Gamma Ray Irradiation to Corn—Formation of Peroxide and Its Effects on Chicks

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Abstract Two feeding experiments with chicks were carried out using gamma ray irradiated diets. In Experiment I, corn was irradiated at the levels of 3 and 6 M rad prior to mixing with other ingredients. In Experiment II, the whole diet containing 65% corn was irradiated at the levels of 0.6, 3 and 6 M rad. Peroxides and carbonyl compounds were formed with increasing dose levels, but the amount of peroxide in the corn was much smaller than that in the irradiated soybean oil reported in the previous study. No marked difference was observed in feed consumption and feed efficiency between control and irradiated diets. In Exp. I, the decreases in digestibility of crude protein and in metabolizable energy content were observed. In Exp. II, hemolysis of chicks developed with irradiation to the diet and encephalomalacia was observed in 4 chicks out of 6 which were fed 6 M rad irradiated diet and all the chicks died within a day or two after showing the symptom of encephalomalacia. It is considered that most serious change with irradiation to the diets containing mainly corn is the destruction of vitamin E.

In the previous paper¹⁾, where the effects of gamma ray irradiation to semi-purified diet containing soybean oil were investigated, extensive formation of peroxides in the irradiated diets and depression of feed consumption, feed efficiency and digestibility with chick were observed. Also, marked dilatation of the small intestine and liver as well as the development of hemolysis was found in these chicks. It was considered that these phenomena were caused by the peroxide or other oxidation products of fat which were formed with gamma ray irradiation.

It is possible that such unfavorable effects appear with a conventional feed as well, when the feed is irradiated. Therefore, the experiments were undertaken with corn which is a cereal grain rich in fat, in comparison with the results in previous report¹⁾. In this paper, effects of dose level on the formation of peroxides and of carbonyl compounds were investigated. For the purpose of elucidating the changes of feeding value due to the irradiation to corn, the performance and vitamin E deficiency symptoms in chicks were observed, and the changes in digestibility of the diet with irradiation were estimated. Whole diet containing 65% of corn as main fat source was also irradiated and effects of irradiation on the diet were investigated.

Materials and Methods

Materials: Corn; A commercial corn was milled with hammer mill.

Composition of experimental diets; Corn 65%, soybean meal 30.2%, DL-methionine 0.2%, mineral mixture 4.1% and vitamin mixture 0.5%. The vitamin mixture containing thiamine

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nitrate 1.38 g, riboflavin 6.88 g, choline chloride 206 g, nicotin amide 3.06 g, calcium pantothenate 3.75 g, folic acid 0.688 g, pyridoxine hydrochloride 1.38 g, vitamin A 2,000,000 IU, vitamin D₃ 400,000 IU and vitamin K₃ 0.053 g per kg. The composition of mineral mixture was shown in previous report¹⁾.

Treatment of experimental diets: The diets were prepared according to the following treatments.

Experiment I (October–November); A) Control (non-irradiated). B) and C) Only corn was irradiated in aluminum case (30×30×15 cm), total absorption dosages being 3 and 6 M rad, respectively. Irradiation time was 16 hrs. The corn was mixed with the other ingredients after irradiation.

Experiment II (January–February); A) Control (non-irradiated). B), C) and D) Whole diet was irradiated with 0.6, 3 and 6 M rad of gamma rays, respectively. Irradiation times were 32, 40 and 8 hrs, respectively. Each diet was irradiated in a cardboard box (30×20×10 cm).

Irradiation procedure and experiments with chicks: Experiments I and II in this study were carried out with the same methods as those for the corresponding experiments described in the previous paper¹⁾.

Storage condition of the diets: All the diets were put into polyethylene bags and were stored in a brooding room, at constant temperature (20–23 C) from 0 to 2nd week, and at room temperature from 3rd to 5th week, until feeding.

Analytical methods: Proximate analyses and determinations of gross energy of the diets and feces, digestion coefficient, peroxide value and degree of hemolysis were carried out by the same methods as described in previous paper¹⁾.

Carbonyl value was determined by the method of Kumazawa and Oyama²⁾, with the absorption at 440 m μ .

Results and Discussion

1. Chemical changes of the diets with gamma irradiation

Chemical changes of the diets with gamma irradiation and the formation of peroxides during storage are shown in Tables 1 and 2.

