

EFFECTS OF A CHEMICAL FEED ADDITIVE AND
FORMALDEHYDE-GAS FUMIGATION ON
SALMONELLA IN POULTRY FEEDS

by *632*

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**THIS BOOK
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INTRODUCTION

Salmonella infections of livestock and poultry continue to be an important problem in the United States and other areas of the world. Domestic animals have been considered the largest single reservoir of Salmonella organisms. Accumulated evidence has indicated that Salmonella contaminated feed may be the major contributing factor in maintaining this reservoir. As Edwards (1958) has noted, it is obvious that any effort to eradicate salmonellosis from domestic animals must take into consideration the continuous seeding of the population through infected feedstuffs.

Wedman (1961) called attention to the potential disease problem resulting from the "cycling" of a number of Salmonella serotypes from farms to processing plants and back to farms by animal by-products incorporated into feeds. Morehouse and Wedman (1961) isolated Salmonella from a wide variety of animal by-products. Recontamination of ingredients after processing was believed to be the principle factor for the presence of Salmonella.

Increasing interest in developing methods to eliminate or reduce Salmonella recontamination of feeds and feed ingredients has been generated by the concern that Salmonella may contaminate products from domestic animals processed for human consumption. Rasmussen et al. (1964) reported that heat treatment reduced the incidence of Salmonella in animal by-products. Mossel et al. (1967) discussed the effect of pelleting and terminal low dose irradiation on Salmonella incidence in feeds. However, the cost of pelleting and extrusion equipment makes control of Salmonella by heat treatment economically infeasible for many producers who

utilize on-the-farm systems of mixing feeds. A possible on-the-farm means of eliminating Salmonella in feed by use of a chemical additive (Endgerm^R) was reported by Westerfeld (1970). He concluded that although the additive did not eliminate Salmonella from contaminated feeds, a reduction in the number of Salmonella initially present may have taken place.

The purpose of this study was to investigate further the capability of Endgerm to reduce Salmonella in feeds, and to investigate formaldehyde-gas fumigation as a possible means of eliminating Salmonella in feeds.

^R Registered trademark for a chemical mixture supplied by Chemical Industries, Des Moines, Iowa.

REVIEW OF LITERATURE

Salmonella are nonsporeforming, Gram-negative rods closely related morphologically and physiologically to the other genera of the family Enterobacteriaceae. They are usually motile, although nonmotile forms occur; attack carbohydrates, forming acid and usually gas; do not ferment lactose, sucrose, or salicin; and do not form indole or liquefy gelatin. They are parasitic upon man and animals and usually produce inflammatory reactions in the intestinal tract (Merchant 1940).

According to Hagan (1943), the bacillus causing human typhoid fever (S. typhosa) was discovered by Ebert in Germany in 1880, but it was not until 1884 that Gaffky isolated it in pure culture and described some of its characteristics. In the course of time many other organisms having characteristics of the typhoid bacillus became known (S. cholera-suis, 1886; S. enteritidis, 1888; S. gallinarum, 1889).

Merchant (1940) related that the name Salmonella was proposed for the genus by Ligières in 1900 in honor of D. E. Salmon, the first Chief of the United States Bureau of Animal Industry. Williams (1965), in describing paratyphoid infections, stated that; "Paratyphoid infections, as the term is used with reference to poultry, denote a large group of acute or chronic bacterial diseases caused by one or more of the normally motile members of the Salmonella genus."

Moore (1895) recorded the first authentic case of paratyphoid infection in domestic poultry in describing an outbreak of infectious enteritis in pigeons. With improvement of culture proced-

ures for the isolation of Salmonella and a better definition of the characteristics of members of the genus, the frequent association of paratyphoid organisms with disease outbreaks in all types of poultry was rapidly established.

Williams (1965) noted that Mazza was one of the first in the world to describe a paratyphoid infection among chickens. In 1889, he isolated an organism that was pathogenic for chickens and pigeons and possessed the biochemical characteristics of a paratyphoid. Rettger et al. (1933) was the first to report the occurrence of paratyphoid infections in turkey poults in the United States.

INCIDENCE

Paratyphoid infections may occur in most species of warm- and cold-blooded animals. Among domestic poultry, paratyphoid infections are most frequently encountered in turkeys and chickens. Most death losses from paratyphoid infections of poultry occur during the first 2 weeks after hatching with the highest losses occurring between the 6th and 20th days. Adult birds infected with paratyphoid organisms generally show no outward symptoms; however, they may serve as intestinal carriers of the infection over long periods of time.

Lee et al. (1936) examined an acute paratyphoid disease that caused 90 percent mortality among poults less than 5 weeks of age. In other flocks the losses were less, ranging from 40 to 70 percent.

Cherrington et al. (1937) reported paratyphoid outbreaks among turkeys from widely separated areas of Idaho in which S.

aertrycke was the common serotype. Poults died rapidly up to 10 days of age. Symptoms were weakness and occasional diarrhea. In one outbreak more than 11,000 of 22,000 poults hatched on one farm died from this infection. Edwards et al. (1948) serotypes 60 different Salmonella types from fowl, mostly from turkeys and chickens. Degree of severity of infection varied greatly with mortality ranging from 5 to 100 percent.

