

Effect of Food Protein Supplements on *Salmonella enteritidis* Infection and Prevention in Laying Hens

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ABSTRACT Reduction of intestinal colonization of *Salmonella enteritidis* (SE) during the grow-out period is crucial to provide safer eggs, minimize economic losses, and reduce the spread of human salmonellosis. In the search for novel elimination and prevention methods based on feed supplementation, the effects of feed supplemented with nonimmunized egg yolk powder (did not contain anti-*S. enteritidis* antibodies), immunized egg yolk powder (with anti-*S. enteritidis* antibodies), egg yolk proteins, egg white, and skim milk powder were examined on laying hens. In the elimination study, the chickens were orally infected with SE then given a supplemented feed of 5, 10, or 15% (wt/wt) of each of the test samples. Fecal samples tested weekly showed an absence of SE after the first week of feeding nonimmunized egg yolk powder

and a gradual decrease with the other samples. In the prevention study, *Salmonella*-free chickens were fed the supplemented feed for 4 wk and then infected orally. Fecal samples tested for 4 wk showed that SE was prevented from colonizing the intestinal tract throughout the test period by nonimmunized egg yolk powder, whereas the other samples only delayed the colonization. None of the fed supplements disrupted the balance of the intestinal microflora, and the counts in the feces remained constant. These results show that the administration of only 5.0% (wt/wt) of nonimmunized egg yolk powder can eliminate and prevent SE colonization in laying hens with no adverse effects. Furthermore, the present results indicate that hen egg yolk contains novel anti-adhesive or immunomodulatory components that may act to prevent SE infection.

(Key words: *Salmonella enteritidis*, laying hen, food supplement, egg yolk, egg yolk antibody)

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INTRODUCTION

Salmonella enteritidis is the cause of the foodborne salmonellosis pandemic in humans, in part because it is the only human pathogen and *Salmonella* serotype that contaminates shell eggs. This contamination routinely causes human illness without causing illness in the infected birds (Petter, 2001). Egg-transmitted salmonellosis is the primary food poisoning risk in the industrialized world (Levine, 1991; Wachsmuth et al., 1991; Hennessy et al., 1996; USDA, 1996). *Salmonella enteritidis* is believed to be vertically transmitted from infected ovaries and oviducts to the eggs of laying hens (Protais, 1989; Suzuki, 1990; Nakamura et al., 1993; Thiagarajan et al., 1994; Methner and Meyer, 1995). Another proposed route is that SE penetrates the eggshell from the chicken feces deposited on the outside of the egg as it passes through the cloaca (Snoeyenbos et al., 1979).

The ability to adhere to and penetrate intestinal epithelial barriers is a common means of entry by *Salmonella*

into poultry (Moulder, 1985; Finlay and Falkow, 1989). The adhesion of the pathogen is mediated by bacterial adhesins, which recognize specific mucosal receptors. Inhibition of adhesion by blocking the receptors with specific adhesin analogues or by steric hindrance (Tuomola et al., 1999) may prevent or eliminate colonization of the intestine by pathogens and thereby prevent the infection.

Elimination from or prevention of SE in broilers and other poultry, especially the table egg-producing layer flocks (USDA, 1988), before they reach the processing plant will improve the chances of products free from this organism (Stern et al., 2001). Research has focused on the development of immunoprophylactic measures, microbiological strategies, and antisalmonella feed additives to prevent intestinal and tissue colonization of table egg-producing flocks by invasive SE (USDA, 1988; Smith, 1989; Schneitz et al., 1990). Passive immunization by oral administration of antibodies from external sources is thought to be useful for the prevention of gastrointestinal infection (Sugita-Konishi et al., 2000). Recently, attention has been focused on egg yolk immunoglobulin Y (IgY) obtained from immunized hens as another antibody

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Abbreviation Key: BG = brilliant green; CE = competitive exclusion; TS = tryptic soy.

source, because of its high productivity (Bartz et al., 1980; Yokoyama et al., 1992; Mine and Kovacs-Nolan, 2002). Sugita-Konishi et al. (2000) reported that these anti-*S. enteritidis* antibodies in egg yolk inhibited *Salmonella* adhesion and infection of human intestinal epithelial cells in vitro. This inhibition was due to the binding of antibodies to some specific antigens on the bacterial surface; however, the specific antibodies were unable to block the adhesion of SE completely and unable to suppress their multiplication inside the cell. Tellez et al. (2001) indicated that a combination of avian-specific probiotic and *S. enteritidis*, *S. typhimurium*- and *S. heidelberg*-specific antibodies have a beneficial effect in reducing the colonization of SE in market-aged broilers; however, this was a combination study with no direct indication if these antibodies alone have any beneficial effect in reducing SE colonization.

