Assessment of *Salmonella* Dublin infection of intestinal porcine epithelial cells (IPEC) in response to Zinc Oxide and a Yeast Mannan Rich Fraction

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Background

In swine, *Salmonella* infections can impair barrier function of the intestinal epithelium. A yeast mannan rich fraction (MRF) that structurally resembles the receptor sites of the intestinal epithelium is utilised to act as a decoy receptor for pathogens like *Salmonella* to adhere to thus preventing infection. Mannan from yeast has been demonstrated to limit infection in animals susceptible to gastrointestinal infection including pigs, poultry and cows by blocking the mechanism by which gram-negative bacteria adhere to and invade the intestine. *Salmonella* species present in pigs are associated with both post weaning diarrhoea and food borne diseases that are transmittable to the human consumers which can have potentially life-threatening consequences to the end consumer.

Objective

This works objective was to assess MRF alongside the industry standard treatment of Zinc Oxide (ZO) *in vitro* to determine its impact on *Salmonella Dublin* infection of a juvenile pig intestinal cell line (IPEC).

Material and methods

IPEC cells were exposed to MRF or Zinc Oxide in the presence of *S. Dublin* (1x108/mL). IPEC cells were lysed in RTL buffer, ruptured and RNA isolated (RNeasy Micro Kit, Qiagen). RNA RIN values above 8 were used to synthesise cDNA (SuperScript® III, Invitrogen). Gene expression for primers sets IL-1β, *TNFα*, *IL-8* and cellular tight Junction genes *Occludin*, *Claudin3* and *Tight junction protein1* (*TJP1*) were assessed by qPCR on the Applied Biosystems 7500 Fast qPCR.

Adhesion of *S. Dublin* to the surface of IPEC intestinal cells was carried out at a 500:1 ratio in the presence of *S. Dublin* alone, with Zinc Oxide or MRF for 1 hour at 37°C. Unattached *S. Dublin* was washed away, IPEC cells were lysed and diluted prior to plating on MacConkey's agar. Colonies were enumerated after incubating over night at 37°C.

Three independent biological replicates were performed for all experiments and statistical analysis carried out using One-way ANOVA, *P≤0.05, **P≤0.01 and ***P≤0.001.

Results

Adhesion of *S. Dublin* to IPEC cells was significantly reduced in response to MRF addition (8.85x105±4.47 x105) compared to Zinc Oxide treated cells (2.07x106±3.50x105, P≤0.001) and the control cells (2.54x106±1.64x105, P≤0.05) (Figure 1). Zinc Oxide treated cells demonstrated no change over the control cell group level of attachment highlighting Zinc Oxide's inability to impair bacterial attachment to the surface of intestinal cells.

Fig. 1 IPEC cells infected with *Salmonella Dublin* with and without treatments

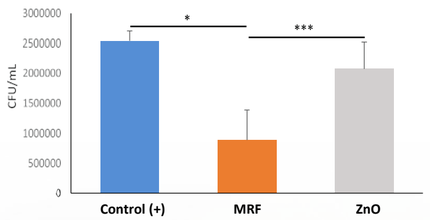

Figure 1, Attachment of *Salmonella Dublin* to IPEC intestinal cells following treatment with MRF, Zinc Oxide or no treatment (Control (n=3).

Junctional genes responsible for barrier function in the intestinal tract demonstrated significantly higher expression in IPEC cells exposed to *S. Dublin* in the presence of MRF for *Occludin* (1.78±0.07, P≤0.01), *Claudin3* (5.22±0.169, P≤0.001) and *TJP1* (2.05±0.06, P≤0.05) compared to the Zinc Oxide treated and infected cell levels for *Occludin* (1.10±0.22), *Claudin3* (1.10±0.32) and *TJP1* (1.44± 0.26) respectively (Figure 2).

