

EFFECT OF A CHEMICAL FEED ADDITIVE
ON SALMONELLA PRESENCE IN
POULTRY FEEDS AND HOST BIRDS

by 1268

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INTRODUCTION

The presence of salmonella in various feeds and feed ingredients, such as animal by-products, is causing great concern to the feed and food production and processing industries. Domesticated animals are a major reservoir of salmonella. Since this reservoir can be maintained through ingestion of contaminated feed to the time the animal is processed, there is a possibility of salmonella contaminated feeds being eaten by man. The possibility of contaminated food and the harmful effect salmonella have on poultry have stimulated investigations on methods to eliminate or reduce the incidence of salmonella contamination of feeds and feed ingredients.

Several workers (Rasmussen et al., 1964; Crane et al., 1967; Adams, 1968; and Quadri, 1970) have reported that heat treatments, such as pelleting and extrusion, reduced the incidence of salmonella in poultry feeds and rendered animal by-products. 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 feed. A control method that is economical and adaptable to on-the-farm mixing is needed. The addition of a chemical bactericide to the feed is a possible method.

The purpose of this study was to: (1) determine the effectiveness of a chemical additive (Endgerm^R) to inhibit the growth of salmonella in poultry feed, and (2) compare the effectiveness of

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

Selenite and Brilliant-green Tetrathionate (B.G.T.) enrichment broths
on the isolation of salmonella from poultry feed and fecal material.

REVIEW OF LITERATURE

Description of Problem

"Paratyphoid infections, as the term is used with reference to poultry, denotes a large group of acute or chronic bacterial diseases caused by one or more of the *normally motile* members of the salmonella genus. Salmonellosis is often used synonymously with 'salmonella infection' as an inclusive term to designate a disease caused by any one or more members of the salmonella genus," (Biester and Schwarte, 1965).

Morphologically and physiologically related to the genera of the family Enterobacteriaceae, salmonella are nonsporeforming, gram-negative rods, usually motile, and produce acid and gas from glucose, maltose, manitol, and sorbitol. They are parasitic to man and animal and usually produce inflammatory reactions in the intestinal tract. (Merchant and Packer, 1967).

Merchant and Packer (1967) reported that genus salmonella was named after D. E. Salmon, who along with Smith, in 1885 isolated the first representative organism of the group from pigs which had died of hog cholera. The second organism of the group was isolated by Gartner in 1888 from a fatal case of gastroenteritis in a young man who had eaten raw meat from a diseased cow.

Chronic intestinal carriers of paratyphoid infection are common; however, the disease seldom occurs in the acute, septicemic form except in young fowl or in mature birds under stress conditions such as virus disease, inadequate diet, or unsanitary environment.

Most death losses from paratyphoid infections of poultry are encountered during the first two weeks after hatching with the highest

losses occurring between the sixth and tenth day. Mortality rates among broods of young birds under natural conditions usually vary from negligible to 10 or 20 percent; however, mortality rates of 80 percent or higher are encountered in severe outbreaks. The pathogenic properties of salmonella are due to endotoxins which are closely associated with the somatic portion of the organisms (Biester and Schwarte, 1965).

Smith and Jones (1966) reported that all known species of salmonella are pathogenic to man, animal or both. Salmonella, often originating in birds or mammals, are responsible for a specific "food-poisoning" in man. These organisms produce a toxin which causes severe gastroenteritis with nausea, vomiting, cramps and diarrhea, which characteristically appears 18-24 hours after ingestion of contaminated food.

Lee et al. (1936) described the symptoms of parathyphoid infection in poults as weakness, unthriftiness, loss of appetite, ruffling of feathers, dragging of wings, sleepiness, diarrhea, and sudden onset of high mortality. These symptoms are very similar in all species of young fowl according to Biester and Schwarte (1965).

Extent of Problem

The extent of the problem of salmonella contamination, in both numbers and species of salmonella isolated in various animals and animal feeds, has been studied by many workers.

Cherrington et al. (1937) reported several outbreaks of parathyphoid in turkeys in widely separated localities in Idaho from a Salmonella aertrycke type organism. The poults died rapidly up to 10 days of age with weakness and occasional diarrhea as the common

symptoms. In one outbreak more than 11,000 of 12,000 poult hatchlings on one farm died from this infection.