Edwards and Bruner (1943), in a 1943 Salmonella infection survey, reported that of 2,285 outbreaks of paratyphoid infection in man and animals, 59 serotypes of Salmonella were isolated. Moran (1960) found that of 1,178 Salmonella cultures typed from animals during 1958, 87.2 percent were from avian sources. Sixty-one types were identified, 40 of which occurred in turkeys and 32 in chickens.

In an experimentally induced paratyphoid infection, Milner and Shaffer (1952) were able to isolate Salmonella in the feces of all of 10 day-old chicks 24 hours after oral inoculation with as few as 10 organisms. In a subsequent study Shaffer et al. (1957) concluded that Salmonella obtained from cloacal swabs (after oral inoculation) were indeed the result of multiplication within the avian host.

TRANSMISSION

The wide distribution of paratyphoid organisms under natural conditions contributes materially to their spread. Evidence has been presented which indicates that poultry feeds which are contaminated with Salmonella may be a source of continual seeding of the

population.

Erwin (1955) collected samples from 206 bags of poultry feed representing the products of 9 commercial companies over a period of 18 months. Three of the feed samples yielded Salmonella which were identified as S. oranienburg. Boyer et al. (1958) conducted bacteriological examinations of samples taken from 51 unopened sacks of poultry feed. From 5 of the mash samples, 6 Salmonella types were isolated. An outbreak of S. thomasville infection in poults was associated with the consumption of the contaminated feed.

The isolation of S. thomasville, S. newbrunswick and S. kentucky from the livers and intestines of poults has been reported by Boyer et al. (1962). In this examination S. thomasville was isolated from an unopened bag of feed of the same batch fed to the poults. In another case, 2 Salmonella serotypes were isolated from the ceca of chicks and the feed fed to them.

Watkins et al. (1959) cultured 200 samples of animal by-products and found 37 (18.5%) samples contaminated with Salmonella. A majority of the samples were contaminated by more than one serotype. In this examination, 28 different Salmonella serotypes were isolated. Moran (1959) reported that during 1959, there was an increase of Salmonella isolations from animal feeds and feed ingredients. This study was based on 2,250 cultures. Forty-six different Salmonella serotypes were found in animal feeds, with 39 different types coming from turkey and 33 from chicken feed.

In a survey by Allred et al. (1967), the Salmonella contamination found in a total of 12,777 samples of feed and feed in-

gredients was 4.2 percent. The categories showing contamination, from highest to lowest, were animal by-products (31.07%), poultry feed (5.23%), fishmeal (4.27%), swine feed (3.13%), oilseed meals (2.28%), cattle feed (0.85%) and grains (0.66%). In an examination of poultry feeds from commercial feed mills over a period of 5½ years, Zindel and Bennett (1968) were able to isolate Salmonella from only 13 (1.6%) of 808 samples.

A survey by Morehouse and Wedman (1961) of disease producing organisms in animal by-products showed 59 serotypes of Salmonella isolated from a wide variety of animal by-products. Recontamination of ingredients after processing was believed to be the principle factor for the presence of Salmonella. Possible sources of recontamination were rodents, wild birds, dogs and human handlers.

Rats and mice are frequent carriers of paratyphoid organisms and their droppings may readily contaminate feed supplies. Wild birds, flies and domestic animals may also be sources of recontamination of poultry feeds. Goetz (1962) reported an incidence in which it was necessary to abandon turkey raising operations in one area because S. typhimurium was indigenous in the wildlife of the area. The organism was isolated from gopher snakes, ground squirrels, and owl pellets on the premises.

In a study of 4 outbreaks of paratyphoid infection in 3 areas of North Central New Jersey during a period of 2 years, Hudson and Tudor (1957) isolated S. typhimurium from several varieties of free-flying birds, including starlings, sparrows, rusty blackbirds and a cow bird originating from the same areas. They called atten-

tion to the possibility that these birds may spread the infection to man and domestic animals. Butler and Mickel (1955) studied samples of wheat from different sources and found that 17.2 percent were contaminated with rodent pellets and 3.9 percent with bird pellets.

Jungerman and Grumbles (1960) isolated Salmonella organisms from 9 of 100 mature healthy dogs. Two of the dogs were infected with more than one Salmonella type. Bruner and Moran (1949) reported 26 Salmonella types that were recovered from dogs. Approximately 40 percent of the cultures were S. typhimurium.

De Las Casas et al. (1968) reported that lesser mealworms, A. diaperinus, when allowed to infect feed artificially contaminated with Salmonella, had an internal Salmonella count which increased from 1 to 2.4 million per insect over a 24-day period. Mealworms from this group were killed and stored in a sterile environment for 45 days. At the end of this period, colony counts ranged from 50 to 435,000 per insect. The capability of the lesser mealworm, alive or dead, as a carrier of Salmonella and other pathogenic bacteria was stressed by the authors. Consequently this insect should be considered as a source of Salmonella contamination of animal feeds.

