KOREAN J. FOOD SCI. TECHNOL.
Vol. 19, No. 5 (1987)

Decontamination of Poultry Feeds by Gamma Irradiation

Myung-Woo Byun, Han-Ok Cho, Jae-Won Lee, Joong-Ho Kwon and Young-Bae Kim*

Division of Food Irradiation, Korea Advanced Energy Research Institute, Seoul
** Department of Food Technology, Korea University, Seoul*

Abstract

The effects of gamma irradiation on microbiological and chemical qualities of four kinds of poultry feeds were investigated. The viable counts of total bacteria in the samples were 10^5 to $10^6/g$. They were reduced by 2 to 3 log cycles after 3 to 5 kGy irradiation, and were completely eliminated with irradiation of 7 kGy. Coliforms and enteric pathogens were contaminated in high levels in all samples, ranging from 1.2×10^4 to $1.7\times10^5/g$ and 4.0×10 to $2.6\times10^3/g$, respectively, They were sterilized by 3 to 5 kGy irradiation. Fungi, ranging from 10^2 to $10^4/g$, mainly osmophiles were identified as *Aspergillus* and *Penicillium*. They were eliminated to a undetectable level by 5 to 10 kGy irradiation. Six kinds of species, including *Aspergillus flavus*, were potential mycotoxin producers. Chemical components, such as proximate compositions, and mineral contents were not affected by the gamma irradiation. However, TBA values and amino acid content seemed to be affected by gamma irradiation.

Introduction

The demand for poultry feed in Korea has been increasing with the expansion of the farm aminal industry. It is estimated that in 1986 more than 2.6 million tons of feedstuffs were used in the breeding of poultry.[1] Damage caused by microorganisms and insects and posioning by pathogens are increasing. On the other hand, *Salmonellae* and other pathogenic microorganisms in poultry feed are an important publich health problem. Elimination of *Salmonellae* and other pathogenic microorganisms from poultry feed could aid in preventing the cycle of infection from feed to poultry to man.

To prevent this damage or poisoning, large quantities of antibiotic subtances, fumigants and other chemical additives have been widely used in feedstuffs.[2-4] These methods present several problems is in terms of ineffectiveness and complexity of treatment, adverse effect on nutritive value, and cost and capacity of treatment. Above all, chemical treatment of feed has had toxic effect on animals and humans; for example, excessive use of antibiotics results in some drug-resistant organisms and residue in animal tissues causes some danger to human health. Recently antibiotics have been prohibited as feed additives in some countries.[20].

The potential of ionizing radiation in controlling microorganism contamination in animal feeds has been indicated at several panels organized by the International Atomic Energy Agency (IAEA) and Food and Agriculture Organization (FAO) in 1962,[5] 1966[6] and 1976[7] and at the first food irradiation symposium in 1966.[8] Particularly valuable were the studies performed in the Netherlands,[9] Japan,[10-12] United Kingdom,[13-14] and in the other countries.[3, 15-19]

Ionizing radiation treatment is considered suitable for the preparation of farm animal feeds. One of the purposes of radiation treatment of feedstuff is to prevent damage caused by fungi and other microbial growth during storage or distribution. Another purpose is to eliminate pathogens such as *Salmonellage* in animal feed. Moreover, it can be useful for producing specified pathogen free (SPF) eggs and meats.

Therefore, radiation treatment has been considered superior to the use of fumigants or chemical additives as regards safety in animal breeding. In Korea, irradiated animal feeds are being used routinely only in the breeding of laboratory animals. They are not currently used in the breeding of farm animals because of uncertainty as to wholesomeness, and because of the shortage of microbiological, nutrititional and economical information on irradiated animal feeds.

Therefore, the present study was a part of recent extensive work undertaken in order to commercialize food irradiation technology in Korea. In this study we investigated the distribution of microorganisms in 4 kinds of poultry feed, and the efficiency of gamma-irradiation

for decontamination. In addition, chemical properties were investigated.

Materials and Methods

Materials

Four kinds of poultry feed were obtained from a local company near Seoul.

Treatment of Gamma-Irradiation

In irradiation, the poultry feeds weighing from 250 to 300*g* were aerobically packed, into pouches laminated with 20 *μm* nylon and 60 *μm* polyethylene. The radiation was effected at room temperature with a dose rate of 40 Gray (Gy)/hr. The dose levels applied were 0, 3, 5, 7, and 10 kGy. The treated smaples were stored together with untreated samples at room temperature for 3 months.

