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Evaluation of the Use of Gamma Radiation for Reduction of Aflatoxin B_1 in Corn (*Zea mays*) Used in the Production of Feed for Broiler Chickens

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Abstract

Corn is one of the main grasses used to produce human or animal food. One of the main problems with the use of corn is the presence of mycotoxins, where aflatoxin B_1 is one of the most harmful for human and animal health. Chemical methods such as the addition of antifungals and sequestrants are used to control this contaminant in food; however, these methods can leave dangerous residues. The aim of this research was to evaluate the effectiveness of irradiation with gamma rays on corn for the control of aflatoxin B_1. For this purpose, three treatments were compared: treatment 1, application of gamma radiation, treatment 2, application of antifungal and treatment 3, combination of gamma radiation and application of antifungal. Corn without exposure to any control of aflatoxin was used as a witness group. Irradiation doses of 2, 6 and 10 kGy were tested, and the dose of 6 kGy was selected as the best since it lowered aflatoxin B_1 more effectively. The corn analyzed in this study was stored during 45 days at 23˚C, and every 15 days. We determined the concentration of aflatoxin B_1, presence or absence of pathogenic microorganisms and insects, and we evaluated the physicochemical characteristics of the grain. Then, the treatments were compared and treatment 1, only irradiation with gamma rays, proved to be significantly more effective in the reduction of aflatoxin B_1 and the total inhibition of the survival of the insects during the 45 days of storage which allowed better preservation of the protein. All treatments controlled the growing of pathogenic microorganisms as *Salmonella* and *Enterobacteriacea*, and kept the humidity of the grain in values lower than 13%. The cost production of a bag of 40 kg of feed was also estimated in USD 22.56; which is 1.80% greater than the cost corresponding to the conventional process that uses mycotoxin sequestrants.

Keywords

Zea mays, Aflatoxin, Gamma Radiation, Poultry Feed

1. Introduction

Corn (*Zea mays*) is one of the most consumed cereals by humans since ancient times and one of the most cultivated grasses worldwide; it constitutes the first cereal in grain production per hectare and the second in total production after wheat. Its easy adaptation has allowed to have crops in tropical and temperate zones, with high productions [1].

According the Food and Agriculture Organization of the United Nations (FAO), the demand for this product by 2030 will increase by 60 million metric tons for human consumption and by 235 million metric tons for animal feed [1].

In the period 2014-2015, corn production recorded a value exceeding one billion tons, with an average annual growth of 3.5% since 2004 worldwide [2].

In Ecuador, the largest production of this grass is concentrated in the province of Los Ríos, with 51% of national production, while Loja with 9% is the province with the lowest production. 57% of the Ecuadorian corn is destined to the poultry industry, 6% is used as raw material in feed for other animals, and 37% is for human consumption [3].

On the other hand, inadequate storage of corn causes losses both to the farmer and industry. Although no specific data are recorded in Ecuador, according to the FAO, in countries of Southeast Asian there are losses of approximately 66% caused mostly by contamination with aflatoxins. In countries such as Mexico, Venezuela, Colombia and Peru, the incidence of aflatoxins in corn ranges from 8% to 82% [4].

In humans, mycotoxins enter the body through the ingestion of contaminated food, skin contact and inhalation of spores. Due to its high liposolubility, these toxins are absorbed by the intestinal tract and, trought microsomal enzymes that are metabolized in the liver. The most toxic aflatoxin is B_1, which is the most important in public health because it is related to the development of hepatocellular carcinoma causing protein malnutrition in more than 118 million children in developing countries [4].

The demands for food products that preserve their organoleptic characteristics, have long lifetimes and are innocuous, they have promoted the incorporation of alternative technologies for the post-harvest treatment of food; a physical method of conservation known as irradiation is one of them [5]. Irradiation is the emission and propagation of energy through space or a material medium. Irradiation is considered as a preservation method of food that allows the sterilization and prolongation of the shelf life of the products [6].