Brady et al. (2002) indicated an antibacterial activity of fractionated hen-egg yolk (lipoproteins) against 2 pathogenic streptococcus strains in vitro. Moreover, Sugita-Konishi et al. (2002) suggested that egg yolk-derived sialyloligosaccharides and its derivatives are useful in preventing *Salmonella* infection when ingested continuously. Microbiological strategies employed early exposure of newly hatched chicks to normal adult intestinal flora (Lloyd et al., 1977; Snoeyenbos et al., 1978; Barnes et al., 1979; Snoeyenbos et al., 1979; Barnes et al., 1980; Impey and Mead, 1989; Nurmi et al., 1992). Nurmi and Rantala (1973) and Snoeyenbos et al. (1978) pioneered the use of normal gut microflora for colonization control [competitive exclusion (CE) or adhesive probiotics, mainly *Lactobacillus* strains]. However, to be effective, it is crucial that the CE culture should be given to chicks very early in life before they are exposed to SE. Several difficulties are being encountered with long-term preservation of the CE cultures and with batch-to-batch variability (Ziprin and Deloach, 1993). Furthermore, colonization cannot be prevented by this method when breeding farms are contaminated (Nurmi et al., 1992; Nakamura et al., 1995), and results suggest that such microbiological strategies may be ineffective in older hens and layer flocks (Corrier et al., 1993).

It is important to develop technologies capable of reducing, eliminating, and preventing the incidence of *Salmonella* colonization in poultry through a novel nonantibiotic approach. In this study, we focused on the eliminating and protective effects of animal-based food supplements against SE infection in laying hens. Hence, we sought to determine the effects of the food protein supplements: egg yolk protein, egg white, and skim milk powder on SE colonization in chickens.

MATERIALS AND METHODS

All animal experiments were performed with the knowledge and approval of the University of Guelph's committee on animal use and care.

SE Isolate

The isolate used for infection was *S. enteritidis* PT4 SA992212 from chickens.² Challenge inoculum was prepared from an overnight culture in tryptic soy (TS) broth (D4552)³ then serially diluted to the specified viable cell concentration of 10⁹ cfu/mL. Inoculum was estimated by spectrophotometry, optical density at 660 nm, and confirmed by colony counts on brilliant green (BG) agar (D0702)³ plates containing 20 µg of novobiocin (B231971).³ One milliliter of the challenge inoculum and 1.0 mL of sterile TS broth as control were used for the oral infection. Methods of the FDA Bacteriological Analytical Manual (Wallace et al., 1995) were used for the detection and identification of SE in the feces of test samples.

Birds

Forty White Leghorn hens 22 to 24 wk old (elimination study), 28 hens (prevention study), and 10 hens (immunized/nonimmunized egg yolk study) that were specific pathogen-free were obtained from the university research station.⁴ Cloacal swabs and fecal samples were collected and tested for SE by the pre-enrichment/enrichment selective plating method (Wallace et al., 1995). BG agar plates containing 20 µg of novobiocin were used, and suspected *Salmonella* colonies were identified biochemically by the lysine iron agar (B211363)³/tryptic sugar iron agar (D4402)³ slants biotyping technique. Then SE was positively identified serologically by the somatic O-antisera group D1 (DF 295147)³ agglutination for serogrouping and serotyping using flagellar H-antisera single factors m and 7 (DF 2548472, DF 2477475)³ (Wallace et al., 1995). Fecal samples were also collected for intestinal microflora estimation before, during, and after supplemented feeding and infection. The microorganisms targeted were total aerobic bacteria, total anaerobic bacteria, *Lactobacillus* spp., and enterobacteriaceae. The culture media used were TS agar (D4452),³ bacto anaerobic agar (253610),³ lactobacilli deMan Rogosa Sharpe agar (D3052)³ and BG agar, respectively. Serial dilutions were prepared in peptone water (PW, D3452),³ and a 0.1-mL sample of each of the 10⁻³, 10⁻⁵, and 10⁻⁷ dilutions was plated on each medium. Plated bacteria were incubated anaerobically in anaerobic chambers with disposable gas generator envelopes for anaerobic environment (B71040)³ or in CO₂-enriched envelopes for *Lactobacillus* spp. (1181440),³ or aerobically, at 30°C for 24 to 48 h. Bacterial colonies on each medium were then counted.

Prior to infection, eggs from each bird were cracked, and egg yolks were aseptically separated from the albumen. Egg yolk samples were tested for antibodies to SE by ELISA using formalin-treated whole SE cells as a coating antigen and primary and secondary antibodies for the colorimetric identification as described previously (Mine, 1997). All birds were negative for SE.

Housing

Birds were housed one per cage in separate wire-bottom, covered metal cage systems in the isolation unit.⁴

²Cornelius Poppe, Health Canada, Guelph, ON, Canada.

³BD diagnostic system, Oakville, ON, Canada.

