Addition of a protected complex of biofactors and antioxidants to breeder hen diets confers transgenerational protection against *Salmonella enterica* serovar Enteritidis in progeny chicks

Christina L. Swaggerty,[*,1] Ramon D. Malheiros,[†] Ludovic Lahaye,[‡] Hector H. Salgado [‡] J. Allen Byrd II[*] Kenneth J. Genovese,[*] Haiqi He,[*] Elizabeth Santin,[‡] and Michael H. Kogut [*]

[*]*U.S. Department of Agriculture, Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, TX, USA;* [†]*Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA; and* [‡]*Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada*

ABSTRACT Addition of vitamins and antioxidants has been long associated with increased immunity and are commonly used in the poultry industry; however, less is known regarding their use in broiler breeder hens. The objective of this study was to determine if feeding a complex of protected biofactors and antioxidants composed of vitamins and fermentation extracts to broiler breeder hens conferred resistance against *Salmonella enterica* serovar Enteritidis (**S. Enteritidis**) in the progeny chicks. Three-day-old chicks from control- and supplement-fed hens were challenged with *S.* Enteritidis and necropsied 4- and 11-days postchallenge (**dpc**) to determine if there were differences in invasion and colonization. Serum and jejunum were evaluated for various cytokine and chemokine production. Fewer ($P = 0.002$) chicks from supplement-fed hens had detectable *S.* Enteritidis in the ceca (32.6%) compared to chicks from control-fed hens (64%). By 11 dpc, significantly ($P < 0.001$) fewer chicks from supplement-fed hens were positive for *S.* Enteritidis (liver [36%]; ceca [16%]) compared to chicks from the control hens (liver [76%]; ceca [76%]). The recoverable *S.* Enteritidis in the cecal content was also lower ($P = 0.01$) at 11 dpc. In additional to the differences in invasion and colonization, cytokine and chemokine production were distinct between the 2 groups of chicks. Chicks from supplement-fed hens had increased production of IL-16, IL-6, MIP-3α, and RANTES in the jejunum while IL-16 and MIP-1β were higher in the serum of chicks from the control-fed hens. By 11 dpc, production of IFN-γ was decreased in the jejunum of chicks from supplement-fed hens. Collectively, these data demonstrate adding a protected complex of biofactors and antioxidants to the diet of broiler breeder hens offers a measure of transgenerational protection to the progeny against *S.* Enteritidis infection and reduces colonization that is mediated, in part, by a robust and distinct cytokine and chemokine response locally at the intestine and systemically in the blood.

Key words: cytokine and chemokine, feed additive, protected vitamins, salmonella, transgenerational protection

INTRODUCTION

Salmonella enterica serovar Enteritidis (**S. Enteritidis**) is one of the leading causes of foodborne illness worldwide (Cummings et al., 2016). Since 1965, the number of foodborne disease outbreaks associated with *S.* Enteritidis has trended upward (Jones and Yackley, 2018), and today, still results in hospitalizations and deaths (Scallan et al., 2018). Infections derived from poultry and poultry products result in a significant number of human illnesses (CDC, 2016). Control of *Salmonella* in food production animals will require a multifaceted approach (Edrington and Brown, 2022).

The use of feed additives in the poultry sector has increased greatly with the removal of antibiotic growth promoters (**AGP**) from the feed. As demand increases for poultry products raised organically or antibiotic-free, alternative approaches that promote growth, maintain flock health, and reduce colonization by foodborne pathogens must be identified. The use of vitamins and other molecules with antioxidant properties have been investigated in poultry undergoing different challenge models (Sahin et al., 2003; Ghazi Harsini et al., 2012; Hu et al., 2019). Therefore, the supplementation of

antioxidant vitamins and fermentation extract induces immunometabolic phenotypic alterations in different tissues to help the animal to cope with early life stressors and improve growth performance (Bortoluzzi et al., 2021).