Therefore, the objective of this work was to evaluate technically and economically the application of an alternative treatment (gamma irradiation) to a product of agricultural origin such as corn (*Zea mays*), to reduce the concentration of aflatoxin B_1 to safe levels and use it in the elaboration of foodstuff for broiler chickens, in order to ensure their innocuousness in consumption.

2. Materials and Methods

The corn (*Zea mays*) that was used in this investigation was supplied by the Collection Center "Asociación Narcisa de Jesus", located in the province of Los Ríos, Ecuador.

A total of 120 kg of the grain that was harvested in physiological maturity was acquired and then dried up to 13% of moisture.

2.1. Comparison of Gamma Irradiation with the Selected Dose, the Application of Antifungal and the Combination of Both Treatments for the Control of Aflatoxin B_1 in Corn (*Zea mays*)

The treatments tested were: treatment 1: only irradiation (S-I) (gamma irradiation at the best dose determined experimentally), treatment 2: only antifungal (S-A) (application of antifungal), and treatment 3: application of antifungal and irradiation (A + I), compared to a control sample without treatments (MC).

- Physical Treatment—Gamma Irradiation

For the irradiation of corn, Cobalt 60 belonging to the Department of Nuclear Sciences (DCN) of the National Polytechnic School (EPN) was used, whose activity in January 2017 was approximately 1310.2 Ci (Curies). The grain bags were placed at a distance of 30 or 40 cm from the source.

In order to select the best radiation dose, a unifactorial experimental design was used. The design variable was the dose and they were tested as levels 2, 6 and 10 kGy. The response variable was the reduction of aflatoxin B_1 content after treatment. The experimental units were bags with 5 kg of corn and four repetitions were made for each dose.

The data were analyzed through a variance analysis (ANOVA) and a multiple range test of Fisher (LSD), with 95% confidence, in the Statgraphics Centurion version XV.II program.

- Chemical Treatment—Antifungal

For the chemical treatment and the combined treatment, a 50:40:10 mixture of propionic acid, ammonium propionate and sodium chloride was used as antifungal, at a rate of 1.3 kg/t [7].

All samples were stored at room temperature at 25˚C, during the 45 days established for this study.

2.2. Physical, Chemical, Microbiological and Toxicological Characterization of Corn

For the physical, chemical and toxicological characterization of corn, five measurements of each treatment were made: the first and second before and after applying the treatments and the others at 15, 30 and 45 days of storage.

For the microbiological analyzes, two measurements were made: the first before the application of the treatments in all the samples; and the second when the 45 days of storage were completed.

For the physical analysis, the method described in the standard NTE INEN 1236:2013 (INEN, 2013) [8] was applied. For the chemical analysis, the NTE INEN-ISO 5983-1 test method (INEN, 2014) [9]] was used.

For the microbiological analysis, the standard NTE INEN 1829-2014 was used (INEN, 2014) [10], and the presence of *Enterobacteriaceae* and *Salmonella* was determined, using the methods ISO 21528-1:2004 (ISO, 2004) [11] and AOAC-RI 061203 (AOAC, 2015) [12].

For the evaluation of the aflatoxin content, the concentration allowed in the NTE INEN 1829-2014 standard (INEN, 2014) [10] was taken as a reference and quantified by means of the AOAC-RI 050901 method (AOAC, 2015), with the use of the ANSR kit Aflatoxin B_1 [13].

2.3. Determination of the Economic Feasibility of the Elaboration of Foodstuff for Broiler Chickens Using Corn (*Zea mays*) Resulting from the Best Treatment for the Control of Aflatoxin B_1

The estimation of the costs related to the elaboration of feed for broiler chickens, based on corn resulting from the best treatment for the control of aflatoxin B_1, was carried out. These costs were compared with those corresponding to the process currently used for the production of a batch of 2000 kg of foodstuff, which include the addition of an antimycotic (propionic acid, ammonium propionate and sodium chloride) and a commercial inert sequestrant (beta-glucans and carbohydrates obtained from yeasts and algae).