Enumeration of Microbial load

Total aerobic bacteria were counted by the surface plate agar method with Difco-TGY agar. Coliforms and enteric pathogens were determined by Difco-Desoxycholate agar and BBL-Salmonellae-Shigella agar, respectively. Fungi were counted by MYG-chloramphenical agar containing malt extract 10*g*, yeast extract 4*g*, glucose 4*g*, agar 20*g*, and chloramphenical 20*mg* per liter (pH 6.0). Osmophilic molds were counted by 15% NaCl-malt agar containing malt extract 50*g*, NaCl 150*g*, and agar 20*g* per liter (pH 6.0). Total aerobic bacteria, fungi and osmophilic molds were counted after 2 to 7 days incubation at 30°C. Coliforms and enteric pathogens were counted after 1 to 2 days incubation at 37°C.[20]

Identification of Mold

Following enumeration of the fungi and osmophilic molds on the agar plate, representative strains were selected and transferred in to DifcoPotato Dextrose agar slants. Identification as to levels of genus and species were performed morphologically, with reference to "The Genus Aspergillus" and "Manual of the Penicilli," with Czapek's Solution agar and MY 20 agar after 7 to 30 days incubation at 30°C.[21, 22]

Chemical analysis

Chemical analysis was chosen for one of the four smaples. Moisture, ash, fiber and pH were analysed according to the AOAC standard methods.[23] Fat, protein and total sugar were determined using the Soxhlet, Kjeldahl and modified Somogyi methods, respectively.[24]

The thiobarbituric acid number (TBA No.) in the samples as an index of lipid rancidity was determined by Turner's method.[25]

Amino acid composition was analysed after acid hydrolysis, followed by a chromatographic separation of the amino acids in the hydrolysates by means of automatic amino acid analyser (Hitachi Model 835).

Mineral content in the samples was determined by using an atomic absorption spectrophotometer (Instrumental Laboratory Inc. Model 457) according to the wet combustion method.[24]

Results and Discussion

Destribution of Microorganisms

Damage to feedstuff caused by insects or microorganisms is considerable in Korea because summer humidity and temperature are high enoguh to allow fungal and bacterial growth. In particular, the damage caused by fungi is more severe during long storage, and the moisture content in feed will influence the growth of mold as mycoflora. In this case, the growth of mold on feed caused damage to its nutritional components, and some species of mold may produce mycotoxins in feed.[2]

The viable cell counts for smaples of different origins show deviations in several contamination, as shown in Table 1. Total bacterial counts in smaples were 3.1×10^5

Table 1. Distribution of microorganisms in poultry feeds

(Colony forming units/*g*)

Samples	Total bacteria	Total fungi	Osmophilic molds	Coliforms	Entric pathogens
I	3.1×10^5	9.6×10^4	7.8×10^4	6.4×10^4	2.6×10^3
II	4.3×10^5	3.0×10^3	2.2×10^3	2.7×10^4	1.1×10^3
III	9.6×10^5	1.3×10^4	1.6×10^3	1.2×10^4	4.0×10^1
IV	8.1×10^5	1.7×10^4	7.6×10^3	1.7×10^5	1.6×10^2

to 9.6×10^5 per gram. The counts of coliforms were high, numbering from 1.2×10^4 to 1.7×10^5 per gram, and those of enteric pathogens counted in all samples were 4.0×10 to 2.6×10^3 per gram.

Osmophilic molds were present at 1.6×10^3 to 7.8×10^4 per gram, mainly comprised by *Aspergillus*. Other kinds of fungi were also counted from 3.0×10^3 to 9.6×10^4 per gram.

As shown in Table 2, osmophilic molds were identified in 9 species, which consisted mainly of species of the *Aspergillus oryzae, A. flavus* groups, *A. versicolor, A. amstelodami, A. candidus, A. terricola, A. niger* and low frequencies of the *A.chevalieri* and *Penicillum regulosum*. Other fungi consisted of *Cladosporium* and *Cepholosporium*.

Aspergillus flavus, A. Versicolor, A. candidus, A. chevalieri and Penicillium rugulosum are known to be potential mycotoxin producers.[26]

In this respect, it seems to be necessary to prevent damage to feedstuff caused by the growth of molds and insects, and to eliminate pathogens.

