A Survey of *Salmonella* Contamination in Modern Broiler Production

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(Received for publication November 8, 1990)

ABSTRACT

A survey of contamination with *Salmonella* was done in the breeder/multiplier and broiler houses, feed mills, hatcheries, and processing plants of two integrated broiler firms. Samples of insects and mice were also collected at each location. Sixty percent (60%) of the meat and bone meal samples collected at feed mills were contaminated. *Salmonella* was isolated from 35% of the mash feed samples tested. The pelleting process reduced *Salmonella* isolation rates by 82.0%. Data collected from breeder/multiplier houses suggested that feed was the ultimate source of *Salmonella* contamination in that environment. *Salmonella* was found in 9.4% of the yolk sac samples collected from day-old chicks in hatcheries. Fecal dropping samples collected in broiler houses about one week prior to slaughter were contaminated at a rate of 5.2%. *Salmonella* was found in 33% of the samples collected from live haul trucks and 21.4% of the whole processed broiler carcasses sampled at processing plants. *Salmonella typhimurium* was the serotype most commonly isolated. The gastrointestinal tract of one of 19 mice sampled was contaminated with *Salmonella*. Data suggest that insects were primarily mechanical carriers. Results suggest *Salmonella* contamination in the U.S. broiler production and processing system has changed little since 1969. The data also underline the contention that effective *Salmonella* control efforts must be comprehensive.

In 1985 the National Research Council (23) stated that illnesses caused by *Salmonella* and *Campylobacter jejuni* were most commonly traced to poultry products. Two years later, the National Research Council (24) stated: "There is conclusive evidence that microorganisms pathogenic to humans (such as *Salmonella* and *Campylobacter*) are present on poultry at the time of slaughter and at retail." Control and eventual elimination of *Salmonella* from poultry products has been the goal of researchers and the poultry industry for over 25 years (17,32).

A number of surveys have been conducted of *Salmonella* contamination in various parts of the broiler production system. In the last comprehensive survey of the U.S.

broiler production system, Morris et al. (22) found that feed samples were most frequently contaminated with *Salmonella*, but that breeder/multiplier flocks could also pass contamination to their progeny. Dougherty (9) studied *Salmonella* contamination in broiler flocks and reported that up to 37.5% of the day-old chicks in his study were contaminated with *Salmonella*, but the contamination rate decreased to about 5.0% in broilers just prior to processing. Dougherty (9) also found that the *Salmonella* serotypes present in feed ingredients were not the same as those in broiler flocks and suggested that contamination in broiler flocks may have originated from either breeder/multiplier flocks or from the hatchery. In a survey of the Australian broiler production system, MacKenzie and Bains (21) found that 82% of the *Salmonella* isolated from broiler carcasses could be traced to feed or feed ingredients. However, these researchers failed to examine day-old chicks or breeder hens. Bhatia et al. (3) reported that *Salmonella* isolation from litter was a reliable indicator of both flock and carcass contamination. Bhatia and McNabb (2) traced *Salmonella* contamination from the hatchery to the processing plant and found the same *Salmonella* serotype in fluff and meconium samples collected at the hatchery, in litter samples at the broiler farm, and on carcasses following processing. Soerjadi-Liem and Cumming (33) sampled the ceca of broiler flocks for contamination with *Salmonella* contamination at the processing plant on a monthly basis for a year and found more contamination in the colder than in the warmer months. These researchers also found that birds raised on old litter were contaminated at much lower rates than were birds raised on new litter. Lahellec and Colin (19) surveyed the French broiler production system and found that *Salmonella* serotypes originating in the hatchery were less prevalent in the final product than those in the poultry house during the rearing of birds. In another study, Lahellec et al. (20) reported that the *Salmonella* serotypes isolated on the chicks' first day in the poultry house were most often present in the final product when broilers were raised on new litter. Goren et al. (13) surveyed the broiler production system in The Netherlands and found a positive

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correlation between *Salmonella* isolation rates from litter at 5 weeks of age and isolation rates from ceca at processing. Preventing exposure of live birds to *Salmonella* under the current U.S. broiler production has been reported to be difficult, if not impossible (38), since *Salmonella* can be isolated from virtually anywhere and most birds contaminated with *Salmonella* show no symptoms of infection (18). *Salmonella* free poultry have been successfully raised only under very controlled conditions (29) and the elimination of *Salmonella* from poultry in large scale production has been judged to be uneconomical (18). A comprehensive approach would appear to be required both to surveys of contamination and to control of *Salmonella* in broilers. Yet Morris et al. (22) reported the last comprehensive survey of the U.S. broiler production and processing system in 1969. Therefore, this study was undertaken to comprehensively examine *Salmonella* contamination in the U.S. broiler production and processing system.