This analysis was done through a cost table in which the costs of raw material, reagents and industrial inputs, production costs, financial, administrative and general expenses, which were provided by an Ecuadorian company of feed production were considered.

3. Results and Discussion

3.1. Determination of the Irradiation Dose in Corn (*Zea mays*) that Allows the Greatest Reduction of Aflatoxin B_1

The necessary irradiation times to reach the doses of 2, 6 and 10 kGy were 23 h 16 min, 69 h 42 min and 116 h 09 min, respectively. These times included pauses to flip the bags when they had received half the required dose, in order to have a greater uniformity.

The statistical analysis of the results showed that the dose of gamma irradiation significantly affected ($p < 0.05$) the content of aflatoxin B_1 in corn, as shown in **Table 1**.

The multiple range test, by the Fisher method (LSD), showed that the doses of 6 and 10 kGy did not show significant differences ($p < 0.05$) among themselves; they allowed a reduction (%) of aflatoxin B_1 of 89.59 ± 4.81 and 95.47 ± 1.29, respectively. While the dose of 2 kGy showed a lower effectiveness in the reduction of aflatoxin B_1, as seen in **Figure 1**.

The best dose of radiation was selected at the value of 6 kGy, due to its high effectiveness and shorter exposure time (69 h 42 min), in relation to the dose of 10 kGy (116 h 09 min), with a distance of 30 cm from the Cobalt 60 source.

Bishnio y Chandra [14] studied the effect of irradiation on peanut seeds contaminated with aflatoxin and found that at doses of 3 to 5 kGy the presence of colony-forming units of *Aspergillus flavus* was significantly reduced and the shelf life of the product was increased by two months [15] found efficiency in the use of gamma radiation for the total reduction in the number of colony-forming units of *Aspergillus flavus* in maize samples that were irradiated with doses of 10 kGy.

Ghanem, Orfi and Shamma [16] studied the effectiveness of gamma radiation treatment for the degradation of aflatoxin B_1 (AFB_1) in peanuts, pistachio, rice, maize and barley. They incubated the sterilized raw materials with *Aspergillus flavus* spores and verified the growth of the fungus. Subsequently, they irradiated

Table 1. ANOVA of the effect of the radiation dose in the reduction of aflatoxin B_1 in corn.

Source	Sum of squares	Df	Mean square	f-ratio	P-value
Between groups	5962.95	2	2981.47	172.41	0.0000
Within groups	155.636	9	17.2929		
Total	6118.59	11			

R-cuad. = 85.49%; R-cuad.(ajusted) = 82.27%.

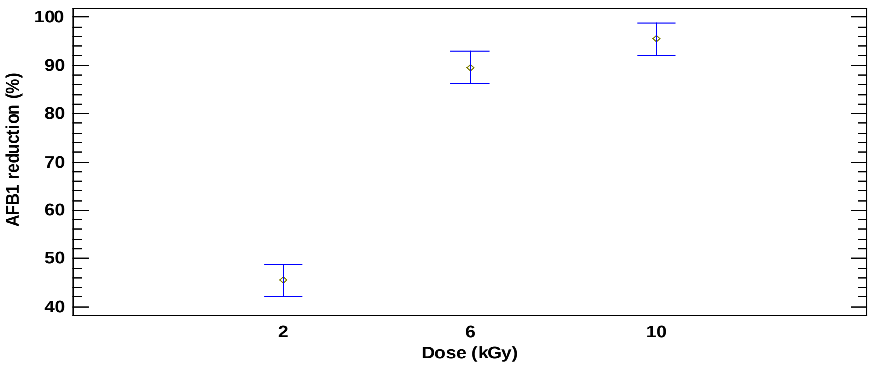

Figure 1. Measurement chart (Fisher, 95%) of the effect of radiation dose in the reduction of aflatoxin B_1.

the products with doses of 4, 6, and 10 kGy. They found that the degradation of AFB_1 was positively correlated with the gamma dose. With the dose of 10 kGy they reached percentages of degradation of the toxin between 45 and 90%. The degradation of AFB_1 showed a negative correlation with the oil content in the samples; the best results were presented in corn, which has a lower lipid content.

