

Destruction of *Salmonella typhimurium* on Chicken Wings by Gamma Radiation

DONALD W. THAYER, CATHLEEN Y. DICKERSON, D. RAMKISHAN RAO, GLENN BOYD, and
CHANDRAMOHAN B. CHAWAN

ABSTRACT

No viable CFU of a streptomycin-resistant *S. typhimurium* were detected on chicken wings inoculated with 100 CFU and treated with 1.8 kGy or greater doses of gamma radiation at 5°C in air. The inoculated *S. typhimurium* did not recover from radiation injury during 3 days of refrigerated storage. Viable CFU were detected on wings inoculated with 1,000 or 10,000 CFU and irradiated with 1.8 kGy but not on those irradiated with 2.7 or greater kGy. The indigenous aerobic mesophilic population on the wings was reduced from 10⁴ to 44 CFU/cm² by 1.4 kGy.

Key Words: Salmonella, poultry, chicken wing, Streptomycin, Gamma Radiation

INTRODUCTION

PROPER HANDLING and cooking can adequately eliminate most risk from *Salmonella*, which might contaminate poultry carcasses and parts. Nevertheless, Dubbert (1988) estimated that 35% of the chicken carcasses in the United States were contaminated with *salmonellae*, and consumer complaints and publicity have increased the need for higher standards of bacterial quality in poultry products (Shane, 1988). The irradiation of poultry was recently approved in the United States for the control of *salmonellae* and other food-borne pathogens (Anon., 1990). Klinger et al. (1986) and Mulder et al. (1977) investigated the use of ionizing radiation to eliminate *salmonellae*, which had contaminated chicken carcasses during processing. Other investigators reported that treating with ionizing radiation poultry meat or carcasses that had been artificially contaminated with various serovars of *salmonellae* effectively controlled that pathogen (Hanis et al., 1989; Licciardello et al., 1970; Previte et al., 1970; Mulder, 1976; Thayer et al., 1990; Thayer and Boyd, 1991a; Thayer and Boyd, 1991b).

Chicken wings are commonly purchased in bulk by consumers as a relatively low-priced food that can be prepared in a variety of appetizing forms. Since bulk chicken wings must be obtained from several birds and are subjected to considerably more handling than are intact poultry carcasses, they may contain a greater indigenous microflora than other, higher-value parts of the chicken. No published information is available on studies of the effects of ionizing radiation on the indigenous microflora or upon *Salmonella*-contaminated bulk-processed chicken wings. Also none is available on challenge studies with chicken parts in which the efficacy of gamma irradiation was tested at several levels of contamination with *salmonellae* cells. The purpose of our study was to determine the gamma radiation dose necessary to eliminate *Salmonella typhimurium* from chicken wings irradiated at 5°C and to determine some of the effects of those treatments on the indigenous microflora of the wings. Specifically, the purposes of this study were to

determine: the dose required to eliminate 100 CFU of *S. typhimurium* per wing, whether the pathogen would recover from radiation injury during refrigerated storage; whether the radiation dose required to eliminate the pathogen is affected by the amount of contamination; and the effects of radiation on the population of indigenous microflora on uninoculated samples.

MATERIALS & METHODS

Experimental design

In the first series of studies, the experimental design included five gamma radiation doses (0, 1.42, 2.83, 4.25, and 5.66 kGy) and two storage times (0 and 3 days) at 5°C for a total of 10 individual treatments. The 3-days refrigerated storage was designed to match storage in the home before use and to determine whether injured cells might recover during such refrigerated storage. Five chicken wings inoculated with *Salmonella* were used/treatment, and each treatment was replicated two times, thus requiring a total of 100 individually treated chicken wings. Uninoculated chicken wings were assayed to determine the effects of the treatments on the indigenous microbial flora. These assays required two wings/dose/storage time and were replicated twice for a total of 40 individual samples.

In the second series of studies, the experimental design included five gamma radiation doses (0, 0.90, 1.80, 2.70, and 3.60 kGy) and four inoculum levels (10, 100, 1000, and 10,000 colony-forming units (cfu) per wing) for a total of 20 individual treatments. Ten chicken wings were used for each treatment, which was replicated twice at different times, thus requiring a total of 400 individually treated chicken wings.

