An update on the preharvest strategies to control multiple *Salmonella* serovars in turkeys

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Take-Home Message

The United States turkey industry is integral to providing animal protein to the growing population around the world. Ensuring the microbiological safety of turkey products is therefore critical for the sustainability of the turkey industry. A common foodborne pathogen, *Salmonella*, has been implicated in several foodborne outbreaks associated with poultry products, including turkeys. The pathogen has become an unavoidable cost in turkey production, and measures targeted to control foodborne *Salmonella* have become inevitable for the industry's viability. In addition to the historically important serovars in turkey production, an increased prevalence of emerging serovars has raised concerns, warranting acceleration of research to find solutions addressing them. Our team at the University of Minnesota has been focusing on various preharvest approaches against different serovars prevalent in turkey production and critical to food safety. We have found potential in two dairy/poultry – origin probiotics and a *Salmonella*-specific vaccine against *Salmonella* Heidelberg, a major *Salmonella* serotype problematic to the turkey industry. Our ongoing investigations are geared to testing these potential solutions against the emerging *Salmonella* Reading and others.

Introduction

The United States turkey industry produced 5.74 billion pounds of ready-to-cook turkey meat valued at more than $4 billion last year. Much of the turkey meat produced (5.22 billion pounds) was domestically marketed, and 558 million pounds of meat was exported (USDA ERS, 2020; USDA, 2021). Given the enormous volume of turkey meat produced and sold, it has become imperative for the industry to take proactive measures to render the products safe for human consumption. A major problem the turkey industry has faced historically is the prevalence of *Salmonella* in production. *Salmonella* is a contaminant in turkey production, as the bird could serve as a natural reservoir for the pathogen. The industry has control methods in place against *Salmonella* in production steps. Despite the control measures adopted, the contamination of turkey products with *Salmonella* has resulted in product recalls causing significant losses to the industry.

***Salmonella* Heidelberg and *Salmonella* Reading**

Through the years, *Salmonella* Heidelberg has become a common serovar in turkey production with high colonization potential and invasive ability in humans compared to the other prevalent serovars such as *Salmonella* Enteritidis (Nair et al., 2018a). In 2011, contaminated ground turkey was linked to a multistate outbreak of *Salmonella* Heidelberg, resulting in 136 illnesses across 34 states. Many *Salmonella* Heidelberg strains implicated in the outbreak were resistant to multiple antibiotics (CDC, 2011). More recently, emerging serovars of *Salmonella* have been associated with outbreaks linked to turkey products. For example, *Salmonella* Reading caused an outbreak from 2017 to 2019 linked to raw turkey products (CDC, 2019). While this serotype has been known for its prevalence in turkey production, it rarely had caused foodborne outbreaks. The current outbreak has raised concerns about other infrequently isolated yet prevalent serovars in turkey production capable of causing foodborne outbreaks

in the future. Hence, appropriate expansion of current and new strategies to include such emerging and potentially emerging *Salmonella* serotypes must be included in devising the control strategies to improve the microbiological safety of turkey products.

Targeted Approaches as Solutions

Currently, many strategies are employed to control *Salmonella* in poultry production, including turkeys. Organic acids, pre and probiotics, vaccination, and phytobiotics have been reported to reduce *Salmonella* in poultry production in academic research and supplier-led field studies. Our research team at the University of Minnesota has been investigating the potential of targeted probiotics, among other strategies, against the common and emerging *Salmonella* serovars in poultry, including turkeys. Some updates from our completed and ongoing projects in turkeys are described in this paper.

Among the targeted probiotic approaches being tested against *Salmonella* serotypes, *Propionibacterium freudenreichii,* a gram-positive probiotic bacterium isolated from fermented dairy products, milk, and dairy cattle, has shown excellent promise in our investigations. These probiotic bacteria had exhibited excellent probiotic qualities in humans and are approved by the FDA as generally recognized as safe (GRAS) for human consumption (Nair et al., 2018a, 2021). Our investigations at the University of Minnesota have also proved its potential beneficial effects in turkeys against *Salmonella* (Nair et al., 2018a, b, 2019, 2020, 2021). Besides *Propionibacterium*, two *Lactobacillus* species derived from the ileum of turkeys (*Lactobacillus salivarius* and *Lactobacillus ingluviei*) have also been found to be promising as targeted probiotics in turkeys for *Salmonella* control (Thomas et al., 2019; Dewi et al., 2021; Peichel et al., 2021).

Prior to our studies in turkeys, we validated the efficacy of both strains for their probiotic potential in lab assays. We found that the *Propionibacterium freudenreichii* strain and both *Lactobacillus* strains could resist a pH as low as 2.5 and bile salts up to 0.3%. This is significant because the probiotic strains must traverse through the adverse digestive and absorptive environment of the poultry gastrointestinal tract (GIT) to render its beneficial effect on the host. Both strains adhered to the avian epithelial cells without invading them, indicating their strong potential to colonize the GIT of poultry without causing invasive infections, a remarkable property of probiotics. Moreover, both strains were susceptible to the common antibiotics used for human therapy and did not exhibit hemolytic activity, indicating that the strains don't have resistance against the tested antibiotics and develop pathogenicity in humans (Thomas et al., 2019; Nair and Kollanoor Johny, 2018a).

