Research Notes P-98
Arm & Hammer Animal and Food Production

CELMANAX reduced adhesion of *Salmonella* and *Campylobacter* to poultry epithelial cells *in vitro*.

This research note summarizes original data presented as abstract #216 at IPSF, Atlanta 2020 by Laney E. Froebel, Lindy K. Froebel, and Tri Duong, Department of Poultry Science, Texas A&M University, College Station, TX.

INTRODUCTION

Most, if not all, interactions of microbial pathogens with their hosts are influenced to an important degree by the pattern of glycans (polysaccharides) and glycan-binding receptors that each expresses.[1] One of the most studied and reported interactions is between gram negative bacteria with type 1 fimbriae and mannan oligosaccharides (MOS) and its effect in reducing colonization in animals under *in vivo* conditions.

However, at the cellular level, this interaction has not been well studied. This study[2] examined the effect of MOS and other similar prebiotics (fructooligosaccharide (FOS), galactoligosaccharide (GOS) and raffinose (RAF)), as well as the effect of mannose, MOS and beta 1-3, 1-6 glucans individually, to reduce adhesion of *Salmonella* and *Campylobacter* using a chicken epithelial cell line *in vitro*.

STUDY OVERVIEW

- An adhesion inhibition assay was performed where both prebiotic and *Salmonella typhimurium* or *Campylobacter jejuni* were added concurrently for co-incubation with the chicken LMH epithelial cell line (Table 1).
- Adherent *Salmonella* and *Campylobacter* were enumerated using XLT-4 and Campy Cefex agar, respectively.
- The prebiotic adhesion inhibition was calculated relative to untreated cells as a percentage.
- Experiment 1 was a dose study with CELMANAX™ Refined Functional Carbohydrates™ (RFCs™). Experiment 2 tested independent components of CELMANAX, and experiment 3 tested common prebiotics, including CELMANAX.

TABLE 1 Composition of purified carbohydrates used.

Compound used in study	Purity %
B1-3, 1-6 Glucan	80
FOS	95
GOS	55
MOS	99.9
RAF	99

FIGURE 1: Dose effect of CELMANAX on inhibition.

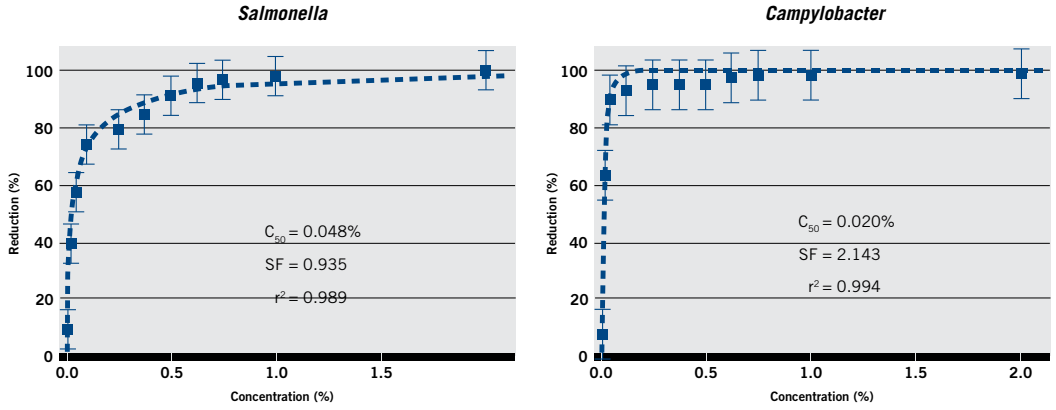


FIGURE 2: Effect of individual components of CELMANAX on inhibition.

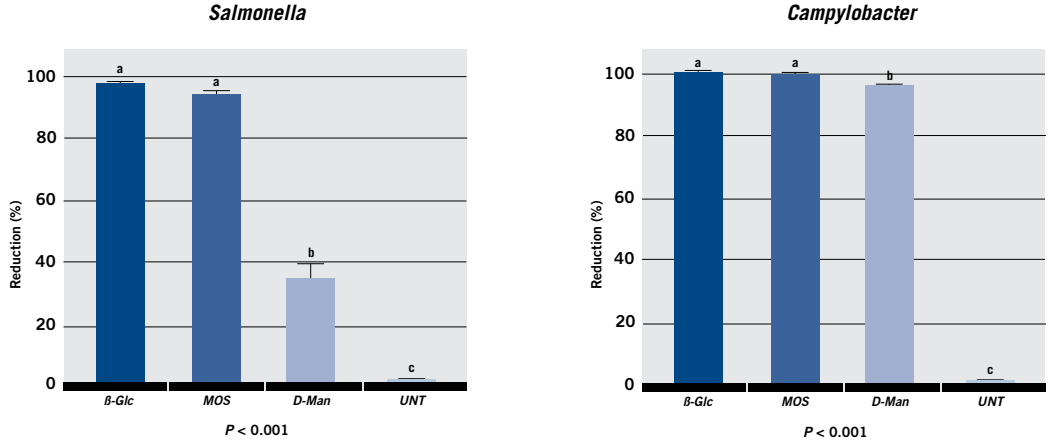
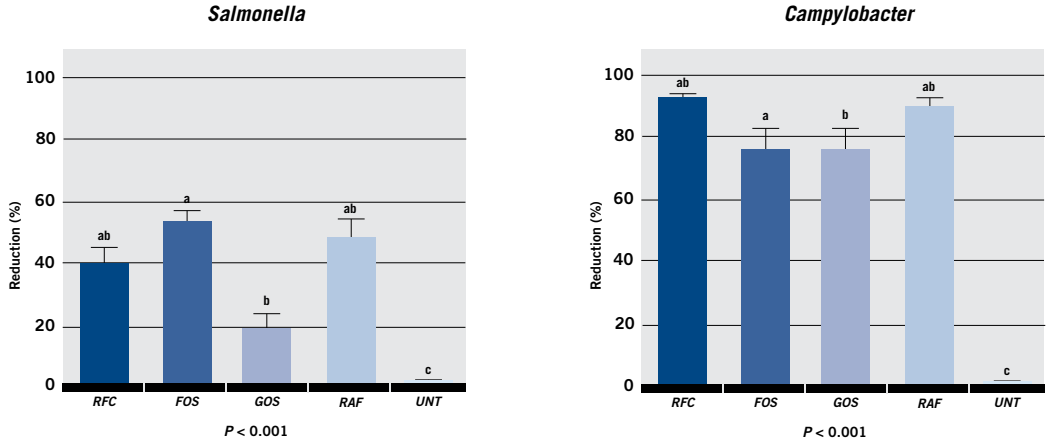