⁴Isolation Unit, Pathobiology Dept. University of Guelph, Canada.

Cages were raised on a metal table, with 3 cages (representing 3 replicates of 1 treatment) on each table, keeping a distance of approximately 60 cm between cages and 100 cm between adjacent tables. Plastic sheets were used under the wire floors of each cage to catch droppings. They were cleaned and replaced daily. Food and water were provided ad libitum.

Feed Preparation

The regular untreated feed⁵ was soy based and pelleted and contained no antibiotics (corn 56%; soybean meal, 16.2%; wheat shorts, 7.1%; pork meal, 6.0%; limestone, 9.5%; dicalcium phosphate, 0.8%; fat, 3.0%; salts, 0.25%; and vitamin-premix, 1.0%; wt/wt). Prior to each feeding study, the feed of each chicken was prepared and stored separately by mixing the regular untreated feed thoroughly with the designated supplement at the required concentration. The nonimmunized egg yolk powder without anti-SE antibodies was prepared from eggs obtained from the university research station⁵ that were cracked after disinfection of the exterior shell surface, and the egg yolks were aseptically separated from albumen. Pooled egg yolks were freeze-dried and crushed into a fine powder. All egg yolk samples were tested for antibodies to SE by ELISA using formalin-killed whole SE cells as a coating antigen. Samples were negative for the aforementioned antibodies. The immunized egg yolk powder (containing anti-*S. enteritidis* antibodies) was prepared as before but from eggs of chickens that were immunized with killed SE cells. In the procedure, 9 White Leghorn hens (20 to 24 wk old) that were specific pathogen free were kept in separate cages in the Poultry Research Unit.⁵ Hens were intramuscularly vaccinated with 0.4 mL of SE inoculum prepared from formalin killed cells (10^9 cfu/mL) in sterile 0.9% NaCl emulsified with an equal volume of complete Freund's adjuvant (9007-81-2).⁶ Hens were given a booster 2 wk after the first injection and then weekly for 2 wk, with a final booster at 6 wk after the first injection. The whole cells of killed *Salmonella* were also used as a coating antigen in the ELISA method to test for antibodies. Pure IgY⁷ was used as a standard to determine the concentration of specific antibodies to SE in the immunized egg yolk powder samples, which was estimated to be 8.0 g/kg powder. The other food supplements, egg yolk proteins (after lipid fractions were extracted by ethanol), and egg white powder were commercially obtained from Taiyo Kagaku Ltd.⁸ Skim milk powder was purchased from a local supermarket. Neither egg yolk proteins nor skim milk powder contained specific antibodies to SE.

Experimental Design

Elimination Study. Feed and water were withheld for 24 h prior to infection. Of the 40 birds, 38 were inoculated orally with 1.0 mL of 10^9 cfu/mL of SE and 2 (negative controls, with 1.0 mL of sterile TS broth, using a syringe and a blunt-end catheter. A booster infection of another 1 mL was given 1 wk after the first infection. Three days postinoculation, the infection was confirmed by testing cloacal swab specimens and fecal samples to monitor SE level. Once the SE level in the feces reached 10^4 to 10^5 cfu/g, supplemented feeding commenced. For 4 wk, each 3 birds in 3 separate cages (representing 3 replicates of 1 treatment) were fed, respectively, a supplemented feed of nonimmunized egg yolk powder, egg yolk proteins, egg white powder, or commercial skim milk powder at concentrations of 5, 10, or 15% (wt/wt). The 2 positive controls (challenged with SE) and the 2 negative controls (unchallenged) were fed the untreated regular feed [3 birds per concentration per supplement \times 3 concentrations \times 4 supplements = 36 birds + 2 positive controls + 2 negative controls = 40 birds].

Prevention Study. For 4 wk, each 3 SE-free birds in 3 separate cages (representing 3 replicates of 1 treatment) were fed respectively a supplemented feed of 5 or 10% (wt/wt) nonimmunized egg yolk powder, 10 or 15% (wt/wt) egg yolk proteins, 10 or 15% (wt/wt) egg white powder, or 5 or 10% (wt/wt) commercial skim milk powder. The 2 positive controls (to be challenged with SE) and the 2 negative controls (to remain unchallenged) were fed the untreated regular feed [3 birds per concentration per supplement \times 2 concentrations \times 4 supplements = 24 birds + 2 positive controls + 2 negative controls = 28 birds]. The singular concentrations in this study were determined based on results obtained from the elimination study above. At the end of 4 wk postfeeding, feed and water were withheld for 24 h and all the chickens except the 2 negative controls were orally infected with 1.0 mL of 10^9 cfu/mL of SE. Postinfection, the chickens were fed the untreated regular feed.

