

ผลของรังสีแกมมาที่มีต่อคุณภาพด้านจุลินทรีย์ของเนื้อไก่ญี่ปุ่น และการเปลี่ยนไมโครฟลอร่าของเนื้อไก่ฉายรังสี

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บทคัดย่อ

ได้ศึกษาผลของรังสีแกมมาปริมาณ 0 ถึง 8 กิโลเกรย์ ที่มีต่อคุณภาพทางจุลินทรีย์ของเนื้อไก่ผลิตในประเทศญี่ปุ่น และการเปลี่ยนแปลงไมโครฟลอร่าของเนื้อไก่ฉายรังสี รังสีปริมาณ 2 กิโลเกรย์ มีผลทำให้เชื้อแบคทีเรียทั้งหมดลดลง 4 log cycles, กลุ่มแบคทีเรียแลคติกลดลง 5 ถึง 6 log cycles, ราและยีสต์ ลดลง 2 Log cycles ส่วนพวกโคลิฟอร์มแบคทีเรียพบว่ารังสีปริมาณ 1 กิโลเกรย์ สามารถลดจำนวนลงจนถึงระดับที่ไม่อาจตรวจพบได้ การตรวจวิเคราะห์ด้านฟลอร่า พบว่าเนื้อไก่ญี่ปุ่นของแต่ละพื้นที่มีโครงสร้างของประชากรจุลินทรีย์ลักษณะจำเพาะจุลินทรีย์กลุ่ม **Flavobacterium** และ **Pseudomonas** เป็นกลุ่มเด่นในไมโครฟลอร่าของเนื้อไก่ญี่ปุ่น การฉายรังสีปริมาณ 2 กิโลเกรย์ มีผลทำให้กลุ่ม **Lactobacillus** และ **Pseudomonas** หายไป สำหรับจุลินทรีย์ที่พบเป็นกลุ่มเด่นในเนื้อไก่ฉายรังสีปริมาณ 2 กิโลเกรย์ และสูงกว่า ได้แก่ กลุ่มของ **Psychrobacter** และยีสต์

ผลการศึกษานี้สนับสนุนการใช้ประโยชน์จากวิธี การฉายรังสีที่ว่า สามารถใช้ปรับปรุงคุณภาพทางจุลินทรีย์ของเนื้อไก่ และแสดงให้เห็นว่าวิธีการนี้ไม่ก่อให้เกิดอันตรายใด ๆ ขึ้นอันเนื่องมาจากการเปลี่ยนแปลงไมโครฟลอร่าในเนื้อไก่ฉายรังสี



Effect of Gamma Irradiation on Microbiological Quality of Japanese Chicken Meat and Microflora Change of Irradiated Chicken

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ABSTRACT

The impact of gamma irradiation with doses between 0 and 8 kGy on microbiological quality of chicken meat produced in Japan and micro flora change of irradiated chicken meat were studied. Radiation at the dose 2 kGy resulted in 4 log cycles reduction of total aerobic bacteria, 5-6 log cycles reduction of lactic acid bacteria and 2 log cycles reduction of fungi and yeasts. For the coliforms, it could be eliminated below detectable level by irradiation dose of 1 kGy. For the chicken flora-analysis, it was found that chicken of each area had their own specific microbial community structure. *Flavobacterium* and *Pseudomonas* were found to be dominant organisms in the microflora of Japanese chicken meat. Irradiation with dose 2 kGy resulted in disappearance of *Lactobacillus* and *Pseudomonas*. The microorganisms which dominated in irradiated chickens with doses of 2 kGy and higher were *Psychrobacter* and yeast.

These studies support the view that radiation improves the microbiological quality of chicken meat and substantiate that radiation does not present hazard resulting from a change in the microflora of irradiated chicken.

1. INTRODUCTION

Chicken meat is one of the important animal protein sources for Japanese. The price of chicken is rather cheaper than the another meat comparing to pork and beef. Normally, there are two types of chicken meat for Japanese consumer. One is imported from the foreign country, the another one is domestic production. For the imported chicken, the price is cheaper than the one produced in the country. It is packed and sent to Japan as frozen products. The microbiological quality of imported chicken will be investigated at the port of entry before distributing to the consumer. For the local chicken, it is anticipated that the microbiological quality will meet the quality standard.