Culture and inocula preparation

A streptomycin-resistant mutant of *Salmonella typhimurium* ATCC 14028 was isolated from the parent by adding an equal amount of fresh Trypticase Soy Broth (TSB) (BBL, Cockeysville, MD) containing 2 mg/mL of streptomycin sulfate (Sigma, St. Louis, MO) to a 24-hr culture in the same medium without streptomycin sulfate at 35°C. A streptomycin sulfate-resistant clone was isolated and designated as *S. typhimurium*^{Sr}. Preliminary experiments established that the antibiotic resistance of this serovar was stable under the experimental conditions used and its growth rate, cultural properties, taxonomic characteristics, and response to gamma radiation were not distinguishable from the parent strain (Thayer et al., 1990). (A streptomycin-resistant strain of *S. typhimurium* was used to avoid confusion during quantitative studies which could result from an unknown and highly variable population of salmonellae that might have contaminated the chicken wings).

S. typhimurium^{Sr} was maintained and cloned on Tryptic Soy Agar (TSA) (Difco, Detroit, MI) and incubated at 35°C. Cultural purity was verified by Gram stain, and by biochemical reactions as determined using a Vitek Automicrobic System® GNI card (Vitek Inc., Hazelwood, MO) (Knight et al., 1990), and confirmed by serologic testing (AOAC, 1990) with polyvalent and individual O-group antisera (Fisher Diagnostics Salmonella Diagnostic Sera, Fisher Scientific, Orangeburg, NY). From a 6-hr culture in TSB, 1 mL incubated at 35°C, was used to inoculate 100 mL of TSB in a 500 mL baffled DeLong culture flask. The inoculated TBS was incubated at 35°C and agitated at 150 rpm for 16 hr. At 16 hr the culture was diluted with sterile 0.1% peptone water to give an appropriate number of cfu per 100 µL inoculum for each chicken wing. The number of cfu per 100 µL inoculum was determined by standard pour plate assay, in triplicate, using TSA incubated for 18 hr at 35°C.

Table 1—Effect of gamma radiation on destruction of streptomycin-resistant *S. typhimurium* inoculated onto chicken wings at a level of about 100 CFU/wing

Dose kGy	0-Day storage at 5°C				3-Day storage at 5°C				
	Replicate		Total	Predicted %	Replicate		Total	Predicted %	Total
	1	2			1	2			
0	5/5 ^a	5/5	10/10	93	5/5	5/5	10/10	91	20/20
1.42	3/5	0/5	3/10	35	2/5	0/5	2/10	32	5/20
2.83	0/5	0/5	0/10	5.5	0/5	0/5	0/10	4.6	0/20
4.25	0/5	0/5	0/10	1.4	0/5	0/5	0/10	1.2	0/20
5.66	0/5	0/5	0/10	7.7	0/5	0/5	0/10	7.7	0/20

^a 5/5 = 5 wings confirmed as positive for *S. typhimurium*^{Sr} out of 5 wings tested.

Sample preparation and packaging

Fresh, nonfrozen wings from chickens 6 wk old at slaughter were purchased from a local poultry supply house and were held at 10°C until use on the same day. Each wing was placed within a sterile No. 400 polyethylene Stomacher® bag and inoculated with 100 µL of an appropriately diluted culture, and the bag heat sealed. The uninoculated controls were packaged in the same manner.

Irradiation

The samples were irradiated at 0.118 kGy/min using a self-contained Cesium-137 gamma source (Shieh et al., 1985). The dose rate was established with standard dosimeters from the National Physical Laboratory, Middlesex, United Kingdom. The absorbed dose was estimated from the change in absorbance at 600 nm of radiochromic detectors (Far West Technology, Inc., Goleta, CA) attached to the sample bags during irradiation. The samples were placed within a uniform portion of the radiation field, and sample temperature was maintained at 5 ± 0.5°C. After treatment the samples were stored at 5°C until analyzed.

Microbiological analysis

Salmonella—In the first series of studies, a two-stage enrichment process was used to recover *S. typhimurium*^{Sr} CFU from each chicken wing. In the first enrichment 100 mL of TSB containing 2 g/L of streptomycin sulfate (Sigma, St. Louis, MO) and 2 g/L of sodium sulfamethazine (Sigma) were added directly to each treated chicken wing within the same Stomacher® bag. Each sample was then incubated for 18 to 24 hr at 35°C. (The purpose of this enrichment was to provide the maximum opportunity for the recovery of any surviving *S. typhimurium*^{Sr}.) After incubation each sample was shaken and streaked onto Brilliant Green Sulfa Agar (BGSA, Difco) and also onto TSA containing 1 g/L streptomycin sulfate. A second-stage enrichment was made by inoculating 10 ml of Selenite Cystine Broth (SCB, Difco), and 10 mL of Tetrathionate Broth (TTB, Difco) with 1.0 mL each from the original enrichment culture. Following incubation for 18 to 24 hr at 35°C each of these cultures was streaked onto BGSA and TSA containing 1 g/L of streptomycin sulfate. At least three colonies typical of *S. typhimurium*^{Sr} were rechecked on TSA without streptomycin sulfate. A positive identification required that the culture give results typical of *Salmonella* on the GNI Card of the Vitek Automatic System, be resistant to streptomycin sulfate, and be confirmed as *S. typhimurium* by its serological reactions. The resistance to streptomycin sulfate ensured that the isolate represented the inoculated culture.