The results of these studies suggest that the irradiation of these products, with doses between 3 and 10 kGy, eliminates fungi that produce mycotoxins and degrade toxins to levels below the maximum values accepted for food.

3.2. Comparison of Gamma Irradiation with the Selected Dose, the Application of Antifungal and the Combination of Both Treatments for the Control of Aflatoxin B_1 in Corn (*Zea mays*)

Three different treatments were compared for the control of aflatoxin B_1: The chemical treatment (S-A) consisted in the addition of commercial antifungal (propionic acid, ammonium propionate and sodium chloride); the physical treatment (S-I) in the irradiation of corn with the selected dose of 6 kGy; while the combined treatment (A + I) corresponded to the application of the commercial antifungal, followed by the gamma irradiation. Additionally, a control sample (MC), with corn without the application of any treatment was used. For this comparison, physical, chemical and microbiological parameters were evaluated.

For the irradiation in the physical treatment and the combined with dose of 6 kGy, a time of 21 h 05 min was required, which included a pause in the middle, to flip the bags of corn, with the aim of achieving a greater uniformity in the dose received. In this case, the bags were placed at 30 cm from the Cobalt 60 source.

3.2.1. Physical Characterization of Corn: Humidity

In the humidity parameter, the ANOVA determined that there was a significant effect of the treatments used on the moisture content in corn ($p < 0.05$). However, the multiple range test by the Fisher method (LSD) allowed us to know that the treatments S-I, S-A and A + I belonged to the same homogeneous group and allowed to maintain a humidity value between 12.13% and 12.20%, while in the control sample, humidity decreased to 12.02%, as shown in **Figure 2**.

In all cases, corn moisture was between 12 and 13%, which is the recommended range to minimize grain deterioration during storage [17]. However, maintaining a higher humidity level within this range of safety would be an economic advantage, since the grain is marketed according to its weight.

The lower humidity value found in MC could be due to a greater presence of insects or *Aspergillus flavus* in the corn samples. This could cause a slight increase in temperature due to breathing, since approximately 673 kcal are generated for each mole of glucose consumed [18]. Exposure to this air at a higher temperature probably removed a greater amount of moisture from the corn with which it had contact.

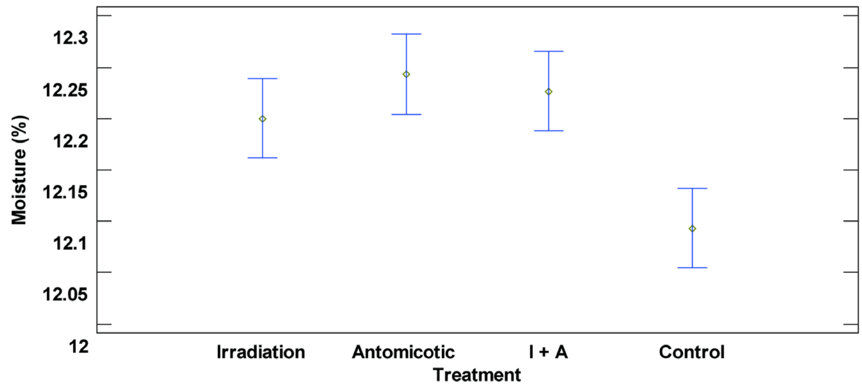

Figure 2. Measurement chart (Fisher, 95%) of the effect of treatments on grain moisture.

3.2.2. Physical Characterization of Corn: Insect Infestation

The presence of live insects in the corn samples of each treatment and the control sample was analyzed, as a result it was obtained that the treatments that included irradiation (irradiation only (S-I) and the combined treatment (A + I)) inhibited in 100% the survival of the insects in the corn, throughout the 45 days of storage.

