EFFECT OF GAMMA IRRADIATION ON SURVIVAL OF PAHOGENIC BACTERIA *Salmonella typhimurium* , AND *Escherichia coli* ON CHICKEN MEAT

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ABSTRACT

The materials used were 96 chicken breast meat samples, weighing about 25 gram and sealed in polyethylene bags, respectively. All of samples were stored at 5 – 6^{0}C over night prior to be used. Cultures were maintained and cloned on Triptych Soy Agar and propagated in 100 ml of triptych soy broth. The sterile meat was inoculated with enough cells of *Salmonella* and *E. coli* for a final population of – 10^8 stationary phase cells/g. The samples were radiated by ^{60}Co sources in 7.6 kGy/hours dose rate at 0^{0}C temperature, and after irradiation, they were stored at refrigerator temperature until being analyzed. All samples were divided into 2 groups namely 48 samples for *S. typhimurium* testing, and 48 samples for *E. coli* testing. Radiation doses were 0 (control), 1, 3, and 5 kGy. The effects of radiation were analyzed on 0, 4, 8, and 12 days of storage. The samples were assayed for colony forming unit/g by standard spread plate procedures using salmonella-shigella agar to *Salmonella*, Mac Conkay Agar for *E. coli and* plate count agar for total microbial with serial dilutions in sterile peptone water 0.1%. The data were analyzed by using analysis of variance from split plot design (4 X 4) with 3 replications. Duncan's multiple range test was used to determine the mean differences. The results showed that the effects of radiation doses were highly significant (P<0.01) on *S. typhimurium* and *E. coli*. The effects of radiation during storage were highly significant (P<0.01) on *S. typhimurium* and were not significant on *E. coli*. There were not significant interactions between radiation doses and storage times on all groups. In conclusions, radiation decreased *Salmonella typhimurium* and *E. coli*, D-value of *Salmonella typhimurium* and *Escherichia coli* was 0.7 and 0.8 kGy, respectively.

(Key Words : *Salmonella Typhimurim*, *Escherichia coli*, Gamma Irradiation, Chicken Meat).

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PENGARUH IRADIASI GAMMA TERHADAP DAYA TAHAN BAKTERI PATOGEN *Salmonella typhimurium* DAN *Escherichia coli* PADA DAGING AYAM BROILER

INTISARI

Penelitian ini bertujuan untuk mengetahui pengaruh perlakuan iradiasi selama waktu simpan 12 hari terhadap daya tahan bakteri patogen *S. typhimurium*, dan *E. coli* pada daging ayam broiler. Sebanyak 96 sampel daging masing-masing seberat 25 gram dibagi dalam 2 kelompok yaitu : 48 sampel untuk uji *S. typhimurium* dan 48 sampel untuk uji *E. coli*. Dosis radiasi yang digunakan adalah 0 (kontrol), 1, 3, dan 5 kGy. Daging yang telah steril (48 sampel) diinokulasi dengan sel *S. typhimurium* dan 48 sampel lain diinokulasi bakteri *E. coli*. Pengaruh perlakuan radiasi dianalisis pada hari ke 0 (kontrol), 4, 8, dan 12 hari. Analisis jumlah koloni setelah perlakuan dengan metode *spread plate* dan data hasil disajikan dalam log. koloni/g. Data yang terkumpul dilakukan analisis variansi pola split plot 4 x 4 dengan 3 kali ulangan. Perbedaan mean diuji dengan *Duncan's multiple range test*. Hasil penelitian menunjukkan bahwa pengaruh radiasi adalah sangat nyata ($P<0.01$) pada bakteri *S.typhimurium* dan *E. coli* pada daging ayam. Pengaruh radiasi selama penyimpanan pada bakteri *S. typhimurium* berpengaruh sangat nyata ($P<0.01$) dan tidak menunjukkan perbedaan pada bakteri *E. coli*. Tidak terdapat interaksi antara waktu simpan dengan dosis radiasi baik pada daging inokulasi *S. typhimurium* maupun inokulasi *E. coli*. Hasil penelitian menunjukkan bahwa perlakuan radiasi dapat menurunkan cemaran bakteri *S. typhimurium* dan *E. coli* dengan *D value* sebesar 0,7 kGy dan 0,8 kGy secara berurutan.

