

# Review: Are we using probiotics correctly in post-weaning piglets?

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*Intensive farming may involve the use of diets, environments or management practices that impose physiological and psychological stressors on the animals. In particular, early weaning is nowadays a common practice to increase the productive yield of pig farms. Still, it is considered one of the most critical periods in swine production, where piglet performance can be seriously affected and where they are predisposed to the overgrowth of opportunistic pathogens. Pig producers nowadays face the challenge to overcome this situation in a context of increasing restrictions on the use of antibiotics in animal production. Great efforts are being made to find strategies to help piglets overcome the challenges of early weaning. Among them, a nutritional strategy that has received increasing attention in the last few years is the use of probiotics. It has been extensively documented that probiotics can reduce digestive disorders and improve productive parameters. Still, research in probiotics so far has also been characterized as being inconsistent and with low reproducibility from farm to farm. Scientific literature related to probiotic effects against gastrointestinal pathogens will be critically examined in this review. Moreover, the actual practical approach when using probiotics in these animals, and potential strategies to increase consistency in probiotic effects, will be discussed. Thus, considering the boost in probiotic research observed in recent years, this paper aims to provide a much-needed, in-depth review of the scientific data published to-date. Furthermore, it aims to be useful to swine nutritionists, researchers and the additive industry to critically consider their approach when developing or using probiotic strategies in weaning piglets.*

**Keywords:** feed additive, gut health, antibiotic alternative, nutrition, swine

## Implications

This review critically examines the use of probiotics in post-weaning piglets, focusing on challenge situations, and proposes potential strategies to increase consistency in probiotic effects. Given the current lack of reproducibility commonly described with probiotic use, this approach could have significant positive effects upon the efficacy of probiotic products and economic viability of the swine industry.

## Introduction

In intensive farming systems, piglets are weaned at much earlier ages (between 3 and 5 weeks) than are those that would be expected in a natural environment (around 17 weeks (Jensen and Recén, 1989)). This early weaning situation is considered one of the most critical periods in swine production, in which the animals have to face multiple stressors. Piglets undergo complex social changes, such as separation from their mothers and littermates (Pluske *et al.*,

1997). In addition, they have to adapt to abrupt changes in the feed regime and in the environment (Weary *et al.*, 2008), leading to a variable period of hypo- or anorexia (Bruininx, 2001). All of this happens at a time when the animals still have an immature immune system (Lallès *et al.*, 2004), low thermoregulation (Le Dividich and Herpin, 1994) and digestive capacities (Lallès *et al.*, 2007a), together with unstable intestinal microbiota (Wang *et al.*, 2013). Weaning is, therefore, a time where the performance of the pigs is seriously affected (Lallès *et al.*, 2007b), and where piglets are predisposed to the overgrowth of opportunistic pathogens like *Salmonella* or *Escherichia coli* (Pluske *et al.*, 1997; Fohse *et al.*, 2016). Altogether, the process is known as a post-weaning syndrome and has been extensively studied and reviewed (Pluske *et al.*, 1997; Lallès *et al.*, 2007a; Heo *et al.*, 2013).

The traditional approach to overcome this situation has been the use of in-feed antibiotics. However, in Europe, the use of antibiotics as growth promoters has been banned (Regulation (EC) No. 1831/2003), and worldwide authorities are also pressing to limit its therapeutic use (National Pork Board, 2015; European Food Safety Authority and European Medicines Agency, 2017). With this context, the pig industry and researchers are making great efforts in trying to find

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biosecurity (Madec *et al.*, 2000), management (Weary *et al.*, 2009; Heo *et al.*, 2013), genetic (Lunney, 2007) and feeding (Pluske *et al.*, 2002; Lallès *et al.*, 2007b) strategies to help piglets overcome the challenges of weaning. Among them, a nutritional strategy that has received increasing attention in recent years is the use of probiotics. It has been extensively documented that probiotics can reduce digestive disorders and improve productive parameters (Ahasan *et al.*, 2015; Bajagai *et al.*, 2016). Still, research in probiotics so far has been characterized as being inconsistent and with low reproducibility from farm to farm. Consequently, although probiotics have demonstrated good potential, many farmers do not consider them to be reliable.