Infection due to Salmonella aertrycke was also noted by Pomeroy and Fenstermacher (1939) in young poult up to five weeks of age. In addition to S. aertrycke, they reported that S. anatum, S. newington, S. montevideo, S. derby, S. senftenberg, S. bareilly and S. bredeney may produce losses among baby poult.

During 1943 in the United States salmonella infection based on 2,090 cultures from 2,285 outbreaks of infection in man and animals, revealed 59 different salmonella types according to Edwards and Bruner (1943).

Edwards et al. (1948), serotyped 60 different salmonella types in fowl with most of the cultures being isolated from turkeys and chickens. Degree of severity of the infection varied greatly with mortality ranging from 5 to 100 percent. Hinshaw (1943) discussing the disease in turkeys pointed out that 31 species of salmonella have been isolated from turkeys in the United States.

Thirty species of salmonella were identified serologically from 241 isolations recovered from 1,148 turkeys by Lukas and Bradford (1954). Of the total cases in which a paratyphoid organism was isolated, 54.0 percent were classified as uncomplicated paratyphoid outbreaks.

Moran (1960) reported on 1855 cultures isolated in the United States from agricultural sources during 1958. Of these cultures, 1733 were members of the genus salmonella with 61 different serotypes represented.

Of the 1733 identified as salmonella, 1178 had been recovered from animals and 555 from processed products of animal origin. One thousand

and twenty-eight of the 1178 cultures from animals were of avian origin. Six hundred and seventy-three were isolated from turkeys, 337 from chickens and 18 from other birds.

In a survey at the Communicable Disease Center (Atlanta, Georgia) from January, 1957 to July, 1961, Moran (1961) reported 6,216 cultures of salmonella had been serotyped from animals. Of the 6,216 cultures serotyped, 2,450 were found in turkeys and 2,266 in chickens.

According to Biester and Schwarte (1965), one of the first to describe paratyphoid infection among chickens was Mazza in 1899. He isolated an organism that was pathogenic for chickens and pigeons and possessed the biochemical characteristics of a paratyphoid.

Schalm (1937) reported a disease among chicks caused by an organism resembling Salmonella typhimurium. Jungherr and Clancy (1939), in routine examination of 1,241 lots of chicks less than 3 weeks old, observed 15 cases of paratyphoid infection.

Williams (1956) reported in Biester and Schwarte's Diseases of Poultry, "an outbreak of paratyphoid infection in chicks due to S. typhimurium. The infection was confined to one large hatchery and mortality varied from 10 percent among some hatches to as high as 90 percent among others. The virulence of the disease was apparently increased by repeated passage of the organism through succeeding generations of chicks."

Erwin (1955) reported what was believed the first recovery of viable salmonella organisms from commercially prepared poultry feed. Three samples of 206 tested resulted in the isolation of Salmonella oranienburg.

Salmonella species of thomasville, tennessee, cubana, kentucky, bareilly, thompson, senftenberg, illinois, and montevideo were isolated by Boyer et al. (1958) from meat scraps and turkeys fed the meat scraps.

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.

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

Pomeroy and Grady (1961) collected 980 samples of various feed ingredients and found 175 samples contaminated with salmonella. Eighty-three samples of meat scraps out of 257 examined were contaminated with salmonella. Thirty-two of 221 meat and bone meal samples showed salmonella contamination.

Burr and Helmboldt (1962) examined 436 animal by-product feed ingredients for the presence of salmonella. Fifty-six (12.8%) of the samples were positive for salmonella. One hundred and forty-five poultry by-product samples were examined and salmonella recovered from 18.6 percent of the samples while 9.9 percent of 161 meat scrap samples were positive.

Boyer et al. (1962) described two cases of salmonella infection in young poults and one case in chicks. Salmonella thomasville, S. newbrunswick and S. kentucky were isolated from the livers and

intestines of poults from the first case, and S. thomasville from an unopened bag of feed from the same batch that had been fed to the poults. Other serotypes were isolated from the livers and/or intestines of the poults in the second case. Two serotypes were isolated from feed samples from unopened bags from the same batch that had been fed to the poults. These two serotypes were isolated from ceca of chicks and the feed fed to them.

Investigating the lowest levels of contamination capable of causing infection, Grumbles and Flowers (1963) demonstrated, by cultural and serologic examination, that as few as 1000 organisms per gram of feed of Salmonella montevideo caused infection in turkeys.

