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Probiotics Work With or Without Antibiotics

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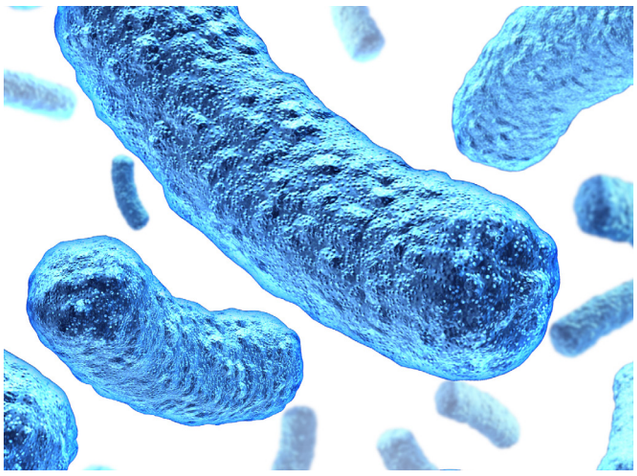


Feed additive antibiotics, including ionophores, are effective in broilers but concerns are increasing as a result of development of antibiotic resistant bacteria and the presence of antibiotic residues in poultry meat. Concurrently, interest in the use of probiotics in broiler production is on the rise. It is known that probiotics benefit poultry through various mechanisms by modulating the microbiome and without the negative connotations associated with antibiotics.

There are regular debates over whether or not probiotics should be used only for Raised Without Antibiotics (RWA) production systems, which include No Antibiotic Ever (NAE) and organic production.

One **MYTH** is that antibiotics provide a performance-enhancing effect by their pure antimicrobial action. Thus, no additional benefit can be achieved with the combined usage of a probiotic and an antibiotic. The misconception is that the mode of action for each of the products is similar and probiotics aren't necessary when an antibiotic is used.

A second **MYTH** is that the simultaneous use of the two products will result in the destruction of the probiotic by the antimicrobial activity of the antibiotic. This also is a misperception or over simplification as to the susceptibility of the probiotic bacteria to the antibiotic. In particular, the spore-forming bacteria probiotics are naturally protected against chemical aggressors (acids) or thermal stress. The non-spore forming probiotics, such as the *Lactobacillus*-based probiotics, could be sensitive. Further sensitivity verification of germinated probiotic bacteria can be analyzed by Minimum Inhibition Concentration (MIC) studies (Table 1).

Table 1. Compatibility of Chr. Hansen probiotics with most common antibiotics.

Antibiotic Name	Therapeutic Class	Recommended in feed Dose (ppm)	*B. subtilis* MIC (µg/ml)
Bacitracin	Polypeptides	10-50	>800
Colistin	Polypeptides	30-40	160
Lincomycin	Lincosamides	5-20	80
Tiamulin	Pleuromutilin	30-50	>800
Virginiamycin	Streptogramin	5-10	16

The **REALITY** is that Chr. Hansen investigated this question seriously years ago. Over the last 10 years Chr. Hansen has conducted many research studies analyzing the additive value of the combined usage of a *Bacillus*-based probiotic with a feed additive antibiotic. Those results consistently demonstrate that Chr. Hansen probiotics deliver performance benefits with or without concurrent usage of a feed additive antibiotic, regardless of the type of antibiotic fed (Table 2).

The **REAL** explanation resides in the different and unique modes of action of effective and ethical *Bacillus*-based probiotics.

Mechanisms of action proven to be associated with these products are: Competitive exclusion (direct and/or indirect), immuno-modulation, bacteriosin production, and enzyme production.

Competitive Exclusion:
Competitive exclusion is a probiotic mode of action that can occur via several different mechanisms. The direct mechanism is the basic space-occupying effect of the probiotic on intestinal cells. The result is less space for pathogenic bacteria to populate the gut. The indirect mechanism is through the production of secondary metabolites which affect the immediate environment of the probiotic bacteria. These metabolites result in the proliferation of lactic acid-producing bacteria, which benefit the intestine.

Bacteriocin or antimicrobial peptide production:
Effective *Bacillus*-based probiotics produce antimicrobial substances that inhibit the growth of pathogens. Growth inhibitory and/or bacteriocidal effects against certain poultry pathogens have been described, based on *in vitro* analysis of a number of probiotic bacteria. Probiotic strains of *Bacillus* spp. are shown to be especially effective (Svetoch et al., 2005; Teo and Tan, 2005; Latorre et al., 2016; Poormontaseri et al., 2017).

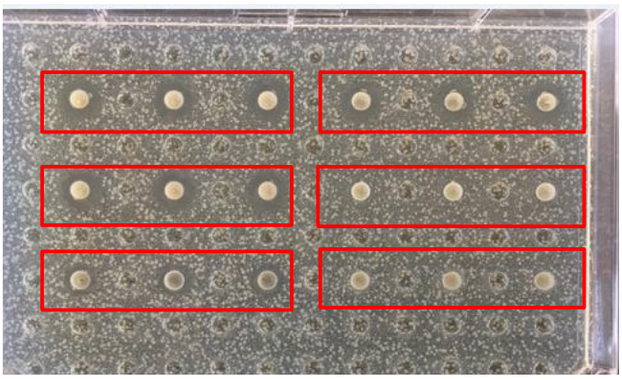
A Case Study: *Bacillus licheniformis* and bacteriocin effects
Bacillus licheniformis produce lichenysin (antimicrobial peptide). However, it is known that other *Bacillus* species produce bacteriocins or bacteriocin-like substances, such as subtilin and coagulin.

