Bacillus subtilis DSM 32315 modulates gut microflora of poultry

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Poultry production plays a very important role in meeting the increasing demand for animal protein in the growing world population. However, this important role also comes with increased pressure to produce poultry in a safe, efficient, and sustainable manner. This pressure is primarily driven by public health concerns of today's consumers, which has led to changes in the sub-therapeutic use of antibiotics for growth promotion (AGP) in many countries. For example, in the European Union, AGP was banned in 2006 (Cogliani *et al.*, 2011) due to concerns about antimicrobial resistance in humans. In the USA, beginning from January 2017, the Food and Drug Administration (FDA) mandated limited use of medically-important antibiotics in food-producing animals to prevention, control or treatment of a specifically identified disease, under veterinary supervision. As a result of these changes, there is a need to identify, develop, and use alternatives to AGP, especially to control enteric pathogens. Probiotics, also known as direct-fed microbials (DFM), which were defined in 2001 by the FAO and WHO as organisms that confer a health benefit on the host, are increasingly more successful as alternatives to AGP due to 1) improved understanding and selection of beneficial probiotic organisms, and 2) their ability to remain viable and elicit specific actions within in the gut.

The Association of American Feed Control Officials (AAFCO) has published a list of microorganisms authorized and determined to be safe by the US FDA for use as DFM (official publication 36.14; Pendleton, 1998). These approved microorganisms include non-bacterial species of fungus and yeast, such as *Aspergillus* or Saccharomyces, as well as spore forming and non-spore forming bacterial species. The non-spore forming DFMs, such as species of *Bifidobacterium*, *Lactobacillus*, *Pediococcus*, and *Enterococcus*, are less stable during feed processing and as a result, they often require modifications or special application processes such as microencapsulation and administration via top-coating of pellets in order to remain viable in feed. The spore forming probiotic are predominately *Bacillus* species and are favored for poultry feed because of their inherent capacity to produce endospores which make them resistant to the high heat and pressure of pelleting. In addition, *Bacillus* species are tolerant to harsh pH environments, and, therefore, can survive digestion in the stomach (Cartman *et al.*, 2008). Once ingested and within the small intestine, endospores can transform into a vegetative form, which are able to germinate, grow, and become metabolically active (Nicholson, 2002). Although *Bacillus* are saprophytes and exists in the soil as a natural habitat, *Bacillus* spores have also been found in the digestive tract of animals (Hong, *et al.*, 2009; Chaiyawan, *et al.*, 2010) including healthy poultry, and some strains are capable of colonizing the gut (Hong, *et al.*, 2009; Chaiyawan, *et al.*, 2010, Wu et al., 2011; Barbosa *et al.*, 2005).

Treatment	Day 11 Ileum		Day 11 Cecum	
	C. perfringens	***Bacillus spp.***	***C. perfringens***	***Bacillus spp.***
Control	4.51	4.75^{B}	6.39^{A}	4.10^{B}
GutCare®	4.26	6.20^{A}	6.06^{B}	6.51^{A}
P value	0.154	<0.001	0.037	<0.001
	Day 18 Ileum		**Day 18 Cecum**	
	C. perfringens	***Bacillus spp.***	***C. perfringens***	***Bacillus spp.***
Control	6.27^{A}	4.71^{B}	6.14	4.58^{B}
GutCare®	5.58^{B}	5.48^{A}	5.89	5.58^{A}
P value	0.039	0.009	0.152	0.002
	Day 35 Ileum		**Day 35 Cecum**	
	C. perfringens	***Bacillus spp.***	***C. perfringens***	***Bacillus spp.***
Control	5.36^{A}	4.77^{B}	6.64^{A}	5.06^{B}
GutCare®	4.73^{B}	6.11^{A}	5.96^{B}	6.39^{A}
P value	0.009	<0.001	0.015	<0.001

Table 1: Effect of B. subtilis DSM 32315 (GutCare® PY1) on C. perfringens populations in ileum and cecum of broiler chickens in a necrotic enteritis challenge