Vitamins are quickly oxidized and may lose their functional activity during feed processing at the mill, or activity may be diminished once the unprotected vitamin is consumed and makes its way through the gastrointestinal tract (**GIT**). Delivery of sensitive ingredients including vitamins to the desired location in the GIT can be a challenge. Microencapsulation is one way to address this challenge as the technology protects fragile ingredients and facilitates passage of the additive(s) through the crop into the lower GIT. Microencapsulation within a lipid matrix improves stability, preserves biological activity, and allows for a slow release of the additive in the desired location to maximize efficacy (Yousaf et al., 2017; Choi et al., 2020). Addition of microencapsulated vitamins or biofactors can improve performance under stress conditions (Bortoluzzi et al., 2021).

There are very few studies in the literature evaluating transgenerational effects of dietary supplementation to the hen and then evaluating the offspring for immunological changes. Addition of omega-3 polyunsaturated fatty acids to the broiler hen's diet results in marginal changes in the chicks (Koppenol et al., 2015). The paternal effect of dietary supplementation with *Astragalus* polysaccharides in broilers shows improved growth within the first 10-days posthatch and a beneficial impact on jejunal morphology in the offspring (Li et al., 2018). A study in layer chickens shows immune stimulation of the hens produces offspring that produce increased antibody responses following immunization suggesting a transgenerational effect (Verwoolde et al., 2022).

Each of these studies demonstrate transgenerational effects in progeny chicks can be achieved by manipulating the dams or sires; however, none evaluated the effects of feeding hens a diet supplemented with a protected complex of vitamins and fermentation extracts and following the progeny under an experimental challenge. Therefore, the objectives of this study were to determine if supplementing a hen's diet with a microencapsulated blend of biofactors and antioxidants composed of vitamins and fermentation extracts resulted in 1) chicks that were more resistant to colonization by *S.* Enteritidis and 2) if the cytokine and chemokine responses were altered compared to chicks from hens on a control diet.

MATERIALS AND METHODS

Broiler Breeder Experimental Design

From d 1 to the end of the experiment, all broiler breeders (Ross 308 AP) were raised and managed according to the commercial supplier's nutritional and management recommendations (Aviagen, 2016). Each

chicken on the study received the standard poultry vaccinations carried out at North Carolina State University outlined in the approved Animal Care Protocol (19-023A) and 2 doses of Vaxxon SRP SE (Vaxxinova, Willmar, MN) at 11 and 18 wk of age. From 57 to 67 wk of age, 448 female and 80 male broiler breeder chicks were divided in 2 dietary treatments (control and supplemented; see below for additional details). There were 8 replicates of 28 females and 5 males each. The birds were housed in floor pens with a partial slatted floor with 12 nests, bell drinkers, female feeders, and a male raised feeder.

The 2 dietary treatments were as follows: T1 was a control feed with no supplementation and T2 was the control diet supplemented with 300g/T of a feed additive comprised of protected biofactors and antioxidants (**P(BF+AO)**). The P(BF+AO) dietary supplement is a complex of vitamins and fermentation extracts microencapsulated in a matrix of triglycerides from hydrogenated vegetable oil. The blend is comprised of vitamins A, D_3, E, B_6, B_{12}, menadione, thiamine, riboflavin, niacin, pantothenic acid, biotin, folic acid, L-tryptophan, and fermentation extracts of dried *Bacillus subtilis, Aspergillus niger*, and *A. oryzae* (Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada). The detailed vitamin and amino acids composition of the blend are provided in an earlier study (Bortoluzzi et al., 2021), but for clarity are also listed here. The minimum supplied per kg of diet for the vitamins and amino acids are: vitamin A, 900 IU; vitamin D_3, 450 IU; vitamin E, 12 IU; vitamin K, 0.135 mg; vitamin B_{12}, 0.00525 mg; biotin, 0.03 mg; thiamine, 0.9 mg; riboflavin, 1.35 mg; pantothenic acid, 3 mg; pyridoxine, 0.75 mg; niacin, 12 mg; folic acid, 0.3 mg.