Other insect and reptile sources of possible contamination of feeds have been reported. Osterlenk and Welch (1942) reported that S. enteritidis can be transmitted through the complete life cycle of flies and that the infection may continue as long as 4 weeks within flies. McNeil and Hinshaw (1944) discussed the part snakes, flies and cats may play in the transmission of paraty-

phoid infections to poultry.

Wedman (1961) called attention to the potential disease problem resulting from the "cycling" of a number of Salmonella serotypes from farms to processing plants and back to farms by animal by-products incorporated into feeds. Niven (1961) pointed out some of the problems that industry encounters in efforts to eliminate Salmonella organisms from rendered animal by-products used in the manufacture of animal feeds. As Edwards (1958) has noted, it is obvious that in any effort to eradicate salmonellosis from domestic animals it is necessary to take into consideration the continuous seeding of the population through infected feedstuffs.

PREVENTION

The classical method employed to reduce mortality in acute outbreaks of paratyphoid infections and to aid in preventing the development and spread of the disease is through medical therapeutics. This is accomplished by the addition of chemicals either to the water or the feed for poultry consumption. All such measures have the disadvantage of being incapable of eliminating the infection from treated birds.

Clark (1946) showed that mortality was reduced when the drug sulfamerazine was placed in the mash 24 hours before intramuscular injection of 0.022 c.c. of Salmonella organisms. Maximum effect was reached with the addition of sulfamerazine in the mash at the level of 0.5 percent. Belding and Mayer (1958) reported that nf-180, a nitrofurantoin, reduced the mortality due to S. san diego by 50 percent when infection and treatment were started

concurrently. After 13 weeks, 34 percent of the infected birds remained carriers. The medication was added to the mash at 0.011 percent for 2 weeks followed by 0.0055 percent for 3 weeks. Bierer and Vickers (1960) found that adding furazolidone in the feed at a level of 0.222 percent, solubilized nf-248 at a level of 0.006 percent in drinking water, or 0.0264 percent furazolidone in the drinking water, drastically reduced mortality from experimentally induced S. typhimurium infection in poults as compared to infected nonmedicated control birds. A nitrofurazone compound, Tiafur, was tested by Lannek et al. (1962) against experimental and spontaneous Salmonella infections in chicks. A suitable dose practically eliminated mortality and clinical symptoms of the disease.

Smith (1955) reported that furazolidone fed at a level of 0.04 percent in the mash continually for 10 days was very effective in reducing the mortality associated with experimental S. typhimurium infection in poults and chicks. It was found that a high proportion of treated birds became carriers even when treatment was started 3 days before infection. Repeating the treatment or increasing the concentration was not found to decrease the carrier rate. Bierer (1963) reported that both 0.011 and 0.0165 percent nihydrazone reduced mortality due to S. typhimurium infection in turkey poults; however, this drug seemed to retard growth at effective therapeutic levels in turkeys.

Ramsey and Edwards (1961) found that 29 percent of the 100 Salmonella cultures they isolated from fowl in 1959 and 1960 were resistant to tetracyclines as compared to 9 of 100 cultures in

1956 and 1957. They attributed this to the use of antibiotics in poultry feeds. Garside et al. (1960) demonstrated a tenfold increase in resistance to chlortetracycline in the case of a single colony inoculum of S. typhimurium administered to chicks receiving the antibiotic in their diet. The organisms were capable of resisting 210 p.p.m. of chlortetracycline after 4 passages. Subsequent passages of the resistant cultures through chicks receiving no drug in their feed revealed that resistance declined gradually, but at the end of 14 weeks some strains were still 4 times as tolerant to the antibiotic as the normal strains. The authors felt that birds receiving antibiotics for the prevention and treatment of Salmonella infections may be a public health hazard because of the large number of infected carrier birds remaining in such flocks.

It has been the occurrence of resistant strains of Salmonella and carrier birds, due to chemical therapeutics, that has prompted investigators to examine methods of destroying Salmonella in the feed rather than in the bird. Several procedures which might be utilized for eliminating Salmonella from animal feeds have been reported but few are economically feasible to be utilized in on-the-farm systems of mixing animal feeds.

Liu et al. (1969b) reported on the relationship of moisture and storage temperature and their effect on Salmonella persistence in meat and bone meal. Moisture levels of 5 to 30 percent had little effect in reducing Salmonella populations in meat and bone meal at 4°C. No significant changes in the test population occurred in meal of 5 and 10 percent moisture at any of the stor-

age temperatures (4, 24-30, 37, and 50°C.) except for a significant decline which occurred in 10 percent moisture meal held at 50°C. A storage temperature of 50°C. caused a sharp reduction in viable cells at all moisture levels except 5 percent.