Table 2. Molds isolated from poultry feeds

Genera/ Speices	Potential mycotoxins[3]	Isolated samples
A.[1] *flavus*	Aflatoxin	I. II. III. IV
A. candidus	Candidulin	I. III. IV
A. versicolor	Cyclopiazonic acid	I. II. III. IV
A. chevalieri	Xanthocillin X	I. II. III. IV
A. niger	Kojic acid	II. IV
A. oryzae		I. II. IV
A. amstelodami		I. II. III
A. terricola		II. III
P.[2] rugulosum	Rugulosin	I. II
Cladosporium		I. III. IV
Cephalosporium		I. III

[1]A: Aspergillus, [2]P: Penicillium
[3]Source: references 26

Table 3. Effect of gamma irradiation on viable counts of microorganisms in poultry feeds

(Clony forming units/*g*)

Samples	Treatments	Total bacteria	Total fungi	Osmophilic moulds	Coliforms	Enteric pathogens
I	Control	3.1×10^5	9.6×10^4	7.8×10^4	6.4×10^4	2.6×10^3
	3 kGy	1.2×10^3	2.3×10^3	3.1×10^2	—	—
	5 kGy	9.8×10^1	—	—	—	
	7 kGy	—	—	—	—	—
	10 kGy	—	—	—	—	—
II	Control	4.3×10^5	3.0×10^3	2.3×10^3	2.7×10^4	1.1×10^3
	3 kGy	6.1×10^3	1.5×10^2	—	4.0×10^1	—
	5 kGy	3.0×10^2	9.0×10^1	—	—	—
	7 kGy	—	—	—	—	—
	10 kGy	—	—	—	—	—
III	Control	9.6×10^5	1.3×10^4	1.6×10^3	1.2×10^4	4.0×10^1
	3 kGy	1.5×10^4	8.9×10^3	—	—	—
	5 kGy	1.0×10^2	2.0×10^2	—	—	—
	7 kGy	—	2.0×10^1	—	—	—
	10 kGy	—	—	—	—	—
IV	Control	8.1×10^5	1.7×10^4	7.6×10^3	1.7×10^5	1.6×10^2
	3 kGy	1.6×10^4	4.5×10^2	—	1.0×10^3	—
	5 kGy	2.4×10^2	2.0×10^2	—	—	—
	7 kGy	—	1.0×10^2	—	—	—
	10 kGy	—	—	—	—	—

Inactivation of microorganisms.

Table 3 shows the effect of gamma irradiation on decontamination of feed. Total bacterial counts could be reduced by over 2 to 3 log cycles with irradiation of 3 to 5 kGy, and the estimated dose for eliminating the total bacterial to below a detectable level ranged from 7 to 10 kGy. Coliforms and enteric pathogens were sterilized completely by 3 to 5 kGy irradiation. Generally, coliforms and enteric pathogens required lower doses for inactivation, compared to that of total bacteria.[27] Osmophilic molds were radiation-sensitive and were eliminated below detectable limits by 5 kGy irradiation. However, fungi were more radiation-resistant, and inactivated by 7 to 10 kGy irradiation. These results are similar to the reports of Kume *et al.*,[11] Ito *et al.*,[10] and Watanabe *et al.*[28]

Effects on Chemical Properties

Table 4 shows the effect of gamma irradiation on the chemical properties of feeds. Proximate compositions of feed were not remarkably changed by the irradiation dose levels, but the TBA values of irradiated feeds were slightly higher than those of nonirradiated ones. In these experiments, the results of the irradiation treatment on proximate components and TBA values were in reasonable agreement with those of Ford[29] and Cho *et al.*[30] It is thought that fats are attacked by free radicals which are formed by gamma irradiation, and then peroxide and other oxidation products are formed under the aerobic condition in a way similar to the auto oxidation process of fat.

Table 5 shows the effect of gamma irradiation on the total amino acid content. Total amino acid content especially aspartic acid, cysteine and serine in irradiated feed decreased slightly with increasing dose levels as compared to nonirradiated feed. Several investigators, reported that the protein quality and its amino acid composition are not significantly affected by irradiation at high doses, whereas the protein quality of autoclaved feed and ethylene oxide fumigated feed are significantly affected. Ford[29] also reported that gamma irradiation of feeds has been shown to have minimal influence on total protein, protein quality and total and available amino acid levels. Autoclaving reduced amino acid availability and consequently protein quality. Limited evidence shows reduction of certain available amino acids following ethylene oxide fumigation.