MATERIALS AND METHODS

Sampling methods

The two integrated broiler firms which cooperated with this study followed typical industry practices, but neither firm had an active microbial control program. Samples were collected from each company on three separate days two weeks apart. Samples were collected from two different breeder/multiplier houses, two different broiler houses, the feed mill, the hatchery, and the processing plant on each sampling day. Insects and mice were trapped at each location and transported to the laboratory for analysis. To ensure that any contamination detected was internal, the external surfaces of insects was sanitized. Insects were either rinsed three times in sterile distilled water or sanitized for 30 s with a 20 ppm chlorine solution and rinsed three times in sterile distilled water before being placed into tryptic soy broth (TSB, Difco, Detroit, MI) for analysis. Captured mice were killed using carbon dioxide, wet thoroughly with 95% ethanol, aseptically opened with sterile scissors, and the entire gastrointestinal tract was aseptically removed. The tract was then cut into 6 to 10 pieces before being placed in TSB for incubation.

In breeder/multiplier houses, subsamples of feed, floor and nest litter were collected from five locations within each house using sterile tablespoons. Composite samples were made by combining subsamples and mixing thoroughly in a sterile, aluminum roasting pan. About 25 g of the material was then transferred directly to TSB for incubation. Eggs were collected by hand and rinses were done using the method of Gentry and Quarles (12).

In broiler houses, samples were collected when birds were 6-7 weeks of age. Composite samples of feed and litter were collected as in breeder/multiplier houses. Water samples (ca. 5 ml) were obtained using a sterile tablespoon from five watering devices into TSB. Live and dead bird rinses were done using the method described by Cox et al. (7). Environmental swabs were collected by wetting a sterile cotton-tipped swab (Fisher Scientific, Pittsburgh, PA) with TSB and rubbing it over an area of about 930 cm² before returning it to the medium for incubation. A new pair of disposable latex gloves were worn during the collection of environmental swabs.

In feed mills, grab samples of feed ingredients (ca. 500 g) were collected at the batch scales, while finished feed samples (ca. 500 g) were collected from pellet coolers and during loading of feed delivery trucks. Environmental swabs were collected as described above.

In hatcheries, composite samples of yolk sac material were

collected from six day-old chicks. Following cervical dislocation, the abdomen of the chick was thoroughly wetted with 95% ethanol, the chick was aseptically opened with sterile scissors and a 1-g portion of excised yolk material was placed in TSB for incubation. Yolk sac materials from six chicks were combined to form one composite sample. Samples of Marek's disease vaccine were collected by allowing the vaccinating apparatus to deliver one dose of vaccine directly into media for incubation. Environmental swabs were collected from hatchers and chick boxes as described except sterile cotton-tipped swabs were used to rub about 6.5 cm² of surface area.

At processing plants, three subsamples of debris were collected from shipment coops using sterile tablespoons. Subsamples were combined to form composite samples, of which three to seven were collected on each sampling day. Four to six composite cloacal swab samples were collected after birds were hung on the processing line just prior to kill. Each composite represented cloacal swabs collected from five different birds. Carcass rinse samples were collected on individual birds at the immersion chiller exit and just prior to packaging. Rinses were done using the method described by Cox et al. (7).