Table 1. Chemical changes of components of corn with gamma ray irradiation and change of peroxide value of whole diets containing irradiated corn during storage

							Exp. I
	Dose M rad	Crude protein %	Crude fat %	Peroxide value			Carbonyl value meq/kg fat
				0-week*	1-week*	2-week*	
					meq/kg fat		
A	0	9.8	4.1	0	—	—	4.1
B Corn	3	9.7	4.2	5.3	—	—	4.8
C	6	9.6	3.8	28.9	—	—	10.3
A	0	21.0	3.1	4.0	7.2	37.8	5.3
B Whole diets**	3	20.9	3.1	7.3	20.1	41.4	8.8
C	6	20.5	2.7	46.8	61.5	75.0	18.0

* After irradiation

** Prepared with the corns indicated in the upper columns

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Table 2. Chemical changes of components of whole diets containing corn with gamma ray irradiation and during storage after irradiation Exp. II

	Dose M rad	Crude fat %	Peroxide value			
			0-week*	1-week* meq/kg fat	2-week*	5-week*
A	0	3.1	4.1	9.3	12.0	14.3
B	0.6	3.1	10.2	16.5	18.0	20.5
C	3	3.1	37.8	49.5	51.5	54.2
D	6	3.0	73.5	70.5	73.0	76.7

* After irradiation

Exp. I: When corn was irradiated at high dose, crude fat content decreased to some extent. Peroxide was formed with increasing dose levels. However, the peroxide values of corn and the whole diet containing corn were far lower than those of semi-purified diets containing soybean oil which showed peroxide value of 273 with irradiation of 3 M rad¹⁾. The peroxide value of corn was almost equal to those of heated ground soybean and of rice bran, *i. e.* 42 with 4.8 M rad³⁾ and 18.3 with 2.25 M rad⁴⁾, respectively.

From these results, it is supposed that peroxides are formed more extensively by irradiation with extracted fat, such as soybean oil, than with fat in texture, such as fat in corn and soybean.

The peroxide value of the diets, which contained mainly corn, was higher than that of corn itself. The reason for this discrepancy cannot be explained so far from this experiment.

Peroxide value of whole diets increased with storage period. In Exp. I, corn was stored in polyethylene bag at 20-23 C, and relative humidity in the bag was estimated to be about 60%, provided that the moisture of atmosphere in the bag was in equilibrium with the moisture content of the corn, 13%.

Carbonyl value of corn increased with high dose irradiation, but its increase was less than that of peroxide value.

The above result was similar to that in previous study⁴⁾. With gamma ray irradiation, it seems that peroxides were formed at first and then carbonyl compounds may have been formed from the peroxides in a similar way to the process in autoxidation of fat⁵⁾.

Exp. II: No or little decrease in crude fat content was found with irradiation. Peroxide was formed with increasing dose levels, even with 0.6 M rad irradiation. The values were higher than those in Exp. I. The difference can be explained by the difference of irradiation time¹⁾. It is also possible that there was some influence of other ingredients when whole diet (Exp. II) was irradiated.

The peroxide values increased more slowly than those of Exp. I during storage, and no marked change was observed in that of 6 M rad irradiated diet. In both experiments, peroxide value of the diets did not increase beyond about 75. It is difficult to explain the reason from this study.

2. Effects of irradiation to diets on chicks

1) Growth test and digestion trial

Exp. I: Feed consumption, body weight gain and feed efficiency in Exp. I are summarized

Table 3. Effects of gamma ray irradiation to corn on chick growth

Exp. I

Period	Dose	Feed consumption	Body weight gain	Feed efficiency body weight gain/feed consumption
	M rad	g/chick	g/chick	
0—2 weeks	0	203	118	0.58 ^a
	3	200	116	0.58 ^a
	6	208	118	0.57 ^b

Number of chicks: 4 groups (20 chicks) in each treatment

Means bearing the different superscripts differ significantly ($P < .05$)

Table 4. Metabolizable energy and digestibility of crude protein as affected with gamma ray irradiation

