



**CHANGES IN ANTI-NUTRITIONAL CONTENTS AND DIGESTIBILITY OF  
GAMMA IRRADIATED SORGHUM GRAIN**

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**ABSTRACT**

Effects of gamma irradiation at doses of 10, 15, 20, 25 and 30 kGy on chemical composition, phytic acid, tannin contents and digestibility of sorghum grain were investigated. Irradiation had no effect ( $P>0.05$ ) on chemical compositions. Tannin and Phytic acid contents were linearly reduced ( $P<0.05$ ) as irradiation doses increased. Tannin content was reduced by 63, 69, 72, 83 and 84% and phytic acid content by 17, 34, 47, 76 and 92%, respectively, at doses of 10, 15, 20, 25 and 30 kGy. Irradiation improved ( $P<0.05$ ) crude protein digestibility of sorghum in the intestinal of cockerels. The results indicated that gamma irradiation has the potential to reduce the anti-nutritional factors and it is the useful processing methods for enhancing the digestibility of sorghum grain. Gamma irradiation as a physical method of preservation proved its efficacy in maintaining the nutritive value of sorghum grain in animal feeding.

**Keywords: Digestibility, Gamma Ray, Tannin, Phytate, Sorghum**

**INTRODUCTION**

Sorghum grain ranks fifth in the world after wheat, rice, corn and barley [1]. The most is used for animal feed and industrial products such as alcohol. Sorghum components, especially its protein and energy, are less digestible than other cereals for non-

ruminants [2, 3, 4], because of anti-nutritional factors such as tannins and phytic acid. Removal of these undesirable components is essential to improve the nutritional quality of sorghum and effectively utilize its potential as animal

feed. Most of various conventional, simple processing methods used to deactivate the antinutrients (e.g., dry heating, cooking, roasting, germination, fermentation) need not necessarily reduce or completely eliminate antinutrients, instead some methods reduce digestibility of grain, which results in significant nutritional losses [5].

Nuclear and related biotechnological techniques have played, a significant role in improving livestock productivity. Radiation processing has been shown to reduce or inactivate some of the anti-nutritional factors in wild leguminous seeds or meals, thereby enhancing their edibility [6, 7]. The effectiveness of gamma irradiation on quality, protein properties and physical structure of sorghum used as food has been investigated [8]. However, little information is available concerning effect of gamma on tannins, phytate and digestibility of sorghum used as feed. Therefore, in this study, it was aimed to evaluate effects of gamma irradiation on chemical composition, anti-nutritional contents and in vivo digestibility of sorghum grain.

## MATERIALS AND METHODS

### Sample Irradiation

Sorghum grain was packed in paper bags 6 × 6 cm and sealed. They were treated to gamma irradiation with doses of 10, 15, 20, 25 and 30 kGy, respectively using a Cobalt-60 gamma cell 220 (Atomic Energy of

Canada Ltd.) at room temperature from a 60 cobalt at Gamma Irradiation Center, Iranian Nuclear Organization, Tehran, Iran. The absorbed dose was measured employing Fricke dosimetry [9]. Similarly packed seed samples without irradiation served as a control.

### In vivo Digestibility

In vivo digestibility of dry matter, crude protein, true protein, starch and energy of untreated and irradiated samples was measured by Rhode Island Red cockerels at 64 weeks of age and similar body weight (average weight, 2.9 kg). Ten cockerels in a completely randomized design were placed into individual metabolic cages with fixed plastic trays for excreta sample collection. The experiment consisted of initial 3 days adaptation period for preliminary adjustment to the feedstuffs, 2 days starvation for exhausting digestive tract, then 1 day, five birds as replicates were fed with 150 g untreated or irradiated samples at 7 am, and following 2 days starvation for complete excretion of undigested material. Temperature was maintained within the 24-28 °C and relative humidity range was 60-65%. Lighting was 16 h light and 8 h darkness. Water was consumed ad libitum. Excreta were collected from individual birds during final 72 h period, weighted and frozen (-18 °C) and stored in an air tight plastic bag in a freezer until samples were

required for analysis. untreated and irradiated samples and excreta were ground through a 1 mm screen in preparation for chemical analysis of dry matter, crude protein, true protein and gross energy.

