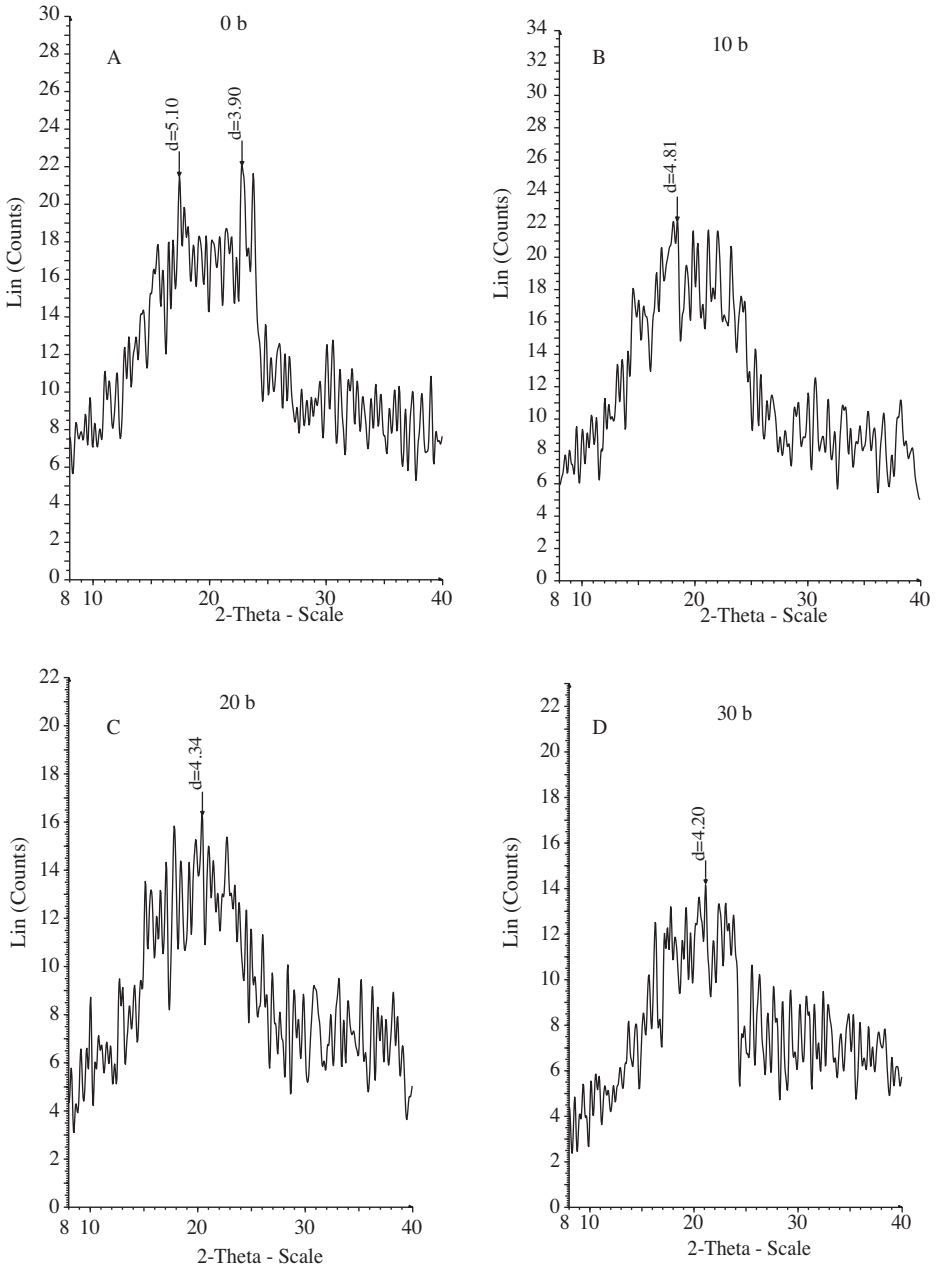

Figure 2. X-ray diffraction patterns of raw barley (A) and barley irradiated at doses of 10 kGy (B), 20 kGy (C), and 30 kGy (D).

irradiations increased the hydrophobicity of protein by exposing nonpolar groups. This is a favorable condition for proteins to approach each other and aggregate (24,26). Evidence for aggregation occurring in barley proteins by irradiation was monitored by electrophoresis technique, as shown in Figure 4. On top of the running gel, accumulated proteins as bands were observed, which were narrow in the raw sample. These bands were more thick and sharp in irradiated samples than in the raw sample. No differences were observed for other bands or protein subunits patterns in the middle of the gel.