When radiation is applied to mixed feed ingredients, such as fishmeal, cottonseed meal and soybean meal, or to mixed feeds, for the elimination of Salmonella, the dose is of the order of 0.6 ± 0.3 Mrad according to Mossel et al. (1967). In similar dose-range-finding tests on decontamination with ^{60}Co gamma rays, Mossel et al. (1967) reported that about 0.5 Mrad were required for reduction in the counts of the most resistant bacteria by a factor of 10^5 .

Rasmussen et al. (1964) reported that heating of Salmonella contaminated feed in a pug-mill at 160°F. for 50 minutes was necessary to destroy the Salmonella. A bioassay revealed that heating to 180°F. did not change the nutritive value of the protein significantly. Crane et al. (1967) reported on the effect of pelleting on Salmonella presence in feed from 8 trials conducted in 1965. Samples of pelleted and granulated formulas containing meat meal were tested for the presence of Salmonella. Twenty-eight percent of the incoming shipments of meat meal were positive for Salmonella while the resultant pelleted and granulated formulas were negative.

Adams (1968) investigated the possibility of inhibiting the growth of Salmonella infected feed by pelleting feed conditioned to 50 and 70°C. Feed was infected with S. typhimurium at a level of approximately 20,000 organisms per gram of feed. Cultures of

the treated feeds and feces from hens fed the treated feeds were negative for Salmonella. Mossel et al. (1967) reported that pelleting at a working temperature of 80°C. usually reduced the bacterial count by a factor of 10^5 , while lower temperatures currently used reduced it by only a factor of 10^3 . It was concluded that a combination of improved sanitation, pelleting at the highest possible temperature and, if still required, terminal low-dose irradiation seemed a promising approach to the manufacture of Salmonella free feeds.

In thermal-death-time studies with Salmonella contaminated feed, Liu et al. (1969a) concluded that feed pasteurization could be accomplished by processing feed at a moisture level of 15 percent or greater and a temperature of 190°F.

EXPERIMENTAL METHODS

Experiment 1

The experimental design for this experiment was based on the hypothesis that any reduction of Salmonella due to the chemical additive would be similar in sterilized and unsterilized feeds, and the persistence of Salmonella in feeds would not be altered by sterilization of the feed samples prior to inoculation with Salmonella. The use of only sterilized feed in this experiment eliminated the problems with the masking effect that other organisms in unsterilized feed have on reducing the ability to count Salmonella accurately.

Feed samples consisting of chick starter, fishmeal, and meat and bone meal were sterilized by autoclaving, in 200-gm. portions, at a guage pressure of 15 p.s.i. and 120°C. for 1 hr. on 2 successive days.

A smooth strain of S. senftenberg 775W was used as the test organism. Each 200-gm. portion of feed was inoculated with a broth culture of Salmonella to provide a contamination level of 2×10^3 cells/gm. of feed in an initial trial and 2×10^6 in subsequent trials. Prior to inoculating the feed samples, concentrations of the broth cultures were determined by optical density using a Bosch & Lomb Spectronic 20. A previously determined optical density curve provided a reasonable estimate of the broth culture concentration and allowed the use of the same inoculation concentration for all treatments.

The chemical additive was a commercially prepared product,

Endgerm, which according to the manufacturer was a composite of acids with the predominant acid being propionic and carried a manufacturer's recommended usage level of 0.1%. The additive was added to the contaminated feed samples at levels of 0.0, 0.1, and 0.2%. A summary of the treatments is shown in Table 1.

Table 1. Treatments used in Experiment 1

Feed sample	Endgerm level	<u>Salmonella</u>
	(%)	(cells/gm.)
Chick starter	0.0	2×10^6
Chick starter	0.1	2×10^6
Chick starter	0.2	2×10^6
Fishmeal	0.0	2×10^6
Fishmeal	0.1	2×10^6
Fishmeal	0.2	2×10^6
Meat and bone meal	0.0	2×10^6
Meat and bone meal	0.1	2×10^6
Meat and bone meal	0.2	2×10^6

The additive and cell suspension were added to the sterile feed samples separately but mixing was accomplished in the same manner for both. The inoculum was thoroughly mixed into 50 gm. of the feed sample and placed, along with the remaining 150 gm., in a Kitchen Maid K4-B mixer and mixed for 10 min. Westerfeld (1970) showed through potentiometric titration of feed samples

containing added ammonium chloride that mixing in this manner provided a uniform distribution of organisms in the feed.

Subsequent to inoculation and mixing, the moisture levels of the feed samples were analyzed with an Ohaus Model 6010 Moisture Determination Balance and adjusted to 10% by the addition of sterilized distilled water. A moisture level of 10% was chosen as it most closely approximated the moisture level of poultry feeds mixed at Kansas State University. Moisture levels were determined by sampling several open sacks of feed and subjecting them to moisture balance analysis. The feed samples tested fell within a range of $11.0 \pm 2.0\%$ moisture.