#### **Total Protein, Fat, Moisture, Ash and Fiber Content**

Moisture content was estimated from the mass of samples before and after they were stored overnight in an oven at 90 °C (AOAC Methods 925.09) [10]. Nitrogen was determined by using an FP-428 LECO apparatus (LECO Corp., Saint Joseph, MI) according to AOAC (Method 984.13). The instrument was calibrated each time with ammonium nitrate as a nitrogen standard. Fat content was determined with an SER 148 Solvent Extractor (Velp Scienti.ca, Milan, Italy) equipped with six Soxhlet posts. The fat extraction was carried out over a 30 min period, with thimbles immersed in boiling petroleum ether followed by 45 min of reflux washing. The samples were then placed in a muffle oven, where they were ashed at 550 °C for 16 h (Method 923.03). The ash content (% dry basis) was estimated as: (mass of ash/mass of the dry sample before ashing) × 100. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to method of van soest [11], using an automatic fiber analyzer (Fibertec System M, Tecator, Sweden). Neutral

detergent fiber was determined with  $\alpha$ -amylase, without sodium sulfite, without washing with acetone, and expressed without residual ash.

#### **Determination of Tannin**

Quantitative estimation of tannin, as catechin equivalent, was carried out using the modified vanillin-HCl method [14]. One g of seed flour was extracted with methanol (10 ml, 26 °C, 12 h), vortexed and decanted. This process was repeated and the supernatant was pooled and made up to 25 ml. The extract (1 ml) was treated with reagent mixture (5 ml) (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1). After 20 min, the color developed was read at 500 nm (Spectronic 21, Miltonroy, USA) using catechin (50–250  $\mu$ g) as standard.

#### **Phytate Content**

Phytate of raw and irradiated samples was determined according to the method described by Wheeler and Ferrel [15]. A standard curve was prepared to calculate the ferric ion concentration. The phytate phosphorous was calculated from the ferric ion concentration assuming 4:6 iron to phosphorous molar ratio.

#### **Determination of in vitro Starch Digestibility**

Starch digestibility was determined according to the method of Hagenimana et al. (2006) with slight change [16]. The

different starch fractions, resistant starch (RS), slowly digestible starch (SDS) and rapidly digestible starch (RDS), of Sorghum grain were measured in triplicate. Starch (100 mg, dry basis, db) or a dry-ground Sorghum sample equivalent to 100 mg of starch (db) were mixed vigorously in deionized water (5.0 mL) then equilibrated in a water bath at 37°C with agitation for 15 min and cooling at room temperature. After that, the mixture was added in 15 ml of phosphate buffer (0.2 M, pH 7.5). Then 1 ml of bacterial  $\alpha$ -amylase (A-3403, Type XII-A, Sigma Chemical Co., St. Louis, MO) were added and incubated at 30 C in a shaking incubator. After incubation, samples were centrifuged for 15 min at 3,000 rpm and supernatant removed. The amount of glucose released after 20 min is defined as RDS. A second measurement glucose released after further 90 min of the incubation is defined as SDS. The starch that remained unhydrolysed after a total of 120 min of incubation was measured as RS. 3, 5-dinitrosalicylic acid method used for analyze the reducing sugar produced at the end of treatment. Measure of the relative rate of starch digestion (The starch digestion index) was calculated as follows:  $SDI = (RDS/TS) \times 100$ .

### Statistical Analysis

All measurements were at least conducted in duplicate. Experimental data were submitted

to a Duncan analysis to determine whether the different treatments yielded significantly different results. The Duncan procedure was applied as described by Steel and Torrie [17], and a difference of p less than 0.05 was considered to be significant.

## RESULTS AND DISCUSSION

### Effects on Chemical Composition

The results for proximate composition of untreated and irradiated sorghum are shown in Table 1. These results indicated that there were no significant differences in protein, ether extract, fiber and ash contents between the irradiated and non-irradiated grains. These results are in agreement with previous works concerning the effect of gamma irradiation on chemical composition of rapeseed and soybean seeds [18, 19].

### Effects on Tannin

Tannin contents of gamma irradiated sorghum revealed a linear dose-dependent decrease ( $p < 0.05$ ) (Table 6). Decrease in tannins was significant at the gamma doses of 10, 15, 20, 25 and 30 kGy compared to control by 63, 69, 72, 83 and 84%, respectively, compared to control. Some reports indicated that gamma radiation also promoted reduction in the tannin contents as the radiation dose increased until a limited dose. The mechanism of gamma irradiation action on tannin has been related to generation of the hydroxyl and superoxide anion radicals and also related to changes in

solubility and chemical reactivity of tannin [20]. Further study is needed to clear its mode of reduction in tannin contents. This reduction in the tannin contents is very favorable, once this antinutritional factor presents the capacity of decreasing the protein digestibility.