Nonimmunized Vs. Immunized Egg Yolk Powder Study. To compare the effect of nonimmunized and immunized egg yolk powder on SE infection, of the 10 birds; 8 were inoculated orally with 1.0 mL of 10^9 cfu/mL of SE and 2 as the negative control, with 1.0 mL of sterile TS broth, using a syringe and a blunt-end catheter. A booster infection of another 1.0 mL was given 1 wk after the first infection. Three days postinoculation, the infection was confirmed by testing cloacal swab specimens and fecal samples to monitor SE level. Once the SE level in the feces reached 10^4 to 10^5 cfu/g, it was monitored for 4 wk, at the end of which supplemented feeding commenced. For 4 wk, each three birds in 3 separate cages (representing 3 replicates of 1 treatment) were fed 15% (wt/wt) nonimmunized or immunized egg yolk powder. The 2 positive controls (challenged with SE) and the 2 negative controls (unchallenged) were fed the untreated regular feed [3 birds per concentration per supplement

⁵Arkell Poultry Research Unit, Guelph, Canada.

⁶Sigma Chemical Co., St. Louis, MO.

⁷Jackson Immuno Research, West Grove, PA.

⁸Taiypo Kagaku Ltd., Yokkaichi, Japan.

× 1 concentration × 2 supplements = 6 birds + 2 positive controls + 2 negative controls = 10 birds].

Bacteriologic Examination. Fecal samples were collected once a week starting 4 d postfeeding in the elimination and immunized vs. nonimmunized studies or 4 d postinfection in the prevention study for 4 wk. *S. enteritidis* was detected, identified, and enumerated as outlined above. Intestinal microflora in fecal samples were also enumerated weekly. At the end of the experimental period, chickens were euthanized. Before and throughout the feeding and infection period, the weights of hens and the number and weight of eggs laid per chicken were monitored weekly for any significant changes in comparison with those of the 2 negative control chickens.

Statistical Analysis. Differences in the mean intestinal bacterial counts between the various diets and that of the control were analyzed by ANOVA.⁹ Means were separated by Duncan's multiple range test. Probabilities less than or equal to $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Experiment 1: Effect of Food Supplements in Preventing and Eliminating SE Infection in Laying Hens

Elimination Study. The number and incidence of SE in the feces of hens during this test period with the effect of the various food supplements are shown in Figure 1. After infection with 1.0 mL of 10^9 cfu/mL of SE, the average log₁₀ level in the fecal samples was 4.9 ± 0.08 cfu/g¹⁰ at wk 2. The supplemented feeding commenced at this level. The incidence of SE in feces decreased rapidly as shown by the mean log₁₀ levels of the counts, and no *Salmonella* were recovered from the feces of those fed the 10 and 15% (wt/wt) nonimmunized egg yolk powder after 1 wk of feeding. However, the incidence decreased gradually with the 5% (wt/wt) and was not detected after 2 wk of feeding (Figure 1A), whereas the control mean log₁₀ levels in the fecal samples remained constant around 5.0 ± 0.27 cfu/g throughout the test period. From the above results, nonimmunized egg yolk powder significantly ($P < 0.05$) reduced the frequency of colonization with SE and was able to eliminate the organism at a concentration as low as 5% (wt/wt) without containing the SE-specific antibodies. On the other hand, egg yolk powder is readily available, practical, and economical as a potential feed supplement. The 10 and 15% (wt/wt) egg yolk proteins (Figure 1B) showed a similar decreasing effect in the incidence of SE in the feces, but complete elimination did not occur until after 2 wk of feeding. Only a slight decreasing effect on SE was detected with the 5% (wt/wt) egg yolk proteins when compared with the