The test population was determined immediately after introducing and mixing Salmonella into the feed samples and at intervals during a 10-day storage period. Populations were determined by transferring 10 gm. of each feed sample into dilution bottles containing 90 ml. of 0.85% sterile saline and plating appropriate serial dilutions on brilliant-green agar using the complete-surface inoculation method. Colony counts were made after incubating plates 48 hr. at 37°C. Populations were calculated from the mean colony counts secured from three test samples prepared at different times. Treated feed samples were sealed in plastic bags during the storage period and reopened only when sampling for population counts. This was a precautionary measure used to reduce contamination and fluctuations in moisture levels of the samples during the storage period.

Experiment 2

A preliminary investigation of formaldehyde-gas fumigation as a means of eliminating Salmonella in poultry feeds was made in this experiment. The experimental design was established to determine the maximum penetrating depth of the fumigant under the conditions used and the effectiveness of fumigation during continuous mixing of feeds.

Unsterilized chick starter was contaminated with S. senftenberg 775W by inoculating 50 gm. of meat and bone meal with the organism which was then mixed into 450 gm. of chick starter to produce a contamination level of 2×10^6 cells/gm. of feed. Mixing was accomplished according to the method explained in experiment 1.

The contaminated feed was placed into 7.62x17.78x3.81 cm. containers at depths from 0.32 to 3.81 cm. In addition, a 500 gm. sample of contaminated feed was placed into the K4-B mixer to facilitate continuous mixing of the samples during fumigation. Fumigation of the contaminated feed samples was done at 37°C. and 60% relative humidity in a small incubator with a 0.28 cubic meter capacity. The fumigant was provided by mixing 4.5 ml. of a 37% solution of formalin and 2.2 gm. potassium permanganate per cubic meter of chamber space. The feed samples and the fumigant were placed in the chamber and the fumigant was allowed a few minutes to fill the chamber completely before starting the experiment. A summary of treatments is shown in Table 2.

Table 2. Treatments used in Experiment 2

Phase	Feed depth <u>1/</u> (cm.)	Fumigation time (min.)
Penetration	0.32	0 <u>2/</u>
	0.32	20
	0.64	20
	1.28	20
	1.91	20
	2.54	20
	3.81	20
Continuous mixing	<u>3/</u>	0 <u>2/</u>
		5
		10
		15
		20

1/ All feed samples used were chick starter contaminated with 2×10^6 Salmonella/gm.

2/ Positive control.

3/ The average depth during mixing of 500 gm. of feed was 5.08 ± 1.28 cm.

Thirty gm. samples were taken from the continuously mixed feed at 5 min. intervals and from the feed of different depths at the end of the 20 min. fumigation period only. Feed samples were introduced into 200 ml. of selenite-brilliant-green broth and incubated for 24 hr. at 37°C. The selective broth cultures were

then streaked on brilliant-green agar and incubated at 37°C. for 24 hr. Suspect colonies were picked and transferred to triple-sugar-iron agar slants (TSI). The TSI slants were examined for typical Salmonella reactions after incubation for 24 hr. at 37°C. Cultures giving typical reactions were examined with Salmonella polyvalent O and Salmonella O group antisera and checked for typical biochemical reactions. Cultures which gave positive results were presumed to be Salmonella.

Fumigation with continuous mixing of the Salmonella contaminated feed was conducted on a larger scale using a Hobart industrial mixer with a capacity of 9.12 kg. of feed. An incubator with 2.34 cubic meter capacity was used as the fumigation chamber.

Salmonella contaminated feed was prepared by inoculating 0.45 kg. of sterilized meat and bone meal with a Salmonella broth culture of known concentration. The contaminated meat and bone meal was then mixed into 8.67 kg. of unsterilized chick starter. The contamination level of the chick starter was approximately 2×10^6 Salmonella/gm.

Data were statistically analyzed by analysis of variance.

RESULTS AND DISCUSSION

Experiment 1

Preliminary in vitro sensitivity tests indicated that a 0.1% concentration of the additive was effective in inhibiting the growth of Salmonella in concentrations up to and including 10^7 cells/ml. in nutrient broth. Higher concentrations of Salmonella were not tested.

In the initial phase, when a calculated Salmonella population of 10^3 cells/gm. was inoculated into the dry feed samples, there was an initial spontaneous reduction of 10^2 to 10^3 cells/gm. of feed (Table 3). As these data show, the chick starter containing 10% moisture proved to be extremely harsh on the Salmonella population. The calculated inoculum of 10^3 cells/gm. of feed underwent spontaneous reduction of sufficient magnitude that at the end of 1-2 days Salmonella were no longer detectable by a standard plate count method.

Several workers have investigated the phenomenon of spontaneous reduction and have concluded that microbial multiplication or reduction is related to the water activity of a substrate. Scott (1953) defined water activity (a_w) of a substrate as the relative humidity of an atmosphere with which a product is in equilibrium. Mossel and Koopman (1965) concluded the lethal effect of dry animal feeds on Salmonella seems to be mainly due to exposing the cells to an environment of different a_w and any gross change in a_w whether in the positive or negative direction affects serious losses in viable cells of Salmonella. Carlson and Snoeyenbos

(1970) found that Salmonella dieoff was directly proportional to elevation in moisture level, except at moisture levels that allowed growth (a_w 's of approximately 0.96 or higher).

