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Immunomodulating effects of probiotics for microbiota modulation, gut health and disease resistance in pigs

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ABSTRACT

Probiotics are live microorganisms that can confer a health benefit on the host, and amongst various mechanisms probiotics are believed to exert their effects by production of antimicrobial substances, competition with pathogens for adhesion sites and nutrients, enhancement of mucosal barrier integrity and immune modulation. Through these activities probiotics can support three core benefits for the host: supporting a healthy gut microbiota, a healthy digestive tract and a healthy immune system. More recently, the concept of combining probiotics and prebiotics, *i.e.* synbiotics, for the beneficial effect on gut health of pigs has attracted major interest, and examples of probiotic and prebiotic benefits for pigs are pathogen inhibition and immunomodulation. Yet, it remains to be defined in pigs, what exactly is a healthy gut. Because of the high level of variability in growth and feed conversion between individual pigs in commercial production systems, measuring the impact of probiotics on gut health defined by improvements in overall productivity requires large experiments. For this reason, many studies have concentrated on measuring the effects of the feed additives on proxies of gut health including many immunological measures, in more controlled experiments. With the major focus of studying the balance between gut microbiology, immunology and physiology, and the potential for prevention of intestinal disorders in pigs, we therefore performed a literature review of the immunomodulatory effects of probiotics, either alone or in combination with prebiotics, based on *in vivo*, *in vitro* and *ex vivo* porcine experiments. A consistent number of studies showed the potential capacity in terms of immunomodulatory activities of these feed additives in pigs, but contrasting results can also be obtained from the literature. Reasons for this are not clear but could be related to differences with respect to the probiotic strain used, experimental settings, diets, initial microbiota colonization, administration route, time and frequency of administration of the probiotic strain and sampling for analysis. Hence, the use of proxy measurements of enteric health based on observable immunological parameters presents significant problems at the moment, and cannot be considered robust, reliable predictors of the probiotic activity *in vivo*, in relation to pig

Abbreviations: CFU, colony forming units; EPS, extracellular polysaccharide; GIT, gastrointestinal tract; GM-CSF, granulocyte macrophage colony-stimulating factor; ETEC, enterotoxigenic *E. coli*; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide; HSP, heat shock protein; IPEC-1, intestinal porcine epithelial cells-1; IPEC-J2, intestinal porcine epithelial cells-2J; IPI-2I, ileal porcine intestinal-2I; LGG, *Lactobacillus rhamnosus* GG; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein-1; PIE, porcine intestinal epitheliocyte; RV, Rotavirus; SCFA, short chain fatty acids; TEER, transepithelial electrical resistance; TNF-α, tumor necrosis factor-α; Tregs, regulatory T-cells; VSV, vesicular stomatitis virus; ZO-1, *zonula occludens*-1

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gut health. In conclusion, more detailed understanding of how to select and interpret these proxy measurements will be necessary in order to allow a more rational prediction of the effect of specific probiotic interventions in the future.