(Kata Kunci : *Salmonella Typhimurium*, *Escherichia coli*, Iradiasi Gamma, Daging Ayam)

Introduction

Many researchers found that poultry may be a natural reservoir for pathogenic and potential fecal-cecal contamination and/or cross contamination of poultry carcasses (Lillard, 1982; Denton and Gardner, 1988). During slaughtering and processing may contribute to the presence of *Escherichia coli* (Conner, 1992) or *Salmonella* (Sulastri, 1994; Andini, 1995) in the retail poultry products . *Salmonella typhimurium* is a major food born pathogen in poultry products, and may be transmitted from contaminated to uncontaminated carcasses during processing (Mead, 1989). *Escherichia coli* is highly virulent bacterium whose transmission to humans has recently been epidemiologically associated with the consumption of contaminated foods. Furthermore, it was reported that *E. coli* is able to readily colonize the ceca of chicken, and is shed in the feces for several months following colonization. Bacterial cross contamination of poultry carcasses occurred in scalding, defeathering, eviscerating and chilling (Berry *et al.*, 1985). Such as in Indonesia, research had conducted in Yogyakarta city on fresh of beef and poultry meat. The result showed that salmonella contamination was about 10^3 to 10^4 Colony Forming Unit per g (CFU/g) (Sulastri, 1994). From the other research, cerotype Salmonella had been found in Jakarta traditional market and Supermarket, namely Salmonella Kentucky, Salmonella agona, Salmonella anatum, Salmonella typhmurium, Salmonella schwarzengrund and Salmonella hadar (Andini *et al.*, 1995).

Several means of the decontaminating poultry and beef carcasses to ensure the absence of *Salmonella* (Kim *et al.*, 1996), *Staphylococcus* aureus (Bayle *et al.*, 1987), *Escherichia coli* (Ogunrinola *et al.*, 1996; Ballestra *et al.*, 1996) and another microbial have been evaluated. These include treatment with chemicals, heat treatment and irradiation.

Many chemical methods have been used in the scalding tank and chiller bath, or for washing chicken carcasses, including chlorine and other chemicals. However, these methods have not been completely acceptable due to residues, discoloration of chicken skin, high cost or variable effectiveness.

Using of irradiation with doses of 4 – 6 kGy a so called radicidation process seem promising. The reason why this process has not gained wide popularity among poultry processors are that most countries either prohibited the use of irradiation for foods or if such treatment is permitted, it requires specific labeling to that effect. In many countries, consumers reactions against irradiated food are rather strong, for which the reason of process has never had and may never have, any wide spread used (Mead, 1989).

The objectives of the research were to investigate the effects of radiation treatment during 12 days storage at 5 – 6^{0}C temperature on survival of *Salmonella typhimurium*, and *Escherichia coli*.

Materials and Methods

Cultures

Salmonella typhimurium ATCC and *Escherichia coli* FNCC-091 were obtained from Inter University Center of Food and Nutrition, Gadjah Mada University, Yogyakarta, Indonesia. All cultures were maintained and cloned on Tryptic Soy Agar (TCA, Difco Detroit, MI). Each cultures was propagated in 100 ml of tryptic soy broth (TSB, Difco, Detroit, MI) at 37^{0}C on water bath shaker incubation for 12-16 hours at 37^{0}C.

Sample preparation

Chicken breast meat was obtained the day of slaughter from Ciomas Adi Satwa Poultry Processing Plant, Jakarta, Indonesia. The chicken breast was carefully trimmed of fat and deboning in the refrigerated room. The chickens breast meat were subdivided into 25 ± 0.2 g amounts and sealed in polyethylene bags. The samples for *Salmonella typhimurium* and *Escherichia coli* testing were sterilized by gamma irradiation to a dose of 10 kGy at 0 ^{0}C. Both the sterile (control) and unsterile meat (inoculated meat) samples were stored at 5-6 ^{0}C prior to be used.

Radiation source and irradiation techniques

The Styrofoam packaging with ice and $^{co}60$ radiation source by dose rate 7.6 kGy/hr were used. Radiation doses used 0, 1, 3, and 5 kGy on the all of group testing. Variations in absorbed doses given to experimental samples were minimized by placement within a uniform portion of the radiation field. Samples were irradiated, at 0 ^{0}C temperature, and after the radiation were stored at refrigerated temperature until being analyzed.

Inoculation of chicken breast meat for determination of D_{10} values

Sterile meat was inoculated with enough cells for a final population of 10^8 stationary phase cells/g (2.5 ml per 25 g meat sample). After inoculation, it was incubated at 37 ^{0}C for 1 hour, then stored in the refrigerator over night until irradiation treatment.

Effect of irradiation on D_{10} value

Inoculated meat samples received radiation doses 0, 1, 3, and 5 kGy at 0 ^{0}C for 4 replications. All samples for each replicate study were inoculated from the same sources. Unirradiated controls (0 kGy) were prepared from the same inoculated meat. Control was used to determine the number (N_0) of colony forming unit (CFU) microbial content prior to treatment with gamma irradiation for samples irradiated. D_{10} value determination was analyzed by graphic methods as IAEA description (IAEA, 1970).