Table 3. Number of Salmonella in chick starter stored at 24-30°C.

Trial	Initial ^{1/} inoculum	Immediately after inoculation	Storage	
			1 day	2 days
(cells/gm. feed)				
1	2x10 ³	1x10 ¹	- ^{2/}	-
2	2x10 ³	1x10 ²	2x10 ¹	-
3	2x10 ³	3x10 ¹	-	-

^{1/} Calculated from optical density for broth culture.

^{2/} Populations of less than 10 cells/gm. could not be detected by the plate count method used.

Because of the initial spontaneous reduction, it was necessary to utilize larger inoculums in subsequent trials to determine if the reductions in the feed samples were due to the additive or to spontaneous reduction and to prolong the survival time of the organisms.

The effect of the additive on Salmonella in the feed samples is shown in Table 4. Plate counts were not taken after the 10th day of storage. After a month storage the samples were tested for the presence of Salmonella, using a standard qualitative test, at which time the samples were discarded. Salmonella were isolated from all of the fishmeal and meat and bone meal samples but not

from the chick starter.

Table 4. Effect of various levels of the additive on Salmonella in feeds

Treatment	Storage period at 24-30°C.				
	0	1	(days) 2	5	10
(cells/gm. of feed) $\frac{1}{2}$					
Chick starter					
+ 0.0% Endgerm	6×10^3	4×10^3	1×10^3	6×10^2	
+ 0.1% Endgerm	7×10^3	5×10^3	1×10^3	5×10^2	$\frac{2}{2}$
+ 0.2% Endgerm	6×10^3	3×10^3	1×10^3	7×10^3	
Fishmeal					
+ 0.0% Endgerm	3×10^4	1×10^4	8×10^3	7×10^3	1×10^3
+ 0.1% Endgerm	4×10^4	1×10^4	9×10^3	8×10^3	2×10^3
+ 0.2% Endgerm	1×10^4	9×10^3	8×10^3	4×10^3	3×10^3
Meat and bone meal					
+ 0.0% Endgerm	3×10^5	1×10^5	6×10^4	3×10^4	6×10^3
+ 0.1% Endgerm	2×10^5	2×10^5	5×10^5	2×10^4	7×10^3
+ 0.2% Endgerm	2×10^5	9×10^4	6×10^4	4×10^4	8×10^3

^{1/} All counts represent the average of three trials.

^{2/} Salmonella present but not detectable by the plate count method used.

An analysis of variance (Table 5) showed no significant difference between levels of the additive in the individual samples, indicating the additive had little effect on the reduction of the

Table 5. Analysis of variance for number of Salmonella in feeds treated with various levels of additive

Feed sample	Source of variance	Degree of freedom	Mean square	"F"
Chick starter	Treatments	2	0.41	1.75
	Days	4	21.74	93.05**
	Error	8	0.23	
Fishmeal	Treatments	2	0.10	1.57
	Days	4	4.62	75.66**
	Error	8	0.06	
Meat and bone meal	Treatments	2	5.40	0.33
	Days	4	253.01	15.27**
	Error	8	16.57	

** Significant ($P < 0.01$)

Salmonella population. The data in Figure 1 indicated the reduction of the Salmonella population was due to spontaneous reduction rather than any deleterious effect of the additive; since the greatest differences in losses of viable cells occurred during inoculation of Salmonella into dry feed samples, after which the rate of reduction appeared to be nearly equal. The drastic change in loss of viable cells in the chick starter after 5 days storage (Figure 1) may be due to experimental error incurred by sampling populations of less than 10^3 cells/gm. of substrate with the plate count method used.

Significant ($P < 0.01$) differences between feed samples in reduction of Salmonella were obtained (Table 6). These differences may be due in part to differences in water activity. Liu et al. (1969b) presented the relationship between a_w and moisture