Progeny Chickens

When the hens were 65 and 66 wk of age, fertilized eggs were collected from hens on the control and supplemented diets and were incubated and hatched under standard conditions (Stromberg, 1975). All progeny studies were under the approved experimental procedures outlined in protocol number 2022-001 and were approved by the USDA/ARS Institutional Animal Care and Use Committee operating under the Animal and Plant Health Inspection Service establishment number 334299. On day of hatch, straight-run chicks from each line were placed in separate floor pens (3 m × 3 m) containing wood shavings, supplemental heat, water, and a balanced, un-medicated corn and soybean meal-based chick starter diet ad libitum. The feed contained 23% protein and 3,200 kcal of metabolizable energy/kg of diet, and all other nutrient levels met or exceeded established requirements (National Research Council, 1994). No medication or other therapeutic interventions were administered over the duration of the study. The experiments were conducted in accordance with the recommended code of practice for the care and handling of poultry and followed the ethical principles according to

the *Guide for the Care and Use of Agricultural Animals in Research and Training* (AgGuide, 2020) and were overseen by the on-site veterinarian.

Progeny Salmonella Challenge Trial

The progeny from the 2 broiler breeder treatment groups was subjected to an in vivo challenge by oral gavage with *S.* Enteritidis to determine if the chicks responded differently to colonization by this foodborne pathogen. This progeny experiment was conducted on 2 occasions. Day-of-hatch chicks ($n = 5$ per group) were sacrificed, and the ceca and liver were cultured to confirm the chicks were free of *Salmonella*. Three-day-old chicks were challenged with *S.* Enteritidis (0.5 mL; 1.3×10^4 cfu/chick). Challenged chicks from each group ($n = 24-25$ chicks per group per replicate; $n = 49-50$ total chicks per group) were euthanized by cervical dislocation and necropsied at 4- and 11-days postchallenge (**dpc**). Mock-challenged controls ($n = 5$ per group) were also sacrificed and necropsied at 4- and 11-dpc. At necropsy, the first 15 chicks from each challenged group had cecal content collected for *S.* Enteritidis enumeration. All chicks in the study (control and challenged) had one cecal pouch and one liver lobe enriched to determine if a chick was positive or negative for *S.* Enteritidis. All chicks on study were bled and serum collected. Additionally, a segment (2−4 cm) of jejunum was collected and flash frozen in liquid nitrogen for evaluation by ELISA to determine if the treatment and challenge resulted in differing cytokine and chemokine production. Additional details are provided in the sections below.

Bacteria Preparation and Recovery

A poultry isolate of *S.* Enteritidis was obtained from the National Veterinary Services Laboratory (Ames, IA), and was selected for resistance to nalidixic acid and novobiocin and maintained in tryptic soy broth (Difco Laboratories, Sparks, MD) containing antibiotics (20 μg/mL nalidixic acid and 25 μg/mL novobiocin; Sigma Chemical Co., St. Louis, MO). A stock culture was prepared in sterile phosphate buffered saline (**PBS**) and adjusted to a concentration of 1×10^9 cfu/mL as previously described (Swaggerty et al., 2005). The challenges were then diluted from the stock culture to the desired concentration. The viable cell concentration of the challenge dose for each experiment was determined by colony counts on XLT4 agar base plates with XLT4 supplement (Difco) and nalidixic acid and novobiocin (**XLT-NN**).

One cecal pouch from each chicken was removed aseptically, and the contents (0.25 g) were serially diluted to 1:100, 1:1,000, or 1:10,000 and spread onto XLT-NN plates to enumerate *S.* Enteritidis. The plates were incubated at 41°C for 24 h, and the number of NN-resistant *S.* Enteritidis cells per g of cecal contents determined. Additionally, one cecal pouch and a liver lobe were collected, placed into separate tubes containing 20 mL Rappaport Vassiliadis broth (Difco), and incubated overnight at 41°C. Following enrichment, 10 μL were streaked onto XLT-NN plates, incubated 24 h at 41°C, then the plates examined for nonlactose fermenting NN-resistant *Salmonella* colonies. For both enumeration and enrichment, representative colonies were confirmed positive by slide agglutination using specific Group D_1 antisera (Difco).

Serum and Tissue Preparation

Whole blood was collected via decapitation (day-of-hatch and 4 dpc) or the jugular vein (11 dpc) into BD Vacutainer SSTcollection tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). The tube was laid flat and allowed to clot at room temperature for 2 to 4 h, then centrifuged for 15 min at $3,500 \times g$, the serum supernatant was transferred to a cryovial, and stored at −80°C until needed.