FIGURE 3: Effect of 0.1% prebiotics on inhibition.



RESULTS

- **Experiment 1:** A direct dose dependent effect of CELMANAX™ was noted on reduction of adhesion of both *Salmonella* and *Campylobacter* to LMH cells (Fig. 1). The half maximum inhibitory concentration of CELMANAX for *Salmonella* and *Campylobacter* was 0.048% and 0.02%, respectively.
- **Experiment 2:** When the individual components of CELMANAX were tested, inhibition was highest with β 1-3, 1-6 glucan and MOS and intermediate with D-Mannose compared to untreated cells (Fig. 2).
- **Experiment 3:** When inhibition property of four prebiotics (CELMANAX, FOS, GOS, RAF) was tested, all prebiotics inhibited *Salmonella* and *Campylobacter* compared to untreated cells. Inhibition of *Salmonella* was greatest with FOS, CELMANAX and RAF, with GOS being intermediate. Inhibition of *Campylobacter* was greatest with CELMANAX and RAF, with FOS and GOS being intermediate (Fig. 3).

CONCLUSION

CELMANAX™, its individual components, and the other prebiotics tested reduced adhesion of *Salmonella* and *Campylobacter* to chicken LMH epithelial cell line in this trial. The IC^{50} for CELMANAX observed for *Salmonella* (0.048%) and *C. jejuni* (0.02%) in this study falls reasonably within the label recommended rate of CELMANAX.

The ability of MOS to inhibit *Salmonella* seen in this study aligns with the literature,[3] but its ability to inhibit *Campylobacter* has not previously been observed. In this trial, β 1-3, 1-6 glucan was found to have as good an adherence inhibition effect on *Salmonella* and *Campylobacter* as MOS.

The adherence inhibition appears to be a property of other prebiotics besides MOS. However, the efficacy and pathogen specificity were different for each prebiotic. This study explains a potential mechanism for the reduction in *Salmonella* and *Campylobacter* colonization reported in CELMANAX-supplemented poultry and livestock in previous studies.[4-9]

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1 Nizet V, Esko JD. Bacterial and Viral Infections. In: Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 39.: https://www.ncbi.nlm.nih.gov/books/NBK1952/

2 Froebel LE, Froebel LK, Duong Tri. Department of Poultry Science, Texas A&M University. Abstract #216. Presented at IPSF, Atlanta, 2020.

3 Baines, et al. Prebiotics and probiotics reduce enterotoxigenic *Escherichia coli* and *Salmonella enterica* infectivity in an *in vitro* bovine cell model. Abstract #108. Presented at the Gut Health Symposium in St. Louis, Mo., 2013.

4 Froebel, et al. Administration of dietary prebiotics improves growth performance and reduces pathogen colonization in broiler chickens. *Poultry Science* 2019;98:6668-6676.

5 Walker, et al. Effect of refined functional carbohydrates from enzymatically hydrolyzed yeast on the presence of *Salmonella* spp. in the ceca of broiler breeder females. *Poultry Science* 2017;96:2684-2690.

6 Walker, et al. The effect of refined functional carbohydrates from enzymatically hydrolyzed yeast on the transmission of environmental *Salmonella* Senftenberg among broilers and proliferation in broiler housing. *Poultry Science* 2018;97:1412-1419.

7 Huff, et al. The effects of yeast feed supplementation on turkey performance and pathogen colonization in a transport stress/*Escherichia coli* challenge. *Poultry Science* 2013;92:655-662.

8 The effect of CELMANAX SCP on fecal pathogen shedding, health, and performance of preweaned Holstein dairy calves. Raabis, Ollivett. Presented at AABP, Omaha, Neb., 2017.

9 Hofacre, et al. Effect of a Yeast Cell Wall Preparation on Cecal and Ovarian Colonization with *Salmonella enteritidis* in Commercial Layers. *J Appl Poult Res* 2018;27:453-460.