The objectives of this study are to investigate the gamma irradiation effect on microbiological quality of Japanese chicken and the change of chicken microflora occurring as the result of gamma decontamination. Several research studies have reported that a dose of about 3-5 kGy can improve the microbiological quality by decreasing amount of microorganisms which contaminated in poultry to meet the quality standard.^(1,2) Therefore, in these study the irradiation dose range of 0.5 to 8.0 kGy are concentrated. The research topics are carried out as follow, viz:

- 1) The survey of microbiological quality of chicken meat produced in various prefectures of Japan.
- 2) The studies of gamma-irradiation effect on the microbiological quality of chicken.
- 3) The microflora study of chicken produced in Japan.
- 4) The study of microflora change of chicken occurring as a result of irradiation.

2. MATERIALS AND METHODS

Material

Ten packs of chicken sample (1kg each) which produced in Iwate, Gunma, Kagoshima and Miyazaki prefecture were purchased directly from the private commercial companys. The chicken meat were contained in the foam tray sized by 20 x26x4 centimeters (widthxlengthxdepth) and covered with plastic film. Upon arriving the laboratory, all of the samples were kept in a freezer (temperature at -18°C).

Microbiological Quality Investigation

Before microbiological investigating, the samples kept in freezer were thawed over night in refrigerator (temperature at 4°C). Ten grams of each pack of the chicken meat were cutted into pieces in a sterile condition and packed in the sterilized bag. Then, 50 ml of sterile water containing 0.85% NaCl and 0.01% Tween 20 was added and homogenized for 1 minute by stomacher. The homogenates were filtered by a sterile gauze bag and diluted to 10^2 to 10^4 times by the sterile water. Each 0.2 ml of suitable dilutions were plated on the surface of each 3 plates of media. The media and incubation condition used in this experiment were as follows :

Nutrient agar; Difco-nutrient agar surface plate method was used for enumerating total aerobic bacteria. The inverted plates were incubated for 3 days at 30°C.

MacCONKEY agar: Difco-MacCONKEY agar surface plate method was used for enumerating coliform group of bacteria. The inverted plates were incubated for 24 hours at 35-37°C.

Glucose yeast extract agar; Compositions of this media were glucose 20g, yeast ex. 10g, MgSO₄·7H₂O 0.2g, KCl 0.1g, K₂HPO₄ 1g, CaCO₃ 5g, agar 20g and water 1 liter. The media were used for enumerating lactic acid bacteria. The plates were incubated anaerobically for 3-5 days at 25°C. The colonies of lactic acid bacteria were recognized by surrounding clear zone.

MYG-chloramphenicol agar; Compositions of this media were malt ex. 10g, yeast ex. 4g, glucose 4g, agar 20g, chloramphenicol 0.02g and water 1 liter (pH 6.0). The media were used for enumerating of fungi and yeasts by incubating plates at 30°C for 3 to 5 days.

Gamma Irradiation

Two chicken samples from Miyazaki prefecture were used for studying the gamma irradiation effect on microbiological quality which irradiated at room temperature. Cutted the chicken meat of each pack samples into pieces, then weighted 100g and homogenized aseptically by homogenizer. Weighted 10g of the homogenized sample and packed into 9 sterilized bags. Then, the samples were irradiated by 150 kCi gamma ray Cobalt-60 source of Takasaki Radiation Chemistry Research Establishment, (JAERI). The dose rate at the irradiation position was 6 kGr/hr according to the Fricke dosimetry. The samples were irradiated at the dose of 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 kGy, respectively.

In addition, one chicken sample from Kagoshima prefecture was used for studying the irradiation effect in frozen condition. For irradiation in frozen condition the samples were packed in a container with dry ice (CO₂). The irradiation doses used in this experiment were the same as dosage mentioned above.

Microbiological Investigation of Irradiated Chicken

After irradiation, all of the samples were investigated for microbiological quality. The media and methods used for enumerating were followed as mentioned above.

Microbiological Identification

The colonies found on the suitable Nutrient agar plates during microbiological quality investigation were identified and tabulated by characteristics of color morphology (size, shape, color and so on). Each typical type of colony was isolated and kept into Nutrient agar slant. All of the isolated colonies were identified by Gram staining and biochemical tests. The microbiological identification were carried out to the genus level. The biochemical tests for the characteristics of genera or species bacteria were applied and followed with the references of Bergey's Manual Determinative Bacteriology⁽³⁾.

3. RESULTS AND DISCUSSION

1) *Microbiological Quality of Chicken which Produced in Japan*

The results of microbiological quality surveyed of chicken produced in various prefectures of Japan were shown in **Table 1**. The count of microorganisms were found as 10^5 to 10^7 cfu/g of total aerobic bacteria, 10^3 to 10^7 cfu/g of lactic acid bacteria and 10^3 to 10^4 cfu/g of fungi and yeasts. The coliforms group which made red colonies on Mac- CONKEY agar were observed in all of the samples from 10^2 to 10^4 cfu/g.

