Technical Bulletin

Anti-inflammatory alternative to antibiotics

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Aiming to find an effective alternative to antibiotics, research groups at university of Ghent (UGent) and Oklahoma (OSU) set up in-vitro and *in vivo* experiments to evaluate the anti-inflammatory activity of Lumance®.

Earlier, it was proposed that Antimicrobial Growth promoters (AGPs) can directly reduce inflammation through inhibition of cytokines production by inflammatory cells, thus saving energy for growth. The inflammatory response shares various molecular mediators and signalling pathways that can ultimately lead to decreased growth performance and endangered health in poultry and other production animals.

Effective alternative to antibiotics

Effective alternatives to AGP can be chosen based on known anti-inflammatory properties or by specific in vitro tests. Among the proposed anti-inflammatory alternatives to antibiotics, plants extract and fatty acids are composed of wide variety of compounds, which are a good source of anti-inflammatory agent. A combination of different alternatives to antibiotic these two compounds may hold the most promising method to substitute antibiotics in animal feeds. In the present study, it is hypothesised that the commercial mixture of plant extract, essential oils, and fatty acids (**Lumance®**) would be synergistically effective in the reduction of inflammation in an *in vitro* and *in vivo* model.

Anti-inflammatory alternative to antibiotics. Photo: Dreamstime

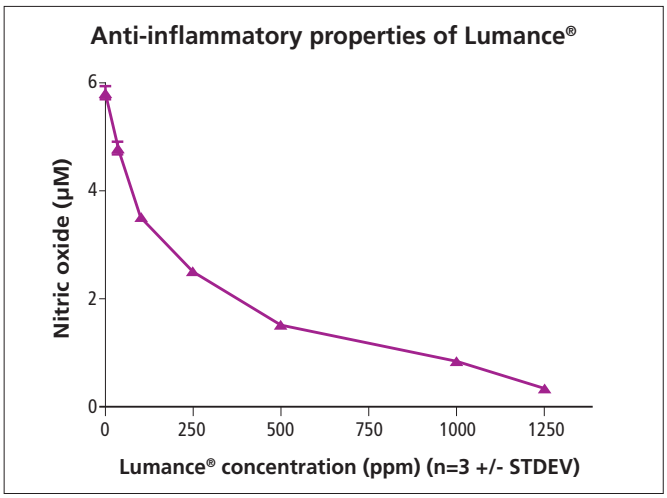
Lumance® (obtained from Innovad®, Belgium) is a complex combining slow release and protection technologies ensuring that medium and short chain fatty acids, esterified butyrate, essential oils, anti-inflammatory compounds and polyphenols are delivered in a gut active way for a powerful and effective anti-inflammatory activity.

Anti-inflammatory activity of Lumance®

I. *In vitro* trial

The anti-inflammatory activity of **Lumance®** was measured by the production of nitric oxide (NO) in an *in vitro* model using the RAW 264.7 assay, essentially as described by Wu et al (2003). In this *in vitro* inflammation model, **Lumance®** inhibited LPS-induced nitric oxide production with an IC50 value of 368 (95 % CI 179, 557) mg/l (**Figure 1**). The inhibition of NO production was not the consequence of cell death because it was established earlier using the tetrazolium salt WST-1 method that cell viability was not affected in any of the treatments.

Figure 1 – The anti-inflammatory effect of Lumance® *in vitro*.



II. *In vivo* gene expression trial

180 1-day-old male Cobb broilers were obtained from a commercial hatchery (Siloam Springs, AR, USA) and grown over a 10-day experimental period. The experiment was carried out in 20 pens with 9 broilers per pen. After 3 days of acclimatisation, all birds were fed a non-medicated corn-soybean basal mash diet supplemented with or without **Lumance®** for 7 days.

On days 2 and 4 birds from each treatment were sacrificed with 1 bird/pen. A segment of the crop and jejunum was harvested from each bird and snap frozen in liquid nitrogen for RNA extraction and subsequent real-time PCR analysis of the expression levels of the chicken ≤-defensin 9 (AvBD9) gene.

Capabilities of HDPs

HDP are immunomodulatory molecules that have evolved to provide broad range of protection against a variety of pathogenic microbes. They are critical components of the innate immune system. HDPs also inhibit LPS induced pro-inflammatory cytokine production such as TNFα, IL-6, IL8 and IL-10. The overall consequence of HDPs function is the control of inflammation along with maintenance of the immune responses required for resolution of infections. Real-time PCR results indicated that, relative to control on day 2, **Lumance®** increased AvBD9 gene expression in the crop by 65-fold, but the induction was almost reduced to the basal level on day 4 **(Figure 2).**

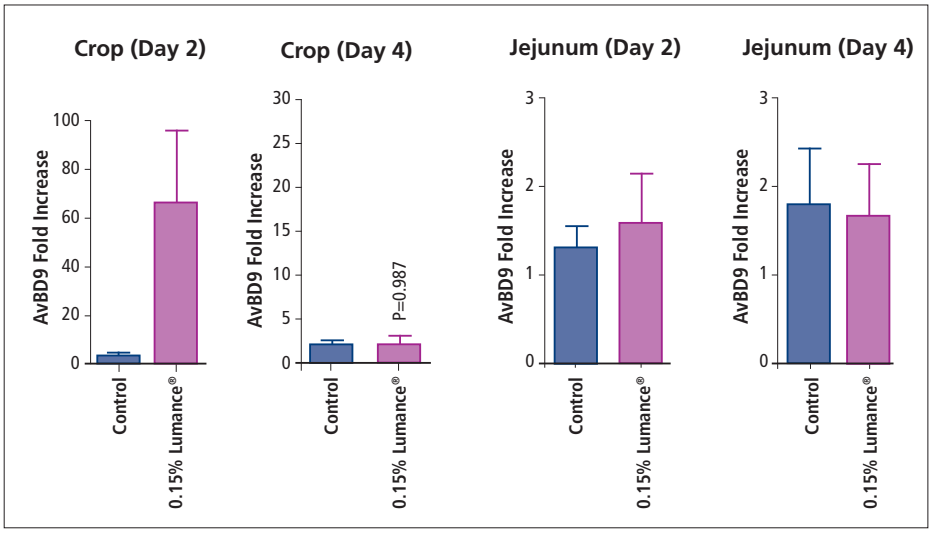
Lumance® showed the highest AvBD9 induction in the crop is likely due to the synergistic effect between short- and medium-chain fatty acids and other plant extracts. However, it is unknown why the ability of the product to induce AvBD9 subsided in the crop by day 4. Although the HDP induction is expected to reduce in the lower GI tract as butyrate gradually gets absorbed and metabolised, it is surprising to see no obvious effect on AvBD9 gene expression in the jejunum on either day 2 or 4.

Promoting gut health and performance

Nevertheless, the HDPs that are induced and released in the upper GI tract by butyrate products are expected to exert a profound impact on the microbiota composition in the lower GI tract, which will in turn alter nutrient digestion and utilisation and subsequent gut health and growth performance.

Taken together, supplementation of **Lumance®** enhanced the HDP gene expression in the GI tract of birds. These products have potential to replace antibiotics for disease control in prevention. However, the product showed the strongest capacity to reduce nitric oxide (NO) production in vitro and to induce HDP expression in the crop and, therefore, have beneficial impact on gut health and reducing inflammation. ■

Figure 2 – Effect of Lumance® on AvBD9 gene expression in the GI tract of broilers.



References available on request.