Laboratory procedures

All media were obtained from Difco Laboratories, Inc., Detroit, MI. Samples of approximately 25 g were transferred directly into 225 ml of TSB, while smaller samples (ca. 1 g) and swab samples were placed in 10 ml TSB. TSB was incubated at 37°C for 24 h and 1 ml of TSB was aseptically transferred to both tetrathionate broth (TB) and selenite cystine broth (SCB). TB was incubated at 42°C for 24 h, while SCB was incubated at 37°C for 24 h. One half ml of both TB and SCB were transferred to M broth (MB) which was incubated at 42°C for 6 h. MB was then tested for the presence of *Salmonella* using the Bio-EnzaBead™ system (Organon Teknika, Durham, NC) as described by Flowers et al. (10). One loop (3 mm) of MB, TB and SCB from positive samples was streaked on MacConkey agar, hektoen enteric agar and bismuth sulfite agar. Suspect colonies were picked to tubes containing 2 ml of TSB, grown for 18 to 24 h at 37°C and tested for ability to utilize urea using methods described by Poelma et al. (26). Urea negative cultures were identified biochemically as *Salmonella* using Micro-Id system (Organon-Teknika). Isolates identified as *Salmonella* were sent to National Veterinary Services Laboratory, Ames, IA for serotyping.

RESULTS

Salmonella were most frequently found in samples collected from feed mills (FM), followed by those collected at processing plants (PP), from insects and breeder/multiplier houses (B/MH) (Table 1). Samples collected at hatcheries (H), from mice and at broiler houses (BH) were less frequently contaminated.

TABLE 1. *Salmonella* in broiler production and processing.

Sampling location	No. samples	Positive samples	
		no.	%
Feed Mills	212	44	20.8
Breeder/Multiplier houses	177	23	13.0
Hatcheries	140	10	7.1
Broiler houses	267	12	4.5
Processing plants	137	22	16.1
Insects	100	13	13.0
Mice	19	1	5.3

In FM samples, meat and bone meal, poultry meal, fish meal, and other ingredients were contaminated with *Salmonella* at rates

of 60%, 40%, 33.3%, and 20.8%, respectively (Table 2). *Salmonella* was not isolated from environmental swabs but was found in one of eight (12.5%) dust samples collected. *Salmonella* was isolated from mash feeds at a rate of 35% and from pelleted feeds at a rate of 6.3%. A comparison of the isolation rates obtained from mash feeds with those from pelleted feeds, indicated that the pelleting process reduced *Salmonella* isolation rates by 82%. *Salmonella typhimurium* was the predominant serotype isolated from pelleted feeds. However, a wide variety of *Salmonella* serotypes was also found in feed and feed ingredient samples (Table 8).

Salmonella isolation rates were similar for feed, nest litter, and nest egg rinse samples collected from B/MH (Table 3). Since breeder birds consumed mash feed and *Salmonella* contamination rates for mash feed were higher than those of pelleted feeds sampled at the FM (Table 2), these data suggest that feed was the source of *Salmonella* contamination in B/MH. The fact that *S. typhimurium* was serotype most commonly isolated from feed, nest litter, and nest egg rinse samples also supports feed as the contamination source. Floor egg rinse and floor litter samples (Table 3) also had similar contamination rates, but *S. typhimurium* was the most common serotype on floor eggs, while *S. indiana* was most common in floor litter.

TABLE 2. *Salmonella* in feed mills.

Sample type	No. samples	Positive samples	
		No.	%
Dust	8	1	12.5
Environmental swabs	18	0	0.0
Fish meal	18	6	33.3
Meat & Bone meal	15	9	60.0
Poultry meal	10	4	40.0
Other ingredients	24	5	20.8
Mash feeds	40	14	35.0
Pelleted feeds	79	5	6.3
All samples	212	44	20.8

TABLE 3. *Salmonella* in breeder/multiplier houses.

Sample type	No. samples	Positive samples	
		No.	%
Cecal droppings	65	8	12.3
Feed	14	2	14.3
Floor egg rinse	12	1	8.3
Floor litter	28	3	10.7
Nest egg rinse	30	5	16.7
Nest litter	28	4	14.3
All samples	177	23	13.0

Salmonella was found in the yolk sac of 9.4% of the chicks sampled in H (Table 4), with *typhimurium* being the most common serotype. *Salmonella heidelberg* was found in one of the 14 swab samples (7.1%) collected from hatchers, but no *Salmonella* were isolated from dead embryos, chick boxes, or vaccinator samples.

Feed, water, and litter samples collected in BH were negative for *Salmonella* (Table 5). Contamination rates of 14.3%, 7.1%, and 5.2% were obtained from dead bird, live bird, and fecal dropping samples, respectively. One of the 42 environmental swabs was positive for *Salmonella*. Samples from BH were collected from birds which were processed less than a week after samples were collected. The serotype most commonly isolated from live birds, dead birds, fecal droppings and environmental swabs was *S. typhimurium*. The failure to isolate *Salmonella* from feed samples collected on the broiler farm suggests that broilers

TABLE 4. *Salmonella* in hatcheries.