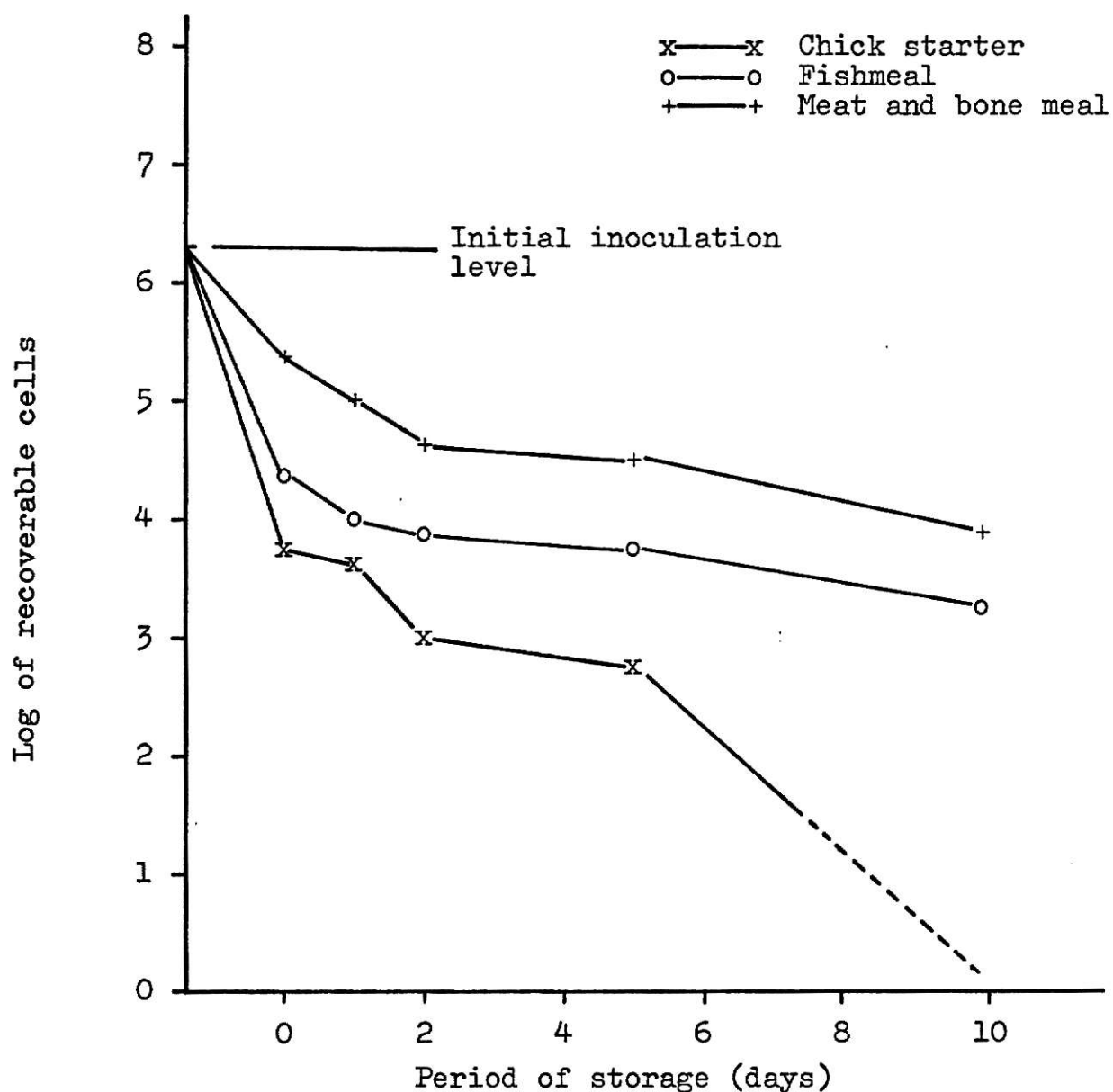


Figure 1. Effect of type of feed and storage time of Salmonella

level of meat and bone meal and chick starter. They found that even though materials of different composition have identical moisture levels, their a_w 's may be different and therefore affect their ability to support microbial growth. Meat and bone meal and chick starter of 10% moisture were shown to have a_w 's of 0.65 and 0.50. respectively.

Table 6. Analysis of variance for number of Salmonella in feeds immediately after inoculation

Source of variance	Degree of freedom	Mean square	"F"
Samples	2	37892.00	21.17**
Error	4	1790.25	

** Significant ($P < 0.01$)

Ineffectiveness of Endgerm in reducing Salmonella populations in dry animal feed may be due in part to the possibility that the chemical was "soaked up" by the individual particles of the feed and never came into contact with the bacterial cells. Also Endgerm may be only effective against growing, multiplying bacterial cells. Liu *et al.* (1969b) showed that Salmonella did not grow in meat and bone meal with a_w 's less than 0.97 (30% moisture content) at 24-30°C. The actual mode of action of Endgerm on a bacterial cell warrants further investigation.

Experiment 2

All feed samples in this experiment were classified as either positive or negative for Salmonella. No attempt was made to enumerate the Salmonella, if present, in the feed. For statistical analysis of the data, a value of one was assigned to cultures negative for Salmonella and a value of zero to those positive for Salmonella.

The results presented in Table 7 show that Salmonella was not

found in fumigated feed in depths up to and including 1.91 cm.

Table 7. Presence of Salmonella in feed fumigated with formaldehyde-gas for 20 min.

Depth of feed (cm.)	No. samples	No. positive for <u>Salmonella</u>
0.32 ^{1/}	9	9
0.32	9	0
0.64	9	0
1.28	9	0
1.91	9	0
2.54	9	6
3.81	9	9

^{1/} Positive control subjected to chamber conditions of 37°C. and 60% relative humidity for 20 min. without fumigation.

Analysis of variance (Table 8) of the data for 1.91 and 2.54 cm. depths (Table 7) showed a significant difference ($P < 0.025$) indicating that the maximum penetrating depth of the fumigant, under the conditions used, was less than 2.54 cm. but at least 1.91 cm.

