IMPROVE POULTRY PRODUCTIVITY

Performance improve by microbiome stabilization in broiler

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Intestinal microbiota is considered a crucial organ that plays an integral role in maintaining the health of the host by modulating several physiological functions including nutrition, metabolism, and immunity. The digestive process is strongly linked to gut microbiota; nutrient absorption, feed digestibility, energy harvest and therefore productivity are influenced by microbiota composition and diversity (Stanley et al., 2013; Mancabelli et al., 2016). The chicken gut microbiota includes hundreds of bacterial species dominated at the phylum level by Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria (Oakley et al., 2014; Clavijo et al., 2018).

It is better known than early colonization of the intestine is of great importance for poultry health and productivity, since it can alter the morphology and physiology of the intestine and susceptibility to infectious diseases (Kers et al., 2018). Once hatched, the gastrointestinal tract of chickens becomes successively colonized by Enterobacteriaceae at first days of age and then Firmicutes (approximately from 7 days of age) (Ballou et al., 2016). However, colonization of gastrointestinal tract with specific bacterial species, belonging to the Enterobacteriaceae or Firmicutes groups is likely a stochastic process driven by the contact with microorganisms coming from the rearing environment and from bacteria present in food and water (Kubasova et al., 2019). Thus, after the initial colonization of the intestine a succession of microorganisms is observed in which the species richness and complexity of the population structure of the microbiota increase as the birds grow, until eventually microbiota reaches a state of maturation and stabilizes. This process normally occurs in commercial broiler chickens around 3 weeks of life (Jurburg et al., 2019; Johnson, et al., 2018). However, although this is the general rule in commercial broiler chickens, development times and succession patterns of intestinal microbiota species can vary greatly depending on the genetic background of the birds and farm management factors (Ding et al., 2018, Ngunjiri, et al., 2019). In this line, the increase in the phylum Proteobacteria, which includes many potentially pathogenic bacteria, correlates with a pro-inflammatory cytokine profile, while the increase in members of the phylum Firmicutes is associated with an anti-inflammatory state. Therefore, gut microbiota is involved in the immune homeostasis of the gastrointestinal tract of birds, and therefore an imbalance in the intestinal microbiota can lead to an immune imbalance and an impact on birds' health.

Quórum sensing

Quorum sensing (QS) is principally related with the bacteria communication but it also contributes to environmental adaptation by facilitating the elaboration of virulence determinants in pathogenic species and plant biocontrol characteristics in beneficial species as well as directing biofilm formation and colony escape. QS also crosses the prokaryotic-eukaryotic boundary in that QS signal molecules influence the behavior of eukaryotic organisms in both plant and mammalian worlds such that, QS signal molecules may directly facilitate bacterial survival by promoting an advantageous lifestyle within a given environmental niche. In this context, QS inhibitors represent an important antimicrobial target that may prevent, suppress, and/or treat infectious diseases. The mechanistic details are different between Gram-negative and Gram-positive bacteria. Gram-negative bacteria utilize N-acylhomoserine lactones (AHLs) while Gram positive bacteria uses oligopeptides and both bacteria groups express AI-2 system, based on furanosyl borate diester (Bouyahya et al., 2017) . Consequently, while antibiotics kill or slow down the growth of bacteria, QS inhibitors or quorum quenchers simply attenuate bacterial virulence, delay the productive impact allows the control of bacteria proliferation, through its immunomodulatory effect.

A large body of work on QS has been carried out in deadly pathogens like Pseudomonas aeruginosa, ***Staphylococcus aureus, Vibrio fischeri, Vibrio harveyi, Escherichia coli, Vibrio, Cholera*** etc. A number of these studies have succeeded in exploiting the bacterial QS system as potential target for treatment of bacterial infections. The inhibition of QS system is believed to be advantageous over conventional antibiotics, because only the communication mechanism between the bacteria is disrupted without killing the individual cells. Hence, this strategy should generate a lower selective pressure and reduce the rate at which AMR develops during the treatment (Williams, 2007).