Pomeroy et al. (1964) pointed out there is definite evidence that contaminated feeds have been sources of salmonella outbreaks in animals and poultry. They agreed with Morehouse and Wedman (1961) that the chief factor resulting in the presence of salmonella in feed ingredients is recontamination after processing.

Control of Problem

The majority of the research on the control of salmonellosis in domestic fowl has been in the areas of prevention by sanitation and reduction of infection by chemicals added to the feed or drinking water.

Belding and Mayer (1958) fed furazolidone (nf-180) to poults to reduce mortality due to S. san diego. When nf-180 was fed concurrently with the infection, mortality was reduced by 50 percent as compared with a group of non-medicated poults. Aureomycin and

9 Sulmet had no appreciable effect in reducing mortality. Treatment with either Aureomycin and Sulmet or nf-180 also reduced the number of carriers to about 16 percent.

Lukas and Bradford (1954) experimented with Terramycin and Aureomycin as controls for parathyphoid infection and found effective concentrations to be 100 p.p.m. of soluble Terramycin in the water and 250 grams of Terramycin or Aureomycin per ton of feed. The majority of the uncomplicated parathyphoid outbreaks during the experimental period were controlled.

Bierer and Vickers (1960) found that adding furazolidone in the feed at a level of 0.022 percent; solubilized nf-248 at a level of 0.006 percent in drinking water, or 0.0264 percent furatadone in the drinking water, drastically reduced mortality from experimentally induced S. typhimurium infection in poult as compared to infected non-medicated control birds.

A nitrofurazone compound, Tiafur, was tested against experimental and spontaneous salmonella infections in chicks by Lannek et al. (1962). A suitable dose practically eliminated mortality and clinical symptoms of the disease. During treatment the birds exhibited a loss of appetite and depressed growth, however, growth seemed adequately compensated after Tiafur had been removed and the disease controlled. They pointed out that reinfection could occur.

Addition of nihydrozone to the feed at the 0.011 percent level was shown to reduce death loss from S. typhimurium 1 to 13 percent (Bierer and Barnett, 1962). When 0.022 percent nihydrazone feed medication was used, only 7 percent mortality occurred, compared to 34 percent in infected non-medicated groups.

Clark (1946), working with sulfamerazine, showed that mortality was reduced when the drug was placed in the mash 24 hours before intramuscular injection of 0.02 c.c. of salmonella organisms. Maximum effect was reached with the addition of sulfamerazine in the mash at the level of 0.5 percent.

Nine sulfonamides were examined by Pomeroy and co-workers in 1948. Sulfadiazine, sulfamerazine, sulfapyrazine, sulfaquinoxaline and sulfamethazine were most effective in reducing mortality from S. typhimurium infection. Sulfadiazine, sulfamerazine and sulfamethazine were effective in reducing mortality from S. typhimurium infection in chicks approximately 50 percent as compared with the control birds.

Rasmussen et al. (1964) investigated the possibility of controlling salmonella in rendered animal by-products with heat. They reported that a temperature of 180°F. for 7 minutes was sufficient to destroy consistently salmonella found in two commercially available, naturally contaminated dry meals. In a third meal, which was unusually high in fat content and salmonella contamination, a temperature of 195°F. for a period of 7 minutes was required for consistent destruction of the salmonella. The results indicated the severity of the heat treatment required to destroy salmonella in naturally contaminated meals will vary considerably for different meals.

Adams (1968) investigated the possibility of inhibiting the growth of salmonella infected feed by pelleting feed conditioned to 50°C. and 70°C. Feed was infected with S. typhimurium at a level of approximately 20,000 organisms per gram. Cultures of the treated feeds and of feces from hens fed the treated feeds were negative for salmonella. The results suggested that pelleting of poultry feed is

a practical method of reducing or possibly eliminating salmonella in poultry feeds.

Crane et al. (1967) reported on the effect of pelleting on salmonella from eight trials conducted in 1965. Samples of pelleted and granulated formulas containing meat meal were tested for the presence of salmonella; while 28.8 percent of the incoming shipments of meat meal were positive for salmonella, the pelleted and granulated formulas were negative. In a survey made early in 1965, a total of 292 feed samples were tested. One hundred and ten were mash samples and 182 were pelleted. Ten percent of the mash samples were positive for salmonella. Of the 182 pelleted feeds tested, no positives were found.