Jejunum samples were homogenized prior to evaluating for cytokine and chemokine production. The lysis buffer solution was formulated by a 1:2 cell lysis buffer supplemented with protease inhibitor cocktail as suggested by the manufacturer (#AA-LYS and #AA-PI, respectively, RayBiotech Life Inc., Peachtree Corners, GA). The jejunal lysate was prepared by adding 100 mg of tissue to BeadBugTM tubes (#Z763799-50EA, Sigma-Aldrich, St. Lois, MO) filled with 1 mL lysis buffer solution and homogenized on speed setting 6 with the Omni International Bead Ruptor Elite (Kennesaw, GA) for 90 s with a 5 s pause between cycles for a total of 3 cycles. The lysate was then centrifuged for 10 min at $840 \times g$, the supernatant was collected and diluted 1:2 in distilled 1× PBS and stored at −80°C until the cytokine and chemokine panel was run.

Cytokine and Chemokine Production

Cytokine and chemokine production was measured in serum and jejunal lysates using the Milliplex chicken cytokine and chemokine panel (Catalog GCYT1-16K-PX12; MilliporeSigma Co., Burlington, MA) according to the manufacturer's instruction. The plates were read and analyzed using a Luminex 200 with xMAP Technology (Luminex Corp., Austin, TX). The required Luminex calibration (LX2R-CAL-K25) and verification (LX2R-PVER-K25) kits were completed prior to sample analysis according to the manufacturer's guidance.

Statistical Analysis

Two challenge trials were conducted, and 24 to 25 birds were used for each group per experiment for a total of 49 to 50 birds per group. The data from both challenge trials were combined for statistical analysis and presentation. The data were analyzed by ANOVA using SigmaStat v4.0 (Systat Software Inc., San Jose, CA) with significance at $P \leq 0.05$.
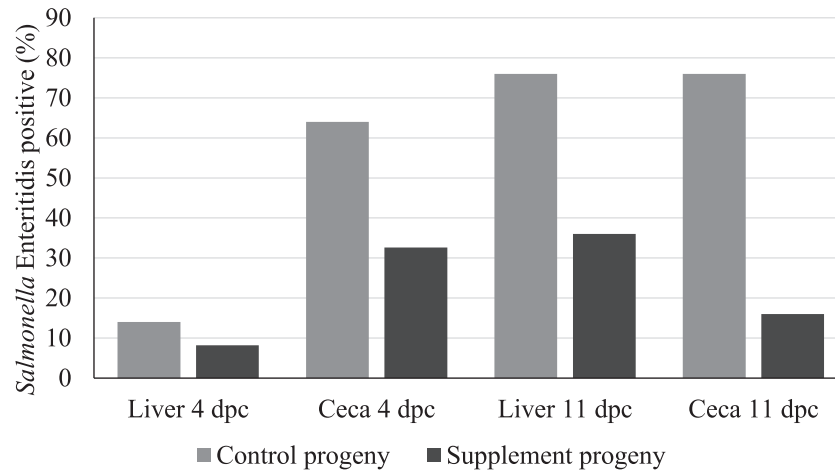
Figure 1. Percentage of *Salmonella* Enteritidis positive liver and ceca samples 4- and 11-days postchallenge in progeny from hens on control and supplemented diets. Three-day-old chicks were challenged orally with *S.* Enteritidis (0.5 mL; 1.3×10^4 cfu/chick). Challenged chicks from each group ($n = 24-25$ chicks per group per replicate; $n = 49-50$ total chicks per group) were necropsied at 4- and 11-days postchallenge (dpc). Mock-challenged controls ($n = 5$ per group) were also sacrificed and necropsied at 4- and 11-dpc. One cecal pouch and one liver lobe was enriched to determine if a chick was positive or negative for *S.* Enteritidis. Data shown are number of positive chicks/total number of chicks in each group.