In the second series of experiments, a simpler preenrichment step was used in which 100 mL of sterile buffered 0.1% peptone water (Difco) was added directly to each wing following treatment within the Stomacher bag (Juven et al., 1984). The peptone water and chicken wing were incubated for 18 to 24 hr at 35°C. All remaining procedures were as described above.

Indigenous microflora—A total of 40 chicken wings were analyzed for the effects of the treatments on their indigenous microbial flora. The surface area of each wing was calculated from its weight using the formula: Surface Area (cm²) = 85.6 + [1.41 x wt. (g)] (Goresline et al., 1959). From 0.1% sterile peptone water (Difco), we added 1 mL/cm² of surface area and the bag with its contents was shaken 30 times. Serial dilutions were then prepared in sterile 0.1% peptone water. Total aerobic mesophiles were determined by standard pour-plate techniques using three TSA plates/dilution. All three plates at a dilution giving 30 to 300 CFU/plate following incubation for 18 to 24 hr at 35°C were counted using a Biotran II automatic colony counter (New Brunswick Scientific, Edison, NJ). *Escherichia coli*

CFU were estimated from the number of typical colonies on the surface of Levine Eosin Methylene Blue Agar (Difco) incubated for 18 to 24 hr at 35°C. Three-day storage samples were analyzed in the same manner as the zero-day storage samples.

Statistical analysis

Data were analyzed as follows: The data for the frequency of survival of *Salmonella* by dose level, inoculum level, and storage day were transformed into their respective arc sine values (Snedecor and Cochran, 1980), and regression techniques were used to fit second-order models (Draper and Smith, 1981). Statistical calculations were performed with the general linear models procedure of the SAS statistical package (Freund et al., 1986; SAS Institute, Inc., 1987). A randomized complete block factorial arrangement of dose and storage days was used to analyze the indigenous microbial flora counts and *E. coli* CFU of wings in relation to increased dose level and storage days. Plate count data of natural flora were transformed into the respective logarithmic value (log number). Linear contrasts were conducted to further separate the effect of irradiation on log number. Significance was recorded at P ≤ 0.01.

RESULTS

THE SURVIVAL of about 100 CFU of *S. typhimurium*^{Sr} on chicken wings decreased significantly as the gamma radiation dose increased (Table 1). Surviving CFU of *S. typhimurium*^{Sr} were not found at absorbed gamma radiation doses exceeding 1.42 kGy. The results obtained with chicken wings stored at 5°C for 3 days were not significantly different from those obtained with samples analyzed immediately following irradiation, and only 25% of the wings tested for *S. typhimurium*^{Sr} at 1.42 kGy. A highly significant effect of radiation dose was found upon analysis of the transformed data. The predicted values indicated that about 5% of the samples receiving a dose of 2.83 kGy would be positive for *S. typhimurium*^{Sr}.

The effects of ionizing radiation on the numbers of aerobic mesophilic CFU found on uninoculated chicken wings were determined (Fig. 1). Except for the samples that were not irradiated, storage for 3 days at 5°C did not significantly alter the results. Gamma radiation doses of 1.42 and 3.92 kGy produced decreases of 2.92 and 3.92 log CFU/cm². The total population of the normal indigenous aerobic mesophilic bacteria on the chicken wings were reduced by a radiation dose of 1.4 kGy from an average of 10⁴ to 44 CFU/cm². No significant recovery (increase in CFU) of the irradiated microflora occurred during the 3 day storage period. The number of CFU typical of *E. coli* increased from 1.9 x 10² to 2.2 x 10⁴/cm² during the three days of refrigerated storage on the nonirradiated controls. No *E. coli* were found in the irradiated samples immediately after treatment, and even after 3 days storage the mean number of *E. coli* CFU in samples that received an absorbed radiation dose of 1.42 kGy was only 22/cm².