Sample type	No. samples	Positive samples	
		No.	%
Chicks (yolk sac)	96	9	9.4
Chick boxes	7	0	0.0
Dead embryos	9	0	0.0
Hatcher	14	1	7.1
Vaccinator	14	0	0.0
All samples	140	10	7.1

TABLE 5. *Salmonella* in broiler houses.

Sample type	No. samples	Positive samples	
		No.	%
Dead bird rinse	14	2	14.3
Environmental swabs	42	1	2.4
Fecal droppings	155	8	5.2
Feed	14	0	0.0
Litter	14	0	0.0
Live bird rinse	14	1	7.2
Water	14	0	0.0
All samples	267	12	4.5

TABLE 6. *Salmonella* in processing plants.

Sample type	No. samples	Positive samples	
		No.	%
Carcasses at chiller exit	56	6	10.7
Carcasses at packaging	14	3	21.4
Cloacal swabs	28	0	0.0
Live haul trucks	39	13	33.3
All samples	137	22	16.1

were not contaminated directly by feed. However, the predominance of *S. typhimurium* in feed samples collected at the feed mill, and in breeder/multiplier flocks, suggests an indirect contamination route.

Salmonella was found in 33.3% of the samples collected from live haul trucks at PP, but was not isolated from cloacal swabs collected from birds just prior to kill (Table 6). Whole processed broiler carcasses were contaminated at a rate of 21.4%. *Salmonella* was found about half as frequently in birds exiting the chiller as in birds at packaging, suggesting that plant workers or equipment cross-contaminated carcasses. A diverse mixture of *Salmonella* serotypes including *S. muenster*, *S. typhimurium*, *S. heidelberg*, and *S. hadar* was isolated from live haul trucks. A variety of *Salmonella* serotypes was also isolated from processed broiler carcasses, but *S. typhimurium* was most commonly isolated.

Insect samples listed in Table 7 were collected from B/MH, BH, FM, H, and PP. Unfortunately, few samples were obtained of most insect species. Nonetheless, *Salmonella* was isolated from Lesser meal worms, American cockroaches, and German cockroaches. However, since *Salmonella* was isolated over 10 times less frequently from insects disinfected with 20 ppm chlorine than from insects not disinfected, these data suggest that insects were primarily mechanical carriers of *Salmonella*. A number of *Salmonella* serotypes were isolated from insects including (in order of isolation): *S. othmarchen*, *S. oranienberg*, *S. thompson*, *S. heidelberg* and *S. infantis*.

S. typhimurium was the serotype most commonly isolated from the B/MH, BH, FM, and PP sampled (Table 8). Since 20 of

TABLE 7. *Salmonella* in insects.

Insects	Disinfected			Undisinfected			Total		
	No. samples	No. pos.	% pos.	No. samples	No. pos.	% pos.	No. samples	No. pos.	% pos.
Lesser meal worm <i>Alphatobius</i> spp.	44	2	4.5	14	6	42.9	58	8	13.8
American cockroach <i>Periplaneta americana</i>	7	0	0.0	7	4	57.1	14	4	28.6
German cockroach <i>Blatella germanica</i>	4	0	0.0	4	1	25.0	8	1	12.5
Caracinops beetle <i>Carcinops pumuiolo</i>	4	0	0.0	4	0	0.0	8	0	0.0
Blow fly <i>Phaenicia sericata</i>	2	0	0.0	2	0	0.0	4	0	0.0
Darkling beetle <i>Alphatobius diaperinus</i>	4	0	0.0	0	0	0.0	4	0	0.0
House fly <i>Musca domestica</i>	2	0	0.0	0	0	0.0	2	0	0.0
Confused flour beetle <i>Tribdium confusum</i>	2	0	0.0	0	0	0.0	2	0	0.0
All samples	69	2	2.9	31	11	35.5	100	13	13.0

TABLE 8. *Salmonella* serotypes isolated from the broiler production and processing system.