### Effects on Phytate

Phytate content of sorghum in this study was 636 mg /100 g dry matter (Table 2). Phytic acid content reduced by 17, 34, 47, 76 and 92%, respectively, at doses of 10, 15, 20, 25 and 30 kGy of gamma irradiation. Phytate values of 0.27% [21], 0.3% [22], 0.886% [23], and 1% [24] have been reported for sorghum. The phytate content of sorghum grains is variable and appears to be dependent on cultivar. Phytate content was significantly reduced on gamma irradiation, especially eliminated at 25 kGy onwards. Similar observations have been made earlier, wherein anti-nutrients of foods or feeds were reduced after exposure to ionizing radiation [7, 25, 26]. The degradation of phytate by radiation can be attributed to cleavage in the structure of phytate or to the formation of inositol and inositol phosphates due to the action of free radicals generated during irradiation. Another possible way of phytate reduction during irradiation could have been through cleavage of the phytate ring itself. Finally, It could be, also, due to the formation of

insoluble complexes between phytate and other components such as phytate-protein and phytate-mineral complexes accordingly to the amount of free phytate was reduced [27].

### Effects on Digestibility

The Table 3 shows the effects of gamma irradiation on the dry matter, gross energy, starch, crude and true protein digestibility of sorghum grain. At doses higher than 15 kGy, digestibilities of dry matter, crude protein, true protein, starch and gross energy increased significantly compared to control. Increase in the protein digestibility can be explained by: 1) in treated sorghum reduction of anti-nutritional factors such as tannins and phytate is the main reason of increase in digestibility. Phytic acid induces a decrease of solubility and protein functionality. Tannins have a detrimental effect on digestibility of protein and amino acids. Due to their hydroxyl groups, tannins may interact with and form complexes with proteins, which may lead to precipitation because of the large size of the tannins [28]. When this antinutritional factor is found at the proportion of 5:1 tannin/protein, all protein is precipitated as consequence of the tannin action [29]. In addition to possibly causing a change in protein conformation, study of Siddhuraju showed that the tannins may also exert steric effects (due to their large size) and prevent enzymes access to

the proteins. Another possible reason for increasing in protein digestibility is modification in the three dimensional structure of sorghum proteins due to irradiation [6]. Studies of Shawrang illustrated that protein denaturation occur by irradiation that lead to improvement in protein digestibility [30, 31]. Phytic acid chelates mineral cations and proteins, forming insoluble complexes, which lead to a reduced bioavailability of trace minerals and reduced digestibility of proteins [6]. 2) Alteration or removal of natural properties of protein that cause decrease in protein hydrophobicity. As mentioned previously, irradiation result in protein denaturation, which exposed hydrophobic amino acids, especially aromatics, which are position groups for active site of pepsin. Furthermore, irradiation cause more peptide bonds exposed to proteolytic enzymes by change in secondary and tertiary structures of protein [4]. Phytate-protein complexes are insoluble and less under the influence of attack by proteolytic enzymes than the same protein; this subsequently affects the functional properties of the protein. Moreover, the partial removal of tannin and phytate probably created a large space within the matrix, which increased the susceptibility to enzymatic attack and consequently improve the digestibility of protein after radiation treatment. Molecular

rearrangement and changes in peptide linkages between the amino groups of amino acids could affect the nutritive availability and the biological utilization of the irradiated proteins. Such changes could interfere with the protein digestibility and/or its biological value.

The phytate molecule is highly charged with six phosphate groups and so is an excellent chelator, forming insoluble complexes with mineral cations and proteins [32]. This leads to reduced bioavailability of trace minerals and reduced protein digestibility. The inhibitory effect of phytate on protein digestibility has been demonstrated in experiments with casein and bovine serum albumin [33]. It was observed that phytate significantly decreased in vitro pepsin digestion of both casein and bovine serum albumin. This observation was attributed to the possible formation of a phytate-protein complex, which is less susceptible to enzymatic attack [34]. Similar results showing the inhibitory effect of phytate on protein digestibility have been reported for lactalbumin, soybean protein isolate and maize zein [35].

The increase in the starch digestibility of sorghum grain following gamma and electron beam irradiation might be due to: 1) Changes the crystallinity of starch as well as microstructure that can be attributed to the formation of free radicals during

irradiation. These substances attack the starch, and disrupt interaction between amylopectin and amylase causing the cleavage of the glucosyl bonds. Thus, the crystallinity of starch molecules declines and it induces effects such as chain scission (which makes the chain shorter) and cross linking [31]. 2) The changes in the morphology and molecular weight of polysaccharides following irradiation have as consequence changes in their solubility that increases/or easy to releasing from other attached components such as proteins or lipid [36]. Shortening of the polysaccharide macromolecular chains can be achieved by various methods such as ultrasound, microwave heating and ionizing irradiation. Among these, an ionizing radiation was found to be effective in degradation by the energy generated from different sources such as a cobalt-60 and an electron accelerator [37, 38]. This result was well supported by the reports related with the depolymerization of polysaccharides by radiolysis on starch [39]. Morphological

studies by scanning electron microscope showed that gamma ray-treated tamarind seed polysaccharides (TSP) showed a reduction in interconnection between bunches of fibers and a flat sheet-like structure increased. 3) The increase in extraction yields and solubility of starch with radiation treatment has also been reported [40]. Similarly, exist an increase in the extraction yields after treating medicinal herbs with gamma irradiation [41]. They observed that the total extraction yield in Korean medicinal herbs, increased by 5–30% with a 10 kGy dose of gamma irradiation. This drastic increment in digestibility were caused by cleavage of the glycosidic bond through free radical formation to from smaller carbohydrate units of dextrans of varying lengths, leading to a reduction in the molecular weight. Therefore, scissions of the chains probably produce short amylose chains, short linear chains from the branches of amylopectin or small branches of amylopectin.