The objective of this review is to critically examine the use of probiotics in the post-weaning phase, focusing on challenge situations, in order to assess whether we are making good use or not of these types of products. Thus, scientific literature related to probiotic effects in experimental models of disease will be reviewed, and subsequently, a discussion about the actual practical approach when using probiotics and how it could be improved will be presented.

Considering the boost in probiotic research observed in the last few years, this paper aims to provide a much-needed, in-depth review of the scientific data published to-date. Furthermore, it aims to be useful to swine nutritionists, researchers and the additive industry to critically consider their approach when using or developing probiotic strategies in post-weaning piglets.

### Use of probiotics against pathogens

A vast amount of research is published yearly in relation to probiotic capacities to improve gastrointestinal health and fight digestive pathogens. It is worth mentioning that the interest of finding probiotic strategies to fight these pathogens not only exists in animal production, but it is also present in human medicine, which, in many cases, uses pigs as a *One Health* approach (Mardones *et al.*, 2017) or as a human model of disease (Meurens *et al.*, 2012). This fact enriches the amount of information available and may be useful for the pig industry. Table 1 recalls main scientific studies published to-date, assessing the use of probiotics against pathogens in piglet experimental models of disease.

#### *Limits on therapeutic use of probiotics*

The first important factor observed is that many authors reported a positive effect by using probiotics, but there is also a considerable amount of research not supporting their use in a disease situation. In general terms, there is a higher number of articles describing beneficial effects with the use of probiotics (>80%) rather than negative effects. However, we must consider that we may have a positive-outcome bias, as many times there may not be industrial interest to publish neutral or negative results (Fanelli, 2012). Still, in view of the current published data, it can be concluded that in the majority of cases probiotic effects against pathogens were positive, although they tended to be rather discrete.

Spectacular improvements, such as eliminating pathogen excretion or important increases in productive parameters have not been reported. Hence, with this background, a first takeaway message would be to stop looking for probiotics as direct replacements for antibiotics, as their effects are not comparable. Alternatively, as proposed by the European Food Safety Authority, probiotics should be considered as zoo-technical additives, in the category of digestibility enhancers or gut flora stabilizers (European Food Safety Authority, 2007). This change of mindset implies that, although with probiotics we may potentially target the same objectives than with antibiotics, when using probiotics our approach should be different. In other words, we should not include a probiotic and expect the same effects than with an antibiotic on its own, but we should combine them with other feed and/or management strategies with a more holistic approach.

#### *Uncertainties around probiotics effects*

Another apparent aspect of the reported results is that it is extremely difficult to discuss and extract conclusions with the data reported to-date because the conditions in which the probiotics have been tested are highly variable. There are important differences in experimental factors such as piglet days of age, treatment concentrations and dosing methods, or other aspects not reflected in Table 1, such as genetics, sanitary status, treatment days or diets. Probiotic effects are known to be treatment specific, depending on the particular strain, dose and context (Bosi and Trevisi, 2010; Li *et al.*, 2012), and host specific, depending on host-related physiological parameters (e.g. health status and genetics) or environment (e.g. sanitary status and diet) (Collado *et al.*, 2007; Mulder *et al.*, 2009; Dinan and Cryan, 2016). Thus, it would be possible that probiotic strains that were not used in a certain trial turned out to be useful in another one, or vice versa. Undoubtedly, this background of uncertainty has made probiotics to be regarded as untrustworthy, being one of the main reasons preventing them from being widespread in the swine industry (Bosi and Trevisi, 2010). A first approach to reduce this variability could be to standardize conditions in which probiotics are studied to have tight control of the variables. However, although this strategy may potentially increase consistency in probiotic research, this would preclude even more the extrapolations of scientific results to the wide array of real-life situations present in pig production. Finally, another approach would be to increase basic research to investigate in-depth the physiological reasons for this variability, with the aim of developing tailored strategies to each situation.

#### *Potential risks of probiotics*

Furthermore, another important point is that results shown in Table 1 suggest that there may be potential risks when using certain probiotics in animals with damaged gut health or pathogen pressure. It has been documented in scientific literature that a baseline of bacterial translocation, possibly due to the increased para/trans-cellular permeability in the

**Table 1** Pig in vivo scientific works evaluating the use of probiotics against digestive bacterial pathogens (*Escherichia coli* and *Salmonella* sp.)

