Asian Journal of Poultry Science

ISSN 1819-3609

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS Asian Journal of Poultry Science

ISSN 1819-3609
DOI: 10.3923/ajpsaj.2020.1.5

Research Article
Reduction of *Salmonella* Enteritidis Colonization in Production Layers Fed High Levels of Mannan and Beta-glucan

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Abstract

Background and Objective: To combat the persistence of *Salmonella* in poultry products, intervention strategies that have efficacy in the digestive tract of birds are needed as part of a comprehensive food safety plan. A study was commissioned to evaluate the ability of a high mannan and β-glucan yeast fraction to prevent *Salmonella* Enteritidis colonization in laying hens. **Material and Methods:** Twenty-four Hy-Line pullets were placed in individual cages and at 17 weeks of age all 24 pullets were challenged orally with 7×10^7 CFU/bird of *Salmonella* Enteritidis. At 18 weeks of age, all ceca and ovaries were aseptically removed and cultured for *Salmonella* Enteritidis prevalence and number by the most probable number (MPN) method. **Results:** There was a numerical reduction of cecal *Salmonella* Enteritidis prevalence that was significant at $p = 0.089$ (16.7% positive) in the yeast fraction group versus the untreated control (58.3%). **Conclusions:** Reduction in the ceca of hens is an important result indicating this yeast cell wall can impact levels of *Salmonella* Enteritidis shed to the environment and thus reduce the potential of *Salmonella* Enteritidis contamination of the eggshell.

Key words: *Salmonella* Enteritidis, *Salmonella* reduction, food safety, yeast cell wall, beta-glucan, mannan, laying hens

Citation: P.T. Price, T. Gaydos, R. Berghaus and C. Hofacre, 2020. Reduction of *Salmonella* Enteritidis colonization in production layers fed high levels of mannan and beta-glucan. Asian J. Poult. Sci., 14: 1-5.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salmonella control measures have been applied in the processing of poultry products for many years at various stages. However, foodborne illness from *Salmonella* remains a concern with 450 deaths and 23,000 hospitalizations per year in the United States[1]. While pinpointing exact costs associated with *Salmonella* is difficult due to unreported or unconfirmed cases of illness, the USDA-ERS reports costs of around $46 million for cases in which the patients visited the physician and recovered. There are cost estimates of around $287 million for hospitalizations and when adding the cost of patients whose lives were lost after hospitalizations, the total estimate approaches $3.6 billion[2]. Of the nearly 2700 different *Salmonella* serovars, *Salmonella* Enteritidis (SE) remains the most prevalent cause of human infection and illness. The US incidence of SE infections in people was 7,830, representing 16% of all *Salmonella* infections in 2016. Interestingly, SE infections also rose by 16% from 2006-2016, raising concern over control measures in all areas of food production[3]. As an industry, poultry production has faced its share of challenges from widespread outbreaks of SE over the last 50 years, such as the 27 outbreaks that occurred in the northeast US associated with shell eggs in the span from[4] 1985-1987. The six-fold increase in SE illness rates from 1976-1995 corresponded with increased consumption of shell eggs and the number of SE positive hens in slaughter survey work[4-6]. The connection to the live bird as a critical source of disease concern should be evident when considering that levels of *Salmonella* illness have not gone down, despite the amount of foodborne illness monitoring and control measure in place[5]. *Salmonella* Enteritidis in particular has been shown to have high survivability in chickens, as He *et al*.[7] showed it was more able to invade macrophages and repress oxidative burst. Additionally, the study showed a high level of organ invasion.