RESULTS

Salmonella Positivity

Progeny chicks from the control- and supplement-fed hens were challenged with *S.* Enteritidis to determine if the feed additive produced transgenerational protection against invasion and colonization by this important foodborne pathogen after 4 and 11 dpc. All day-of-hatch and mock-infected controls were negative for *Salmonella* throughout the duration of the study (data not shown). As shown in Figure 1, chicks from the control-fed hens (32/50; 64%) were significantly ($P = 0.002$) more likely to have detectable *S.* Enteritidis in the ceca at 4 dpc compared to chicks from treated hens (16/49; 32.6%). Compared to the ceca, the percentage of *S.* Enteritidis positive liver samples at 4 dpc was markedly lower for both groups of chicks but was not significantly ($P = 0.36$) different from one another (14 and 8.2% for chicks from control- and supplement-fed hens, respectively).

The differences between progeny from control and supplement-fed hens were even greater at 11 dpc (Figure 1). Chicks from the hens on the supplanted diet were significantly ($P = 3.0 \times 10^{-5}$) less likely to have detectable levels of *S.* Enteritidis in the liver compared to chicks from control-fed hens (36 and 76%, respectively). Similarly, the ceca were less likely ($P = 3.9 \times 10^{-11}$) to be *S.* Enteritidis positive in chicks from supplement-fed hens (16%) compared to those on the control diet (76%).

Salmonella Recovery

At 4 dpc, the recoverable *S.* Enteritidis in chicks from hens on the supplemented diet tended to be lower than those from hens on the control diet (7.8×10^1 cfu/g and 4.3×10^3 cfu/g, respectively), but the difference was not statistically significant ($P = 0.07$; Table 1). Whereas by 11 dpc, there was a significant difference in the number of *S.* Enteritidis that were recovered. As shown in Table 1, chicks that were from hens fed the control diet had 6.4×10^4 cfu/g of recoverable *S.* Enteritidis while chicks from hens fed the supplemented diet only had 1.4×10^1 cfu/g that was recovered ($P = 0.01$).

Cytokine and Chemokine Production in Jejunum

A panel of cytokines and chemokines was measured in jejunum samples collected from chicks produced from control and supplement-fed hens under nonchallenged conditions compared to 4 and 11 dpc with *S.* Enteritidis. Only cytokines that were significant are shown (Table 2). There were no differences ($P > 0.05$) in any of the cytokines and chemokines evaluated following the challenge in chicks from hens fed the control diet. Differences ($P < 0.05$) were observed in jejunum samples collected from chicks from supplement-fed hens following challenge compared to those administered a mock-infection. Specifically, production (pg/mL) of IL-16, IL-6, MIP-3α, and RANTES was significantly ($P < 0.05$) higher following *S.* Enteritidis challenge in chicks produced from hens fed the supplement (Table 2) indicating a differential immunological response in the offspring of hens on the 2 diets.

Table 1. *Salmonella* Enteritidis recovered from the cecal content of chicks produced by hens on control and supplemented diets.

	Control hen progeny	Supplemented hen progeny	P value
4 dpc[1]	[2]$4.3 \times 10^3 \pm 2.2 \times 10^3$	$7.8 \times 10^1 \pm 5.1 \times 10^1$	0.07
11 dpc	$6.4 \times 10^4 \pm 9.4 \times 10^4$	$1.4 \times 10^1 \pm 8 \times 10^0$	0.01

[1]Days postchallenge.
[2]Recovered cfu of *Salmonella* Enteritidis per gram of cecal content $\pm$ SEM.

Table 2. Jejunum cytokine and chemokine production in progeny from control- and supplement-fed hens 4- and 11-days post-*Salmonella* Enteritidis challenge.

Target[1] (dpc)	Control (pg/mL)	Control + SE (pg/mL)	[2]Fold-change	P value	Supplement (pg/mL)	Supplement + SE (pg/mL)	Fold-change	P value
IL-16 (4)	2394.8 ± 420.4	2859.7 ± 379.6	1.2	0.5	1668.2 ± 235.6	3218.4 ± 440.8	1.9	0.01
IL-6 (4)	1261.0 ± 243.1	996.1 ± 195.2	â 1.3	0.43	673.1 ± 107.9	1357.9 ± 226.4	2.0	0.02
MIP-3α (4)	7567.1 ± 1974.9	8988.6 ± 2145.3	1.2	0.65	3783.8 ± 879.1	8168.9 ± 1762.4	2.2	0.05
RANTES (4)	23504.3 ± 10022.6	45522.8 ± 6401.7	1.9	0.10	18598.6 ± 5827.2	40021.8 ± 7361.3	2.2	0.04
IFN-γ (11)	334.0 ± 35.7	281.4 ± 26.4	â 1.2	0.26	436.4 ± 90.6	190.3 ± 32.3	â 2.3	0.03

[1]dpc = days postchallenge.