From the results, it was shown that the quality of some chicken meat samples did not meet the microbiological quality standard. Because the total aerobic count found in some samples were up to 10^6 and 10^7 cfu/g and found also coliform bacteria, eg. the sample of Miyazaki, Kagoshima and Gunma. It can be concluded that the slaughterhouse of chicken in that area were still lacked of good management and sanitary handling. Therefore, it is necessary to improve the sanitation and management of the slaughterhouse to meet sanitary handling. Because of unsanitary handling is a common cause of contamination, not only with the high count of microorganism but also with pathogenic bacteria.

2) *The Gamma-Irradiation Effect of Chicken*

2.1 Gamma-irradiation effect of chicken irradiated at room temperature

The results of irradiation effect of Miyazaki chicken sample I and II irradiated at room temperature were shown in **Fig. 1** and **Fig. 2**, respectively. It was found that amount of microorganisms were reduced by the gamma-irradiation treatment. The amount of coliforms could be eliminated below detectable level by irradiation at the dose of 1.0 kGy. Irradiation dose of 2.0 kGy resulted in 4 log cycles reduction of total aerobic bacteria, 5 to 6 log cycles reduction of lactic acid bacteria and 2 log cycles reduction of fungi and yeasts.

2.2 Gamma-irradiation effect of chicken irradiated at frozen condition

In this experiment, the results showed that the characteristics of irradiation survival curves of chicken irradiated at frozen condition were similar to the one irradiated at room temperature. Coliforms group were eliminated below detectable level by irradiation dose of 0.5 kGy. In addition, irradiation at dose of 2 kGy resulted in 2 log cycles reduction of total aerobic bacteria and 3 log cycles reduction of lactic acid bacteria, fungi and yeasts. The irradiation survival curves of microorganisms in chicken irradiated at frozen condition were shown in **Fig. 3**. From these study, the dosage at 2 kGy appeared to be sufficient for improving the microbiological quality of chicken.

3) *Microflora of chicken which produced in Japan.*

Flora analysis of chicken from various prefectures of Japan were carried out. The microorganisms found in chicken sample of each prefecture were identified till to the genus level. Characteristics of bacterial genera isolated from Japanese chicken and the chicken microflora were shown in **Table 2** and **Table 3**, respectively. It was found that chicken of each prefecture had their own specific microbial community structure. *Flavobacterium* and *Pseudomonas* were found in all prefecture sample used in this experiment. The Iwate flora were consisted of *Lactobacillus*, *Pseudomonas*, *Micrococcus*, *Flavobacterium* and *Arthrobacter*. For the Gunma flora,

Flavobacterium, *Arthrobacter* and *Acinetobacter* were predominant. Besides, *Pseudomonas* was the predominant genera among the Miyazaki flora and *Lactobacillus*, *Pseudomonas* and *Xanthomonas* were predominant in Kagoshima flora.

4) Flora change of irradiated chicken.

The results of flora analysis of non-irradiated and irradiated chicken were shown in **Table 4**. It was found that the dominant microorganisms of non-irradiated chicken were *Lactobacillus* and *Pseudomonas*. Irradiation at the dose of 2 kGy resulted in disappearance of *Lactobacillus* and *Pseudomonas*. The dominant organisms of irradiated chicken at the dose of 2 kGy and higher were yeast and *Psychrobacter*. From this studies, it can be concluded that irradiation of chicken with doses of 2 to 4 kGy dose not present a hazard resulting from a shift in the microflora.

4. CONCLUSION

From the microbiological quality survey of chicken meat produced in Japan, the results showed that the chicken quality of some area did not meet the quality standard. Irradiation treatment can be used for eliminating microorganisms in chicken. Dosage of 2 kGy appeared to be sufficient for improving the microbiological quality of chicken. The microflora of Japanese chicken showed that each prefecture had their own specific microbial community structure. *Flavobacterium* and *Pseudomonas* were the common bacterial genera which can be found in the chicken. The dominant organisms of chicken irradiated with the dose 2 kGy and higher were *Psychrobacter* and yeast.

This studies substantiated that the microbiological quality of chicken can be improved by irradiation and does not present a hazard resulting from a shift in microflora.