When Salmonella contaminated feed was fumigated during continuous mixing, a 5 min. fumigation time (Table 9) was sufficient in eliminating Salmonella from all feed samples. The average depth of the feed during mixing was 5.08 ± 1.28 cm. for the 500 gm. samples and 20.32 ± 2.54 cm. for the 9.12 kg. samples. Since

Table 8. Analysis of variance for recovery of Salmonella in feed fumigated for 20 min. at 1.91 and 2.54 cm. depths

Source of variance	Degree of freedom	Mean square	"F"
Samples	1	2.00	8.00*
Error	8	0.25	

* Significant ($P < 0.025$)

the analysis of variance (Table 8) indicated that any elimination of Salmonella in feeds of 2.54 cm. depth or greater would be due to some factor other than the penetration of the feed by the fumigant, it was concluded that mixing did in fact increase the effectiveness of fumigation under the conditions used.

Samples examined for Salmonella immediately after fumigation were positive for Salmonella indicating that action of the fumigant on the bacterial cells was not immediate or irreversible. Fumigated samples examined 12 hr. after fumigation were all negative for Salmonella while the controls remained positive.

Additional criteria for fumigation with formaldehyde-gas, other than that given in the experimental procedure, would be that fumigated feed be held for sufficient periods of time to allow the fumigant to act upon the bacterial cells.

Moisture content analysis of the fumigated feed showed only a 1% increase after 20 min. fumigation. An appreciable increase in moisture level of feed fumigated under the conditions of this experiment would not be expected.

Table 9. Presence of Salmonella in feed fumigated during continuous mixing

Sample <u>1</u> / size	Fumigation time	No. samples	No. positive for <u>Salmonella</u>
(kg.)	(min.)		
0.50	0 <u>2</u> /	9	9
0.50	5	9	0
0.50	10	9	0
0.50	15	9	0
0.50	20	9	0
9.12	0 <u>2</u> /	6	6
9.12	5	6	0
9.12	10	6	0
9.12	15	6	0
9.12	20	6	0

1/ Average depth was 5.08 ± 1.28 cm. for the 0.50 kg. samples and 20.32 ± 2.54 cm. for the 9.12 kg. samples.

2/ Positive control subjected to chamber conditions, without fumigation, for 20 min.

Although formaldehyde-gas fumigation appears to be a promising method for eliminating Salmonella from feed, there are several unanswered questions which must be investigated before this method is recommended for other than experimental use. At present there is no information on the quantity of formaldehyde residuals remaining in the fumigated feed and the effects of fumigated feed on poultry.

SUMMARY

Chick starter, fishmeal, and meat and bone meal were artificially contaminated with S. senftenberg 775W and treated with levels of 0.0, 0.1, and 0.2% Endgerm. Enumeration of the numbers of Salmonella present was made by a standard plate count method during a 10-day storage period.

The results of this study show that the chemical additive, Endgerm, was not responsible for reduction in Salmonella populations in the feed samples. Reductions of Salmonella populations in the treatments were attributed to spontaneous reduction rather than any deleterious effect of the chemical additive. Because of the masking effect that other organisms present in feed have on the enumeration of Salmonella, enumerations of Salmonella in unsterilized feed treated with Endgerm were not attempted. It was hypothesised that sterilization of the feed would not affect the action of Endgerm on the Salmonella populations.

Evidence is presented suggesting that fumigation with formaldehyde-gas may be an effective means of eliminating Salmonella in feed and feed ingredients. Fumigation, without mixing, was effective in eliminating Salmonella in feed held at depths up to and including 1.91 cm. A 5 min. fumigation time during continuous mixing was sufficient to eliminate Salmonella in 9.12 kg. of feed.

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EFFECTS OF A CHEMICAL FEED ADDITIVE AND
FORMALDEHYDE-GAS FUMIGATION ON
SALMONELLA IN POULTRY FEEDS

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Chick starter, fishmeal, and meat and bone meal were artificially contaminated with S. senftenberg 775W at a level of 2×10^6 cells/gm. of feed and treated with a chemical feed additive (Endgerm^R) at levels of 0.0, 0.1, and 0.2%. A standard quantitative test for Salmonella was made on the treated feed samples during a 10-day storage period to determine the inhibitory effect of Endgerm on the presence of Salmonella in poultry feeds.

Endgerm had no significant effect on elimination of Salmonella in feeds at levels of 2×10^6 organisms/gm. Reduction of Salmonella populations in treated feeds was attributed to spontaneous reduction rather than any deleterious effect of the chemical. Degree of reduction of Salmonella populations differed between type of feed with meat and bone meal being the least deleterious and chick starter the most.

Salmonella contaminated feed was fumigated with formaldehyde-gas at 37°C. and 60% relative humidity. A standard qualitative test for Salmonella was made on the fumigated feed samples. Fumigation for 20 min. was effective in eliminating Salmonella in feed depths up to and including 1.91 cm. A 5-min. fumigation period during continuous mixing of Salmonella contaminated feed was sufficient to eliminate Salmonella in 9.1 kg. of feed.