Highly significant effects were found for the radiation dose, the log of the inoculum, and the interaction between the two factors. No samples tested positive for *S. typhimurium*^{Sr} following gamma radiation doses of 2.7 or 3.6 kGy (Table 2). After treatment with a radiation dose of 1.8 kGy no viable CFU of *S. typhimurium*^{Sr} were detected on wings inoculated with either 10 or 100 CFU/wing. Viable *S. typhimurium*^{Sr} were

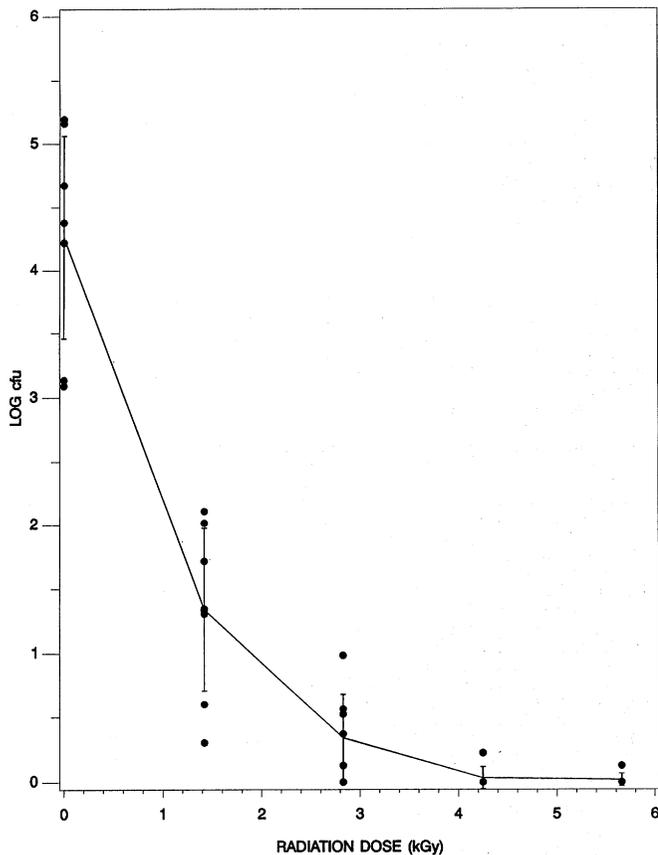


Fig. 1—Response of the indigenous mesophilic microbial flora on chicken wings to gamma radiation. The vertical bars represent \pm one standard deviation. For statistical calculations (0 on log scale) a CFU value of 1 was arbitrarily assigned where no CFU were detected.

detected on chicken wings inoculated with either 1,000 or 10,000 CFU/wing following irradiation with 1.8 kGy. Viable *S. typhimurium*^{Sr} were not detected on chicken wings inoculated with 10 CFU/wing after a gamma radiation dose of 0.9 kGy or greater. Using the second order model developed from these data the percentage of samples (wings) that would be expected to test positive for viable CFU of *S. typhimurium*^{Sr} was predicted (Table 2).

DISCUSSION

The use of an antibiotic-resistant strain of *S. typhimurium* ensured that any survivors isolated after radiation treatment represented the known test population and not a highly variable population of natural *salmonellae* contaminants. The antibiotic resistance was also used to aid in isolating the surviving *S. typhimurium*. The entire wing was incubated in the enrichment broth assuming that even if the pathogen should be firmly attached to the chicken as it multiplied, at least some cells would be released into the broth.

Quantitative studies of the effects of inoculum size are based on the probability that the dilution process would produce the desired inoculum in a given volume, in this case, 100 μ L. The initial population was estimated from past experience and could be confirmed only as part of the study. Due to the nature of the dilution process we would expect to obtain inocula of about 1,000 or 10,000 CFU much more reliably than inocula of 100 or 10 CFU per 100 μ L. With very small inocula, such as 10 CFU per 100 μ L, we expected that some wings might receive more than 10 CFU and that

some might receive none. However, in our study, 3–100 μ L aliquots from the inocula for replicate 1 and replicate 2 contained 11, 15, and 10 and 13, 18, and 8 CFU, respectively. Even if the wings all were to receive exactly 10 CFU, the probability of recovery of the inoculum would be expected to be lower than for wings receiving 100 or 1,000 CFU inocula. Added to these uncertainties is the unknown effect of the indigenous microflora on a much smaller population of *S. typhimurium*. Thus, it was not considered surprising that in (Table 2) only nine of 20 nonirradiated chicken wings inoculated with 10 CFU of the pathogen were confirmed as having viable *S. typhimurium* CFU. The statistical procedures were chosen for their ability to accommodate such variations. As a result, some predicted values, (e.g. those in Table 1) for a dose of 5.66 kGy, appear high. Actually, they are within the 95% confidence level for the analysis, and there were no significant differences in predicted results (Table 1) at doses of 2.83, 4.25, and 5.66 kGy.