Increase in the energy digestibility of irradiated barley can be explained by a decrease in the viscosity of digesta in the intestine of cockerels due to breakdown of β-glucans, increase in liberation of starch granules from the protein matrix due to protein denaturation, an increase

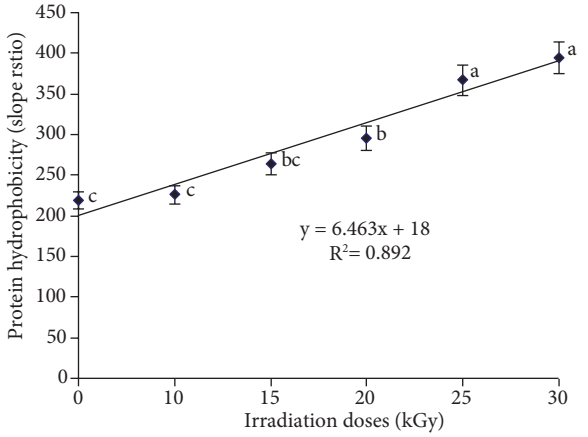

Figure 3. The protein hydrophobicity of raw and irradiated barley grain.

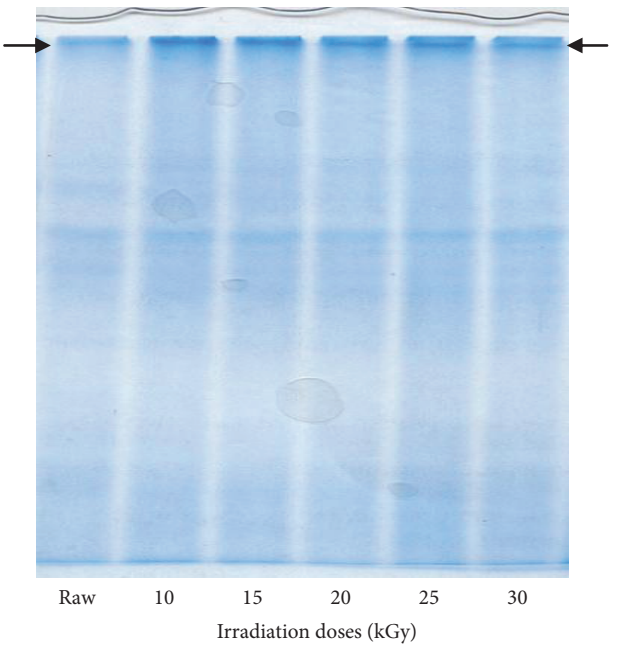

Figure 4. Protein subunit patterns of raw and irradiated barley grain.

in the availability of the starch chains to enzymes, and a depolymerization of starch.

Increase in the protein digestibility can be explained by a decrease in protein hydrophobicity, denaturation, and a decrease in the digesta viscosity. As mentioned previously,

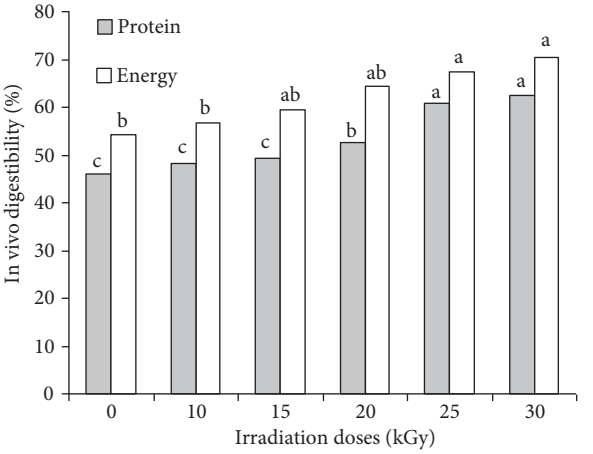

Figure 5. The digestibility of protein and energy of raw and irradiated barley grain. Bars with the same letter do not significantly differ (P > 0.05).

irradiation results in protein denaturation, which exposed hydrophobic amino acids, especially aromatics, which are position groups for active sites of pepsin and trypsin enzymes (27). Studies by Shawrang et al. (26,28) illustrated that protein denaturation occurring by irradiation could lead to improvement in intestinal protein digestion.

This study documented the effect of electron beam irradiation at doses of 10, 15, 20, 25, and 30 kGy on the chemical composition, β-glucan content, water extract viscosity, crystallinity of starch, protein subunit patterns, and in vivo digestibility of barley grain. Irradiation had no effect on total contents of protein, starch, and β-glucan, but decreased water extract viscosity and the crystallinity of starch and increased protein hydrophobicity. In vivo digestibility of energy and protein was increased as the irradiation dose increased. Therefore, irradiation results in a better nutritional quality of barley grain under investigation in respect to an increase in the bioavailability of its components.

Acknowledgments

The authors are grateful to the Yazd Radiation Processing Center, Nuclear Science and Technology School, Atomic Energy Organization of Iran for the irradiation operations. This study was financially supported by Deputy of Research and Technology, Atomic Energy Organization of Iran (project no: A87A050; 21.10.2008).

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