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

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Background and objectives

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 intestines. Enterotoxigenic *Escherichia coli* (ETEC) cause post weaning diarrhoea (PWD) which results in poor weight gain and potential death at great economic cost to the farmer. A yeast mannan rich fraction (MRF) was assessed alongside the industry standard treatment of Zinc Oxide *in vitro* to determine its impact on ETEC infection on an IPEC intestinal cell line.

Material and methods

IPEC cells were exposed to MRF or Zinc Oxide alone or in the presence of *E. coli* ($1x10^8$/mL). 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*, *ZBP-1*, *IL-8* and *IL-2* were assessed by qPCR on the Applied Biosystems 7500 Fast qPCR.

Adhesion of *E. coli* to the surface of IPEC intestinal cells was carried out at a 500:1 ratio in the presence of *E. coli* alone, with Zinc Oxide or MRF for 2 hours at 37°C. Unattached *E. coli* 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.

Results

Gene expression for inflammatory genes *Z-DNA Binding protein* ($P \leq 0.05$), *IL-1β* ($P \leq 0.001$) and *TNFα* ($P \leq 0.05$), was significantly reduced following *E. coli* infection and treatment with MRF compared to infected cells treated with Zinc Oxide. Similarly, chemoattractant genes *IL-2* ($P \leq 0.001$) and *IL-8* ($P = 0.35$) demonstrated significantly lower or a trend towards a significant drop respectively for IPEC cells exposed to *E. coli* with MRF treatment when compared to the Zinc oxide treated and infected cells. Adhesion of *E. coli* to IPEC cells was significantly reduced in response to MRF addition compared to Zinc Oxide treated cells ($P \leq 0.001$) and the control cells ($P \leq 0.05$). Zinc Oxide treated cells demonstrated no change over the control cell groups level of attachment highlighting Zinc Oxide's inability to impair bacterial attachment to the surface of intestinal cells.

Conclusion

Both on a physical and molecular level bacterial infection of intestinal cells was only impaired by MRF addition. With the ban on Zinc Oxide, yeast MRF may prove to be a suitable alternative to Zinc Oxide for improved gut health in young pigs.