Similar results were obtained in a study on the effect of gamma radiations in rice weevil carried out by Romero F. [19], in which it was determined that there is absolute inhibition in the development of insect´s eggs in corn at 0.05 and 0.07 kGy and total elimination of adult insects in corn with a dose of 0.07 kGy.

According to Casp & Abril [20] irradiation with low doses made for the misinsection of grains is effective, since the insects die with this range of doses in all their stages of development, including the eggs deposited inside the grain. Additionally, this treatment alters the DNA of the insects, so that they become unable to reproduce and, therefore, there are no risks associated with the development of resistance, unlike the use of insecticides [21].

In the only antifungal treatment (S-A) and in the control sample (MC), insects were found at the end of the storage period. A live insect of the genus *Prostephanus truncatus* was detected in the MC treatment at 30 days of storage; and at 45 days, live insects of the same genus were detected in two replications of the S-A treatment, and in the three MC repeats.

The appearance of these insects in the mentioned time agrees with that stated by [22] who claim that insects spawn different amounts of eggs inside or on the surface of the corn. In particular, the corn borer (*Prostephanus truncatus*) spawns about 400 eggs during its reproductive life, and its development time is estimated to be 4 to 6 weeks.

The infestation during the storage of corn can generate losses for various reasons. First, insects feed on the grain; some species consume the endosperm, which affects the quality and weight of the product, while others feed on the germ, which decreases germination and viability rates if used as seed. The insects, in addition, contaminate the corn with its excrement, dead bodies or parts of them. Additionally, they increase the risk of bacterial or fungal infections, since they carry spores and generate wounds in the stored grains, facilitating the development of these microorganisms [23]. Finally, the presence of insects can cause an increase in the temperature of the product, which leads to higher humidity and more favorable conditions for the development of fungi [24].

3.2.3. Chemical Characterization of Corn: Protein

It was found that neither the applied treatments nor the storage time had significant influence ($p > 0.05$), on the protein content in the corn. The amount of protein, in all cases, complied with the requirement established in the Ecuadorian regulation NTE INEN 187: 2013 "CEREALS AND LEGUMINOUS. CORN IN GRAIN. REQUIREMENTS" (INEN, 2013), with values in the range between 8.00 ± 0.10 and 8.15 ± 0.09, throughout the storage time [25].

Ionizing radiation can alter the composition and nutritional value of foods; the scope of these changes depends on the nature, type, variety and composition of the products, the dose and type of irradiation applied and the environmental conditions before and after the process. The results obtained in this investigation suggest that the doses of gamma radiation tested did not produce significant changes; this agrees with other studies such as that of Oliveira [26], who irradiated rice with doses of 6.5 to 7.5 kGy, and claim that there was no alteration in their nutritional quality. Similarly, Farkas [27] stated that treatments with doses between 2.0 and 7.0 kGy do not affect the nutritional properties, sensory quality or physical properties of food.

The preservation of protein content in irradiated corn represents an advantage for its use as a feed ingredient. According to Rodríguez *et al.*, [28] the consumption of protein at adequate levels, during the initiation and completion stages of broilers, is fundamental to achieve the required weight, at low cost.

3.2.4. Microbiological Characterization of Corn: *Enterobacteriaceae* and *Salmonella*

The microbiological analysis of *Enterobacteriaceae* and *Salmonella* in the treated corn was carried out in two stages: the first before the application of the treatments to a sample taken from all the bags with corn; and the second one was carried out at the end of the experimentation, after 45 days of storage. In both stages, zero colony-forming units (CFU) of enterobacteria and absence of *Salmonella* were found for all treatments.

The microbiological quality of the corn used in this work can be attributed to the good practices used during the harvest and post-harvest of the grain, as well as storage conditions. According to Bejarano *et al.*, [29], moisture, temperature and storage time are critical factors in the control of microorganisms. However, occasionally there have been problems associated with the growth of bacteria in the grain stored for periods of time greater than 4 months. In this context, the irradiation of corn would have the additional advantage of inhibiting the development of pathogens.