References	Probiotic	Pathogen	Animals		
	Strain, dose per pig and dosing method	Strain and dose per pig	Days old: weaning → Inoculation	Benefits	Main results
De Cupere <i>et al.</i> (1992)	(a) <i>Bacillus cereus</i> var. <i>Toyo</i> ( $1 \times 10^9$ cfu/g) (b) <i>Lactobacillus</i> spp. ( $7.5 \times 10^7$ cfu/g) (c) <i>Streptococcus faecium</i> ( $5.6 \times 10^8$ cfu/g) Included in feed	<i>Escherichia coli</i> 0141 K85 ( $10^9$ cfu)	28 → 30	No	No improvements on clinical symptoms or mortality. No improvements on fecal <i>E. coli</i> shedding
Shu <i>et al.</i> (2001)	<i>Bifidobacterium lactis</i> HN019 ( $10^9$ cfu/day) Oral administration	<i>E. coli</i> sp.	21 → natural acquisition	Yes	Reduced diarrhea scores and fecal shedding of <i>E. coli</i> . Improved animal performance. Increased T-cell differentiation and pathogen-specific antibody titers
Bhandari <i>et al.</i> (2008)	<i>Bacillus subtilis</i> ( $6 \times 10^8$ cfu/kg) Included in feed	<i>E. coli</i> K88 ( $4 \times 10^{10}$ cfu)	17 → 24	Yes	Reduced diarrhea scores and mortality. Modulated microbial diversity.
Lessard <i>et al.</i> (2009)	(a) <i>Pediococcus acidilactici</i> (b) <i>Saccharomyces cerevisiae</i> (c) <i>P. acidilactici</i> + <i>S. cerevisiae</i> Lactation ( $10^9$ cfu). Oral administration Weaning ( $10^9$ cfu/kg). Included in feed	<i>E. coli</i> O149: F4 K88 ( $10^9$ cfu)	21 → 49 + 50 + 51	Yes	Before challenge: (a) increased T-cell differentiation. After challenge: (a, b, c) Reduced bacterial translocation. (b) Increased ileal immunoglobulins
Zhang <i>et al.</i> (2010)	<i>Lactobacillus rhamnosus</i> GG ( $10^{11}$ cfu/day) Oral administration	EPEC 149: K91, K88ac ( $10^{10}$ cfu)	18 → 26	Yes	Reduced diarrhea scores and fecal coliform shedding. Modulated microbial diversity. Increased jejunal immunoglobulins. Modulated systemic inflammatory cytokines
Bhandari <i>et al.</i> (2010)	<i>E. coli</i> ( $4.5 \times 10^{12}$ cfu) Included in feed (daily mix) <sup>1</sup>	<i>E. coli</i> K88 ( $1.2 \times 10^{11}$ cfu)	21 → 27	Yes	Reduced EPEC in ileum. Improved animal performance
Wang <i>et al.</i> (2009)	<i>Lactobacillus fermentum</i> I5007 ( $2 \times 10^9$ cfu) Oral administration	<i>E. coli</i> K88ac ( $2 \times 10^9$ cfu)	21 → 21	Yes	Increased T-cell differentiation and ileum cytokine expression
Konstantinov <i>et al.</i> (2008)	<i>Lactobacillus sobrius</i> DSM 16698 ( $10^{10}$ cfu) Included in feed (daily mix) <sup>1</sup>	EPEC K88 O149 F4 ( $1.5 \times 10^{10}$ cfu)	21 → 28	Yes	Reduced levels of EPEC in the ileum, improved performance and increased diarrhea
Krause <i>et al.</i> (2010)	<i>E. coli</i> ( $1.5 \times 10^{11}$ cfu) Included in feed (daily mix) <sup>1</sup>	<i>E. coli</i> K88 ( $1.4 \times 10^{10}$ cfu)	17 → 24	Yes	Increased animal performance and microbial diversity. Reduced diarrhea scores (in presence of raw potato starch)
Daudelin <i>et al.</i> (2011)	(a) <i>Pediococcus acidilactici</i> MA18/5 M (b) <i>S. cerevisiae</i> SB-CNCM I-1079 (c) <i>P. acidilactici</i> + <i>S. cerevisiae</i> Sows: gestation ( $3 \times 10^9$ cfu) + lactation ( $6 \times 10^9$ cfu). Included in feed (daily mix) <sup>1</sup> Piglets: lactation ( $1 \times 10^9$ cfu). Oral administration Weaning: $2 \times 10^9$ cfu/kg. Included in feed	EPEC 0149 F4 ( $5 \times 10^9$ cfu)	21 → 28	Yes	(a, b) Reduced EPEC attachment to intestinal mucosa. (a,c) Induced ileum cytokine expression
Trevisi <i>et al.</i> (2011)	<i>L. rhamnosus</i> GG ( $6 \times 10^9$ cfu) Included in feed (daily mix) <sup>1</sup>	EPEC F4 ( $1.5 \times 10^{10}$ cfu)	21 → 28	No	Reduced animal performance. Increased diarrhea scores. Reduced serum immunoglobulins. Tended to a worse histomorphology