Statistical analysis

The data were analysed by analysis of variance as split plot design. The significant means were determined by Duncan's Multiple Ring Test (Sudjana, 1994). We calculated linear correlation coefficients between radiation doses and storage time.

Result and Discussion

Salmonella typhimurium

Average of number of *Salmonella typhimurium* by radiation treatment and storage time effect is shown in Table 1. Statistical analysis of variance showed that the radiation doses effected very significantly effect ($P<0.01$) on the poultry meat inoculated by *Salmonella typhimurium*, while effect of radiation among storage times were not significant and there was no interaction between doses radiation and storage times. Multiple regression analysis effect of radiation on the *Salmonella typhimurium* was $R^2 = 0.94$.

Radiation treatment until 5 kGy indicated that *Salmonella typhimurium* decreased colony of *Salmonella* by 7.19 $\log_{10}$ for 12 days storage time. D_{10} for *S. typhimurium* was 0.7 kGy, this result of research was lower than was studied by Andini *et al* (1995*) who reported that D-10 value was 1.038 kGy. Anhungfu *et al.* (1995) reported that irradiation was effective in reducing *S. typhimurium* on inoculated chops and hams. Low dose (0.75 to 0.90 kGy) irradiation reduced the number of cells by 1 log for chops and by 3 log for hams, D_{10} value of *Salmonella typhimurium* was 0.4 – 0.7 kGy.

Table 1. Averages of number of *Salmonella typhimurium* (log. Colony/g)

Doses (kGy)	Storage time (days)				
	0	4	8	12	average
0	7.992	8.050	7.413	7.720	7.794^{a}
1	5.572	5.427	2.867	2.780	4.162^{b}
3	2.258	0.000	0.000	0.000	0.564^{d}
5	1.725	0.667	0.000	0.000	0.598^{c}
Average	3.391^{a}	3.536^{ab}	3.566^{c}	2.626^{b}	

^{a,b,d,c} Different superscripts in the same row or column were high of significant differences ($P< 0.01$).

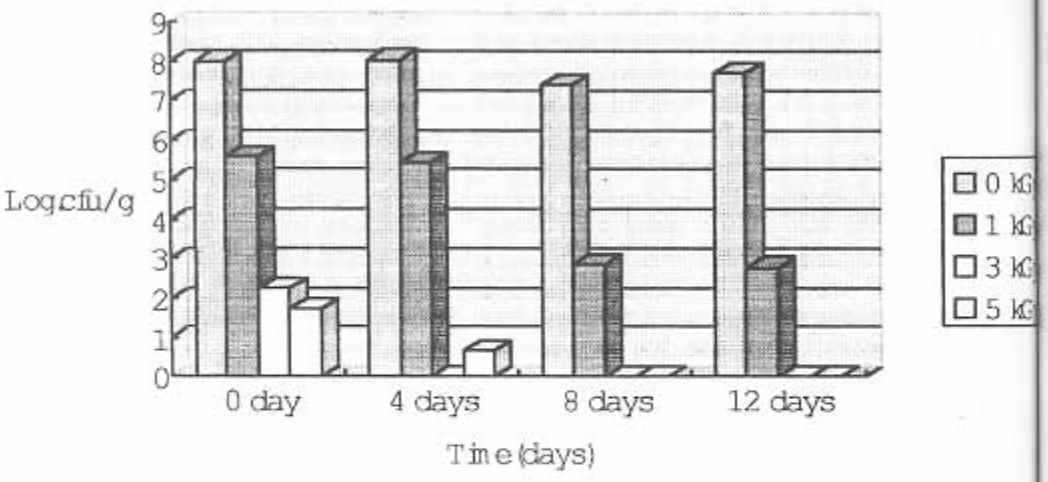

Fig. 1. Effects of gamma irradiation on the *Salmonella typhimurium* growth until 12 days storage.

Thayer *et al.* (1992) studied the effect of radiation on pathogen i.e. bacteria *Salmonella typhimurium* on chicken wings. The result showed that dose of 2.83 kGy was able to decontaminate *Salmonella typhimurium* cell. The other study suggested that decontamination or elimination of *Salmonella* sp. required low radiation doses Dickerson *et al.* (1991) used radiation doses of 2 – 3 kGy, Lamuka *et al.* (1992) used radiation doses of 2.5 kGy and they concluded that using dose of 4.5 kGy and stored at 4^0C could reduce and eliminate salmonella content.