Ionizing radiation is capable of eliminating microorganisms by different mechanisms, such as the destruction of cell membranes, the inactivation of enzymes and even damage to the genetic material [30]. Additionally, microorganisms that have suffered sub-lethal damage due to the incidence of ionizing radiation have a greater sensitivity to abiotic stress factors, which reduces their probability of survival dramatically [31]. On the other hand, it has been proven that treatments with doses between 2 and 7 kGy and have been effective for the elimination of pathogenic non-spore-forming bacteria such as *Salmonella*, *Campylobacter*, *Listeria monocytogenes* and *Escherichia coli* [27].

3.2.5. Toxicological Characterization of Corn: Aflatoxin B_1

The analysis of aflatoxin B_1 was performed in all treatments and control, the results showed that the treatments applied had a significant effect on the aflatoxin content ($p < 0.05$). The multiple range test, of Fisher method (LSD), showed that there are significant differences in the content of aflatoxin B_1, as shown in **Figure 3**.

No significant differences were found between the three treatments, but there were significant differences with respect to the control sample (MC). The irradiation at 6 kGy (S-I), with an average of 8.75 ppb and the combined treatment (A + I), with an average of 15.30 ppb, were significantly more effective for the control of aflatoxin B_1 than the absence of treatment.

This reduction of aflatoxins in the irradiation process can be due to the radiolysis of water present in the grains, in which highly reactive free radicals are formed and can attack the terminal furan ring of AFB_1, generating products of low biological activity. In similar studies, the AFB_1 reduction was determined to be 75% and 100% after irradiation in contaminated peanut meal [4].

The aflatoxin values in ppb, of the two treatments that were significantly more effective, varied throughout the experimentation; in the case of the treatment only irradiation (S-I), the effectiveness was of 90% to 100% after irradiation until45 days of storage; in the case of the combined treatment (A + I) the effectiveness value was variable, ending with 77.03% after 45 days of storage.

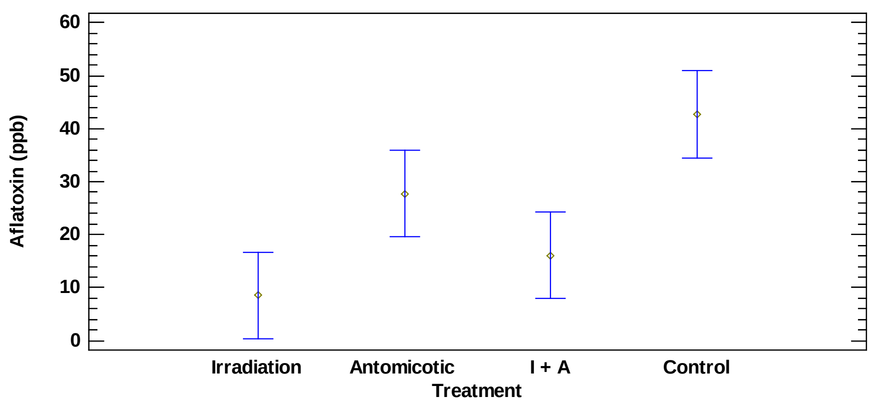

Figure 3. Measurement chart (Fisher, 95%) of the effect of the treatment applied on the content of aflatoxin B_1.

Due to its high effectiveness and economic benefits, irradiation with a dose of 6 kGy (S-I) was determined as the best treatment.

The statistical analysis of these data allowed to establish that the irradiation process is effective in the reduction of aflatoxin B_1, an important advantage in relation to other methods because the consumption of feed with high parameters of aflatoxin B_1 affects the metabolism of the birds, by decreasing the activity of several enzymes in the digestion of starch, lipids and proteins [32]. As a result of the consumption of aflatoxins in their food, birds can reduce growth and feed conversion. Subsequently, seizures of meat in slaughterhouses and increased mortality can occur, all of which would be reflected in economic losses [32].