Completed Studies

Our first study determined the efficacy of *Propionibacterium freudenreichii* against *Salmonella* Heidelberg in 2-week-old turkey poults. *Propionibacterium* was tested in two studies against *Salmonella* Heidelberg colonization of poult ceca and dissemination to liver and spleen. The four treatment groups were: negative control (NC), *Propionibacterium freudenreichii* control (PF), *Salmonella* Heidelberg control (SC), and a test group (PFS; PF + SH). The poults in the PF and PFS groups were supplemented with probiotics for 2 weeks in drinking water. On day 7, SC and PFS groups were inoculated with *Salmonella* Heidelberg at 10^6 CFU/ml. After 7 days, the cecum, liver, and spleen were collected for determining surviving *Salmonella* Heidelberg populations. Results indicated that *Propionibacterium* significantly reduced cecal colonization of *Salmonella* Heidelberg and the dissemination of the pathogen to the liver, compared to the SH challenge controls ($P<0.05$) (Nair et al., 2018a).

Following our successful investigations in poults, we determined *Propionibacterium*'s efficacy against *Salmonella* Heidelberg in growing and finishing turkey hens. Turkeys in the PFS group received *Propionibacterium* in drinking water until 6 weeks (growing turkey study) or 11 weeks (finishing turkey studies) of age. Turkeys in SC and PFS groups were inoculated with *Salmonella* Heidelberg as crop gavage. Results indicated that *Propionibacterium* significantly reduced *Salmonella* Heidelberg colonization in the PFS group by 1.3 $\log_{10}$ CFU/g (growing turkey study) and >2.0 $\log_{10}$ CFU/g (finishing turkey studies) in the turkey ceca ($P\leq0.05$). In addition, *Propionibacterium* supplementation reduced the dissemination of *Salmonella* to the internal organs ($P\leq0.05$) (Nair et al., 2019, 2020).

With the samples collected from the growing and finishing turkey studies, we explored the potential for microbiome modulation in the turkey cecum by *Propionibacterium* supplementation in conjunction with the *Salmonella* Heidelberg challenge, using Illumina MiSeq. Results indicated that the species richness and abundance were similar among the treatment groups. Treatments caused clustering of the samples among each other ($P<0.05$). Firmicutes were the predominant phylum in the growing and finishing turkey cecum. The *Salmonella* challenge resulted in modulation of *Streptococcus*, *Gordonibacter*, and *Turicibacter* ($P<0.05$) in the SH groups compared with the PC and NC groups. They are known to be associated with inflammatory responses in birds and mammals. The supplementation of PF increased the relative abundance of carbohydrate-fermenting and short-chain fatty acid-producing genera in the PC group compared with the S group ($P<0.05$), indicating its beneficial effects in turkeys (Nair et al., 2021).

Our next step was to determine if the effect of *Propionibacterium* was pronounced with other preharvest interventions against *Salmonella* Heidelberg in turkeys. In addition to the PF group, we included two additional treatment groups, a mannanoligosaccharide prebiotic (MO; 0.2% via feed) group, and a *Salmonella*-specific vaccine (VC; AviPro Megan Egg) group and tested them against *Salmonella* Heidelberg in 7-week-old commercial turkeys. These treatments were either applied alone or in combination with the others. PF and MO were supplemented from day 1 throughout the study, and the vaccine was applied as per the manufacturer's recommendations. Turkeys were challenged with *Salmonella* Heidelberg (10^6 CFU/turkey) at week 6. Results indicated that PF, MO, and VC treatments resulted in 1.0-, 1.5-, and 2.0- $\log_{10}$ CFU/g reduction in the cecal colonization of *Salmonella*, respectively, compared to the SH control at 2-days after inoculation ($P<0.05$). The combination of the three interventions resulted in an average 2.3 $\log_{10}$ CFU/g reduction ($P<0.05$) at 2 days after inoculation. At 7-days post-inoculation, the VC treatments were highly effective, resulting in the complete reduction of *Salmonella* Heidelberg when applied alone or in combination ($P<0.05$). In addition, VC treatments completely inhibited the dissemination of *Salmonella* to the liver and spleen ($P<0.05$) (Nair et al., 2018b).

Ongoing Studies

With the promising results with *Propionibacterium* and vaccine application on *Salmonella* Heidelberg, we are currently focusing on research targeting biocontrol of the emerging *S*. Reading using the *Propionibacterium,* a *Lactobacillus* probiotic strain isolated from turkey ileum and vaccination. In this study, *Lactobacillus salivarius* UMNPBX2 was supplemented through drinking water for the entire study period. Besides the turkeys in the NC group, all turkeys were inoculated with *S*. Reading. The study results indicated that the VC group resulted in the highest reduction across all studies (2.3 log reduction; $P<0.05$). *Lactobacillus* and the combination resulted in significant reductions ($P<0.05$). All treatments significantly reduced the dissemination of *Salmonella* Reading to the liver and spleen and reduced recovery from the crop (Peichel et al., 2021). Based on the results from these investigations, we are expanding our studies to other *Salmonella* serotypes and testing other emerging preventative approaches for improving preharvest food safety.

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