Bacteriocins are cationic (positive charged) peptides that display hydrophobic or amphiphilic properties and, in most cases, the bacterial membrane is the target of their activity. Several models have been proposed demonstrating the mechanism of action of these cationic peptides. The thrust of this action involves the formation of channels through which ions can pass and (or) the disruption of bacterial cytoplasmic membranes This has a lethal effect on bacteria via the formation of pores in the bacterial membrane. The three principal steps required for this effect are: 1) binding of peptides to the bacterial membrane 2) peptide aggregation within the membrane 3) formation of channels.

Bacteriocins must cross the negatively-charged outer wall of Gram-negative bacteria, which contains lipopolysaccharides (LPS), or the outer cell wall of Gram-positive bacteria, which contains acidic polysaccharides.

Recently, Chr. Hansen discovered a combination of *Bacillus* strains, the first to demonstrate the inhibitition of Gram-negative bacteria such as *Salmonella* spp. and *Escherichia coli* (Figure 1).

Figure 1. Example of direct *in vitro* pathogen inhibition of **GALLIPRO® Fit** against *S.* Typhimurium.



Enzyme production:

Bacillus-based probiotics can be a factory of digestive enzymes. Those enzymes are released in the intestinal content by the germinated probiotics. Once released they will continue to act locally, transforming the undigestible nutrients into digestible nutrients. Many of these enzymes are summarized in the table below.

Table 2. Example of enzymes produced by *Bacillus subtilis.*

Enzymes produced by *B. subtilis* determined by ApiZYM and API20E	Substrate
Alkaline phosphatase	Phosphorus (LPS of Gram neg. bact)
Estearase (C4)	Fat
Estearase lipase (C8)	Fat
Leucine arylamidase	Protein
Cystine arylamidase	Protein
Acid phosphatase	Phosphorus
α -galactosidase	NSP*
β -galactosidase	NSP*
α -galactosidase	Carbo**
β -galactosidase	NSP*

*NSP: non-starch polysacharides **Carbo: other carbohydrates

Source: Chr. Hansen, Innovation CD News M1006

The practical differences between antibiotics and probiotics

Probiotics have a versatile and dynamic mode of action

Antibiotics are chemical molecules acting effectively on specific types of bacteria. Depending on the therapeutic class, the antibiotic affects either the bacterial cell wall, the cellular protein synthesis, or DNA synthesis of the bacteria. Probiotics can have a similar mode of action, (ex: lichenysin of *Bacillus licheniformis*). However, other beneficial effects on intestinal health associated with probiotics are absent with antibiotics. For instance, antibiotics do not modulate the intestinal immune system. Antibiotics do not produce enzymes that can digest the undigestible. Antibiotics do not occupy space on intestinal cells, thereby naturally excluding pathogen attachment.

There is no need for probiotic rotation

Long-term usage of antibiotics may result in antibiotic resistance by pathogens. This complicates the choice for poultry specialists and results in frequent rotation. Chr. Hansen continually evaluates the efficacy of our strains with pathogen inhibition assays. So far, there is no development of resistance or acquisition of resistance genes by pathogenic bacteria in relation to a probiotic being used as a feed additive.

Chr. Hansen probiotics are proven to have a complementary efficacy to antibiotics

Covering a span of over 10 years, Chr. Hansen has completed many research studies on the combined usage of their probiotics with commonly used antibiotics (Table 3). Studies consistently show an additive effect of probiotics with antibiotics. MIC (Minimum Inhibitory Concentration) studies show compatibility between feed additive antibiotics and Chr. Hansen probiotics. These effective probiotics can be used in RWA or NAE production systems, as well as conventional production systems which utilize feed additive antibiotics, including ionophores.

Table 3. Summary of 10 years of Chr. Hansen research on combined use of probiotics with antibiotics and ionophores.

Year	Institute	Probiotic Tested	Antibiotics Used	Combination Effect of Probiotic/Antibiotic on:			Combo Effect
				Weight	FCR	Mortality	
2005	Customer Experience, BR	GalliPro® GalliPro®MS	Avilamycin 10ppm Lasalocid 60ppm (S/G) Monensin 100ppm (F)	Yes	Yes	Yes	Yes
2010	Auburn University, Alabama, US	GalliPro® GalliPro®Tect	Virginiamycin 5ppm Monensin 90ppm	Yes	Yes	Yes	Yes
2010	University of Viçosa, BR	GalliPro®	Salinomycin 55ppm Bacitracin 50ppm	Yes	Yes*	=	Yes
2010	Southern Poultry Research, Georgia	GalliPro® GalliPro®Tect	Salinomycin 60ppm BDM 50ppm	Yes*	Yes*	NA	Yes
2010	Southern Poultry Research, Georgia	GalliPro® GalliPro®Tect	BDM 50ppm (S/G) Virginiamycin 20ppm (F)	=	Yes*	NA	Yes
2014	Chinese Academy of Agri. Sciences	GalliPro®	Virginiamycin 20ppm	Yes	Yes	Yes	Yes + (Yield)
2015	Bangladesh Agri. University	GalliPro®	Lincomycin 15ppm	Yes	=	No	Yes + (Yield*) under Heat stress challenge)
2016	Masagounder *et al.*	GalliPro®	Virginiamycin 5ppm BDM 50ppm Monensin 80ppm	Yes*	Yes*	NA	Yes (both Ross and Cobb*)

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