EXPERIMENTAL PROCEDURE

Experiment 1

A slurry of 800 mls. of sterile water and 200 gms. of meat and bone meal was sterilized in an autoclave at 15 p.s.i. gauge pressure and 120°C. for three, 40-minute periods. Five mls. of a pure culture of Salmonella heidelberg, obtained by inoculating Nutrient Broth (DIFCO) with a sample of stock culture, were added to the slurry and incubated for 48 hours at 37°C. Plate counts were made of the slurry in sterile water dilutions of 10^{-4} , 10^{-6} , and 10^{-7} on Nutrient Agar (DIFCO) by a standard counting method (Pelczar and Reid, 1965) using the Quebec Counter.

The slurry was added to 200 pounds of meat and bone meal and mixed for 5 minutes in a horizontal mixer. Plate counts of inoculated meat and bone meal samples, taken from the beginning, middle and end of the mix, were made to determine whether there was an uniform distribution of approximately 3×10^6 organisms per gram of mix. The artificially contaminated meat and bone meal was incorporated into an all vegetable protein, salmonella free, chick starter ration so as to provide levels of 500 and 2000 salmonella organisms per gram of complete feed. The two contaminated rations were divided in half so that each contamination level was available for the addition of the chemical additive Endgerm. Endgerm, according to the manufacturer, is a composite of acids with the predominant acid being proprionic acid. Endgerm was added at the 0.1% level and the rations mixed for 5 minutes in a horizontal mixer. A sample of the chick starter ration, sterilized by autoclaving at a gauge pressure of 15 p.s.i. and 120°C. for two, 3-hour periods, served as a control.

A summary of the treatments is shown in Table I.

Table I. Treatments used in Experiment 1.

Treatments	Battery level
Chick starter	1 (top)
Chick starter + 500 org./gm. + Endgerm (0.1%)	2
Chick starter + 2000 org./gm. + Endgerm (0.1%)	3
Chick starter + 500 org./gm.	4
Chick starter + 2000 org./gm.	5 (bottom)

Duplicate groups (15 chicks per group) of day-old White Leghorn cockerels were fed the experimental rations and water ad libitum. The chicks were randomly assigned to compartments in an electrically heated battery brooder. Treatments were assigned to levels of the battery in the order shown in Table I. Treatment assignments were not randomized because in a preliminary study randomization placed the treatments in such a manner that the treatment of 2000 organisms per gram of feed was located directly above the control feed. Two days after the experiment started, fecal matter from the birds fed the control feed was found to be contaminated with Salmonella heidelberg. Contamination of the fecal material from the control birds suggested the feed had been contaminated. Cultures of the control feed were positive for Salmonella heidelberg. The problem of "spill-over" contamination was reduced by assigning the treatments as indicated in Table I.

The presence of salmonella in the fecal material of test birds was used as the criteria for the presence of salmonella in the feed. Several workers (Forsythe et al., 1967; Boyer et al., 1962; Grumbles and Flowers, 1963) have reported that salmonella was recovered from birds fed feed, artificially and naturally, contaminated with salmonella.

Two fecal samples per pen were obtained twice a week during the first three weeks of the trial and once during the fourth week. Twenty-four hours prior to the time of collection, the dropping pan of each pen was cleaned and the surface covered with Kraft paper to reduce possible contamination from the previous day's excreta. At each collection period, the fecal samples were collected at random from each pan with sterile wooden spatules. One sample was placed in a capped culture tube containing 10 mls. of Selenite enrichment broth and the other in a capped culture tube containing 10 mls. of Brilliant-green Tetrathionate (B.G.T.) enrichment broth. The samples were incubated in the enrichment broths for 8 to 12 hours at 37°C.

After incubation, one 5 mm. loopful from each sample was streaked on Brilliant-green agar (B.G.) plates and incubated for 24 hours at 37°C. Plates were examined after the 24-hour incubation period and colonies that showed typical salmonella growth were planted on Triple Sugar Iron (T.S.I.) agar (DIFCO) by stabbing the butt and streaking the slant. The T.S.I. slants were incubated for 24 hours at 37°C. The slants that showed gas and H₂S formation and an unchanged slant were transferred to Urea broth (DIFCO) to differentiate between salmonella and proteus. The Urea cultures were incubated for 24 hours at 37°C. All samples that showed a faded pink

color of Urea broth were considered as positive for salmonella.