Table 1 (Continued)

References	Probiotic	Pathogen	Animals	Benefits	Main results
	Strain, dose per pig and dosing method	Strain and dose per pig	Days old: weaning → Inoculation		
Li <i>et al.</i> (2012)	<i>L. rhamnosus</i> ACTT 7469 – High ( $10^{10}$ cfu) and low dose ( $10^{12}$ cfu) Oral administration	ETEC F4 K88 ( $10^{10}$ cfu)	21 → 28	Yes	High and low dose reduced fecal coliform shedding and improved diarrhea scores (low dose was more effective)
Guerra-Ordaz <i>et al.</i> (2014)	<i>Lactobacillus plantarum</i> JC1 B2028 ( $2 \times 10^{10}$ cfu) Included in feed (daily mix) <sup>1</sup>	ETEC K88 ( $1.2 \times 10^{10}$ cfu)	25 → 33	Yes	Improved ileal histomorphology. Reduced systemic inflammatory cytokines. Improved fermentation profile in ileum and colon
Zhu <i>et al.</i> (2014)	<i>L. rhamnosus</i> ACTT 7469 – High ( $10^{12}$ cfu) and low ( $10^{10}$ cfu) dose Oral administration	ETEC F4 K88 ( $10^{10}$ cfu)	21 → 28	Yes	Both doses improved diarrhea scores. Modulated ileal T-cell differentiation. High dose increased serum cytokine expression
Zhou <i>et al.</i> (2015)	<i>Bacillus licheniformis</i> DSM 5749 + <i>B. subtilis</i> DSM 5750 – high ( $8 \times 10^8$ cfu) and low ( $4 \times 10^8$ cfu) dose Oral administration	ETEC 0149 F4 K88 ( $10^{10}$ cfu)	21 → 28	Yes	Increased serum and ileal T-cell differentiation. Low dose: increased jejunal cytokine expression
Trevisi <i>et al.</i> (2015)	<i>S. cerevisiae</i> CNCM I-4407 ( $5 \times 10^8$ cfu/kg) Included in feed ( $2 \times 10^{11}$ cfu/kg) Oral administration	<i>E. coli</i> 0149 F4ac ( $10^8$ cfu)	24 → 31	Yes	Reduced diarrhea scores. Reduced fecal ETEC shedding. Modified blood metabolic profile
Yang <i>et al.</i> (2016)	<i>B. licheniformis</i> DSM 5749 + <i>B. subtilis</i> DSM 5750 – high ( $8 \times 10^8$ cfu) and low ( $4 \times 10^8$ cfu) dose Oral administration	ETEC/VTEC/EPEC F4+ ( $10^{10}$ cfu)	21 → 28	Yes	Increased intestinal cytokines and epithelial barrier integrity
Zhang <i>et al.</i> (2017)	<i>B. licheniformis</i> DSM 5749 + <i>B. subtilis</i> DSM 5750 – high ( $4 \times 10^9$ cfu), moderate ( $8 \times 10^8$ cfu) and low ( $4 \times 10^8$ cfu) dose Oral administration	ETEC 0149 F4 K88 ( $10^{10}$ cfu)	21 → 28	Yes	Modulated microbiota and improved histomorphological parameters
Barba-Vidal <i>et al.</i> (2017a)	<i>B. longum</i> subsp. <i>Infantis</i> CECT7210 ( $10^9$ cfu) Oral administration	ETEC K88 ( $5 \times 10^9$ cfu and $5 \times 10^{10}$ cfu)	21 → 26 + 27	Yes	Reduced intestinal colonization of pathogens. Stimulated local immune response. Effects on feed intake, microbial fermentation and intestinal architecture showed a differential pattern between challenged and non-challenged animals (not favorable in challenged animals)
Trevisi <i>et al.</i> (2017)	<i>S. cerevisiae</i> CNCM I-4407 ( $5 \times 10^8$ cfu/kg) Included in feed	<i>E. coli</i> 0149 F4ac ( $10^8$ cfu)	24 → 31	Yes	Improved intestinal architecture. Limited early activation of gene sets related to impairment of jejunal mucosa
Casey <i>et al.</i> (2007)	<i>Lactobacillus murinus</i> DPC6002 and DPC6003, <i>Lactobacillus pentosus</i> DPC6004, <i>Lactobacillus salivarius</i> DPC6005, and <i>Pediococcus pentosaceus</i> DPC6006. Probiotic mix ( $4 \times 10^9$ cfu) or fermentate ( $4 \times 10^{10}$ cfu). Oral administration	<i>Salmonella typhimurium</i> ( $10^8$ cfu)	N/A → N/A + 15 + 16 + 17	Yes	Reduced diarrhea scores. Increased animal performance. Reduced fecal <i>Salmonella</i> shedding