Traditionally, carcass processing or egg processing has been the stage at which most *Salmonella* intervention measures have been applied, such as washing off any and all fecal contamination, spraying or dipping with antimicrobials and significantly lowering temperatures[8-10]. Current application of interventions in the US poultry industry consists of some mixture of biosecurity, nutritional and feed substrate management, health additives and biologicals. Focusing particularly on the application of health additives, it has been observed that there are varying levels of effectiveness in *Salmonella* control by organic acids, probiotics and prebiotics. Bourassa *et al*.[11] found that feeding propionic acid showed no difference in *Salmonella* litter or bird samples but showed that utilizing propionic acid in feed+formic acid in water, or formic acid in feed alone for the entire grow-out resulted in up to an 83% drop in prevalence. Use of butyric acid in various protected forms has been shown to have positive effects, such as reduction of SE shedding in broilers monitored in the crop, ceca and liver[12]. Use of probiotic products has shown promise as well, with *Bacillus* spp. being commonly chosen for commercially available products due to the spore forming heat stability of the organism. In a study with 192 Hy-Line layers, Upadhaya *et al*.[13] showed that both *Bacillus subtillus* and *Bacillus methylotrophicus* treatments reduced *Salmonella* positive layers over 1×10^1 CFU g^{-1} in a *Salmonella* Gallinarum challenge. *Bacillus subtillus* has also been effective in achieving reductions in *Salmonella* Heidelberg, though Hayashi *et al*.[14] showed that doses of 250 g and 500 g T^{-1} did not show any dose differentiation. Yeast products are a well-documented prebiotic source for controlling a variety of foodborne pathogens in poultry production[15-17]. While yeast are capable of competing for nutrients with bacteria, the derivatives of yeast such as the indigestible cell wall serve as substrates to beneficial bacteria[17]. In many cases, the manufacture of these carbohydrates is targeted to concentrate high mannose and β-1,3 glucan products for animal performance. However, certain pathogenic bacteria have demonstrated an affinity for binding or adhering *in vitro* to these cell wall materials. Bacteria with mannose-binding fimbriae could potentially show reduced colonization in the digestive tract of production poultry[18]. This study is focused on demonstrating the potential of a high concentration mannan and β-glucan cell wall to reduce intestinal colonization of laying hens by *Salmonella* Enteritidis.

MATERIALS AND METHODS

Study was carried out at the Southern Poultry Research Group facility in Nicholson, GA between the dates of May to July, 2018. Twenty-four, 9-week-old Hy-Line W-36 pullets were purchased from a commercial layer company. A commercially available yeast cell wall product was added to the pullet feed at 1 pound t^{-1}. Birds were provided with mash feed formulated to meet or exceed NRC standards and water *ad libitum* throughout the duration of the trial. The unit for each treatment was 12 cages of a battery, therefore each cage became a replicate. Birds were randomly assigned to treatments. There was 1, 12 cage battery per treatment giving each treatment group 1 sample time (7 days post-challenge). At 14 weeks-of-age the pullet day length was increased to 16 h to stimulate ovarian development. At 18 weeks-of-age each bird was orally challenged with 1 mL of 7×10^7 CFU/bird

of a nalidixic acid resistant strain of *Salmonella* Enteritidis. There was ambient humidity and controlled lighting to induce egg production at the appropriate age. On 7 days post-challenge all hens were humanely euthanized by cervical dislocation, ceca and ovaries aseptically removed, weighed and placed into sterile plastic sampling bags (Fisher Scientific) for *Salmonella* isolation. Each ovary clutch was placed in one bag, mashed together and cultured. All samples were stored on ice and taken to the onsite Southern Poultry Research Group, Inc. Laboratory for *Salmonella* analysis.

***Salmonella* isolation and identification:** Twenty milliliters of tetrathionate (Hajna) broth was added to each boot sock. Individual organ samples were weighed and tetrathionate broth was added to each ceca and ovary sample at 1 part sample to 9 parts broth to produce a 1:10 wt/vol dilution. Samples were mixed using a stomacher for 1 min and incubated for 24 h at 42°C. A loop full of incubated media was struck to Xylose Lysine Tergitol-4 (XLT-4) plates containing 25 µg of nalidixic acid mL^{-1} to facilitate selection of the antimicrobial-resistant challenge organisms and plates incubated for 24 h at 37°C. Suspect *Salmonella* colonies were confirmed and sero grouped using poly-O Salmonella-specific antiserum.

***Salmonella* enumeration via most probable number method:** *Salmonella* on all ceca and ovary samples were enumerated using a modification of the most probable number (MPN) method of Berghaus *et al*.[19]. A 1 mL sample of pre-incubation 1:10 tetrathionate broth from each sample was transferred to 3 adjacent wells in the first row of a 96-well 2 mL deep block. A 0.1 mL aliquot was transferred to 0.9 mL of tetrathionate broth in the 2nd row. This process was repeated for remaining rows producing 5, 10-fold dilutions. Blocks were incubated for 24 h at 42°C. One microliter of each well was transferred onto XLT-4 agar containing nalidixic acid with a sterile multichannel pipette and plates were incubated for 24 h at 37°C. The final dilution of each sample was recorded and MPN calculations were performed as previously described. Suspect *Salmonella* isolates were confirmed by poly-O antisera. The lower quantitative limit of the MPN was 0.3×10^0 MPN mL^{-1} and the upper quantitative limit was 1.1×10^5 MPN mL^{-1}.