The mineral content of feed under gamma irradiation

Table 5. Effect of gamma irradiation on the amino acid content in poultry feed[1]

Amino acid	Irradiation dose (kGy)			
	Control	3 kGy	5 kGy	7 kGy
Aspartic acid	0.83	0.75	0.76	0.75
Threonine	0.25	0.22	0.22	0.21
Serine	0.40	0.36	0.35	0.34
Glutamic acid	1.33	1.55	1.31	1.30
Glycine	0.46	0.41	0.42	0.40
Alanine	0.47	0.44	0.44	0.42
Cysteine	0.28	—	—	—
Valine	0.36	0.39	0.33	0.32
Methionine	0.21	0.24	0.22	0.22
Isoleucine	0.21	0.21	0.19	0.19
Leucine	0.66	0.67	0.65	0.64
Tyrosine	0.14	0.14	0.14	0.11
Phenylalanine	0.96	0.90	0.91	0.90
Lysine	0.44	0.42	0.42	0.41
NH_3	0.27	0.26	0.27	0.25
Histidine	0.13	0.13	0.14	0.11
Arginine	0.38	0.38	0.35	0.34
Total	7.78	7.47	7.12	6.91

[1]Total amino acid content is expressed as the percentage on the basis of dry weight

Table 4. Effect of gamma irradiation on some chemical properties in poultry feed

Treatments	TBA (mole/g)	pH	Proximate composition (%)					
			Moisture	Crude fat	Crude protein	Ash	Crude fiber	Total sugar
Control	8.20×10^{-6}	6.92	12.16	2.71	15.31	6.23	4.47	59.10
3 kGy	8.42×10^{-6}	6.91	12.14	2.70	15.33	6.18	4.50	59.12
5 kGy	8.80×10^{-6}	6.90	12.15	2.72	15.34	6.22	4.46	59.08
7 kGy	9.02×10^{-6}	6.91	12.18	2.74	15.32	6.19	4.47	59.09
10 kGy	1.06×10^{-5}	6.91	12.16	2.73	15.30	6.20	4.48	59.13

Table 6. Effects of gamma irradiation on the mineral content in poultry feed[1]

Treatments	Mineral content (mg/100 g, dry wt.)						
	Na	K	Ca	Mg	Cu	Fe	Zn
Control	145.6	627.6	427.1	268.8	0.78	387.2	15.4
5 kGy	145.6	625.3	426.6	266.5	0.80	387.2	14.9
10 kGy	145.8	626.4	424.9	267.6	0.80	388.4	14.8

[1]Minerals were analyzed with A.A. immediately after irradiation and each value is the mean of triplicate experiments

are shown in Table 6. The mineral content of feed was not affected by gamma irradiation. This result is similar to reports by Cho *et al.*[30] Zhang *et al.*[33] also reported similar results using poultry feeds, that minerals are unaffected by 6 to 8 kGy irradiation.

The above facts indicate that the gamma irradiation of feed would be a welcome asset in the decontamination of microorganisms and nutrients.

References

1. Korean Federation of Animal Feeds: *The Present Condition of Mixed Feeds Production and Raw Materials Use* (1986)
2. Ito, H. and Iizuka, H.: *Decontamination of Animal Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), IAEA, Vienna, p. 15 (1979)
3. Nadudvari, I.: *Decontamination of Animal Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), IAEA, Vienna, p. 33 (1979)
4. Ito, H., Kume, T., Takehisa, M. and Iizuka, H.: *Japan Food Irradiation*, **15**, 11 (1980)
5. IAEA: *Radiation Control of Salmonellae in Food and Feed Products* (Proc. Panel Vienna, 1962), Tech. Rep. No, 22, IAEA, Vioenna (1963)
6. IAEA: *Microbiological Problems in Food Preservation by Irradiatim* (proc. Panel Vienna, 1966), IAEA, Vienna (1967)
7. LAEA: *Elimination of Harmful Orgarnisms from Food and Feed by Irradiation* (Proc. Panel Zeist, 1967), IAEA, Vienna (1968)
8. IAEA: *Food Irradiation* (Proc. Symp. Karlsruhe, 1966), IAEA, Vienna (1966)
9. Mossel, D.A.A., de Groot, A.P.: *J. Sci. Food Agric.*, **18**, 362 (1967)
10. Ito, H., Kume, T. and Takehisa, M.: *Nippon Nogeikagaku Kaishi*, **55**, 1081 (1981)
11. Kume, T., Ito, H. and Takehisa, M.: *Nippon Nogeikagaku Kaishi*, **55**, 1197 (1981)
12. Ito, H., Begum, A., Kume, T. and Takehisa, M.: *Nippon Nogeikagaku Koishi*, **57**, 9 (1983)
13. Coates, M.E., Ford, J.E., Gregory, M.E. and Paterson, J.S.: *Lab. Anim.* **3**, 39 (1969)
14. Ley, F.J., Bleby, J., Coates, M.E. and Paterson, J.S.: *Lab. Anim.*, **3**, 221 (1969)
15. Lapidot, M.: *Decontamination of Animals Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), LAEA, Vienna, p. 43 (1979)
16. Eggum, E.O.: *Decontamination of Animals Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), IAEA, Vienna, p. 55 (1979)
17. Sandev, S. and Karaivanov, I.: *Decontamination of Animal Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), IAEA, Vienna, p. 83 (1979)
18. Adamiker, D.: *Decontamination of Animal Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), IAEA, Vienna, p. 113 (1979)
19. Sickel, E.: *Decontamination of Animal Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), IAEA, vienna, p. 133 (1979)
20. Cho, H.O., Byun, M.W., Kwon, J.H., Kim, S.W., Yang, J.S., Kim, Y.J. and Lee, J.W.: *Annual Research Report*, KAERI/RR-523/86, p. 18 (1987)
21. Raper, K.B. and Fennel, D.I.: *The Genus Aspergillus*, Robert E. Krieger Pub. Co., Huntington, New York (1973)
22. Raper, K.B., Thon, C. and Fenel, D.I.: *Manual of the Penicilli*, The Williams and Wilkins Co. (1949)
23. AOAC: Official Methods of Analysis, 13th ed., *Association of Official Analytical Chemists*, Washington, D.C. (1980)
24. Kohara: *Handbood of Food Analysis*, Kenpakusha, Japan (1977)