Exp. I

Dose	Digestibility of crude protein	Metabolizable energy content	ME intake	Body weight gain/ME intake
M rad	%	Kcal/g feed	Kcal/chick	g/Kcal
0	90.6 ^a	2.84 ^a	577	4.89
3	89.8 ^{ab}	2.71 ^b	542	4.67
6	88.9 ^b	2.73 ^b	567	4.81

The figures show the average of 4 groups (20 chicks)

Means bearing the different superscripts differ significantly ($P < .05$)

Table 5. Effects of gamma ray irradiation to whole diets on chick growth

Exp. II

Period	Dose	Feed consumption	Body weight gain	Feed efficiency body weight gain/feed consumption
	M rad	g/chick	g/chick	
0—2 weeks	0	207	117	0.56
	0.6	199	112	0.56
	3	214	120	0.56
	6	206	115	0.56
3—5 weeks	0	785	283	0.36
	0.6	751	278	0.37
	3	798	316	0.40
	6	—*	—*	—*

Number of chicks: 0—2 weeks; 3 groups (15 chicks)

3—5 weeks; 2 groups (6 chicks)

* Four chicks of this treatment died from encephalomalacia

in Table 3. No significant difference was detected in feed consumption and body weight gain between chicks fed control and irradiated diet, and the difference in feed efficiency was slight.

Digestibility of crude protein and metabolizable energy (ME) content in Exp. I are shown in Table 4. Digestibility of crude protein was decreased a little with high dose irradiation, and ME content was decreased with irradiation at the levels of 3 and 6 M rad.

Exp. II: The results of growth test in Exp. II are summarized in Table 5. No significant

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difference was detected in feed consumption, body weight gain and feed efficiency between control and irradiated diets so far as determination was possible. In 3rd-5th week, the difference in feed efficiency between control and the irradiated diets seems not to be very little, but no statistic significance was detected. This may be partly due to the small number of the chicks used in this period.

READ *et al*⁶⁾, feeding corn irradiated at levels of 3 and 6 M rep, observed no marked difference in weight gain, caloric consumption and caloric efficiency of rat between irradiated corn and control, and no gross symptom of toxicity. UMEDA *et al*⁷⁾, using semi-purified diets containing irradiated milo, found no significant difference in feed intake and feed conversion between control and the diet irradiated at level of 5 M rad, but they observed that ME content of the diet containing irradiated milo was significantly reduced. No gross symptom of toxicity due to irradiated feed ingredients was found in any of their chicks.

From these results, it is considered that high dose irradiation to corn causes no marked decrease in feed intake and feed efficiency, but causes the reduction of ME content and of crude protein digestibility. It seems that the decrease in digestibility is attributable to the formation of polymerized fat and peroxidized fat-protein complex. A detailed discussion on these phenomena was given in the previous report¹⁾.

When semi-purified diet containing soybean oil was irradiated at levels of more than 3 M rad, feed consumption, body weight gain and feed efficiency with chicks fed the diets proved to be significantly inferior to those of control¹⁾. The difference in the effects of irradiation on feed value between the diets containing corn in this experiment and the diets containing soybean oil in the previous one¹⁾ can be explained by lower peroxide production in the former.

2) Organs of chicks fed irradiated diets

In Exp. II, the chicks were slaughtered after 5-week experiment and their organs were weighed. The results are shown in Table 6. No significant difference was detected among all the diets. However, in previous study¹⁾, the chicks fed 3 and 6 M rad irradiated diets containing soybean oil showed marked dilatation of the small intestine and liver. It is supposed that the degree of dilatation in organs of chicks fed irradiated diet depends on the amount of peroxide formed.

Table 6. Weight of organs of chicks fed irradiated diets Exp. II

Dose (M rad)	0	0.6	3	6*
Liver	2.80	3.16	2.70	2.95
Gall bladder	0.11	0.12	0.12	0.15
Pancreas	0.51	0.43	0.48	0.49
Spleen	0.19	0.37	0.30	0.19
Proventriculus	0.61	0.63	0.61	0.60
Gizzard	2.49	2.62	2.67	2.58
Small intestine + large intestine	4.49	4.01	4.17	3.85
Caecum	0.68	0.74	0.72	0.62
Body weight	442 g	441 g	504 g	482 g

Weight of organs is expressed by the percentage to the total body weight of
average 3 chicks