Szabó <i>et al.</i> (2009)	<i>E. faecium</i> NCIMB10415 (Microencapsulated). Sows ( $10^9$ cfu/kg), suckling piglets and weaned piglets ( $2.5$ to $3 \times 10^8$ cfu/kg) Included in feed	<i>S. typhimurium</i> DT104 ( $6 \times 10^9$ cfu)	14 → 28	No	Increased colonization and fecal shedding of <i>Salmonella</i> . Increased serum immunoglobulins
Walsh <i>et al.</i> (2012)	<i>E. faecium</i> + <i>B. subtilis</i> + <i>B. licheniformis</i> ( $10^9$ cfu/L for each strain) Included in drinking water	<i>S. typhimurium</i> ( $10^{10}$ cfu)	19 → 25	No	Increased coliform shedding, no effect on <i>Salmonella</i> scores. Prevented decrease in animal performance
Kreuzer <i>et al.</i> (2012)	<i>E. faecium</i> NCIMB10415 (Microencapsulated). Sows, suckling piglets and weaned piglets ( $10^9$ to $5 \times 10^9$ cfu). Included in feed	<i>S. typhimurium</i> DT104 ( $2 \times 10^{10}$ cfu) 38 days	28 → 38	No	Reduced animal performance. No effect on fecal <i>Salmonella</i> shedding. Increased pathogen translocation
Yin <i>et al.</i> (2014)	(a) <i>Lactobacillus zeae</i> (b) <i>Lactobacillus casei</i> Fermented feed ( $10^9$ cfu/ml)	<i>S. typhimurium</i> DT104 ( $10^6$ to $10^7$ cfu) 31 days	28 → 31	Yes	(a, b) Improved diarrhea scores. Decreased rectal temperature, serum haptoglobin concentrations and fecal <i>Salmonella</i> shedding. (a) Reduced pathogen translocation
Naqid <i>et al.</i> (2015)	<i>L. plantarum</i> B2984 ( $10^{10}$ cfu/day) Included in feed (daily mix) <sup>1</sup>	<i>S. typhimurium</i> SL1344 ( $10^8$ cfu)	28 → 35	Yes	Increased serum immunoglobulins
Upadhaya <i>et al.</i> (2017)	a) <i>B. subtilis</i> RX7 ( $1 \times 10^9$ cfu/g) b) <i>Bacillus methylotrophicus</i> C14 ( $1 \times 10^9$ cfu/g) Included in feed	<i>S. typhimurium</i> ( $10^{11}$ cfu)	28 → 39	Yes	(a, b) Decreased <i>Salmonella</i> fecal shedding. Modulated microflora, serum systemic inflammatory cytokines and stress biomarkers
Barba-Vidal <i>et al.</i> (2017c)	<i>B. licheniformis</i> CECT 4536 ( $10^9$ cfu/kg) Included in feed	<i>S. typhimurium</i> ( $5 \times 10^8$ cfu)	24 → 31	Yes	Reduced the colonization and fecal shedding of <i>Salmonella</i> . Positive effect on some behavioral displays
Barba-Vidal <i>et al.</i> (2017a)	<i>B. longum</i> subsp. <i>Infantis</i> CECT7210 ( $10^9$ cfu) Oral administration	<i>S. typhimurium</i> ( $2 \times 10^9$ cfu and $6 \times 10^9$ cfu)	24 → 32 + 34	Yes	Reduced pathogen shedding. Stimulated local immune response. Effects on feed intake, microbial fermentation and intestinal architecture showed a differential pattern between challenged and non-challenged animals (not favorable in challenged animals)
Barba-Vidal <i>et al.</i> (2017b)	<i>B. longum</i> subsp. <i>infantis</i> CECT7210 and <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BPL6 ( $10^9$ cfu) Oral administration	<i>S. typhimurium</i> ( $5 \times 10^8$ cfu)	28 → 35	Yes	Reduced pathogen shedding. Decreased rectal temperature. Decrease of diarrhea scores. Worsened voluntary feed intake, villous: crypt ratio and fermentation profiles. Stimulation of the intestinal immune system
Ahmed <i>et al.</i> (2014)	(a) <i>Lactobacillus reuteri</i> avibro ( $10^{10}$ cfu/kg) b) <i>B. subtilis</i> + <i>B. licheniformis</i> ( $3.2 \times 10^9$ cfu/kg) Included in feed	<i>S. typhimurium</i> KCTC2515 ( $3 \times 10^9$ cfu) + <i>E. coli</i> KCTC2571 ( $1 \times 10^9$ cfu)	28 → 28	Yes	(a, b) Increased animal performance and nutrient digestibility. Reduced <i>Salmonella</i> and <i>E. coli</i> shedding