5. REFERENCES

1. Mossel, D.A.A. (1983). Essentials and perspectives of the microbial ecology of foods. In: Food Microbiology: Advances and Prospects, T.A. Roberts and F.A. Skinner (Eds), Academic Press, London, p. 1-45.
2. Nouchpramool K., Y. Prachasitthisak, S. Charoen, S. Kanarat, K. Kanignuntha and S. Tangwongsupang. (1986). Improvement of bacteriological quality of frozen chicken by gamma radiation. OAEP-1-124, Dec. 1986.
3. Bergey's Manual of Determinative Bacteriology. Jojn G. Holt..[et.al.] (Eds), 9th ed. Williams & Wilkins.

Table 1 Microbiological quality of chicken meat which produced in various prefectures of Japan* (CFU/g).

<i>Sample No.</i>	<i>Total aerobic bacteria</i>	<i>Coliforms</i>	<i>Lactic acid bacteria</i>	<i>Fungi and Yeasts</i>
<i>I-1</i>	2.1×10^5	1.0×10^2	6.3×10^3	2.1×10^3
<i>I-2</i>	3.3×10^5	1.5×10^2	1.5×10^4	2.2×10^3
<i>I-3</i>	3.8×10^5	8.3×10^2	1.4×10^4	3.0×10^3
<i>I-4</i>	5.1×10^5	1.8×10^2	1.5×10^4	3.7×10^3
<i>M-1</i>	5.2×10^7	1.8×10^4	1.3×10^7	3.0×10^4
<i>M-2</i>	9.8×10^6	3.4×10^4	2.3×10^6	5.6×10^4
<i>K-1</i>	2.0×10^6	1.8×10^4	4.0×10^5	2.2×10^4
<i>K-2</i>	2.5×10^6	2.2×10^4	1.2×10^5	3.8×10^4
<i>G-1</i>	1.0×10^6	7.5×10^3	2.3×10^4	1.3×10^4
<i>G-2</i>	1.1×10^6	6.7×10^3	2.0×10^4	1.8×10^4

* The chicken samples were purchased from Iwate(I), Miyazaki(M), Kagoshima(K) and Gunma(G) prefecture.

Table 2 Some characteristics of bacterial genera isolated from chicken meat (from 104 of isolated strains)

<i>Genus</i>	<i>Morpholog</i>	<i>Gram staining</i>	<i>Motility</i>	<i>Oxidase test</i>	<i>Catalase test</i>	<i>Glucose</i>
<i>Flavobacterium</i>	Rods	-	-	+	+	-
<i>Pseudomonas</i>	Rods	-	+	+	+	-
<i>Acinetobacter</i>	Rods	-	-	-	+	-
<i>Xanthomonas</i>	Rods	-	+	-	+	-
<i>Lactobacillus</i>	Rods	+	-	-	-	F
<i>Arthrobacter</i>	Rods	+	-	-	+	-
<i>Psychrobacter</i>	Cocci	-	-	+	+	-
<i>Micrococcus</i>	Cocci	+	-	-	+	-
<i>Staphylococcus</i>	Cocci	+	-	-	+	F

F = Anaerobic fermentation of glucose.

Table 3 Microflora of chicken produced in various prefectures of Japan
(in % of total).

<i>Microorganism genera</i>	<i>I-1</i>	<i>I-2</i>	<i>I-3</i>	<i>I-4</i>	<i>G-1</i>	<i>G-2</i>	<i>M-1</i>	<i>M-2</i>	<i>K-1</i>	<i>K-2</i>
<i>Flavobacterium</i>	6.1	25.0	4.3	3.5	36.1	2.9	-	3.8	3.5	1.6
<i>Pseudomonas</i>	26.5	-	15.5	13.0	1.3	-	92.2	69.8	6.2	40.1
<i>Micrococcus</i>	14.3	14.6	1.7	51.5	4.4	-	-	-	0.7	-
<i>Lactobacillus</i>	53.1	49.0	78.5	31.4	-	-	7.8	26.4	46.2	47.7
<i>Arthrobacter</i>	-	11.4	-	0.6	55.2	59.4	-	-	-	10.6
<i>Yeast</i>	-	-	-	-	3.0	-	-	-	-	-
<i>Acinetobacter</i>	-	-	-	-	-	30.6	-	-	-	-
<i>Psychrobacter</i>	-	-	-	-	-	7.1	-	-	-	-
<i>Xanthomonas</i>	-	-	-	-	-	-	-	-	43.4	-

I = Iwate prefecture. M = Miyazaki prefecture.
G = Gunma prefecture. K = Kagoshima prefecture.