An additional differential test was made by incubating those samples that were positive for the T.S.I. and Urea tests in 5 sugars with fermentation tubes for 24 hours at 37°C. The sugars used were glucose, maltose, lactose, sucrose and deucitol. Samples fermenting glucose, maltose and deucitol were considered positive for salmonella. The serotyping of suspected salmonella colonies was done by the Kansas State Board of Health, Topeka, Kansas.

All birds that died during the first four weeks were examined for salmonella, according to the preceding procedures, by preparing cultures from samples of the liver, spleen, and duodenum.

At the end of four weeks the contaminated feed was withdrawn and the birds given salmonella-free feed. Two weeks after cessation of feeding the contaminated feed, two birds from each replication were sacrificed and examined for salmonella according to procedures outlined for fecal samples.

At eight weeks of age (four weeks after the birds were taken off contaminated feed) the remainder of the birds were sacrificed and processed by standard procedures. Prior to sacrifice, swabs were taken at random from feathers adjacent to the vent and from the cloaca and examined for salmonella. Examination procedures were the same as the procedures for the fecal material.

The initial plan for experiment 1 was to include an examination of the various samples of feed, particularly those containing Endgerm, to determine if Endgerm affected the rate of destruction of salmonella. However, because the presence of other organisms masked the ability to count salmonella accurately in unsterilized feed by the methods

used in this study, this portion of the experiment was abandoned and restudied in experiment 2.

Experiment 2

Experiment 2 was based on the hypothesis that: (1) any destructive capacity of Endgerm on salmonella would be similar in sterilized and unsterilized feeds; and (2) the use of sterile feed would eliminate problems with the masking effect that organisms in unsterilized feed have on reducing the ability to count salmonella accurately.

A chick starter ration was autoclaved at a gauge pressure of 15 p.s.i. and 120°C. for two, 3-hour periods. The sterilized feed was divided into five lots. One lot served as a sterile control and the other four were used to test the bactericidal ability of Endgerm on four levels of contamination from Salmonella heidelberg. The levels studied were 500, 1000, 1500, and 2000 organisms per gram of feed. The levels were obtained by mixing known concentrations of Salmonella heidelberg with 454 gms. of chick starter. Four levels of contamination also were prepared in unsterilized chick starter to serve as a check for the presence of salmonella. The treatments were mixed in a sterilized Kitchen Aid Model K4-B mixer for 10 minutes.

A potentiometric titration technique was used to determine the length of time required to obtain uniform dispersion of organisms through the feed. In this technique an ammonium chloride solution, rather than inoculum, served as the agent. This technique gave an indication of the uniformity of distribution of chloride ions in each sample of feed. The treatment samples were divided into 22-gram samples for titration. Each sample was added to a solution containing

250 mls. of distilled water and 1 ml. of nitric acid and allowed to stand for 10 minutes. The solution was then titrated with 0.1N silver nitrate until the pH meter read 2.72 (Luhman, 1955). Titrations were performed on the four contamination levels and statistically analyzed for the degree of dispersion.

Data from potentiometric titration of feed samples containing added ammonium chloride, were statistically analyzed to determine if the mixer adequately distributed the organisms through the feed. Results of the analysis are shown in Table II.

Table II. Coefficient of variance analysis of the potentiometric titration.

Mixing time (minutes)	Mean	Standard deviation	Coefficient of variance
5	.988% NH_2Cl	.1244	12.58
10	.411% NH_2Cl	.0271	6.60

A coefficient of variance value of 6.60 for 10-minute mixing period indicated this time period was adequate for uniform distribution of organisms in the feed. (Pfost, 1966).

RESULTS AND DISCUSSION

Experiment 1

All cultures of fecal material were classified on the basis of either positive or negative for salmonella. No measure of approximate numbers of organisms was made. For statistical analysis of the data, a value of one was assigned to cultures positive for salmonella and a value of zero to those negative for salmonella. Data were statistically analyzed by the analysis of variance.

Results of the fecal examinations are shown in Table III.

Table III. Presence of salmonella in fecal material of chicks fed feed containing several levels of Salmonella heidelberg and Endgerm.