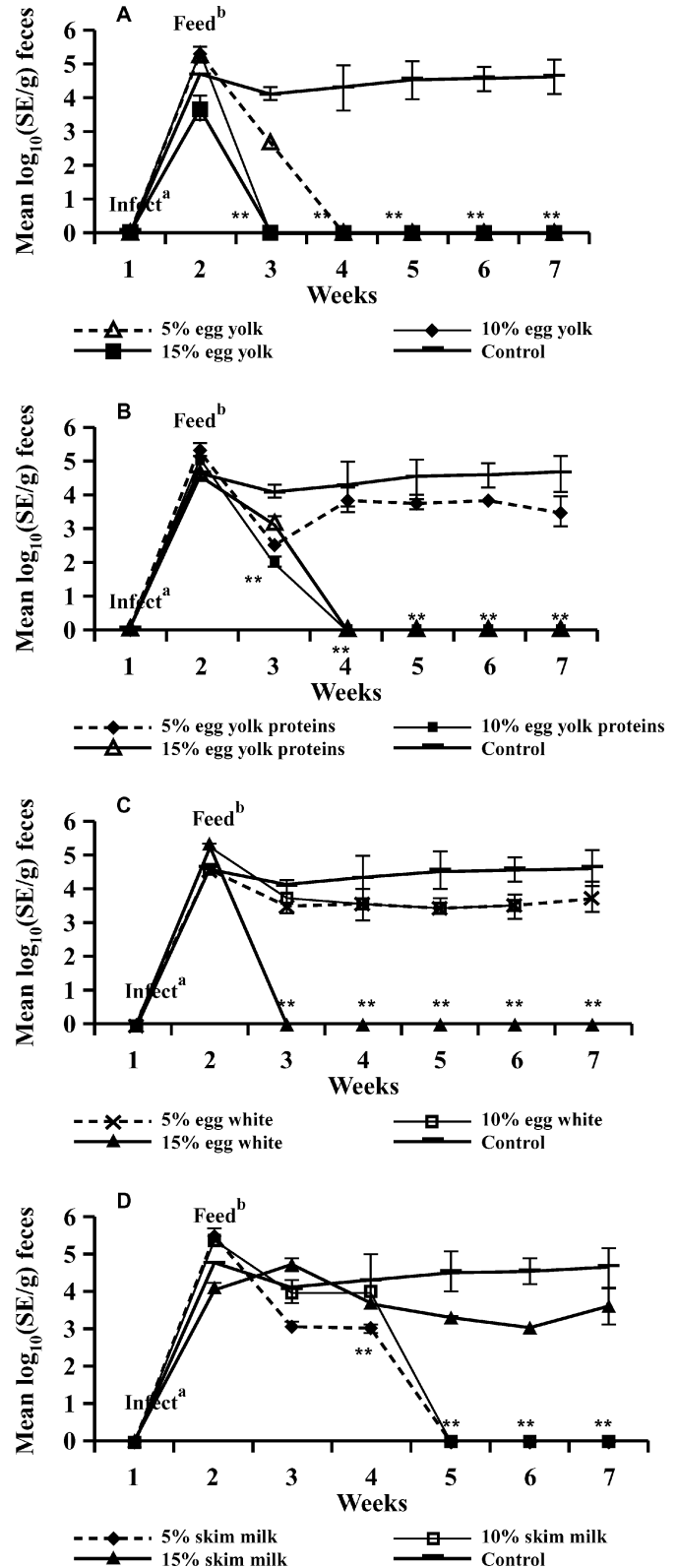


FIGURE 1. Panel A: effect of feeding nonimmunized egg yolk powder on elimination of *Salmonella enteritidis* from the intestine of laying hens. Panel B: effect of feeding egg yolk proteins on the elimination of *Salmonella enteritidis* from the intestine of laying hens. Panel C: effect of feeding egg white on the elimination of *Salmonella enteritidis* from the intestine of laying hens. Panel D: effect of feeding skim milk powder on the elimination of *Salmonella enteritidis* from the intestine of laying hens. ^a Birds were infected with 10^9 cfu/mL SE. ^bSupplemented feeding with the various concentrations commenced. Error bar indicates standard error of the mean. **Significant ($P < 0.05$) compared with the control.

⁹SPSS version 8.0 for Microsoft Windows, SPSS, Chicago, IL.

¹⁰Log₁₀ colony-forming units per gram ± standard error of the mean.

control mean \log_{10} counts in the feces. These results suggest that egg yolk proteins do have an elimination effect, more statistically significant ($P < 0.05$) at higher concentrations of the protein. The above observations also indicate that the proteins in the egg yolk play a role against the colonization of SE, but other components present in the egg yolk also may contribute to this activity.

Passive immunization by oral administration of specific antibodies has been an attractive approach against gastrointestinal pathogens in human and animals. The potential application of orally administered egg yolk antibodies for that purpose has been studied extensively in the past and documented in several reviews (Larsson et al., 1993; Hatta et al., 1997; Mine and Kovacs-Nolan, 2002). However, the present work indicated that egg yolk itself contains novel anti-infectious factors besides IgY, and they can eliminate SE from an infected chicken's gut. This report is the first to demonstrate such new biological function of egg yolk components.

The incidence of SE in feces rapidly decreased with the 15% (wt/wt) egg white supplement and was evident after 1 wk of feeding (Figure 1C). However, just a slight reduction in the mean \log_{10} counts was observed with the 5 and 10% (wt/wt) egg white powder. Unsurprisingly, egg white proteins at higher concentrations showed an elimination effect, but it is unclear whether this effect is an anti-adhesive or the result of the various antimicrobial factors present in egg white (mainly lysozyme and ovotransferrin), which are well known as natural antimicrobial agents (Ibrahim, 1997). To our knowledge, there is no report on the exclusive effects of egg white components against SE in the animal or human gut. Further studies are required to identify the inhibitory mechanism of egg white and determine whether its activity is antimicrobial or a novel anti-adhesive.