**Table 1: Chemical Compositions of Irradiated Sorghum Grain (as g/100 g Dry Matter)**

Treatments	Dry matter	Ash	Crude protein	Ether extract	Crude Fiber	Starch
Untreated (control)	91.9	4.2	11.8	6.9	19.5	76.9
<b>Gamma irradiation</b>						
10 kGy	98.6	4.3	11.6	7.2	19.1	77.1
15 kGy	98.4	4.5	12.1	6.8	18.8	77.0
20 kGy	99.3	4.6	11.9	6.7	18.5	75.8
25 kGy	98.5	5.1	11.7	6.6	18.1	77.0
30 kGy	99.7	4.1	12.0	7.2	17.9	77.2
SEM	1.07	1.23	0.97	0.94	2.15	2.1

Note: Means in the same column without superscripts are not significantly different ( $p > 0.05$ ); SEM: standard error of the means

Table 2: Anti-Nutritional Contents of Untreated and Irradiated Sorghum Grain (mg/100 g DM)

Treatments	Tannin	Phytic Acid
Untreated (control)	82.5 <sup>a</sup>	636 <sup>a</sup>
Gamma irradiation		
10 kGy	30.2 <sup>b</sup>	530 <sup>b</sup>
15 kGy	25.6 <sup>bc</sup>	421 <sup>c</sup>
20 kGy	23.1 <sup>c</sup>	334 <sup>d</sup>
25 kGy	13.7 <sup>d</sup>	155 <sup>e</sup>
30 kGy	13.2 <sup>d</sup>	53 <sup>f</sup>
SEM	5.31	86.2

Note: Means in the same column with superscripts are significantly different ( $p < 0.05$ ); SEM: standard error of the means

Table 3: Digestibility of Untreated and Irradiated Sorghum Grain (mg/100 g DM)

Treatments	Dry matter	Organic matter	Crude protein	True protein	Gross energy	Starch
Untreated (control)	48.2 <sup>d</sup>	54.3 <sup>c</sup>	34.8 <sup>e</sup>	42.0 <sup>e</sup>	51.8 <sup>e</sup>	72.5 <sup>a</sup>
Gamma irradiation						
10 kGy	49.3 <sup>cd</sup>	54.8 <sup>c</sup>	35.9 <sup>e</sup>	43.7 <sup>de</sup>	52.3 <sup>de</sup>	74.1 <sup>b</sup>
15 kGy	49.8 <sup>cd</sup>	56.4 <sup>bc</sup>	38.0 <sup>cde</sup>	46.3 <sup>cde</sup>	54.6 <sup>cd</sup>	76.0 <sup>c</sup>
20 kGy	51.1 <sup>bcd</sup>	58.5 <sup>ab</sup>	40.9 <sup>bcd</sup>	49.9 <sup>bc</sup>	54.8 <sup>cd</sup>	77.1 <sup>cd</sup>
25 kGy	53.0 <sup>ab</sup>	59.7 <sup>a</sup>	43.9 <sup>ab</sup>	53.0 <sup>ab</sup>	55.2 <sup>c</sup>	77.6 <sup>d</sup>
30 kGy	54.6 <sup>ab</sup>	60.0 <sup>a</sup>	46.9 <sup>a</sup>	56.4 <sup>a</sup>	56.3 <sup>bc</sup>	79.1 <sup>e</sup>
SEM	2.89	1.93	3.14	3.97	1.83	1.88

NOTE: Means in the same column with superscripts are significantly different ( $p < 0.05$ ); SEM: standard error of the means

## CONCLUSION

The present study reveals that Gamma irradiation has the potential to reduce the anti-nutritional factors and enhance digestibility of sorghum grain. Decrease in anti-nutritional factors such as tannins and phytate is the main reason of increase in digestibility of treated sorghum grain. Phytic acid induces a decrease of solubility and protein functionality. Effect of gamma irradiation on phytate elimination was higher than tannins and it was dose dependent. Gamma irradiation is useful processing methods for decreasing the anti-nutritional compounds and improving nutritive value of sorghum in animal feeding.

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