Treatments	No. samples ^{1/}	Positive for salmonella
		%
Chick starter	28	0
Chick starter + 500 org./gm. + Endgerm (0.1%)	28	61
Chick starter + 2000 org./gm. + Endgerm (0.1%)	28	79
Chick starter + 500 org./gm.	28	68
Chick starter + 2000 org./gm.	28	68

^{1/} Samples from each of ten lots of birds collected during a 4-week period.

Salmonella were isolated from 61 percent or more of the samples from all treatments except the control group (Table III).

Statistical analysis of the data is shown in Table IV. Data from the control were not used in the analysis of variance because

all observations were negative for that treatment. This meant that all observations were given a value of zero. With no variance within the control treatment, such data had to be excluded from the analysis of variance. The control was used to show any external appearance differences that might occur between birds fed salmonella and those not fed salmonella. These data show no significant difference between treatments, indicating that Endgerm was not effective in eliminating Salmonella heidelberg at levels of 500 and 2000 organisms per gram of feed. Although no quantitative test was made, it is possible Endgerm may have reduced the number of organisms present in the feed.

Table IV. Analysis of variance of recovery of salmonella from fecal material as influenced by type of enrichment broth and Endgerm.

Source of variation	Degrees of freedom	Mean square	"F"
Treatment	3	.213	.268
Replication	27	.405	.509
Error term	81	.795	

Data on the effect of Selenite and Brilliant-green Tetrathionate enrichment broths on the recovery of salmonella from fecal material of chicks are shown in Table V.

Table V. Effect of Selenite and Brilliant-green Tetrathionate enrichment broths on recovery of salmonella from fecal material

Treatment	No. samples per enrichment broth	Percent recovery	
		Selenite	B.G.T.
Chick starter	14	0	0
Chick starter + 500 org./gm. ^{1/}	28	50	79
Chick starter + 2000 org./gm. ^{1/}	28	61	86
Total	70	44	64

^{1/} Values represent combined data from treatments with and without Endgerm.

These data show that B.G.T. enrichment broth was more effective, in terms of actual percentage of salmonella recovered, than the Selenite enrichment broth. However, an analysis of variance (Table IV) shows there was no significant difference between enrichment media (replications) in recovery of salmonella.

Results from examination of various tissues of birds at the end of four weeks of feeding contaminated feed are shown in Table VI.

Table VI. Cultures of tissues from birds fed contaminated feed for four weeks.

Treatment	No. cultures per tissue		No. cultures positive for salmonella	
	Liver-spleen	Duodenum	Liver-spleen	Duodenum
Chick starter	4	4	0	0
Chick starter + 500 org./gm. + Endgerm (0.1%)	4	4	0	1
Chick starter + 2000 org./gm. + Endgerm (0.1%)	4	4	2	0
Chick starter + 500 org./gm.	4	4	1	2
Chick starter + 2000 org./gm.	4	4	3	1

These data indicate that regardless of treatment, with the exception of the control, at least one bird from each treatment showed tissue contamination by Salmonella heidelberg. These data further substantiate the ineffectiveness of Endgerm to eliminate Salmonella heidelberg from the feed at levels of 500 and 2000 organisms per gram of feed.

Table VII shows the data collected after the birds were taken off contaminated feed and fed salmonella-free feed.

Table VII. Presence of salmonella in cultures from birds after removal of contaminated feed.

Age of birds (weeks)	Weeks off salmonella feed	No. samples	No. positive for salmonella
6	2	20 ^{1/}	0
8	4	20 ^{2/}	0

^{1/}Liver-spleen and duodenum cultures.

^{2/}Feather and cloacal cultures.

Cultures were taken from the liver, spleen and duodenum of the birds at 6 weeks of age while feather and cloacal swabs were taken from the birds at 8 weeks of age. All cultures were negative for Salmonella heidelberg at both ages. This suggests that withdrawal of feed contaminated with salmonella and feeding them salmonella-free feed is a possible method of reducing the incidence of salmonella contamination in the host bird, therefore, reducing contamination of meat during processing. These data may also suggest there is some period of time when natural organism "die-off" would correct problems in salmonella contamination. The natural "die-off" hypothesis was suggested by Snoeyenbos, 1969.

Experiment 2

Although results in experiment 1 indicate Endgerm did not eliminate salmonella from chick feed, it may have reduced the level of salmonella contamination. Results of an effort to determine the ability of Endgerm to reduce the numbers of salmonella organisms in the feed are discussed.

Examination for the reduction of organism numbers was based on the plate count technique (Pelczar and Reid, 1965) of samples inoculated at four levels of contamination. Results are shown in Table VIII.

