Anti-inflammatory feed additives can be an alternative to zinc oxide against post-weaning diarrhoea

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Background and objective

During the post-weaning period, inflammatory cytokines increase along the entire intestinal tract (Pié et al., 2004). Mucosal integrity is disrupted by inflammation with the loss of the barrier function in the gut (Grilli et al., 2015a), leading to post-weaning diarrhoea or enhancing its severity. Organic acids (OA) and pure botanicals (PB) are commonly used as feed additives in swine. They have a positive impact on live performance and an intrinsic antimicrobial activity (Grilli and Piva, 2012; Grilli et al., 2010). Moreover, they reduce the inflammatory status and down-regulate inflammatory genes expression in the intestinal mucosa (Grilli et al., 2015b). This can eventually lead to a reduction of clinical signs of diarrhoea in piglets. The aim of our studies was to assess if a blend of OA and PB can face an inflammatory challenge with a preventive or therapeutic effect on intestinal cells.

Material and methods

Study 1 was designed to determine if a treatment with OA and PB could reverse the damage induced by an inflammatory insult. To do so, Caco-2 cells were cultured in basal medium with two different concentrations (0.2 and 1 g/L) of a balanced blend of OA and PB for 15 days. At day 0, cells were challenged bacterial LPS and inflammatory cytokines for 24 hours.

Study 2 was designed to determine if a treatment with OA and PB could prevent the damages associated to the same inflammatory insult. This time Caco-2 cells were cultured in the basal medium with OA and PB for 14 days prior to the challenge.

In both experiments, transepithelial resistance (TER) was measured as an indicator of mucosal integrity. Gene expression analysis of tight junctions (TJ) markers (ZO1 and occludin) was performed by qPCR. Data were analyzed with 1-way ANOVA (qPCR) or ANOVA repeated measures (TER) and each treatment had 6 independent replicates (n=6).

Results

Study 1: LPS challenge at day 0 significantly decreased TER by 40% in as little as 24 hours. Over the course of the experiment, the control recovered up to 95% of the initial TER value. Treated cells, instead, reached 112-115% of the initial TER value in just 5 days. The treatment with OA and PB increased TJ markers expression in a dose-dependent fashion.

Study 2: Over the course of the experiment, treated cells had higher TER compared to the control. LPS challenge at day 14 significantly decreased the control TER by 44%, while TER was only reduced by 17% in the 0.2 g/L treatment. The highest dosage (1 g/L) completely protected cells against the inflammatory challenge, with a TER at day 15 of 103% compared to the beginning. The treatment with OA and PB at 1 g/L significantly increased TJ markers expression.

Discussion and conclusion

Our results demonstrate that OA and PB improve resistance of intestinal cells to inflammatory challenge both in a preventive or therapeutic way. This is of pivotal importance in the post-weaning period as piglets need protection at the mucosal level to improve barrier function and intestinal health. In fact, preserving barrier function prevents bacterial translocation, local and systemic inflammation and ensures a better absorption of nutrients. These *in vitro* studies open the door to an in-field feed additive alternative to ZnO at pharmacological dosages.

References:

Pié et al. (2004). J. Nutr. 134(3):641-647; Grilli et al. (2015a). Animal. 9(11):1760-1768; Grilli et al. (2015b). BMC Vet. Res. 11:96; Grilli and Piva. (2012). In: On-farm strategies to control foodborne pathogens. Nova Science publisher.; Grilli et al. (2010) Livest. Sci. 133:173-175;