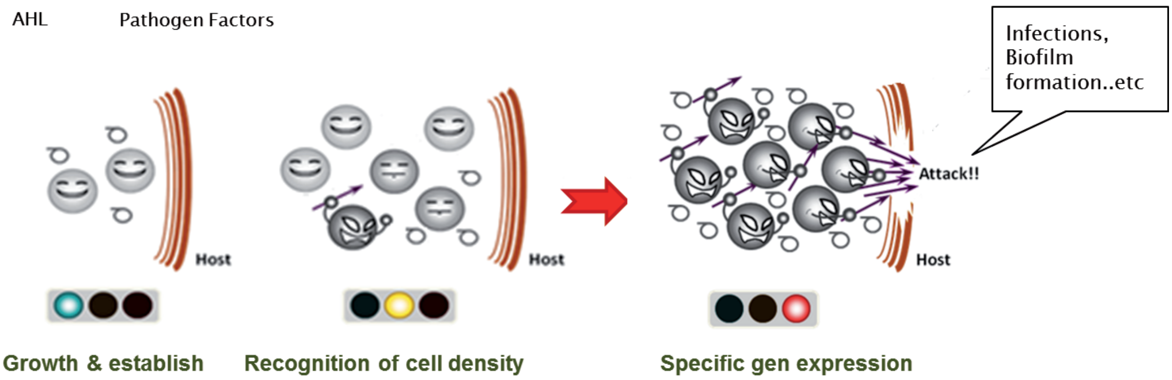

Figure 1: Proliferation process intermediate by quorum sensing communication among pathological bacteria.

Aditivos anti-quórum sensing

The secondary metabolites of medicinal plants such as terpenoids (essential oils) and flavonoids are shown to be effective against pathogenic bacteria even at low concentrations (Bouhdid et al., 2008). Generally, that biological compounds usually target the bacterial system via three different ways: stops the signaling molecules from being synthesized, degrades or modify the signaling molecules and/or target the signal receptor (Koh et al., 2013, Chan et al., 2011). It is for that, during this century has been high study the impact of plant extracts, especially essential oils, in the QS and its implication in pathogens proliferation. Thus, authors like Burt et al. (2014) explored the capacity of carvacrol for prevent the proliferation and biofilm development of *Chromobacterium violaceum ATCC 12472, Salmonella enterica subsp. Typhimurium DT104, Staphylococcus aureus 0074 and Pseudomonas aeruginosa*, as important pathogens in human health. In that publication, they demonstrated that carvacrol showed a preventive functionality against all of them, except Pseudomonas aeruginosa (Figure 2). This inhibitory effect of carvacrol was observed at sub-lethal concentrations (0.5 mM) where no effect was seen on total bacterial numbers, indicating that carvacrol's bactericidal effect was not causing the observed inhibition of biofilm formation. Since QS is an essential part of biofilm formation, the effect of carvacrol on QS was also studied. In this line, Sub-MIC concentrations of carvacrol reduced expression of cvil (a gene coding for the N-acyl-L-homoserine lactone synthase), production of violacein (pigmentation) and chitinase activity (both regulated by QS) at concentrations coinciding with carvacrol's inhibiting effect on biofilm formation. These results indicate that carvacrol's activity in inhibition of biofilm formation may be related to the disruption of QS.

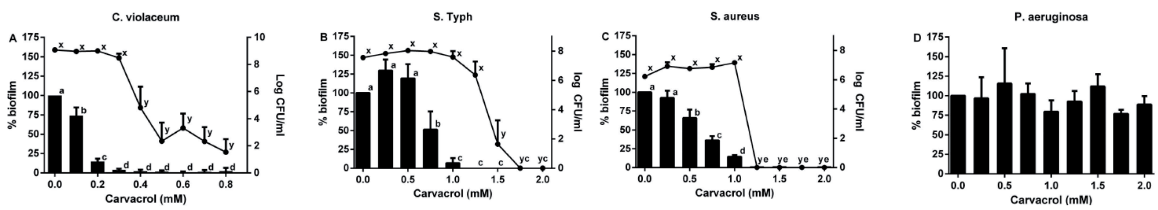

Figura 2:

Carvacrol reduces biofilm formation by *C. violaceum, S. Typhimurium* and *S. aureus* but not *P. aeruginosa*. Percentage biofilm formation measured as OD (590 nm) after crystal violet staining after 24 h incubation in the presence or absence of **carvacrol** (0–0.8 mM) (bars, left axis) compared to colony counts (log cfu/ml) from the same samples (connected dots, right axis) (Burt et al., 2014).