N/A = not available.

<sup>1</sup>Daily mix: probiotic suspended on a daily basis and mixed with feed.



enterocyte determined by inflammatory stress, is normally associated with weaning (Lallès *et al.*, 2004). This permeability has been reported to be affected by probiotic treatments such as in Trevisi *et al.* (2008), who reported an increase in translocation with a *Bifidobacterium animalis* and fructo-oligosaccharide treatment in post-weaning piglets. Consequently, an elevated risk of sepsis could be forecast in post-weaning animals when using probiotics (Verna and Lucak, 2010). Moreover, it has also been reported that some probiotics may have immune-suppressive effects in the host (Siepert *et al.*, 2014). This effect has no disadvantageous consequence in a healthy context. Nevertheless, in the need of a rapid humoral response, the immune activation is less efficient (Bosi and Trevisi, 2010) and therefore would also be deleterious in a disease situation. Thus, in a context of increased permeability, it can be hypothesized that some probiotics could impair the immune response and increase risk of sepsis in some animals, despite the observed reductions in pathogen loads.

As stated before, the increase of basic research on probiotics is fundamental to improve the use-criteria of probiotics in the field and to obtain reproducible outcomes. Such a tailored use of probiotics requires a great amount of knowledge of probiotic intrinsic capacities and also of how probiotics modify ecological dynamics of the intestinal microbiota, depending on factors like sanitary status, genetics or feeding practices, among others. Fortunately, there has been great technological development during the last few decades. Nowadays, we have a sufficient amount of quality trials to begin to characterize the strains in relation to their mechanisms of action and interactions with the hosts. This is interesting because it opens a door to knowledge-based treatments, taking into account the context in which they are applied.

