A220, an all-natural feed additive, reduced *Salmonella* intestinal colonization in broilers and tuned down SPI-1 Type III Secretion System (TTSS) virulence machinery

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Antibiotic resistance concerns have driven the need for alternative solutions to antibiotics in reducing *Salmonella* colonization in broiler chickens at the farm level. A220, is an all-natural feed additive that features a proprietary blend of essential oils, fatty acids and an enterosorbent mineral. This formula has been shown to neutralize a variety of key virulence factors of pathogenic bacteria in addition to exerting direct bacteriostatic/ bacteriocidal effects. This study was This study was aimed to evaluate *in vitro* and *in vivo* effects of A220 on *Salmonella enterica* sv. *Typhimurium* (ST) infection in broiler chickens.

An *in vitro* digestion model simulating the pH and enzymatic conditions of 3 gastrointestinal segments (crop, proventriculus and intestine) was first used to evaluate the antibacterial effects of A220 on ST. In the *in vivo* trial, one-day old male broiler chicks were randomly allocated to one of three groups (n=30 chickens), i.e., challenged control with non-treated feed and A220 supplemented at 0.25% and 0.5% in feed. All groups were challenged with ST (10^6 cfu/bird) via oral gavage on d9. In the *in vitro* trial, A220 significantly reduced total CFU of ST recovered in the proventriculus and intestinal segments compared with control ($P < 0.05$). *In vivo*, A220 0.25 and 0.5% reduced ($P < 0.05$) the total cfu recovered and total prevalence of ST in the ceca. A220 at both doses significantly reduced gut permeability after ST challenge as measured by the serum FITC-dextran levels ($P < 0.05$). Further, A220's effects on ST's *Salmonella* pathogenicity island-1 virulence network development were explored via treating ST at subinhibitory concentration (1 mg/mL) of A220 *in vitro*. Compared to the control, A220 downregulated ST hilA and invF mRNA expression, which further blocked expression of key downstream effectors involved in ST invasion. Collectively, A220 had the potential to reduce ST colonization in broiler chickens and preserve the functional integrity of the intestinal barrier of chickens during ST challenge. This is at least in part attributed to its antivirulence properties by interfering with SPI-1 TTSS virulence machinery.

Key Words: *Salmonella Typhimurium*, intestinal colonization, virulence gene expression, *Salmonella* pathogenicity island-1, natural antimicrobial