Stressors like pathogens and toxins increase the gut permeability leading often to chronic inflammation and disease such as Necrotic Enteritis.

Deoxynivalenol (DON) increases the gut permeability and predisposes for Clostridium perfringens induced Necrotic Enteritis in broiler chickens. In other words, DON can replace coccidiosis (Eimeria) as a predisposing factor for NE.

Transepithelial electrical resistance (TEER) is a widely accepted technique to measure the integrity of tight junction dynamics in cell culture models and the IPEC-J2 cell line is a well-established model for studying intestinal barrier functions.

We here demonstrated that Lumance® enhanced significantly the tight junctions even under bacterial enterotoxin (LPS) challenge in IPEC-J2 cell.

Introduction

The intestinal tract is lined by a simple epithelium, consisting of a monolayer of epithelial cells which constitutes the most extensive and important barrier between the body’s internal milieu and the external environment (Groschwitz & Hogan, 2009). The epithelium is unique because it not only absorbs nutrients but also harbors many microbes and substances including harmful pathogens and toxins that have potential to threaten animal health (Oshima and Miwa, 2016). Besides the selective absorption and protection, the intestinal epithelium spatially segregates the gut microbiota and the host immune system to avoid unnecessary immune responses that lead to intestinal inflammation (Okumura & Takeda, 2017).

The role of the epithelium in maintaining the delicate balance between absorbing digestive nutrients and preventing entry and subsequent response of harmful contents, is essential for animal health and productivity (Salim & Söderholm, 2011). Stressors, pathogens and toxins, among others, may increase the permeability of this natural barrier, facilitating the invasion of harmful macromolecules and micro-organisms, leading to chronic inflammatory response and pathogenesis of several diseases such as necrotic enteritis (Chida, An, & Soda, 2009).

For instance, it was demonstrated that mycotoxin deoxynivalenol (DON) has a cytotoxic effect on epithelial cells, leading to an altered intestinal barrier function, which results in an increased permeability of the intestinal wall to enteric bacteria such as Clostridium perfringens induced necrotic enteritis in broiler chickens (Antonissen et al., 2014). This coincides with negative effects on villus height, tight junctions, mucus, oxidative stress and reducing the ability to digest and absorb the nutrients which unavoidably lead to important economic losses for the producer (Antonissen et al., 2015). Therefore, maintenance of intestinal homeostasis and barrier function, along with other supportive...
mechanisms is critically important especially in periods of stress and disease. A combination of different active ingredients having the ability to act synergistically with positive effects on intestinal barrier function, immune response, epithelial cell proliferation and bacterial pathogenesis may hold the most promising approach to promote and protect gut health. Lumance® (Innovad, NV/SA Belgium) is a complex blend, combining target-release butyrate, fatty acids, plant extracts and essential oils. In our previous studies, we demonstrated the synergistic anti-inflammatory and anti-bacterial properties of Lumance® in in-vitro and in-vivo (Khadem et al., 2017, 2018 a,b). The aim of this study was to investigate the effect of Lumance® on the intestinal barrier function and integrity. To this, the intestinal porcine epithelial cell line (IPEC-J2), originally from the jejunum of a neonatal piglet, was used as the experimental model (Figure 1).

**Materials and methods**

The Intestinal porcine epithelial cell (IPEC-J2) were cultured in a humidified incubator at 37°C under 5% CO₂ in 25 cm² cell culture flask (Corning Inc., Corning, NY). Cells were grown in Dulbecco’s modified Eagle’s medium: Nutrient Mixture F-12 (DMEM/F12; Sigma-Aldrich, St. Louis, MO) with 5% fetal bovine serum (FBS; Mediatech. Inc., Manassas, VA) and 1% penicillin-streptomycin mixture (Mediatech. Inc., Manassas, VA). Cells were seeded into 12-well cell culture plates (BD Falcon, Corning Inc., Corning, NY) at 10⁵ cells/ml to form a confluent monolayer within 4 days, and then switched to the same medium without FBS to induce differentiation. Lumance® (Innovad NV/SA) was dissolved in DMEM/F12 to make the working solution (1,000 ppm). Cells were treated with Lumance® throughout the differentiation period (day 1 to 5). Medium was replaced every 2 days and epithelial cell integrity was assessed through the measurement of trans-epithelial electrical resistance (TEER) test (Sambuy et al, 2004). On day 5 post-differentiation, cells were treated with 10 µg/ml LPS on the apical side to mimic bacterial contact with intestinal epithelial cells in the lumen, and TEER was measured at 0, 12, 24 h, respectively. TEER measurements were conducted using a Millicell ERS-2 Voltohmmeter® (Millipore, Billerica, MA) and the values were expressed as Ohm per well (Ω/Well).

**Results and discussion**

The LPS exposure significantly decreased TEER at 24 h compared to the control group (Figure 2) (P < 0.05), indicating that LPS impaired the epithelial barrier integrity. No differences were seen between the LPS and the control groups after 12 h. This could be due to the low dose of LPS used in this in-vitro model. Further testing in a dose-response manner with LPS challenge is required though to confirm this. However, pretreatment of IPEC-J2 cells with Lumance® at the 1000 ppm concentration during differentiation for 5 days significantly increased TEER (P < 0.05) at 0, 12 and 24 h after LPS challenge when compared with the control treatment. Thus, treatment of the epithelial cells with Lumance® prevented LPS induced paracellular permeability as shown by the increased TEER at 24 h.

It is well established that the intestinal epithelial barrier selectively restricts harmful macromolecules and micro-organisms and regulates the epithelial permeability and that the disruption of the intestinal barrier induced by toxins and pathogens leads often to the development of severe intestinal inflammation, digestive disorders, leaky gut syndrome and diarrhea (Yan & Ajuwon, 2017, Schlegel et al., 2012). Several studies so far have revealed that specific fatty acids, plant extracts and essential oils may exert positive influences on intestinal barrier function,
The synergistic effect of Lumance® is superior to any of its single components. Poultry World Magazine.

In conclusion, we demonstrate that a commercial blend of plant extracts, fatty acids and essential oils (Lumance®) and LPS on epithelial cell integrity by measuring TEER at 0, 12 and 24 h of LPS stimulation in IPEC-J2 cells.

We here studied the effect of a commercial blend of plant extracts, fatty acids and essential oils (Lumance®) and LPS on epithelial barrier integrity with and without LPS-induced impairment in IPEC-J2 cell. These results provide new insights into the mechanisms underlying the beneficial effects of Lumance® on gut health and may have important implications toward the leaky gut syndrome as well as diarrhea related prevention and treatment in production animals.

References: