Technical Bulletin

The synergistic effect of Lumance® is superior to any of its single components

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Introduction

Excessive use of antibiotics over the last few decades has led to the current legislation whereby antibiotic use in animal production should be rationalized. As a first step, Antibiotic Growth Promoters (AGPs) were banned from use within the EU (Bengtsson and Wierup, 2006). Use of non-antibiotic growth promoters has been rising as a result of the pressure to maintain a cost effective and economical animal production. Due to consumer demand, such pressure escalated to total reduction of antibiotic use in farm animals destined for human consumption.

It is critical to develop cost effective antibiotic alternative strategies to ensure the long-term sustainability of animal production (Cheng et al., 2014). Although extensive research has been carried out on ingredients that exert antibacterial effect individually, less work has been done to understand how multiple ingredients may work in combination.

Combination of different ingredients has demonstrated synergistic activity (Diao et al., 2015; Souza et al., 2009). Ideally, alternatives to AGPs should have the same beneficial effect as AGPs. Recent findings demonstrate that controlling and reducing inflammation are the main mechanisms behind the growth promoting activity of AGPs, which explains the consistent effect of AGPs as compared with the varying effects found in some alternatives (Khadem et al., 2014; Niewold, 2007). Therefore, it is reasonable to state that effective growth promoters must be inhibitors of inflammatory response.

A combination of different anti-inflammatory compounds may hold the most promising method to substitute antibiotics in animal feed. There are three major reasons:

1) antibiotic alternatives fail to cover all the beneficial effects that antibiotics show;
2) there is a synergistic effect among different alternatives that will reduce the effective dosage required (e.g., organic acids and essential oils);
3) the host immune response should be enhanced with an integrated approach that will completely replace antibiotics (Yang et al., 2015).

Several recently published studies have shown that the combined use of different antibiotic alternatives has better effects on the performance and health of animals when compared to single components (Walia et al., 2017; Liu et al., 2017). However, the literature available on the ability of these combinations to suppress the inflammatory response is relatively scarce. In the current study, the single and combined anti-inflammatory activity of three major non-antibiotic AGP alternatives; fatty acids, essential oils and plant extracts were evaluated in an *in-vitro* model.

Materials and method

Evaluation of synergistic anti-inflammatory activity of fatty acids, essential oils and plant extracts in a well-proportioned mixture.

Lumance® (obtained from Innovad® SA/NV Belgium) is a complex product, combining slow release and protection technologies ensuring that fatty acids, essential oils, plant extracts and anti-inflammatory compounds and polyphenols are delivered in a gut active way for a powerful and effective anti-inflammatory control. The anti-inflammatory activity of three main ingredients, fatty acids (FA), essential oils (EO) and plant extracts (PE) individually (similar doses as used in **Lumance®**) and in a commercial mixture (**Lumance®**) was tested using the RAW 264.7 assay, essentially as described by Wu et al 2003. Briefly, the monocytic murine cell line RAW 264.7 grown in cell-culture flasks in Dulbecco's modified Eagle's medium until 70-80% confluence, and then scraped off, resuspended in the same medium and seed into a ninety-six-well plate (100µl/well) with a cell density of 1×10^6 cells/ml. Then, 50 µl/well of FA, EO, PE (similar dose as used in **Lumance®**)

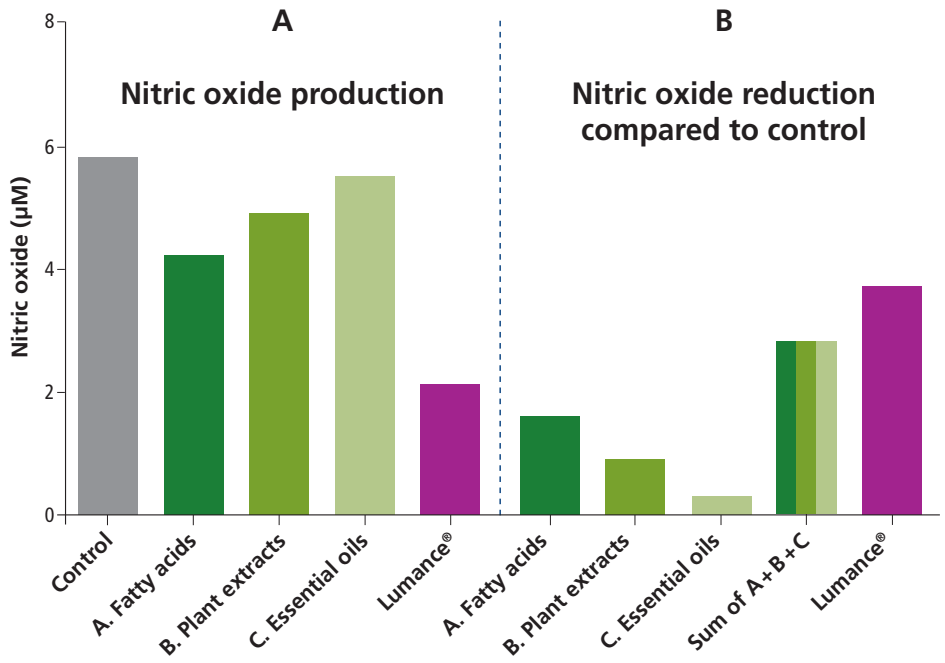
and **Lumance®** were added and incubated for 4 h at 37°C. In the control group, 50 µl/well of medium was added without any additives. Subsequently, 50 µl of medium or 50 µl of medium containing lipopolysaccharide (LPS; 50 ng LPS/ml) were added. Then, 100 µl of medium were taken and pipetted into another ninety-six-well plate. The inflammatory response was measured by the production of NO_2-. NO_2- production was measured with Griess reagent using a serial dilution of $NaNO_2$ as a standard.

Results

The addition of FA, EO, PE alone (with the same concentration as used in **Lumance®**) did not show any significant influence on reducing the NO production while the combination of these compounds had a significant effect on reducing the NO production *(Fig. 1, A)*. *Figure 1, B* shows the measured reduction in NO production by these three ingredients and the sum of them compare to the whole mixture. Although the results showed that these three compounds moderately lowered NO production and inflammation, their combination was more effective.

The current study suggests that the anti-inflammatory activity of antibiotic alternatives can be enhanced in a synergistic fashion by combining them with low doses of FA, EO and PE.

Future *in vivo* animal studies are needed to help translate these results to the real practice. In conclusion, the potential benefits of using a combination of different anti-inflammatory compounds could be quite obvious. Hopefully, a greater chemo-preventive effect could be achieved by compounds targeting different signaling mechanisms.



Anti-inflammatory effects of Lumance® and its ingredients

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