3.3. Determination of the Economic Feasibility of the Elaboration of Feed for Broiler Chickens from Corn (*Zea mays*) Resulting from the Best Treatment for the Control of Aflatoxin B_1

3.3.1. Estimating the Irradiation Costs of Corn

To estimate the irradiation cost of corn, a reference value of a commercial irradiator was taken, which corresponds to 0.07 USD / kg of the irradiated product [33].

In addition, it was considered that the corn was transported via land, with a cost of 1.25 USD/qq of product transported; with respect to corn costs, the minimum support price was taken, which is 14.90 USD/qq of corn; the quintal has 45.36 kg, with 13% humidity and 1% impurities [3]. The cost of irradiated corn is 0.43 USD/kg of product.

3.3.2. Feed Production Costs for Broilers Chickens

The production cost was determined for a 2000 kg batch of feed for broilers chickens, from irradiated corn; this value was contrasted with the costs corresponding to the process that is currently used for the preparation of feed in a feed processing plant.

The cost of the raw material of the current feed production process, including the addition of an antifungal powder and an inert sequestrant, is worth 1014.38 USD. The costs of production include direct and indirect costs of the process of preparation of feed, which consider the costs of raw materials, reactive and industrial inputs, labor and indirect manufacturing costs.

The total production cost of a 40 kg bag of feed for broiler chickens is 22.16 USD.

For the elaboration of feed from irradiated corn, the costs of raw materials were taken, in this analysis the costs of adding the powder antifungal or inert sequestrant currently used are not considered, since they would no longer be necessary. This cost is 1045.78 USD.

The total production cost of a 40 kg bag of feed with irradiated corn would be 22.56 USD. **Table 2** compares the costs of balanced feed production for broiler chickens plus additives and irradiated corn. It can be seen that the second is 1.80% more expensive than the first.

Table 2. Compares the of total costs for the preparation of a 40 kg bag of feed for broilers with irradiated and no irradiated corn.

Feed	Total cost (USD)	Difference (USD)	Difference (%)
Corn no irradiated	22.16	0.40	1.80
Corn irradiated	22.56		

This difference of 0.40 USD could be justified if the benefits of irradiation are considered compared to the use of additives (antifungal and sequestrant) in the preparation of feed, since it allows the elimination of insects that could be present in the corn and be developed during storage, as well as chemical waste.

Additionally, transport and irradiation items could decrease if larger amounts of corn are irradiated or if a strategic irradiation center near the industrial zone is implemented.

4. Conclusions

The dose selected for the irradiation of corn was 6 kGy, since it allowed a reduction of aflatoxin B_1 in an average of 89.58% and despite not presenting significant differences with respect to 10 kGy, it is cheaper.

The physical (humidity and infestation), chemical (protein content) and microbiological (*Enterobacteriaceae* and *Salmonella*) characteristics of the corn treated with irradiation (S-I), antifungal (S-A) and by the combined treatment presented values that comply with national regulations.

The S-I treatment was the most effective in the reduction of aflatoxin B1 present in corn, with a final value of 0 ppb after 45 days of storage. In addition, it allowed a complete elimination of insects up to 45 days of storage taken for this study and an inhibition of the development of pathogenic microorganisms (*Enterobacteriaceae* and *Salmonella*), without affecting the protein content.

It was determined that a bag of 40 kg of feed for broiler chickens from irradiated corn would cost 22.56 USD that would be 1.8% higher than the cost of the current feed which includes the addition of an antifungal and an inert sequestrant.

Although corn treated by irradiation would have a higher cost, it would have advantages over untreated corn, especially related to the elimination of pathogenic microorganisms and insects, which turn out to be a problem when the grain is stored for long periods of time. Additionally, the irradiation leaves no residue in the treated material.

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