Table 4 Microflora of non-irradiated and irradiated chicken
(in % of total)*.

<i>Microorganism genera</i>	<i>Irradiation dose (kGy)</i>						
		0.5	1.0	1.5	2.5	3.0	4.0
<i>Pseudomonas</i>	46.0	-	-	-	-	-	-
<i>Lactobacillus</i>	54.0	94.1	84.0	35.0	-	-	-
<i>Acinetobacter</i>	-	5.9	15.2	29.0	-	-	-
<i>Staphylococcus</i>	-	-	0.4	6.0	-	-	-
<i>Yeast</i>	-	-	0.4	30.0	25.4	57.1	66.7
<i>Psychrobacter</i>	-	-	-	-	74.6	42.9	33.3

* = The chicken samples used in these experiment were purchased from Miyazaki prefecture.

Fig. 1 Irradiation effect on microorganisms of chicken
(Sample from Miyazaki prefecture I)

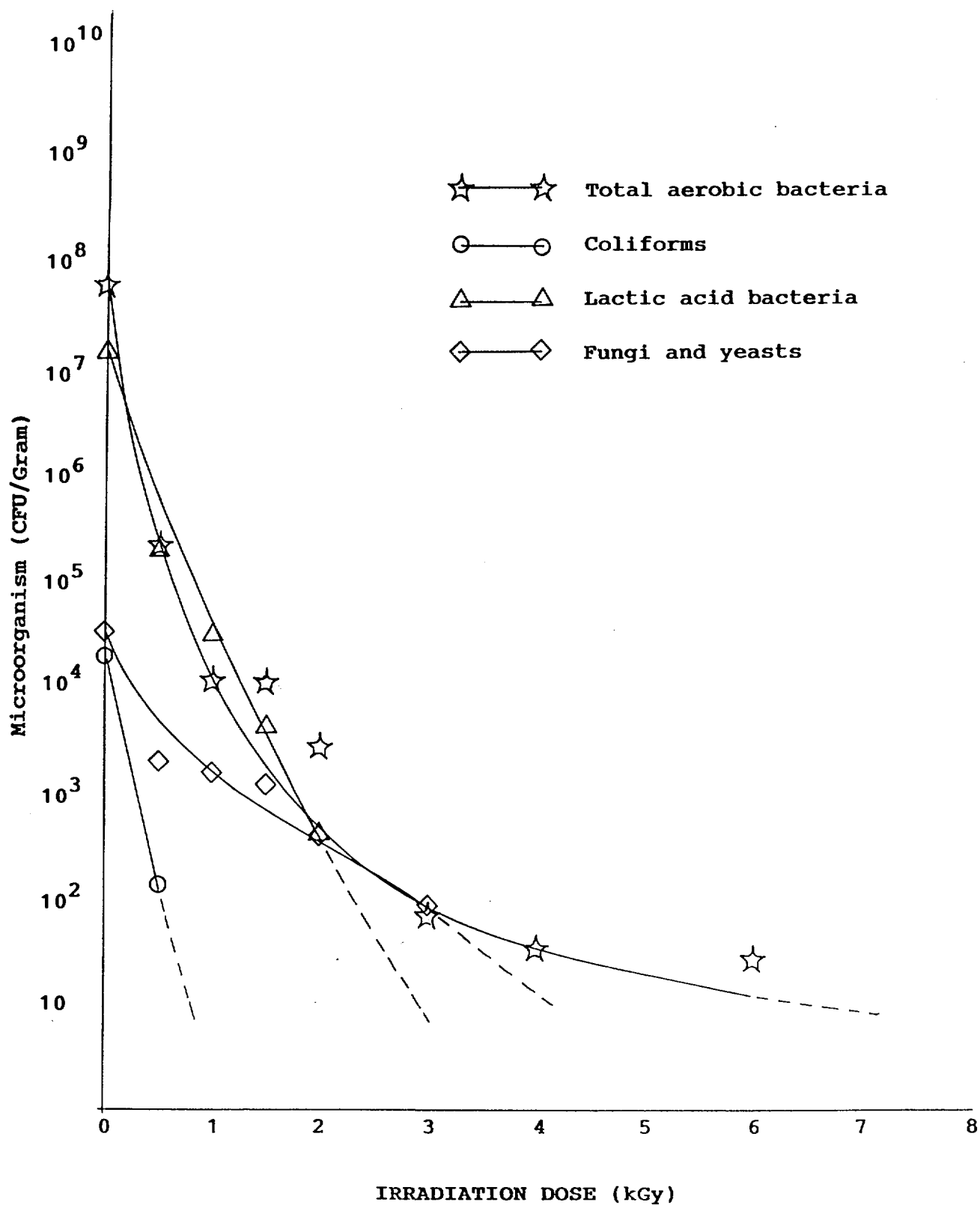


Fig. 2 Irradiation effect on microorganisms of chicken
(Sample from Miyazaki prefecture II)