Serotype	Breeder House	Broiler House (Number of isolates)	Feed mill	Hatchery	Processing	Totals
Adelaide			13			13
Agona			2			2
Anatum	7	5				12
Binza			3			3
Broughton			5			5
Cerro			3			3
Hadar		3	1		7	11
Havana			4			4
Heidelberg	6	6	3	6	6	27
Indiana	9		2			11
Infantis			3			3
Lexington			1			1
Mbandaka			4			4
Muenster					6	6
Neumuenster			3			3
Newington			4			4
Ohio			1			1
Orangienburg			1			1
Othmarchen			2	5		7
Thompson			3			3
Typhimurium	12	10	30	6	10	68
Untypable	7			4		11
Worthington			3		5	8
Total	41	24	91	21	34	211

the 23 serotypes isolated were found in feed, these data suggest that feed was a great repository of serotypes. However, these data also suggest that effective *Salmonella* control efforts must be comprehensive. Nonetheless, since *S. heidelberg* and *S. typhimurium* were the only two serotypes isolated from B/MH, BH, FM, H, and PP, perhaps only a limited number of serotypes were capable of transmission from one location to another.

DISCUSSION

The following percentages of samples positive for *Salmonella* in this study (Table 1) and the last comprehensive survey (22) are as follows: FM samples, 20.8 vs. 21.5%; B/MH samples, 13.0 vs. 11.8%; H samples, 7.1 vs. 1.9%; BH samples, 4.5 vs. 7.8%; and PP samples, 16.1 vs.

12.7%. These data suggest that little has changed since the last comprehensive survey with respect to *Salmonella* contamination in the U.S. broiler production and processing system.

Salmonella was most frequently isolated from feed and feed ingredients (Table 1). While this finding confirms the findings of other researchers (21,22), the *Salmonella* isolation rates from B/MH cecal droppings (12.3%) (Table 3), from day-old broiler chicks (9.4%) (Table 4), and from live birds at broiler farms (7.1%) (Table 5) suggest that the B/MH environment was also important in the transmission of contamination to the final product. Furthermore, the variety of serotypes isolated from processed broiler carcasses suggests that the complete elimination of *Salmonella* from feed would not mean the elimination of this pathogen from the final product. The ubiquitous nature of *Salmonella* contamination in the broiler production system (38) necessitates a comprehensive approach to control. Nonetheless, a detailed examination of contamination in the various sampling locations would also appear to be necessary.

The *Salmonella* contamination rate observed in mice (5.3%) (Table 1) is similar to that observed by Dack (8) who found that 1.2% of the rat and mice excreta samples tested were positive. This result was also not surprising since the most commonly isolated serotype (*S. typhimurium*) (Table 8) causes disease in mice (36).

The contamination rate we report (Table 2) for meat and bone meal (60%) is comparable to that (67%) previously reported for animal protein products (30), but is lower than the contamination rate reported by others (21,22) for meat and bone meal (87.2% and 72%, respectively). Estimates of the number of *Salmonella* organisms found in animal feed ingredients range from 1 to 40 *Salmonella* organisms/100 grams (25,35). However, Gabis (11) has suggested that rendered animal by-products become contaminated with *Salmonella* following cooking as a result of microbial growth niches in the postcooking environment. High numbers of many different microorganisms (including *Salmonella*) may be found in these growth niches. As rendered products are conveyed from the cookers to finished product storage areas, varying amounts of these highly contaminated materials slough off or fall into the cooked product (11). Thus, the number of *Salmonella* organisms contaminating animal feed ingredients may be highly variable.

Pelleting of feeds has been suggested as a method of decontaminating *Salmonella* containing feeds (37). However, Blankenship et al. (4) indicated that whether or not the pelleting process eliminates *Salmonella* from poultry feeds is dependent upon: feed moisture, time and temperature of conditioning, number of *Salmonella* present, efficiency of heat and moisture transfer in feeds and the thermal resistance of the *Salmonella* strain involved. Our data (Table 2) and those of Shrimpton (30) indicate that pelleting reduces *Salmonella* isolation rates in feeds by 82.0% and 81.1%, respectively. Thus, the pelleting process is effective at reducing *Salmonella* isolations from feeds but does not completely eliminate the organism from feeds. It should also be noted that one *Salmonella* organism in 10 g of feed has been reported capable of causing colonization

in chicks of 4 d of age or less (34).

