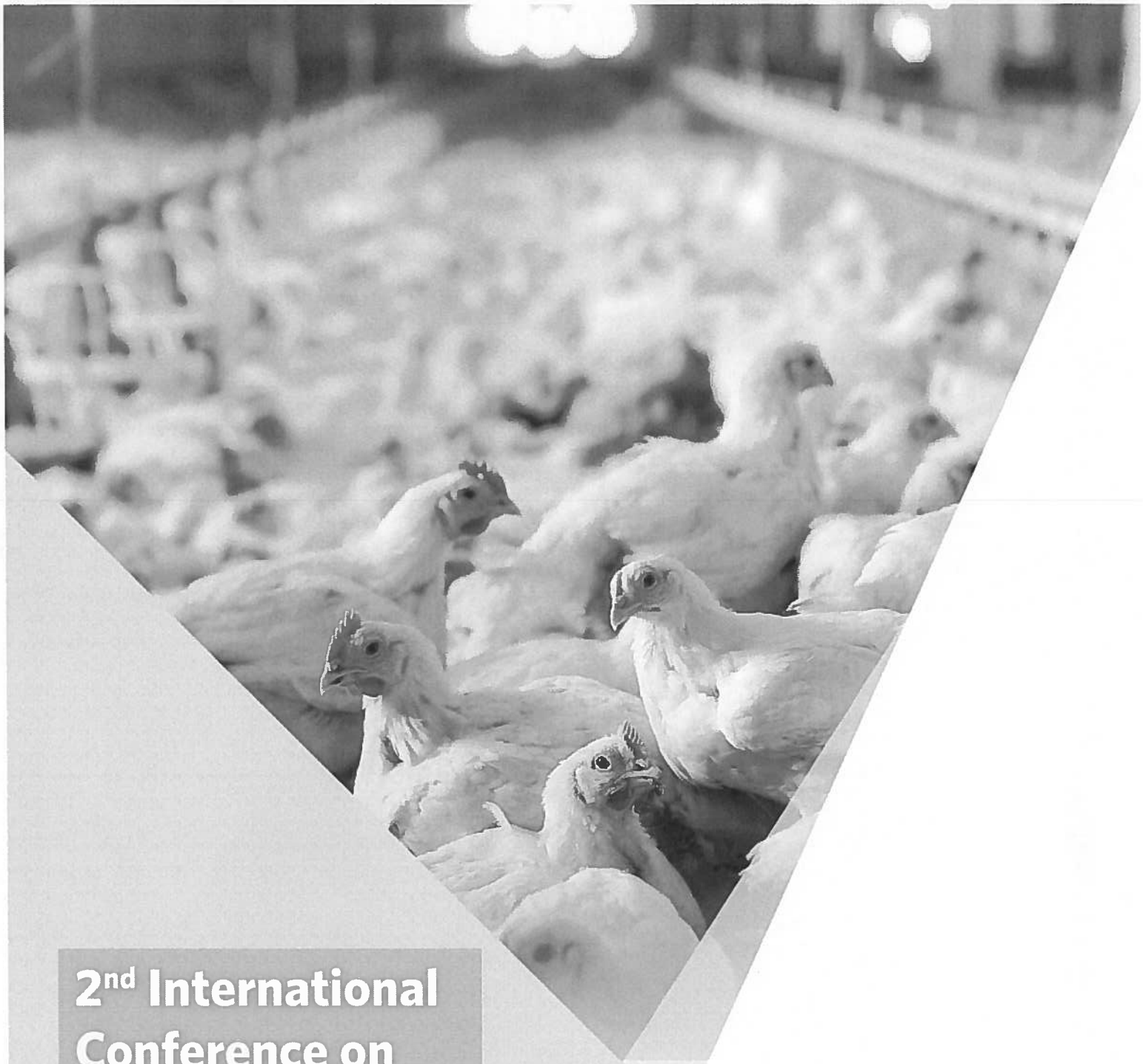

2nd International Conference on Necrotic Enteritis

July 11-12, 2018
Denver, Colorado, USA

Program

2nd International Conference on
Necrotic Enteritis in Poultry

Evaluation of the *in vitro* and *in vivo* antibacterial effects of NeutraPath™

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A formulated feed additive, NeutraPath consists of a synergistic blend of antimicrobial fatty acids, essential oils and a proprietary bacterial toxin adsorbent that is optimized for pathogen control in livestock production. An *in vitro* assay was used to determine minimal inhibitory concentrations (MICs) of NeutraPath™ against *Clostridium perfringens*, *Escherichia coli* O157, *Salmonella* ser. Heidelberg and *Vibrio parahaemolyticus*. With MICs of 3 to 5 mg/ml, NeutraPath exhibits strong antibacterial activity *in vitro*.

Two studies using an experimental model of *C. perfringens*-induced necrotic enteritis (NE) evaluated the *in vivo* antibacterial effects of NeutraPath. Day-old male Cobb 500 broilers were randomly assigned to treatments: non-challenged control, challenged (CH) control, BMD (55 ppm bacitracin) and NeutraPath (0.17% active ingredients). Except for the non-challenged group, birds received ~10^8 CFU of *C. perfringens* on three consecutive days.

In Study 1, NeutraPath, but not BMD, showed a trend in reducing ileal *C. perfringens* populations, as enumerated by cultivation-based MPN method (P=0.056). This reduced bacterial load may explain the observed improvement in mortality (27% vs. 54%, P<0.05) and NE-related lesion scores (0.6 vs. 1.2, P<0.05) between NeutraPath treatment and CH control.

To further investigate the decreased bacterial load, Study 2 used an ELISA to quantify *C. perfringens* and its □-toxin in cecal contents. Results showed NeutraPath reduced *C. perfringens* populations 7.7-fold (P<0.05) and □-toxin levels 4-fold (P<0.05) compared to CH control. These reductions were associated with significantly improved mortality (26.6% vs. 4.7%, P<0.05).

Collectively, these studies show NeutraPath exerts potent *in vitro* and *in vivo* antibacterial effects that reduce bacterial load and can be used to control *C. perfringens*-induced necrotic enteritis in broilers.