### How to improve the use of probiotics in early life stages

In view of the present situation, it goes without saying that improving the use of probiotics in the swine industry relies on a drift from an empirical use to a more knowledge-based strategy. This section provides a few suggestions to be considered in the use of probiotics in early life stages, and in particular in post-weaning disorders. However, its aim is solely to provide a starting point for the reader to critically evaluate the use of probiotics, rather than a dissertation on their use.

To start with, assessment of the probiotic strains should be done in a wide range of health conditions. As commented by Bosi and Trevisi (2010), the identification of strains with positive effects in a broad range of gut health situations, and even capable of working in different species is economically interesting for the additive industry. However, in some cases, although specific strains had demonstrated positive effects in a normal physiological situation, they were reported to be detrimental in challenge situations in piglets (see Table 1). Hence, in our opinion, it would be highly recommended to characterize the possible risks of using a probiotic in a disease context, building clear differences whether probiotic usage is intended as a therapy or as prophylaxis. For

instance, in human studies, a clear distinction is made between research aimed at maintaining health and that which aimed to treat a disease, and this difference has important implications when designing trials and in regulatory affairs (Hill *et al.*, 2014).

A second issue to address is the capacity of probiotics to modulate microbiota. As commented before, until today one major interest when using probiotics has been to replace antibiotics via production of *in situ* antimicrobial compounds or enzymes to cure infections (Patil *et al.*, 2015). Although some particular strains may have demonstrated effects here (Bhandari *et al.*, 2008; Cheikhoussef *et al.*, 2008), their usefulness in this aspect is limited and spectacular improvements such as eliminating pathogen excretion are rarely reported (see Table 1). However, probiotics become much more powerful and valuable when we use them as 'preventive' health promoters and gut microbiota stabilizers (Simmering and Blaut, 2001). There is an increasing amount of scientific publications supporting that probiotic effects in gut ecology and/or immune stimulation may provide support to keep animals healthy (Zhang *et al.*, 2010; Klaenhammer *et al.*, 2012; Prieto *et al.*, 2014; Zacarías *et al.*, 2014). In addition, new selection criteria based on the mechanisms of action of the strains can allow the apparition of other probiotics that have not been previously considered in animal production but can enhance gut health and make it more robust. Besides, to increase control on their effects, probiotic strategies should be more focused. Strains should be selected depending on the objectives being looked for, and not as if probiotics were beneficial for everything. Effects should target specifically to a site. Targeting, for example, M cells if applications seek to boost intestinal immunity by enhancing development of secretory IgA (Corthésy *et al.*, 2007), or targeting the hypothalamic–pituitary–adrenal axis if we want to improve animal well-being and reduce effects of common stressors (Hardy *et al.*, 2013; Zhou and Foster, 2015). In addition, some specific probiotic strains adapted to the colonic environment could be good candidates to fight gut dysbiosis (Corthésy *et al.*, 2007), but other strains could be better to enhance productive performance based on their enzymatic hydrolysis properties (Kim *et al.*, 2007) or biosynthetic pathways for amino acids' new synthesis (Pridmore *et al.*, 2004). Hence, further assessment and classification of commercial probiotics in relation to their mechanisms of action are desirable, to be able to implement strategies that are more precise and oriented to specific needs of these animals.

Another point to take into account is the variability in the response to a probiotic, depending on the host or the herd in which it is introduced. It has been described how a probiotic strategy may have 'responder' and 'non-responder' individuals in a homogenous group of animals, and also how different microbial environments can determine variability among herds (Klaenhammer *et al.*, 2012; Arora *et al.*, 2013; Starke *et al.*, 2013). For instance, it has been described how the genetically determined different presence of sugar complexes along the host gut surface may facilitate the adhesion

