Targeted preharvest interventions against *Salmonella* in turkeys

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The problem of *Salmonella* in turkey production

Salmonella is a foodborne zoonotic pathogen prevalent in poultry production, including turkeys. The pathogen is a constant threat to human public health because of its potential to cause outbreaks involving illness cases, hospitalizations, and deaths. Poultry is a significant source of clinical, non-clinical, and antibiotic-resistant variants of *Salmonella* (CDC, 2021). *Salmonella* colonized in the cecum of turkeys and disseminated to the internal organs are considered a potential food safety hazard through products (USDA FSIS, 2021). Appropriate management practices and antimicrobial interventions during the preharvest phase can effectively control *Salmonella* and improve postharvest control of the pathogen. Moreover, the emergence of drug-resistant *Salmonella* in production environments has necessitated antibiotic stewardship as a responsibility of the turkey producers and integrators. The USDA recommends nonantibiotic interventions such as vaccines, prebiotics, probiotics, and organic acids in production (USDA FSIS, 2021). Although individual interventions have proven benefits, shifts in the dynamics of predominant serotypes encountered on farms, new serotypes emerging with more invasive capabilities, and multiple serotypes potentially colonizing in turkey gut demand innovative and broad approaches for *Salmonella* control. Our lab investigates several nonantibiotic interventions for the preharvest safety of turkeys, of which updated research is briefly presented in this document.

Non-host derived probiotics for *Salmonella* reduction

Several probiotics are investigated for their potential in pathogen control independently and in combinations through various commercial preparations. However, consistency in results is an essential determining factor for industry application. Our laboratory investigates a non-host derived probiotic, *Propionibacterium freudenreichii* subsp. *freudenreichii*, a dairy origin GRAS-status bacteria commonly used in cheese production, for its activity against *Salmonella* in turkeys. The bacterium shows antibacterial activity against many foodborne pathogens like *Escherichia coli* and *Listeria monocytogenes* (Nair and Johny, 2018). Previous studies from our lab showed that *Propionibacterium freudenreichii* subsp. *freudenreichii* can reduce multiplication, motility, cell association, and invasion of three major *Salmonella* serotypes in poultry (Nair and Kollanoor-Johny, 2017). The probiotic potential of *Propionibacterium freudenreichii* subsp. *freudenreichii* in turkeys was also tested by determining factors like its resistance to gastric pH and bile salts, hemolytic activity, invasive characteristics, and antibiotic susceptibility. The results from this study indicated that the probiotic is tolerant to low pH and bile salts with no hemolytic activity and invasive properties noted (Nair and Johny,

2018). In the *in vivo* studies, *Propionibacterium freudenreichii* subsp. *freudenreichii* was tested against *Salmonella enterica* Heidelberg in three different age groups. In these studies, turkeys were supplemented with the probiotic through drinking water. A reduction of 1.6 to 2.2 $\log_{10}$ of *Salmonella enterica* Heidelberg was obtained in the probiotic-supplemented groups compared to the positive control when bacteria were recovered at two weeks of age (Nair and Johny, 2018). Studies using turkey challenge models at the age of week 7 showed promising results in preventing invasion of *Salmonella enterica* Heidelberg to the spleen and liver. Only 37.5 % of liver and spleen samples were positive in *Propionibacterium freudenreichii* subsp. *freudenreichii* treated groups compared to the 87.5 % positive samples in the control group (Nair et al., 2019). Studies with finisher turkeys at 12 weeks of age indicated a 2 to 2.6 $\log_{10}$ reduction in *Salmonella enterica* Heidelberg populations in the cecum of probiotic-supplemented turkeys compared to the control groups (Nair et al., 2021b). Cecal microbiome studies in turkeys revealed that supplementation of *Propionibacterium freudenreichii* subsp. *freudenreichii* could modify microbial flora by increasing the carbohydrate fermenting and short-chain fatty acid-producing bacteria in the gut of turkeys (Nair et al., 2021a).

Host-derived probiotics for *Salmonella* control

Although several host-derived probiotics are investigated for their many benefits in the hosts, *Lactobacillus* species attracts special mention due to their historical safety margin and health benefits evident in animals and humans. Among the several species of *Lactobacillus*, *Lactobacillus salivarius* was investigated as a competitive exclusion bacterium against *Salmonella enterica* Enteritidis, *Salmonella enterica* Typhimurium, and *Salmonella enterica* Kentucky in the cecum of chickens. A dose of 10^6 to 10^8 log *Lactobacillus salivarius* through oral gavage was found to reduce 2 to 2.5 $\log_{10}$ *Salmonella* in the cecum of chickens (Zhang et al., 2007). Pascual et al. (1999) demonstrated that a single oral gavage with 10^8 *Lactobacillus salivarius* could result in 100% *Salmonella*-free chickens at 21 days post-inoculation. However, the authors recommended multiple doses per their observation that the *Lactobacillus* populations significantly diminishes between 21- and 28-days post-inoculation (Pascual et al., 1999).