[2]All fold-change are increased unless noted with â indicating the SE challenge resulted in a decrease from the nonchallenged chicks. Values in bold font are opposite direction in the tissue collected from chicks produced from control hens compared to those from supplement-fed hens. n = 8 to 10 samples per group.

Cytokine and chemokine production in jejunum samples were also determined 11 dpc and the data are summarized in Table 2. As seen at 4 dpc, the progeny from control-fed hens had comparable ($P > 0.05$) levels of cytokine and chemokine production regardless of infection status. Production of IFN-γ was significantly ($P = 0.03$) decreased (2.3-fold) following *S.* Enteritidis challenge in progeny from the supplement-fed hens.

Cytokine and Chemokine Production in Serum

Serum samples were also evaluated for cytokine and chemokine production at 4 and 11 dpc. Fewer changes (both number of cytokines and their concentrations) were observed in the serum samples compared to the jejunum samples. IL-16 and MIP-1β were significantly ($P < 0.03$) higher following *S.* Enteritidis challenge in progeny from control-fed hens whereas there was no difference observed in the offspring from the supplement-fed hens (Table 3). No differences were observed in the serum samples collected 11 dpc (data not shown).

DISCUSSION

The global animal feed additive market including livestock, swine, poultry, and aquatic species was estimated to be near $20B USD in 2017 and by 2025 is expected to surpass $31B USD (Bhandalkar and Roy, 2019). This substantial increase is driven, in part, by increased consumer demand for antibiotic-free products, increased risk of antibiotic resistant bacteria, and the industries' need to find suitable antibiotic alternatives that will promote health and performance. Recent reviews highlight studies showing the effectiveness of antibiotic alternatives (Mehdi et al., 2018) and the important role of gut health in poultry (Adedokun and Olojede, 2019).

Moreover, addition of feed additives to the diet can be one component of a comprehensive preharvest food safety regime to reduce the instance of foodborne pathogens, including *Salmonella*, entering the food chain (Jeni et al., 2021; Biagini et al., 2022). Adult chickens that are infected with *S.* Enteritidis do not typically show clinical signs of disease; however, young birds are highly susceptible and may experience acute clinical disease or death (Gast and Porter, 2020). Recent reviews highlight the development of the innate immune response in embryos and newly hatched chicks (Alkie et al., 2019) and the positive impact of breeder hen diet on embryonic development and chick survivability (Taha-Abdelaziz et al., 2018) stressing the importance of strong development and chick quality.

In the study presented herein, addition of a microencapsulated blend of biofactors and antioxidants to the diet of broiler breeder hens provided transgenerational protection against *S.* Enteritidis invasion (Figure 1) in progeny chicks. All chicks used in this study were provided a control diet that met industry standards and contained no supplementation. The only difference was the diet (supplemented or control) that was fed to the breeder hens. Most studies using feed additives and *Salmonella* challenges in poultry are directly evaluating the benefits to the birds that are provided the treatments and not the offspring of the treated breeders' hens (Lowry et al., 2005; Grilli et al., 2011; Kogut et al., 2013; Abudabos et al., 2017). There are limited studies evaluating transgenerational immunity in chickens and tend to focus on antibody production (Leandro et al., 2011; Verwoolde et al., 2022), cytokine responses (Koppenol et al., 2015), or tolerance to endotoxin (Li et al., 2018) whereas the study presented herein, to our knowledge, is the first to follow chicks under a bacterial challenge. In addition to reducing the number of positive chicks, the number of *Salmonella* that were recoverable was also significantly reduced (Table 1) in

Table 3. Serum cytokine and chemokine production in progeny from control- and supplement-fed hens 4-days post-*Salmonella* Enteritidis challenge.