Escherichia coli

The result of gamma radiation treatment on chicken breast meat was shown in Table 2.. Analysis of variance of the data indicated that effect of storage times were not significant, gamma radiation doses were highly significant ($P< 0.01$). There were no interaction effects between radiation doses and storage times. Many factors affected the effectively of irradiation treatment namely water content in food, food composition, pH and oxygen (Supriyadi, 1992). Multiple regression analysis effect of radiation on the *Escherichia coli* was $R^2 = 0.98$.

Table 2. Average number of *E. coli* cells (log. Colony/g)

Doses (kGy)	Storage time (days)				
	0	4	8	12	average
0	7.625	7683	8.683	7.778	7.942^a
1	6.488	5.679	6.107	5.461	5.934^b
3	3.380	2.285	2.603	2.650	2.730^c
5	2.075	1.159	2.594	2.392	2.055^d
Average	4.892	4.202	4.997	4.570	

^{a,b,d,c} Different superscripts in the same column were highly significant differences ($P< 0.01$).

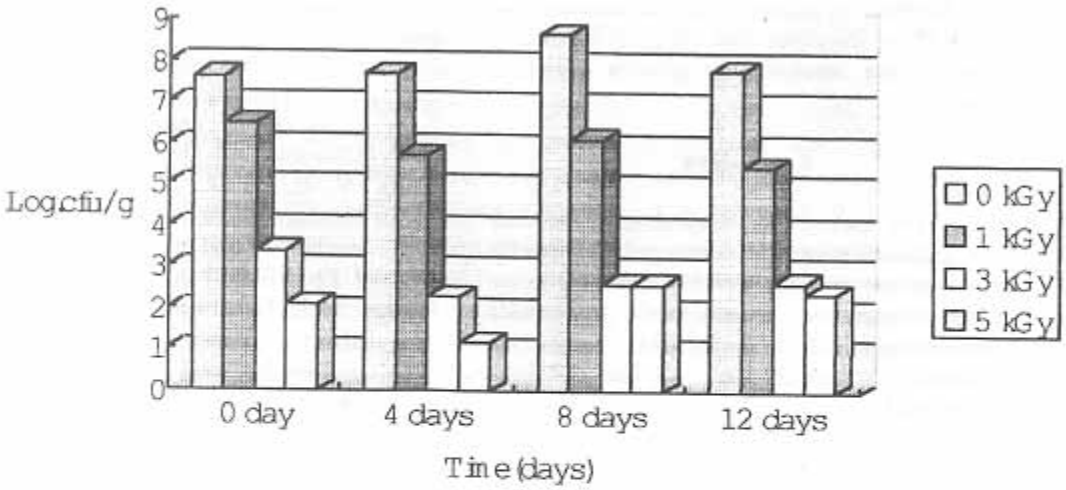

Fig.2. Effects of gamma irradiation on the *Escherichia coli* growth until 12 days storage.

Destructions of *E. coli* in this research might be caused by damaged of cells membrane, inhibition of phosphorilase oxidative, therefore the bacteria cell could not grow (Supriyadi, 1988). D_{10} value for *E. coli* by graphic method was 0.8 kGy, radiation treatment by 3 kGy decreased *Escherichia coli* contaminant by 4.24 cyclus log. An hungfu *et al.* (1995[b]) indicated that at least 1 log reduction of *E coli* was found, but the cells continued to grow during storage at 7^0 and 25^0 C. Thus low dose irradiation could provide increased safety for the products but only if the products were not heavily contaminated.

Thayer and Boyd (1993) found D-value of 0.28 kGy for *E. coli* O157 : H7 irradiated in vacuo on finely ground lean beef at 5^0C and a value of 0.27 kGy on either lean ground beef or mechanical deboned chicken meat. Grand and Patterson (1991) obtained a D value of 0,34 kGy for non pathogenic strain of *E coli* irradiated in the presence of air on fresh pork fillets. Our research found D-value of 0.8 kGy , this result might be caused by a high number of inoculated and different meat medium on the research.

Chen *et al.* (1996) on their research reported that irradiation by 1 kGy dose on *E. coli* bacteria eliminated this contaminant by 10^2 CFU/g, therefore they concluded that this bacteria are sensitive by gamma radiation treatment.

Conclusion

The result of the research indicated that the radiation treatment destructed *Salmonella typhimurium* and *Escherichia coli* of poultry meat contaminant. D-values of *Salmonella typhimurium* and *Escherichia coli* by irradiation treatment were 0.7 and 0.8 kGy respectively.

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