On the other hand, essential oil blends, such as carvacrol, thymol and cinnamaldehyde, have selective antimicrobial properties (Lee et al., 2002; Guo et al., 2004). Consequently, their use at optimum dosage, also affect QS, has been shown to have efficacy towards reducing the colonization and proliferation of ***Clostridium perfringens*** and controlling coccidia infection and, consequently, they may help to reduce necrotic enteritis (Guo et al., 2004; Mitsch et al., 2004; Oviedo-Rondón et al., 2005, 2006a, 2010), guarantee success with their use in a sustainable poultry production (Choct, 2009; Smith, 2011). In the same line, Trevisan et al. (2018) demonstrated a high sensibility of ***Salmonella Typhimurium*** to Carvacrol inclusion, with a MIC of 312 g mL-1.

Finally, the interference of this compounds in the QS and their direct antimicrobial potential, allow an increase the effectivity of synthetic antimicrobials, when are combined with essential oils. Thus, Backman et al. (2011) confirmed the cinnamaldehyde and its impact in *Vibrio angillarum* metabolism, reducing drastically the protease activity (Figure 3) and linked it with an improvement in the effectivity of chloramphenicol and doxycycline, when they are combined (Figure 4). Allowing a higher bacteria control and reducing the antimicrobial dosage required for it complete control.

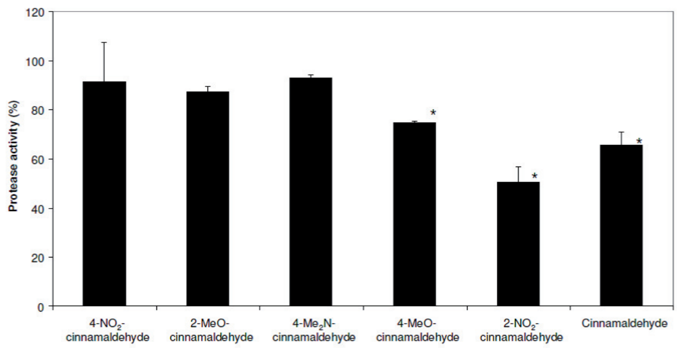

Figure 3:

Effect of cinnamaldehyde and cinnamaldehyde derivatives on the protease activity of *Vibrio anguillarum* LMG 4411. Cinnamaldehyde and cinnamaldehyde derivatives were tested at 100 µM, except 4-NO2-cinnamaldehyde (25 µM).

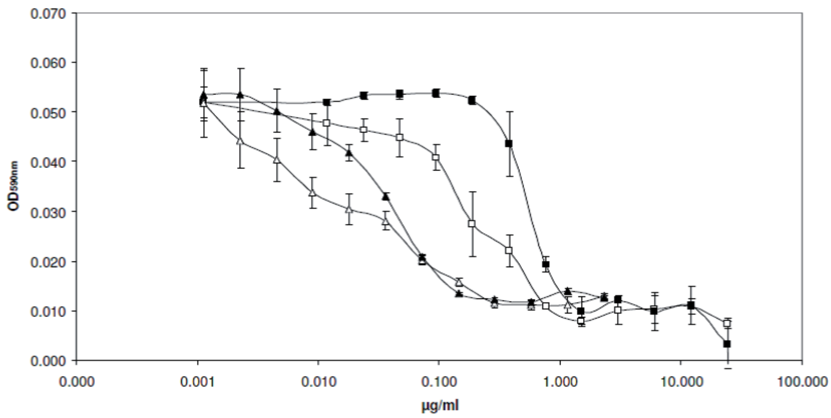

Figure 4:

Effect of cinnamaldehyde on antibiotic susceptibility of *Vibrio vulnificus* LMG 16867 Effects of chloramphenicol (squares) and doxycycline (triangles) on the growth of *Vibrio vulnificus* LMG16867 in the presence (open symbols) and absence (solid symbols) of cinnamaldehyde (100 µM) are presented. (Backman et al., 2011)

Performance improvement in broiler

The effect of Emerald (essential oil combination, based in carvacrol, cinnamaldehyde, thymol and eugenol) as Natural Growth Promotor, was study in an Indian commercial broiler farm. The aim of this study was to evaluate dietary effects on growth performance, mortality and time to slaughter weight in commercial broiler production.