25. Turner, E.W., Paynter, W.D., Montie, E.J., Bessert, M.W., Struck, G.M. and Olson, F.C.: *Food Technol.*, **8**, 326 (1954)
26. Thomas, D. Wyllie and Lawrence G. Morehouse: *Mycotoxic Fungi, Mycotoxicoses*, Marcel Dekker Inc., New York and Basel (1977)
27. Byun, M.W., Kwon, J.H. and Cho, H.O.: *Korean J. Food Sci. Technol.*, **16**, 47 (1984)
28. Watanabe, H., Ito, H., Shibabe, S. and Iizuka, H.: *Nippon Nogeikagaku Kaishi*, **45**, 500 (1971)
29. Ford, D.J.: *Decontamination of Animal Feeds by Irradiation* (Proc. Advisory Group Meeting, Sofia, October 1977), IAEA, Vienna, p. 69 (1979)
30. Cho, H.O., Byun, M.W., Kwon, J.H. and Lee, J.W.: *Kor. J. Food Hygiene*, **2**, 3 (1987)
31. Eggum, B.O.: *Z. Tierphysio. Tierernahr. Futtermittelkd.* **25**, 204 (1969)
32. Ford, D.J.: *Br. J. Nutr.*, **35**, 267 (1976)
33. Zhang, S.T., Ou, Z., Li, W., Wu, G.R., Zhen, J.B. and Ma, A.Y.: IAEA-SR-129/p-l-l, p. 59 (1986)

Received July 27, 1987

감마선 조사에 의한 가금용 사료의 살균

변명우 · 조한옥 · 이재원 · 권중호 · *김영배
한국에너지연구소 식품조사연구실 · *고려대학교 식품공학과

간접식품으로서 4종의 양계용 배합사료의 미생물 오염정도와 방사선조사에 의한 살균효과 및 화학성분의 변화를 조사한 결과는 다음과 같다.

4종의 양계용 배합사료의 총 세균의 오염은 $10^5 \sim 10^6$ g범위였으며 7 kGy의 방사선조사로서 거의 완전 살균되었고, 대장균 및 장내 병원성세균도 전 시료에서 $1.2 \times 10^4 \sim 1.7 \times 10^5$/g과 $4.0 \times 10 \sim 2.6 \times 10^3$/g 검출되었으며, 이들은 방사선감수성이 높아 3~5kGy 조사로서 완전 사멸되었다. 곰팡이의 오염은 $10^2 \sim 10^4$/g 정도로서 이들의 대부분은 내삼투압성 곰팡이였으며 5~10 kGy의 조사로서 검출한계 이하로 사멸되었다. 또한분리된 곰팡이의 대부분이 *Aspergillus* 속이었으며, 분리된 11종의 곰팡이중 *Aspergillus flavus* group을 포함한 6여종이 잠재적으로 독소를 생성하는 균이었다. 화학성분의 변화에 있어서 일반성분 및 무기질 함량은 방사선조사의 영향이 없었으나 TBA가는 다소 증가하였으며, 아미노산 함량은 조사선량의 증가에 따라 약간의 감소를 나타냈다.

이러한 결과는 동물사료에 5~10 kGy 정도의 방사선조사로서 화학적성분에 큰 영향을 주지않고 유해 미생물을 충분히 사멸시킬 수 있어 간접식품으로서 사료의 위생적 생산과 장기 안전저장을 가져올 수 있을 것으로 생각된다.