1. Introduction

The value of dietary modulation and nutritional strategies to enhance gut health of pigs is becoming increasingly apparent. While a frequently used term in relation to human and animal health, the precise scientific definition of 'gut health' is still lacking. An absolute state of optimal gut health is probably practically impossible to define, as gut health is a dynamic and relative concept. Bischoff (2011) proposed five major criteria for a healthy gastrointestinal tract in humans, being: 1) effective digestion and absorption of food, 2) absence of gastrointestinal illness, 3) normal and stable intestinal microbiota, 4) effective immune status, and 5) a status of well-being. However, it is worth noting that many of the terms used ('effective', 'normal', 'well-being') are, in themselves, relative terms and difficult to define. A definition of a healthy gut has to be accompanied by a measure of the overall health and welfare of the animal. Whereas the interest in immune modulation in relation to human gut health has primarily addressed severe inflammatory diseases such as inflammatory bowel disease and colon cancer, the focus of pig gut health has been both in relation to prevention of infectious diseases and performance of the animals, *i.e.* nutrient utilization and growth performance (Heo et al., 2013; Pieper et al., 2016). Weaned piglets commonly suffer from gastroenteritis caused by enterotoxigenic *Escherichia coli* (ETEC). The European legislation has banned the use of in-feed antibiotics as growth promoters since 2006, and the high reduction of antibiotics use has been shown to be effective in limiting the prevalence of antibiotic resistance genes in the gut microbiota of European pigs compared to Chinese pigs (Xiao et al., 2016). However, the use of sub-therapeutic antibiotics for prevention of enteric diseases among weaning pigs has continued the concerns regarding the increasing emergence of antibiotic resistant bacteria. There is still a demand for the development of alternatives to antibiotics while preserving health in farm systems. Probiotics, especially, have been primarily used as feed-additives to prevent infectious intestinal diseases and to improve performance of livestock (Guo et al., 2006). In their review, Lallès et al. (2007) concluded that manipulation of the prebiotic composition of the weaning diet may be the most promising way to improve gut health in weaned piglets, and that positive results have also been produced with probiotics fed to piglets or to sows. The major responses appeared to be mediated through early changes in the gastrointestinal microbiota, including enhanced number of beneficial bacteria and/or decreased number of potentially pathogenic bacteria together with favorable fermentation products. Measureable, reproducible effects of dietary pre- and probiotics on intestinal physiology and mucosal immunology were limited or difficult to interpret (Lallès et al., 2007). However, subsequent and more recent studies have been conducted with probiotics to study the effect on intestinal immune responses under challenge of the pigs (*e.g.* Yang et al., 2016), and more scientific knowledge is available on the fundamental mechanisms of the potential immunomodulatory effects of the feed additives.

The purpose of the present paper was to review the literature in order to synthesize the knowledge concerning the immune modulating effects and mechanisms of action of probiotics, either alone or in combination with prebiotics in relation to gut health, with special emphasis on the fine balance between gut microbiology, immunology and physiology, and the potential prevention of intestinal disorders in pigs. *In vitro* (intestinal pig cell lines) and *in vivo* investigations on pigs were considered in the literature search. General criteria of including peer-reviewed journal articles in English and selectively including book articles or chapters, as well as grey literature such as PhD theses and dissertations were used.

2. Definitions

The widely accepted definition of probiotics was formulated by a FAO/WHO Commission of experts in 2001: "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). Most of the species ascribed as having probiotic properties belong to the genera *Lactobacillus* and *Bifidobacterium*, commonly found in the gastrointestinal tract (GIT) of humans and animals and thus generally regarded as safe. However, also members of other bacterial genera can have probiotic activity, indeed most of the probiotic strains used in pig farms belong to *Bacillus*, *Enterococcus* and *Saccharomyces* genera. Such strains are selected mainly on the basis of their good producibility on larger scales and high viability and stability during storage and feed preparation (Ohasi and Ushida, 2009).

Amongst various possible mechanisms of action, probiotics are believed to exert their effects by production of antimicrobial substances, competition with pathogens for adhesion sites and nutrients, enhancement of mucosal barrier integrity and immune modulation (O'Hara and Shanahan, 2007; Bermudez-Brito et al., 2012). Thus, the beneficial activities of probiotics are ascribable to three main core benefits: supporting a healthy gut microbiota, a healthy digestive tract and a healthy immune system (Hill et al., 2014).

It is widely recognized that the health benefits of probiotics are highly strain-specific, thus different strains belonging to the same species can have different effects. For such reason, multi-strain mixtures may be more effective than single strains by complementing each other's health effects and exerting synergistic activities (Timmerman et al., 2004). Prebiotics are "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that have the potential to improve host health" (Gibson and Roberfroid, 1995).

Prebiotics can also be fermented in pig large intestine (Jensen, 1998). From that derives the capacity of prebiotics to positively modulate the composition and/or activity of gut microbiota that confer benefits upon host wellbeing and health (Gibson, 2004;