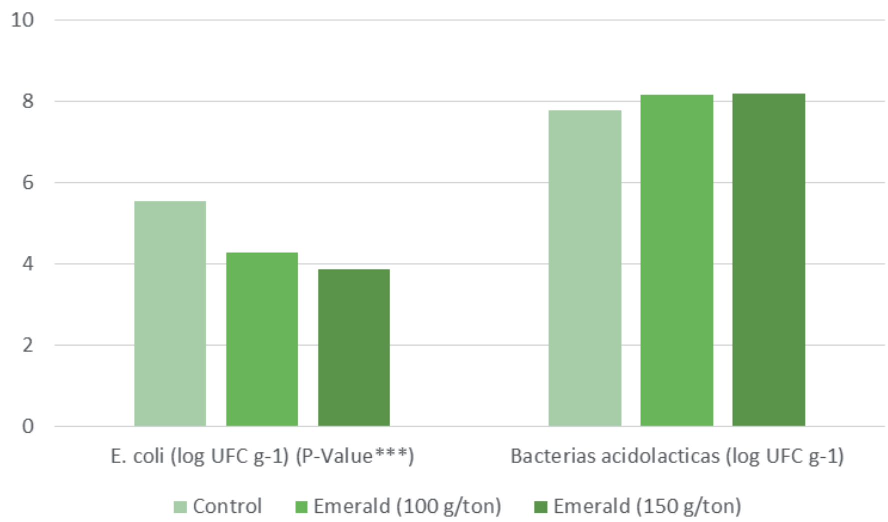

Figure 5: **Effects of dietary addition of EMERALD on growth performance of broilers.**

According with the Figure 5, the addition of 150 g/ton Emerald to the diet resulted significantly higher body weight gains (60.6 vs 57.0 g/d, P<0.01) and better FCR compared with control group (P<0.05). It is also relevant that EMERALD at 100 g/t reduces statistically the FCR compared with control group. This performance improvement could be linked with an improvement in the gastrointestinal health and microbiome stability(Figure 6), where the Emerald inclusion reduced significantly the E. coli content (P<0.001) whiles not affect the Lactic acid bacteria prevalence.

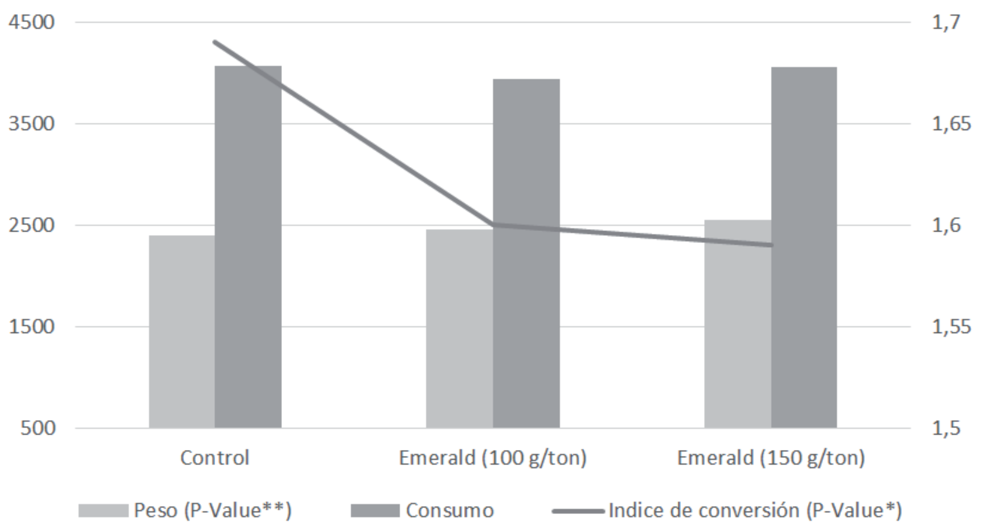

Figura 6: Effects of dietary addition of EMERALD on E.coli and LAB numbers in intestinal contents of broilers.

As conclusion, gastrointestinal stability is key to maximizing animal performance and health. In this line, the microbial control produced by essential oil inclusion in feed, by quorum sensing inhibition, may be useful as enhancer of growth performance and microbiota stabilization, by reducing pathogen prevalence; with a positive effect in mortality and global profit.