Statistical analysis: *Salmonella* prevalences in ceca and ovary samples were compared between treatment groups using Fisher's exact test. *Salmonella* MPNs in culture-positive ceca samples were compared between treatments using linear regression. A Tobit censored regression model was also used to compare treatment groups with respect to *Salmonella* MPNs in ceca samples while considering culture-negative samples to be censored at a lower limit of -0.5 $\log_{10}$ MPN g^{-1}. For the comparison of *Salmonella* MPNs, samples with a negative culture result by the MPN method but a positive result by enrichment were arbitrarily assigned an MPN equal to one-half the minimum detection limit of the MPN assay. MPNs were log-transformed prior to statistical analysis. All statistical testing assumed a two-sided alternative hypothesis and $p<0.05$ was considered significant. Analysis were performed using commercially available statistical software (Stata version 15.1, StataCorp LLC, College Station, TX).

RESULTS AND DISCUSSION

None of the ovaries were culture positive for *Salmonella* Enteritidis in either treatment or control groups. The YCW treatment group had 16.7% *Salmonella* Enteritidis positive ceca compared to the control group 58.3% *Salmonella* Enteritidis positive ($p = 0.089$). The estimated marginal mean of *Salmonella* load in the ceca was low and similar between the YCW treatment 0.55 MPN g^{-1} and control 0.20 MPN g^{-1} ($p = 0.199$). These data are displayed in a dot plot in Fig. 1. When analyzing the MPN results using a Tobit censored regression the MPN g^{-1} of the level of SE in the ceca was reduced by 0.73 logs compared to the control ($p = 0.109$). The control group had MPN g^{-1} of -0.37 compared to the YCW treatment -1.17 MPN g^{-1}. *Salmonella* spp. can bind to mannose via the type-1 binding fimbriae. The cell wall fraction of *S. cerevisiae* has been shown to bind a variety of gram

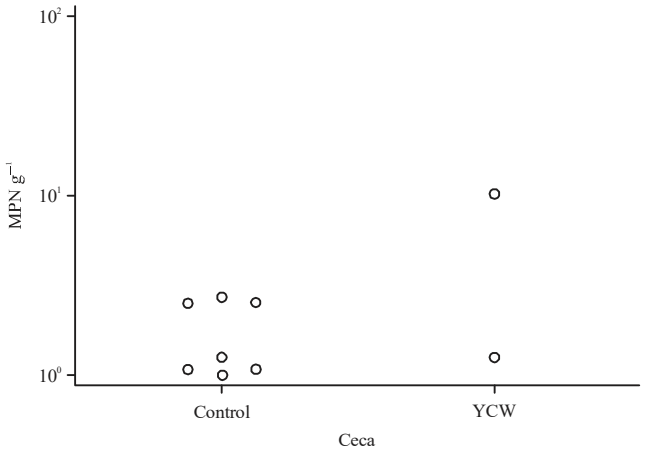

Fig. 1: Dot plot of *Salmonella* MPNs in culture-positive ceca samples collected from each treatment group control and yeast cell wall (YCW) 7 days post-challenge

negative organisms[18]. Reduction in *Salmonella* Enteritidis positive hens will reduce the overall load in the environment leading to reduced risk of eggshell contamination and transmission of foodborne illness. Yeast cell wall numerically reduced the prevalence of *Salmonella* Enteritidis in the ceca of the layer type hens. While there is limited data, generally the ability of feed additive products to reduce *Salmonella* in live birds is measured through enumeration as prevalence is not often reduced. The ability to show a full log reduction is often viewed as a threshold of biological significance when cecal prevalence is near 100%[20]. Prior work in cecal culture for *Salmonella* with the product in this study showed not only a log less CFU g^{-1} than control ($p<0.015$), but also 20% lower prevalence[21]. The use of YCW as a prebiotic in layer diets may decrease the prevalence of *Salmonella* Enteritidis leading to lower contamination of the environment effectively reducing the risk of the zoonotic transmission of *Salmonella* Enteritidis.

CONCLUSION

The use of YCW in layer diets can be part of a multi-hurdle approach to reduce the prevalence and tends to reduce the load of SE in layer chickens. Reducing the prevalence and load of SE positive hens reduces the total load of SE in the environment, likewise reducing the risk of contamination of eggs and eggshells entering the market.

SIGNIFICANCE STATEMENT

This study discovered that a high-quality yeast cell wall fraction can be beneficial for the reduction of *Salmonella* Enteritidis colonization in laying hens. This study will help the researcher to uncover opportunities for mitigating food safety risk in live production.

ACKNOWLEDGMENT

This trial was funded by Phileo by Lesaffre.

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