Similarly, skim milk also showed a slight elimination effect but only with low concentrations at 5 and 10% (wt/wt). Such concentrations had a gradual decreasing effect (as shown in Figure 1D) on the incidence of SE in feces and no SE was evident after 3 wk of feeding. With the 15% (wt/wt), there was an initial increase in the mean \log_{10} counts after 1 wk of feeding, and then a steady slight decrease was observed in the counts afterward. This initial increase perhaps suggests that, at a high concentration skim milk and its components may act as growth factors for the organism. The results from skim milk can be explained because the nonfat component of milk has powerful antimicrobial activities, in particular lactoferrin, and oligosaccharides in milk have preventive effects against gastrointestinal pathogens as documented by several researchers (Cravioto et al., 1991; Naidu, 2000). Finally, commercial skim milk powder contains about 5% (wt/wt) lactose (Holland et al., 1989), and it has been shown that lactose is effective in the prevention of *Salmonella typhimurium* infection (Corrier et al., 1990). Therefore, in future work we will demonstrate whether the effect is due to the functional properties of lactose or to the other milk components.

Prevention Study. The results of SE detection in the feces of the hens in this study are shown in Figure 2. After the feeding period of 4 wk with the various concentrations of the aforementioned food supplements, the chickens were infected with 1.0 mL of 10^9 cfu/mL of SE and then given the untreated control (regular) feed. The mean \log_{10} counts in the fecal samples of the chickens that were fed the nonimmunized egg yolk powder supplement showed that SE was not evident with the 5 and 10% (wt/wt) supplement concentrations (Figure 2A) during the 4-wk test period when compared with the mean \log_{10} counts of the control hens. From the above results, egg yolk powder treatment was able not only to significantly ($P < 0.05$) reduce the frequency of SE colonization but also to prevent this organism from colonizing the intestinal tract at a concentration as low as 5% (wt/wt) without containing the SE-specific antibodies. These data are significant because the adhesion of SE in the intestinal epithelial cells is crucial to the initial phase of infection (Ofek and Doyle, 1994) and so blocking this adhesion in the intestine may prevent infections if the laying hens were exposed to the organism during their lifespans. The preventive effect of egg yolk proteins on the incidence of SE in the feces is shown in Figure 2B. The organism was not detected for up to 2 wk in the fecal samples of the chickens that were fed the 10 and 15% egg yolk proteins, but then the mean \log_{10} levels of SE increased to 4.7 ± 0.43 cfu/g at 4 wk. These results indicate that the extracted egg yolk proteins alone may not be as effective in blocking SE from colonizing the intestinal tract as the complete egg yolk supplement.

The results of the egg white protein supplement are shown in Figure 2C. No SE was detected in the feces of the chickens that were fed 15% (wt/wt) egg white powder during the 4-wk test period; however, with the 10% egg white, the preventive effect was for up to 2 wk. The mean \log_{10} levels increased to 4.7 ± 0.20 cfu/g at 4 wk. Ultimately, similar results were evident with the 5 and 10% (wt/wt) skim milk powder, as shown in Figure 2D. The nonimmunized egg yolk powder seemed to be the most effective among all the samples tested in eliminating and preventing SE from colonizing the intestinal tract of laying hens.

Experiment 2: Effect of Nonimmunized Egg Yolk Powder versus Immunized Egg Yolk Powder in Eliminating SE in Laying Hens

As documented by many researchers, egg yolk antibodies have attracted considerable attention for the prevention and treatment of viral and bacterial gastrointestinal infections. However, one important question arose from our present work. Is the active component in egg yolk, the egg yolk antibodies, or other components? To address this question, we compared nonimmunized egg yolk and immunized egg yolk powder in terms of their activity in the elimination of SE in laying hens. The comparative effect of the immunized and nonimmunized egg yolk powder on the number and incidence of SE in the feces