Junctional gene expression

Fig. 2A *TJP1* gene expression of IPEC-J2 cells in response to *Salmonella Dublin* infection with treatments

Fig. 2B *Occludin* gene expression of IPEC-J2 cells in response to *Salmonella Dublin* infection with treatments

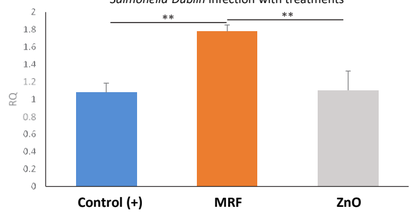

Fig. 2C *Claudin 3* gene expression of IPEC-J2 cells in response to *Salmonella Dublin* infection with treatments

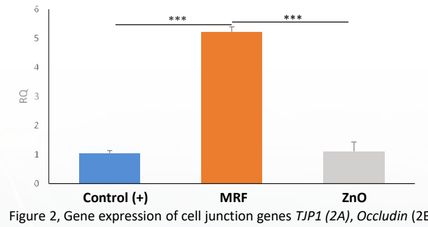

Figure 2, Gene expression of cell junction genes *TJP1 (2A)*, *Occludin* (2B) and *Claudin3* (2C) from IPEC intestinal cells infected with *Salmonella Dublin* and treated with MRF, Zinc Oxide or no treatment (Control), (n=3).

Proinflammatory gene *TNFα* was significantly reduced following *S. Dublin* infection and treatment with MRF (0.039±0.006, P≤0.001) compared to infected cells treated with Zinc Oxide (201.56±56.89), *IL-1β* demonstrated no change between treatments although *IL-8* gene expression was significantly reduced in both Zinc Oxide (0.499±0.13, P≤0.01) and MRF (0.61±0.043, P≤0.05) treated cells over the control (Figure 3).

Inflammatory gene expression

Fig. 3A *TNFα* gene expression from IPEC-J2 cells in response to *Salmonella Dublin* infection with treatments

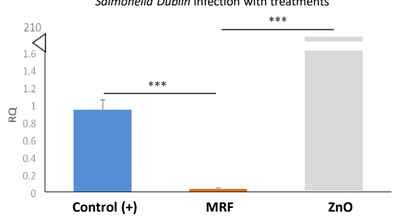

Fig. 3B *IL-1β* gene expression from IPEC-J2 cells in response to *Salmonella Dublin* infection with treatments

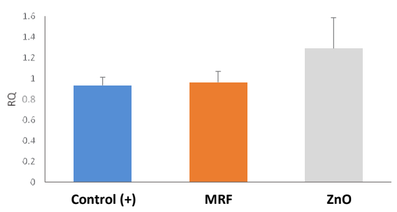

Fig. 3C *IL-8* gene expression from IPEC-J2 cells in response to *Salmonella Dublin* infection with treatments

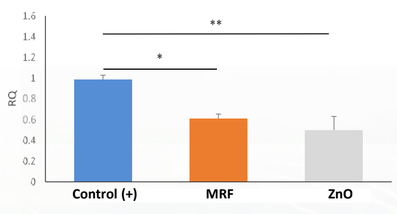

Figure 3, Gene expression of inflammatory markers *TNFα* (3A), *IL-1β* (3B) and *IL-8* (3C) from IPEC intestinal cells infected with *Salmonella Dublin* and treated with MRF, Zinc Oxide or no treatment (Control), (n=3).

Conclusion

Yeast mannan oligosaccharides have previously been highlighted for its ability to agglutinate *Salmonella* species (Spring et al., 2000). It was also demonstrated in a recent study that MRF could reduce *E. coli* attachment to intestinal cells. The reduction of *E. coli* attachment to intestinal cells by MRF addition appeared to have a direct impact on lowering inflammatory markers for infection on a gene and protein level (Browne et al., 2019) and similarly observed here.

- Both on a physical and molecular level *S. Dublin* infection of intestinal cells was more significantly impaired by MRF addition.
- Genes associated with tight barrier function between cells was significantly higher in MRF treated cells over both the control and Zinc oxide treated cells which coincided with the greater reduction of bacterial attachment to intestinal cells with MRF addition.
- Key gene inflammatory markers for infection were reduced with the addition of MRF over both the control and Zinc oxide treatments which correlates to the lower level of *S. Dublin* attachment following MRF addition to intestinal cells.

With the ban on Zinc Oxide, yeast MRF may prove to be a suitable alternative to Zinc Oxide in managing pathogen load and infection in young pigs.

References

Spring P, Wenk C, Dawson KA, Newman KE. The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. Poult Sci. 2000; 79: 205–211.

Browne N, Traynor A, Horgan KA. Mannan rich fraction from yeast modulates inflammatory responses in intestinal cells (HT-29) exposed to Escherichia coli. Journal of Applied Animal Nutrition, In Press, 2019.