Table VIII. Effect of Endgerm on various levels of salmonella organisms in sterile feed.

Treatments	No. of organisms per gram
Chick starter	0
Chick starter + 500 org./gm. + Endgerm	0
Chick starter + 1000 org./gm. + Endgerm	0
Chick starter + 1500 org./gm. + Endgerm	0
Chick starter + 2000 org./gm. + Endgerm	0

The data show Endgerm completely inhibited salmonella growth in sterilized feed. The unsterilized feeds were positive for salmonella (data not shown), however; the presence of other organisms made the counting of salmonella organisms impossible.

One explanation of these results is that moisture content may have been lowered sufficiently during sterilization to deprive the organisms of adequate moisture for growth. Moisture content of the sterile and unsterilized feeds was analyzed with an Ohaus Model 6010 Moisture Determination Balance. The determination was analyzed at 80 watts for 15 minutes with the heating element $1\frac{1}{2}$ inches above the feed samples. Results showed the sterilized feed had a moisture content of 14.5 percent and the unsterilized feed, 12.7 percent moisture. The moisture content of the feeds being relatively equal suggests this was not the reason for these results.

There is the possibility the effectiveness of Endgerm is altered when the feed, to which it is added, is contaminated with other enteric organisms. The Endgerm may be tied up by the other organisms, inhibiting its ability to eliminate or reduce salmonella count. This may be one reason for the results shown in Table VIII. This hypothesis warrants further investigation.

SUMMARY

One hundred and fifty day-old White Leghorn cockerels chicks were fed chick starter artificially inoculated with Salmonella heidelberg and containing a chemical additive Endgerm. Cultures of fecal material were examined for salmonella to determine the inhibitory effect of Endgerm on the presence of salmonella in host birds.

The results of this study show that the chemical additive Endgerm was not effective in eliminating the presence of Salmonella heidelberg in feeds at levels of 500 and 2000 organisms per gram. Difficulty was encountered in making a quantitative evaluation of salmonella because numerous other enteric organisms masked the ability to obtain an accurate count of salmonella by the conventional plate count technique. This precluded determining if Endgerm was effective in reducing the number of organisms in the feed.

There is some evidence to suggest that withdrawal of contaminated feed from the birds may be a means of reducing or eliminating salmonella from host birds.

Data show no significant difference between Selenite and Brilliant-green Tetrathionate enrichment broth in the isolation of Salmonella heidelberg from fecal material.

Evidence is presented suggesting the effectiveness of Endgerm is altered when the feed, to which it is added, is contaminated with other enteric organisms. The Endgerm may be tied up by the other organisms, inhibiting its ability to eliminate or reduce salmonella count.

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EFFECT OF A CHEMICAL FEED ADDITIVE
ON SALMONELLA PRESENCE IN
POULTRY FEEDS AND HOST BIRDS

by

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AN ABSTRACT OF A MASTER'S THESIS

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Day-old White Leghorn cockerels were fed sterilized and unsterilized feed artificially contaminated with Salmonella heidelberg at levels from approximately 500 to 2000 organisms per gram of feed, with and without a chemical feed additive (Endgerm^R) at 0.1% level. A standard qualitative test for the presence of salmonella was made on samples of fecal material and feed to determine the inhibitory effect of Endgerm on the presence of salmonella in host birds. Selenite and Brilliant-green Tetrathionate pre-enrichment broths were compared to determine their effectiveness in recovery of salmonella from fecal material.

Endgerm had no significant effect on elimination of salmonella in feeds at levels of 500 and 2000 organisms per gram. No visible pathological conditions attributable to salmonellosis were evident in chicks.

Cultures taken from birds, two and four weeks after withdrawal of contaminated feed, were negative for salmonella. This suggests that withdrawal of feed contaminated with salmonella and feeding them salmonella-free feed is a possible method of reducing the incidence of salmonella contamination in the host bird.

There was no significant difference between Selenite and Brilliant-green Tetrathionate pre-enrichment broths in the recovery of salmonella from fecal material.

Endgerm completely inhibited salmonella growth at levels of approximately 500 to 2000 organisms per gram in sterilized feed but not in the unsterilized feed. These results may indicate the effectiveness of Endgerm is altered when the feed, to which it is added, is contaminated with other enteric organisms.