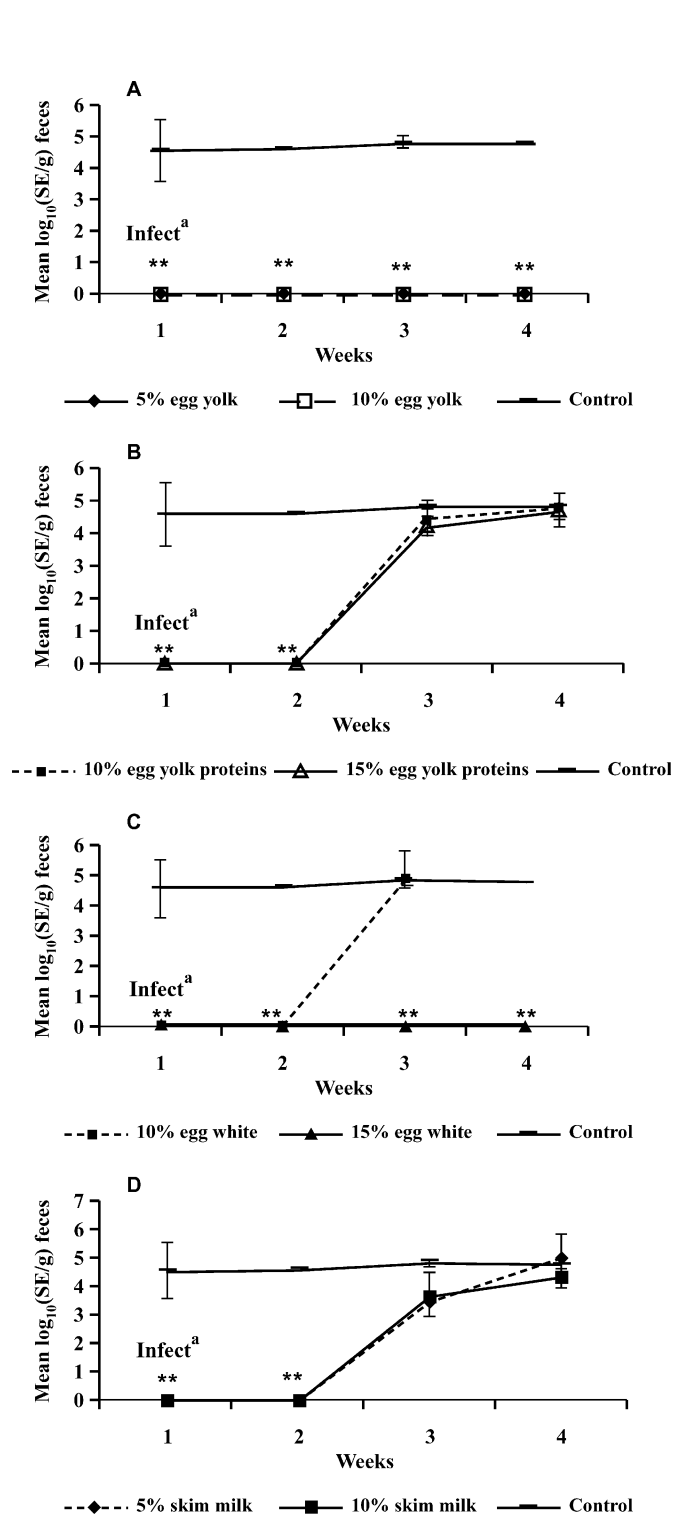


FIGURE 2. Panel A: effect of feeding nonimmunized egg yolk powder on the prevention of *Salmonella enteritidis* from the intestine of laying hens. Panel B: effect of feeding egg yolk protein on the prevention of SE from the intestine of laying hens. Panel C: effect of feeding egg white on the prevention of *Salmonella enteritidis* from the intestine of laying hens. Panel D: effect of feeding skim milk powder on the prevention of *Salmonella enteritidis* from the intestine of laying hens. ^aBirds were infected with 10^9 cfu/mL SE after being fed for 4 wk with supplemented feed containing the designated concentration; the control group was fed untreated regular feed. Error bar indicates standard error of the mean. ******Significant ($P < 0.05$) compared with the control.

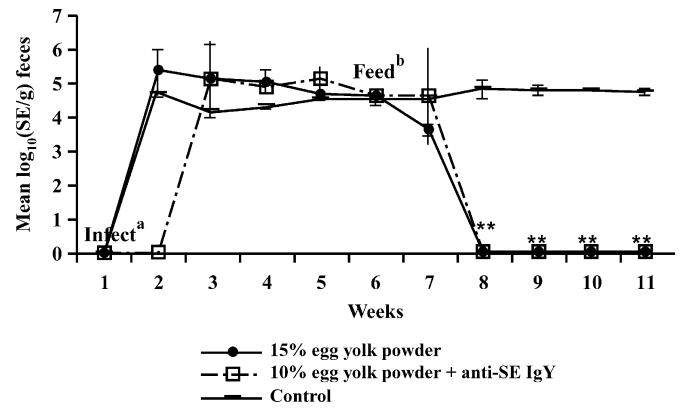


FIGURE 3. Effect of feeding immunized egg yolk powder with anti-*Salmonella enteritidis*-specific antibodies versus nonimmunized egg yolk powder on the elimination of SE from the intestine of laying hens. ^aBirds were infected with 10^9 cfu/mL SE. ^bSupplemented feeding with the 2 treatments commenced; the control group was fed untreated feed. Error bars indicate standard error of the mean. ******Significant ($P < 0.05$) compared with the control.

are shown in Figure 3. A rapid decrease in the mean \log_{10} levels of the counts and an elimination of the organism were observed when feeding 15% (wt/wt) nonimmunized egg yolk powder ($P < 0.05$) when compared with the control group. Incidentally, a similar effect was observed in the mean \log_{10} levels of SE in the feces of those hens that were fed immunized egg yolk powder supplement that contained anti-*S. enteritidis* antibodies (specific IgY, 8 g/kg of yolk powder). These results indicated that other components in the egg yolk powder may be important factors in eliminating SE from chicken gut, and the anti-*S. enteritidis* antibodies are not primarily the active anti-adhesive factors against SE elimination in laying hens.