Target	Control	Control + SE	[1]Fold-change	P value	Supplement	Supplement + SE	Fold-change	P value
IL-16	43.5 ± 13.7	89.3 ± 28.2	2.1	0.02	62.4 ± 17.3	75.6 ± 7.6	1.2	0.53
MIP-1β	14.3 ± 4.5	29.6 ± 4.0	2.1	0.03	16.9 ± 5.1	20.2 ± 2.4	1.2	0.59

[1]All fold-change are increased unless noted with â indicating the SE challenge resulted in a decrease from the nonchallenged chicks. n = 8 (nonchallenged) and 10 (SE-challenged) per group.

the chicks from the breeder hens on the dietary supplement. It has been proposed that reducing the level of foodborne pathogen colonization, such as by *Salmonella* spp., in live birds entering the poultry processing plant may "contribute to overall improvement in food safety" and one approach is to use alternative feed additives (Ricke, 2023). The current study was carried out for 2 wk, so additional studies will need to be performed to determine if the reductions in *S.* Enteritidis positivity and recovery are maintained over the course of a normal broiler grow-out period.

It is clear from the colonization and enumeration differences between chicks from control- and supplement-fed hens that distinct immunological responses occurred following the *S.* Enteritidis challenge. Activation of microbial killing mechanisms and costimulatory molecule production are required for antigen presentation and activation of the acquired immune system, and coordination of these intracellular signaling pathways is accomplished, in part, by the release of cytokines and chemokines (Medzhitov and Janeway, 1997; Takeuchi and Akira, 2007). Differences in cytokine (IL-16, IL-6, IFN-γ) and chemokine (RANTES [CCL5], MIP-3α [CCL20], MIP-1β [CCL4]) production differed between chicks produced from the supplement-fed hens compared to those from control-fed hens (Tables 2 and 3). Following challenge, increased jejunal expression of IL-16, IL-6, MIP-3α, and RANTES were found after 4 dpc followed by a decrease in IFN-γ after 11 dpc. Expression (mRNA) of cytokines and chemokines in chicken intestinal tissues following challenge with *Salmonella enterica* spp. has been reported for years and shows increased expression of various cytokines and chemokines including, but not limited to, IL-6, IL-1β, IFN-γ, CXCLi8, and CCL4 (Beal et al., 2004; Setta et al., 2012; Shanmugasundaram et al., 2021). Expression of MIP-3α (CCL20) has also been reported in chicken primary macrophages in response to *S.* Typhimurium (Huang et al., 2019). Similarly, increased expression of intestinal IL-6 and IFN-γ in the serum of S. Enteritidis-infected chickens is associated with enhanced immunity and protection (Song et al., 2020). The strong inflammatory and chemotactic responses early (4 dpc) indicate a robust cytokine- and chemokine-mediated immunological response is responsible, in part, for the decreased colonization and invasion. Maybe even more important is the chemotactic response likely recruited activated immune cells which significantly reduced the actual numbers of *S.* Enteritidis by 2 to 4 logs, which agrees with an earlier study showing recruitment of heterophils is responsible for reducing systemic *S.* Enteritidis numbers (Swaggerty et al., 2005). Future histological studies could be explored to confirm the specific cell type(s) that are reducing the *Salmonella* numbers.

In conclusion, it has been suggested that pathogen reduction in animal agriculture will require a combination of intervention strategies to effectively reduce the number of foodborne illnesses (Doyle and Erickson, 2012). We have demonstrated a level of transgenerational protection against *S.* Enteritidis colonization

in young chicks by feeding the breeder hens a diet supplemented with a microencapsulated blend of biofactors and antioxidants that is mediated, in part, by a robust and distinct cytokine and chemokine response locally at the intestine and systemically in the blood. Reducing early colonization by *S.* Enteritidis may translate to fewer foodborne pathogen-causing bacteria that enter the food chain via contamination at the processing plant, thereby ultimately reducing the risk to the consumer.

DISCLOSURES

L.L., H.H., and E.S. are Jefo employees but did not participate in the analysis of this study. All other authors declare no conflict of interest.

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