Lactobacillus salivarius is also known for improving body weight, intestinal morphometry, and intestinal integrity in turkey poults. *Lactobacillus salivarius* was used to ferment soybean meal in the turkey diet and fed prophylactic and therapeutic regimens after a *Salmonella enterica* Typhimurium challenge. It was found that fermented soybean meal with *Lactobacillus salivarius* increased villi length, villus surface area, villus crypt ratio, and crypt depth (Morales-Mena et al., 2020). Recent studies from our lab had reported a significant reduction in multidrug-resistant *Salmonella enterica* Heidelberg in the cecum of turkeys when a combination of *Lactobacillus salivarius* UMNPBX2 and *Lactobacillus Ingluviei* UMNPBX19 was supplemented through drinking water (Thomas et al., 2019). Additionally, Peichel et al. (2021b) recently reported the antibacterial activity of *Lactobacillus salivarius* UMNPBX2 against outbreak causing *Salmonella enterica* Reading isolates in turkeys.

Vaccines for *Salmonella* control

Vaccination has been historically used to prevent several human and animal diseases. They work on the principle of developing immunity in the host. Vaccination is a promising pre-harvest strategy to control *Salmonella* in turkey flocks. A commercially available, USDA-approved *Salmonella* vaccine (AviPro Megan Egg, Elanco), containing live cultures of *Salmonella* enterica Typhimurium, is approved for turkeys. It is effective against *Salmonella enterica* Typhimurium in turkeys when vaccinated at day 1 of age and followed by revaccination at week 3 of age. However, studies on cross-protection to other serovars and the duration of immunity are limited in turkeys. Recently, Peichel et al. (2021a) reported cross-protection of AviPro Megan egg vaccine against *Salmonella enterica* Reading in three different age groups of turkeys. Approximately 2 $\log_{10}$ reductions of *Salmonella* in the cecum and significantly fewer *Salmonella* positive liver, spleen, and crop samples indicate its cross protectivity against an emergent and drug-resistant clone of *Salmonella enterica* Reading (Peichel et al., 2021a).

Studies on AviPro *Salmonella* Duo containing metabolically mutant strains of *Salmonella enterica* Typhimurium and *Salmonella enterica* Enteritidis showed that it could reduce *Salmonella* colonization in the cecum and invasion to internal organs (Hesse et al., 2018a). The researchers also found high antibody titers in turkeys receiving multiple boosters (Hesse et al., 2018a). Similarly, studies on the clearance of vaccine strain showed that it is more likely to re-isolate both *Salmonella enterica* Enteritidis and *Salmonella enterica* Typhimurium vaccine strains until day 14 of vaccination after the first dose. But with each booster dose, re-isolation was reduced as antibody titers increased (Hesse et al., 2018b). Similarly studies investigating BBS 866, a live attenuated *Salmonella enterica* Typhimurium vaccine, proved to provide cross-protection against *Salmonella enterica* Heidelberg in turkeys (Bearson et al., 2019).

Ongoing Investigations

As discussed earlier, preharvest interventions using probiotics and vaccines have been proven to have independent antibacterial effects against multiple *Salmonella* serovars. A potentially enhanced activity exists if these interventions are used in combinations, especially against the outbreak causing *Salmonella* strains in turkey production. In a recently completed study from our lab, Dewi et al. (2021) used a combined and targeted approach using turkey-derived *Lactobacillus* strains and an essential oil ingredient, *trans* cinnamaldehyde against *Salmonella enterica* Heidelberg in turkey poults. This combination treatment resulted in more than 2.5 $\log_{10}$ *Salmonella enterica* Heidelberg in the cecum compared to a lower magnitude of reduction obtained from individual treated groups (Dewi et al., 2021). In another study from our lab, Peichel et al. (2021b) combined *Lactobacillus salivarius* with *Salmonella* Typhimurium vaccine for enhanced benefits in turkeys. These results suggest more studies on choosing targeted combination approaches against *Salmonella* in turkey production. Currently, our lab group is testing the combined effect of probiotics and vaccines against other emerging *Salmonella* serovars in turkey production. We are also invested in understanding better the mechanisms of action of the combination approaches using molecular approaches.

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