This is because egg yolk powder whether containing the anti-*S. enteritidis* antibodies or not, significantly ($P < 0.05$) reduced the frequency of colonization by SE at a concentration as low as 5% (wt/wt) in the feed. Other researchers have concentrated on the egg-derived anti-*S. enteritidis* antibodies alone as the active components in preventing SE infection in poultry and did not use nonimmunized egg yolk powder as a control. In addition, the preventive effect of the orally administered IgY on gastrointestinal infections caused by the enteric pathogens, *Salmonella* spp. in particular, has been studied at length (Sugita-Konishi et al., 1996; Yokoyama et al., 1998). However, findings in this study suggest that egg yolk components have synergistic effects to prevent *Salmonella* infections in laying hens. It has been reported that, in mice, egg yolk derived sialyloligosaccharide, sialylglycopeptide and asialo-yolk derived sialyloligosaccharide have antibacterial properties and provide protection against gastric diseases such as *Salmonella* infection by preventing bacteria from binding to the intestine rather than by activating macrophages (Sugita-Konishi et al., 2002). However, the concentration of these sialyloligosaccharide components in the egg yolk powder is very low to exhibit an anti-adhesive activity. Therefore, it seems

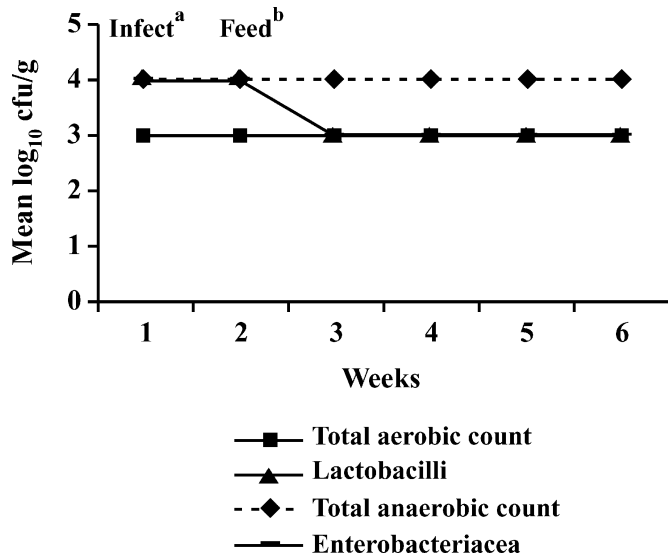


FIGURE 4. The number of the intestinal microflora after ^ainfection with *Salmonella enteritidis* and ^bfeeding with 10% (wt/wt) egg yolk powder supplement.

that egg yolk has novel anti-adhesive factors, yet to be discovered. The identification of these factors is now in progress in our laboratory.

Microflora

Imbalances in the intestinal microflora sometimes induce physiological damage or different pathogenic diseases, which result in great losses from the industry (Ishihara et al., 2000). In this study, the influence of egg yolk feed supplement on intestinal microflora balance was investigated during the elimination study. The microflora mean log₁₀ levels in the fecal samples of all the chickens that were fed the supplement at different concentrations are shown in Figure 4. Fecal samples tested throughout the study showed that none of the supplements, at any concentration, had a negative effect on the intestinal microflora of the chickens. The total bacterial counts (total aerobic, total anaerobic, and lactobacilli counts) remained constant throughout the infection and feeding period except for a slight decrease in the enterobacteriaceae counts from 4.0 to 3.0 cfu/g. The enterobacteriaceae level decreased slightly after SE was eliminated from the intestine as SE belongs to this group suggesting a decrease in number of SE.

Studies have shown that SE colonizes the intestinal tract of laying hens and causes foodborne illness in humans, as fecal matter may contaminate shell eggs and further processing. Our findings demonstrate that adding dried, nonimmunized egg yolk powder at a concentration of 5% (wt/wt) to poultry feed can help to control SE colonization in the intestinal tract of laying hens. From our results, it is also suggested that administration of egg yolk powder without anti-*S. enteritidis* antibodies does not disrupt the intestinal microflora balance and has a preventive effect on SE infection. Future experiments

should determine how this technique could be developed as a practical intervention method for reducing human exposure to *Salmonella* as well as determining the exact active components in the egg yolk powder that are exhibiting such a beneficial effect.

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