* The figures are averages of 2 chicks

Table 7. Developments of hemolysis and of encephalomalacia in chicks fed irradiated diets

Exp. II

Dose M rad	Average of hemolysis at the end of 5 week* %	Fragile erythrocytes at the end of 5-week fragile**/total	Development of encephalomalacia	
			0—2 weeks dead/total	3—5 weeks dead/total
0	0.14	0/3	0/15	0/6
0.6	4.50	2/3	0/15	0/6
3	70.19	3/3	0/15	0/6
6	84.77	3/3	0/15	4/6

* Average of 3 chicks

** Number of chicks whose hemolysis was more than 3.1%¹⁰⁾

3) Hemolysis and development of encephalomalacia

It has been reported that vitamin E deficiency causes nutritive encephalomalacia and hemolysis in chicks⁹⁾. Degree of hemolysis of chicks at the end of 5th week in Exp. II and development of encephalomalacia during experiment are shown in Table 7. The symptoms of encephalomalacia in this study were the same as explained in previous paper¹⁾.

Degree of hemolysis was intensified with increasing irradiation to the diets, even at 0.6 M rad. Fragile erythrocytes were not found in the chicks fed control diet, but they were observed in all the chicks examined in the groups fed 3 and 6 M rad irradiated diets.

Though no encephalomalacia was observed in any treatments in 0-2nd week, symptoms of encephalomalacia developed in 4 chicks out of 6 which were fed 6 M rad irradiated diet in 5th week. All the chicks suffering from encephalomalacia died within a day or two after showing the symptoms. These results suggest that vitamin E in the diets was destroyed with gamma ray irradiation.

In previous study¹⁾, hemolysis and symptoms of encephalomalacia in chicks fed irradiated semi-purified diets were also observed. COATES *et al*⁹⁾ observed that the content of tocopherol in chick mash composed mainly of corn and wheat reduced by half with 5 M rad irradiation.

The effects of gamma ray irradiation to corn and the diets containing mainly corn can be explained as follows.

With irradiation, peroxides were formed at first, and then carbonyl compounds, polymerized fat and peroxidized fat-protein complex could be formed from the peroxides.

Two phenomena were observed as the effects of the above compounds on chicks. One is the decreases in digestibility of crude protein and in ME content. It seems that the depression in digestibility is attributable to the formation of polymerized fat and of peroxidized fat-protein complex.

The other is the development of hemolysis and of encephalomalacia. All the chicks suffering from encephalomalacia died. This phenomenon suggests that vitamin E in the diets was destroyed by peroxides, which is considered to be the most serious change with irradiation to corn.

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とうもろこしに対する放射線照射 ——過酸化物の生成および雛に対する影響——

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とうもろこしに対する放射線照射の影響を調べるため, 2回の実験を行った。実験 I では, とうもろこしに対し, 3および6 M rad の γ 線を照射した後, 他の成分と配合した。実験 II では, とうもろこしを 65% 配合した飼料に, 0.6, 3 および 6 M rad を照射した。照射後, 飼料成分の変化を調べるとともに, 雛に給与して, その影響を検討した。その結果, 過酸化物およびカルボニル化合物の生成量は照射量を増すにつれ増加したが, 前報において, 大豆油を配合した半精製飼料で得た値に比べて著しく少なかった。雛に給与した場合, 発育試験では, 飼料摂取量および飼料効率に対して, 照射による影響は認められなかった。消化試験の結果, 実験 I において, 6 M

rad を照射した場合は, 粗蛋白質の消化率はわずかに低下し, 代謝エネルギー (ME) 含量が減少したが, 3 M rad では ME 含量のみが減少した。また, 大豆油照射の場合に認められた腸管等の肥大は, 本実験では認められなかった。雛の溶血性は照射量の増加にしたがって高まった。6 M rad 照射区では, 試験開始後 5 週間目に 6 羽中 4 羽が, 脳軟化症にかかり死亡した。以上の結果から, とうもろこしに対する γ 線照射の影響として, 6 M rad 以下の照射量では雛の発育成績に著しい影響はないが, 照射によって生成された過酸化物により飼料中のビタミン E が破壊され, 溶血性の上昇, 脳軟化症の発症などビタミン E 欠乏症を惹起する可能性があると考えられる。