Bacillus subtilis probiotic

Bacillus subtilis is the most commonly used probiotic in the feed industry. A variety of *B. subtilis* strains have been shown to reduce gut colonization by pathogenic bacteria such as *Escherichia coli* (Wu et *al*., 2011), *Salmonella* spp. (Park and Kim, 2014), and *Clostridium perfringens* (Gebert, *et al*., 2007). This pathogen inhibition can be attributed to the mechanisms of action by which *B. subtilis* creates favorable environments for beneficial bacteria; such as production of certain enzymes that aid nutrient digestibility (Chen *et al*., 2005; Giang *et al*., 2011; Mittal *et al*., 2011), competitive exclusion (Barbosa *et al*., 2005), alteration of cell to cell signaling in pathogenic bacteria (Hughes and Sperandio, 2008), activation the innate immune cells (Rhee *et al*., 2004), enhancement of immune regulation and host response (Lee *et al*., 2010), and production of metabolites or inhibiting substances that can enhance lactic-acid bacteria, especially species of *Lactobacillus* (Hosoi *et. al*., 2000; Wu *et al*., 2011; Shim *et al*., 2012). However, not all strains of *B. subtilis* are equally effective probiotics. Strains differ in their tolerance to feed pelleting and their capacity to remain viable through the harsh conditions of the digestive tract, including low pH and the presence of bile. Additionally, the ability to exert beneficial effect on the gastrointestinal (GI) microflora through defined mechanisms of action varies between *Bacillus subtilis* strains (Fioramonti *et al*., 2003).

GutCare® PY1- The right probiotic for today's challenges

Evonik's new probiotic, GutCare® PY1 (*Bacillus subtilis* strain DSM 32315) was developed through a defined multi-parameter selection process specifically considering the key challenges currently facing the poultry industry. This selection process included several *in-vitro* and *in-vivo* tests that defined the strain's ability to remain viable through feed processing and digestion, germinate into an active form with the ability to produce secondary metabolites. The secondary metabolites specifically produced by the *B. subtilis* DSM 32315 strain were shown to inhibit the growth of pathogenic bacteria colonizing

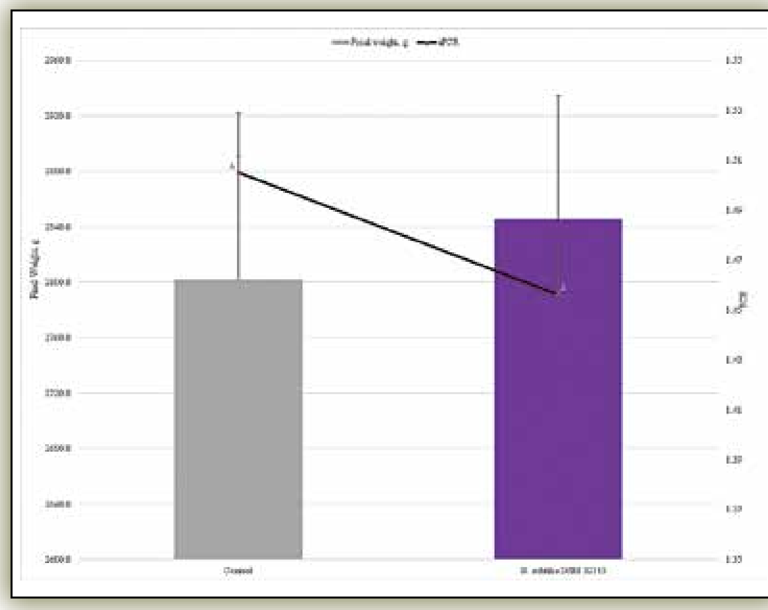

Figure 1: Effect of B. subtilis DSM 32315 (GutCare® PY1) on performance of broiler chickens in a necrotic enteritis challenge.

the gut, including *Clostridium perfringens*. *C. perfringens* is a natural gut bacterium, but can cause disease in poultry, such as sub-clinical or clinical necrotic enteritis (NE), colangiohepatitis, and gangrenous dermatitis. NE is particularly detrimental to poultry. The disease occurs when pathogenic strains of *C. perfringens* proliferate in the GI tract of poultry and produce toxins that result in necrotic lesions in the intestines. Performance losses therefore, not only occur due to reduced feed intake and costs of the immune response elicited to the pathogen, but also due to reduced digestive and absorptive capacities of the gut. The economic damage to the poultry industry globally, due to NE, is estimated to be between $4-6 billion/year (Wade and Keyburn, 2015). The development of a probiotic solution to replace the potentially unsustainable AGPs often used to control the disease was a key initiative in the screening and development of the GutCare® PY1 strain. To validate the effects of our new probiotic, two NE challenge trials were conducted.