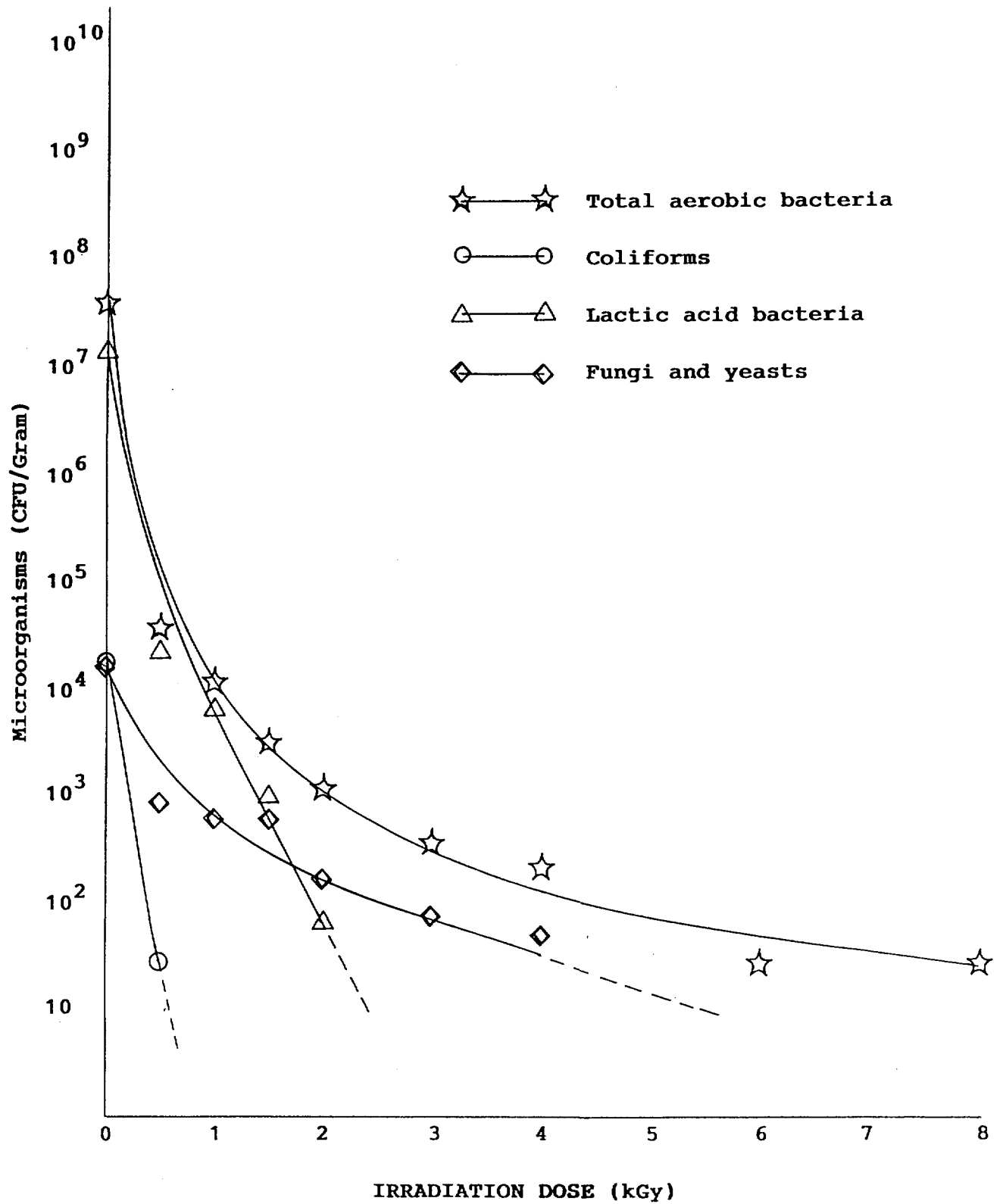


Fig. 3 Irradiation effect on microorganisms of chicken
(The sample was irradiated in frozen condition)