Klinger et al. (1986) investigated the radiation dose necessary to completely destroy *salmonellae* and other pathogens on chicken carcasses. They found that broiler, breast, and leg meat was free from *salmonellae* after treatment with doses as low as 2.0 kGy. They isolated *salmonellae* from unirradiated chicken meat used in the study. Kiss and Farkas, (1972) and Ouwerkerk, (1981) observed that *salmonellae* were eliminated from chicken carcasses by the application of 2–5 kGy and 3.0 kGy, respectively. Hanis et al. (1989), however, reported that a dose of 5 kGy did not eliminate *S. typhimurium* from artificially contaminated deboned chicken meat. Lamuka et al. (1991) found that a gamma radiation dose of 2.6 kGy eliminated all naturally occurring *Salmonella* from mechanically deboned chicken meat. Jay (1986), reported that a dose of 2.5 kGy was highly effective in destroying *salmonellae* on refrigerated and frozen chicken carcasses. Lamuka et al. (1990) did not find naturally occurring *salmonellae* on whole chicken carcasses treated with a gamma radiation dose of 2.5 kGy under commercial conditions. In our study, no viable CFU of *S. typhimurium*^{Sr} were detected on wings inoculated with 100 CFU after radiation doses of 1.8 kGy or greater. However, 25% of the wings that received a dose of 1.42 kGy had detectable viable CFU of *Salmonella*^{Sr}. Thayer and Boyd (1991b) developed predictive equations for the survival of *S. typhimurium*^{Sr} on chicken legs and mechanically deboned chicken meat. Their equation predicted that a dose of 1.4 kGy would destroy 2.7 logs of CFU. Samples inoculated with either 1,000 or 10,000 CFU of *S. typhimurium*^{Sr} in our study required radiation doses exceeding 1.8 kGy to completely destroy the pathogen. Thus, the actual dose required would be between 1.8 and 2.7 kGy. This would agree with results of the predictive equation that a dose of 2.5 kGy would destroy 10,000 CFU of *S. typhimurium*^{Sr}/cm² of skin. This dose does not impart off-flavors to the broiler chicken according to Lamuka et al. (1990) and Stevens (1988). In the study by Stevens (1988), the total population of aerobic mesophilic bacteria on chicken wings was reduced by a radiation dose of 1.4 kGy from an average of 10⁴ to 44 CFU/cm². Katta et al. (1991) reported that a radiation dose of 2.5 kGy eliminated 99% of the indigenous microflora from chicken carcasses.

Bryan (1979) reported that the lowest dose of several species of *Salmonella* producing a clinical response in healthy adult humans was 10⁵ CFU. As noted above, a gamma radiation dose of 1.4 kGy would be expected to reduce the population of *S. typhimurium*/cm² by 2.7 logs. Thus, a population of 1,000 CFU/cm² would decrease to approximately 500 cells, well below the estimated infectious dose, and a very large amount of raw irradiated chicken would be necessary for an infectious dose to a healthy adult. Further, it has been demonstrated that radiation-injured *S. typhimurium* are much more sensitive to the effects of cooking than non-irradiated *S. typhimurium* (Thayer and Boyd, 1991c). We thus conclude that

Table 2—Effect of inoculum size on the survival of streptomycin-resistant *S. typhimurium*^{sr} to treatments with gamma radiation

Radiation dose kGy	Inoculum level (CFU/wing)															
	10				100				1,000				10,000			
	A ^a	B	Tot ^b	Pred	A	B	Tot	Pred	A	B	Tot	Pred	A	B	Tot	Pred
0	4/10	5/10	9/20	48%	10/10	7/10	17/20	80%	6/10	10/10	16/20	96%	10/10	9/10	19/20	100%
0.9	0/10	0/10	0/20	13%	8/10	1/10	9/20	36%	9/10	7/10	17/20	55%	10/10	10/10	20/20	100%
1.8	0/10	0/10	0/20	2%	0/10	0/10	0/20	10%	2/10	0/10	2/20	19%	3/10	0/10	3/20	24%
2.7	0/10	0/10	0/20	0%	0/10	0/10	0/20	3%	0/10	0/10	0/20	5%	0/10	0/10	0/20	5%
3.6	0/10	0/10	0/20	2%	0/10	0/10	0/20	3%	0/10	0/10	0/20	3%	0/10	0/10	0/20	0%

^a A and B represent replicates.

^b Tot = total of columns A and B; Pred = prediction of percent survivors.

irradiating poultry wings to 1.5 kGy \geq 3.0 kGy should provide significant protection against the presence of *salmonellae* and also greatly reduce the population of indigenous microflora.

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