

# In vitro killing activity of Innovad’s Novibac® CF 60 against *Clostridium perfringens*, *Salmonella typhimurium* and *Escherichia coli*

May 2018, University of Ghent by Dr. Alireza Khadem

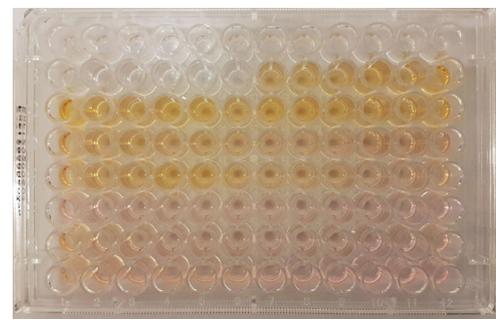
## Background

Growing concerns over resistance to antibiotics in pathogenic bacteria has resulted in increasing public and government pressure to reduce the use of antimicrobial growth promoters (AGPs) in animal feed. A negative result of AGPs removal is potential increase in incidence of certain diseases in poultry, such as necrotic enteritis and avian salmonellosis. Therefore, effective alternative strategies to prevent and control bacterial contamination of raw materials and finished feeds, as well as reduce the pathogenic bacteria in the gut of animals are highly essential. Among the proposed antimicrobial alternatives to antibiotics are organic acids such as propionic and formic acid. Propionic acid is mainly used as preservatives for feed, whereas formic acid is mainly used to improve nutrition and health in animals. A combination of different alternatives to antibiotic may hold the most promising method to substitute antibiotics in animal feeds.

## Experimental Protocol

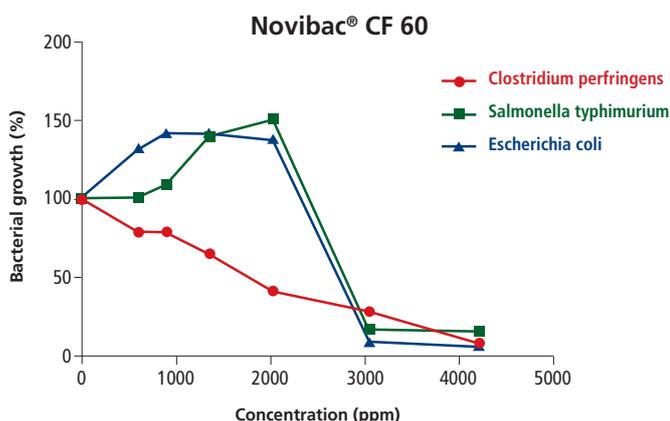
The antibacterial activities of **Novibac® CF 60** against *C. perfringens*, *Salmonella typhimurium* and *Escherichia coli* isolated from the intestine of chickens were studied in-vitro. The spray dried free flowing mixture of calcium formate, calcium propionate and citric acid, from Innovad® NV/SA Belgium were added at different concentrations (ranging from 600 to 4200 mg/kg) to a media inoculated with *C. perfringens*, *S. Typhimurium* and *E. coli*. The antimicrobial susceptibility testing was performed using the broth microdilution

assay based on the ISO 10932/IDF 223 standard protocol. Bacterial growth was verified by measuring the OD at 580nm and the minimal inhibitory concentrations were determined.



## Results

**Novibac® CF 60** effectively inhibited the growth of these three bacteria. The most susceptible organisms were *S. Typhimurium* and *E. coli*, with MICs of 3000 mg/kg, followed by *C. perfringens* with MIC of 4200 mg/kg. The mechanism of this beneficial effect was attributed to the ability of these acids to pass across the cell membrane of the bacteria, dissociate in the more alkaline interior and acidify the cell cytoplasm. However, these results show that **Novibac® CF 60** at low concentration (less than 2000 ppm) increase the growth of *S. Typhimurium* and *E. coli*. It can be hypothesized that organic acids at low concentration serve as a carbon source for the endogenous microbiota and at high concentration can exhibit toxic effects on bacteria.



## Conclusion

These results suggest that **Novibac® CF 60** at the recommended dose of 3000 ppm act as preservatives for grains or feed and have a potential role in beneficially altering gut microflora by reducing pathogenic bacteria.

In conclusion, the susceptibility data obtained in this study provides strong support for the claim that **Novibac® CF 60** is an effective treatment for a range of infectious bacteria in feed and animal.