Clostridium perfringens challenge Trial 1

In the first study conducted in Finland, the NE challenge was induced in each bird with an oral inoculation of *Eimeria maxima* oocysts at 12 days of age as a predisposing factor for the inoculation of the birds with a pathogenic field strain of *C. perfringens* at 16 days of age. The challenged broilers were either fed a control diet or a diet supplemented with GutCare® PY1 at 500g/MT (1 x 10^6 CFU/g feed) for 35 days. Molecular analysis of microbial populations in the ileum and cecum of birds at different time points showed that feeding GutCare® PY1consistently and significantly ($p < 0.05$) increased populations of *B. subtilis* and decreased populations of *C. perfringens* (Table 1). In the same study, the GutCare® PY1 fed group showed an improvement in body weight and feed conversion ratio, in comparison to the group fed the control diet (Fig. 1). Furthermore, using a scale of 0 (no lesion) to 4 (severe lesion), the incidence of foot pad lesion was lower in the GutCare® PY1 fed group (1.77) in comparison to the group fed the control diet (2.29).

Clostridium perfringens challenge Trial 2

In a second trial conducted in Thailand, NE challenge was induced with an inoculation of each bird at 9 days of age with sporulated *Eimeria* spp. oocysts as a predisposing factor, followed by the inoculation of a pathogenic strain of *C. perfringens* on days 14, 15 and 16. Similar to the first trial, it was shown that feeding GutCare® PY1 significantly ($p < 0.05$) decreased populations of *C. perfringens* in the duodenum, ileum and cecum, at day 35. In addition, litter moisture was decreased by 2.9 % in the GutCare® PY1 fed group compared to the control group (Fig. 2). This finding is in line with literature showing that an imbalance in the gut microflora related to *C. perfringens* is associated with wet litter (increased litter moisture), which is correlated to an increased risk for developing foot-pad lesions (Taira *et al.*, 2014).

Meta-analysis of different trials

These studies highlight the effectiveness of GutCare® PY1 in reducing the performance related detriments associated with *C. perfringens* infection. This is attributable to at least one specific mechanism of action; the production of different secondary metabolites that inhibit *C. perfringens* populations in the GI tract. However, it is hypothesized that GutCare® PY1 may have multiple mechanisms of action by which it can improve the intestinal microbial balance and performance of poultry. A meta-analysis of eight different trials conducted across the world (Thailand, India, Finland and the USA) with broilers raised under various conditions showed that birds fed GutCare® PY1 had consistently improved body weight gain and feed conversion ratio compared with birds fed control diets (Doranalli *et. al*, 2017).

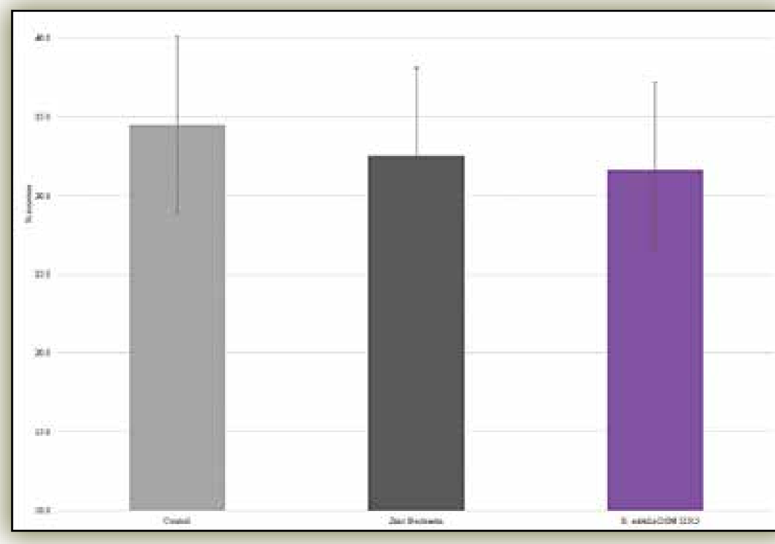

Figure 2: Effect of B. subtilis DSM 32315 (GutCare® PY1) on litter moisture of broiler chickens in a Clostridium perfringens challenge

GutCare® PY1 is a superior probiotic with natural highly resistant spores-forming bacteria that is stable during feed processing and storage. It may reduce the threat of pathogenic bacteria colonization of the gut especially *C. perfringens*, resulting in a more balanced intestinal microbial population and improved growth performance. It therefore offers an effective and sustainable replacement to AGPs in the maintenance of healthy flocks and the optimization of performance in poultry. ■

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