Snoeyenbos (31) found that after 30 d of egg production in B/MH, *Salmonella* were more frequently isolated from nest than from floor litter. Our data substantiate these findings (Table 3) and suggest that the contamination of nest litter may have been an important vehicle in the transmission of contamination from feed to the final product. However, Humphrey and Lanning (16) found that *Salmonella* isolation rates from breeder/multiplier feed were reduced by the addition of 0.5% formic acid to feed, but isolation rates obtained from broiler ceca collected at the plant remained the same. These researchers suggested that measures to reduce contamination in broiler houses would be required to reduce *Salmonella* isolation rates in broiler ceca at the processing plant.

The contamination rate (9.4%) we report for yolk sac contamination in the hatchery (Table 4) is slightly higher than the 6.6% and 5.0% reported by Bhatia et al. (3) and Lahellic and Colin (19), respectively. The results obtained from hatchers are comparable to those of Lahellic and Colin (19) (6.3%), but both isolation rates are considerably lower than those of Cox and Bailey (6) (88%). However, these data, in reality, are not comparable, since Cox and Bailey collected samples from chick belts, egg fragments, and paper pads, while samples were collected from hatchers during the other two studies.

The *Salmonella* isolation rate we report from fecal droppings (5.2%) in BH (Table 5) is higher than that reported from a study (9) conducted on old litter (2.5%), but it is lower than those reported by two other studies (19,20) conducted on new litter (13.2% and 22.9%, respectively). Since *Salmonella* are isolated less frequently from flocks reared on old litter than those reared on new litter (33), perhaps litter type can account for the discrepancies in these data.

The isolation rate we report from live haul trucks (33.3%) (Table 6) is similar to that reported by Goren et al. (13) (29.6%), but both rates are lower than those reported by Rigby et al. (28) (86.6%). Goren et al. (13) found a high degree of similarity between the *Salmonella* serotypes isolated from transportation crates and those isolated from the skin of processed broilers.

The failure to isolate *Salmonella* from cloacal swabs is analogous to the data reported by Rigby and Pettit (27) and Bailey and Cox (1), both of whom concluded that more recoveries were made from samples taken from the ceca than from cloacal swabs. Since Bailey and Cox (1) have shown that cloacal swabs do not detect $<10^4$ organisms, these results suggest that most birds were carrying $<10^4$ *Salmonella* as they entered the plant. These data are consistent with data reported by Campbell et al. (5), who found that 93.5% of the carcasses sampled at final rinse contained 30 or fewer *Salmonella* organisms per carcass.

The 21% contamination rate we reported for whole processed broiler carcasses (Table 6) is lower than the 37% reported by Green et al. (14). Since broiler flocks sampled during the colder months have higher rates of *Salmonella* contamination than flocks sampled during the warmer months (33), seasonal effects may account for these differences. Our data were collected during the warmer months,

while Green et al. (14) do not mention season of the year.

Greenberg (15) found a high percentage of contamination in slaughterhouse flies and observed that *Salmonella* persisted from the maggot to the adult stage. While *Salmonella* was isolated from lesser meal worms, American cockroaches, and German cockroaches (Table 7), our data suggest that *Salmonella* was not maintained internally in the insects sampled. Since Greenberg (15) has shown that *Salmonella* can be recovered from tagged flies at distances up to 3 miles from their point of origin, the question of whether contamination of insects is internal or external may be a moot point. Nonetheless, our data suggest that insects simply mechanically transport *Salmonella* organisms. While the data collected certainly do not discount the role of insects in the transmission of *Salmonella*, the data do suggest that when the primary sources of *Salmonella* are eliminated, the organism will also disappear from the insect population.

In summary, the data reported here are similar to those reported over 20 years ago (22). *Salmonella* was most frequently isolated from feeds and feed ingredients (Table 1). Yet the similarity in contamination rates observed in cecal droppings collected from breeder/multiplier houses (Table 3), day-old chicks (Table 4), and live birds in broiler houses (Table 5) suggests that the breeder/multiplier house environment is an important vehicle for the transmission of contamination from feed to the final product. These data underline the need for a comprehensive approach in the control of *Salmonella* contamination in the broiler production and processing system.

ACKNOWLEDGMENTS

The technical assistance of Timothy Hurley and George Gartrell is greatly appreciated. This work was supported by Southeastern Poultry and Egg Association Grant #M2. The authors gratefully acknowledge the support.

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