on the glycocalyx of some enteropathogens, possessing specific colonization factors (such as *E. coli* F18 and K88) and, possibly, of commensal bacteria (Krogfelt, 1991; Lee *et al.*, 2013). Moreover, the emerging '-omic' technologies clearly open a window to refine our approach and understand better the interactions between a probiotic strain and the ecosystem in which it is going to be introduced. It is expected that by increasing our understanding in pig microbiome knowledge, we will identify key microbial groups of the piglets gut with an important role in maintaining a productive and disease-resistant ecosystems (Kim and Isaacson, 2015). In addition, we will eventually be able to identify the most appropriate strain (or strains) to use as specific probiotic treatments for a particular situation depending on the targeted microbial ecosystem (Sanders *et al.*, 2013). For instance, two enterotype-like clusters have recently been identified in pig microbiota significantly correlated with performance (Ramayo-Caldas *et al.*, 2016). Likewise, to correlate probiotic effects to specific enterotypes would reasonably reduce the variability of empirical use. On the other side, our understanding in probiotic interactions with the host and in particular with the intestinal cells gene expression has greatly improved in recent years. For example, a common mechanism for the anti-inflammatory activity of several probiotics has been described to be regulated by the micro-organisms pattern recognition receptors *toll-like receptor 2 (TLR-2)* (Villena *et al.*, 2012; Tomosada *et al.*, 2013). In addition, it has been described how selective pressures among European pig populations have derived into specific *TLR-2* gene variants (Darfour-Oduro *et al.*, 2016). Overall, this is interesting because it provides a common mechanism for the anti-inflammatory activity of several probiotics (including different strains such as *Lactobacillus* spp. and *Bifidobacterium* spp.) (Tomosada *et al.*, 2013). Moreover, it provides a potential biomarker for the screening and selection of new immune-regulatory strains, to be used efficiently at a population level to enhance immunity.

Furthermore, another possibility to potentiate probiotic effects would be to combine probiotics with complementary actions, with many beneficial examples reported in the bibliography (Casey *et al.*, 2007; Lessard *et al.*, 2009; Zhou *et al.*, 2015; Barba-Vidal *et al.*, 2017b). Probiotic combinations can be multi-strain probiotics, containing more than one strain of the same species or closely related species (for instance, *Lactobacillus acidophilus* and *L. casei*), or multispecies probiotics, containing strains of different probiotic species that belong to one or more genera (e.g. *L. acidophilus*, *Bifidobacterium longum* and *Enterococcus faecium*) (Timmerman *et al.*, 2004). It has been suggested that the greater variety of probiotic genera present within a mixture may reduce its effectiveness, through mutual inhibition by the different species, antimicrobial compounds or competition for either nutrients or binding sites (Chapman *et al.*, 2011 and 2012). However, multispecies probiotics have also been related to a broader spectrum of activity (e.g. inhibition of a wider variety of pathogenic bacteria), and if well-designed, a greater amount of synergism and symbiosis

when different probiotic effects are combined (Timmerman *et al.*, 2004). Hence, although bacterial combinations have a high potential, beneficial properties of different strains are not always additive (Chapman *et al.*, 2011). This is not an easy field of research and bacterial interactions inside the pig gut ecosystem should be further explored to be able to construct effective strategies. Still, unfortunately *in vivo* studies comparing single strains to probiotic combinations are still rare. Additional approaches to strengthen effects could be the addition of specific prebiotic substrates (symbiotic concept) to selectively improve the growth of the introduced strain (Shenderov, 2011; Arboleya *et al.*, 2016) or to promote a microbiota more favorable for the probiotic to exert its action (Guerra-Ordaz *et al.*, 2014). Another option to improve and to specifically select the effects of a probiotic would be the genetic manipulation of the strain (Bjerre *et al.*, 2016; Xu *et al.*, 2016). However, introduction of GMO in the animal feed is nowadays a very controversial issue.

The way a probiotic is administered to the piglets can also be a critical point to consider as, sometimes, reduced stability and viability of the probiotic cells can limit the use of the potentially most beneficial strains. Some bacterial genera are particularly sensitive to be introduced in the dry feed, as they cannot stand chemical–physical conditions of the feed or the manufacturing process (Angelis *et al.*, 2006). In this sense, the development of acclimatization procedures or protective coating to enable them to stand environmental aggression (Sewell, 2016) is a promising field of development for the use of probiotics as in-feed additives. Still, dry feed is not the only way a probiotic can be administered to piglets. Daily administration of fresh probiotic as a solid or liquid suspension by mixing it with the feed (top dressing) is a common procedure in research trials (see Table 1). However, although it may be a good strategy to increase the viability of probiotics when delivered, it is a highly time-consuming routine difficult to be implemented in commercial pig farms. Alternatively, fermented milk, suspension in milk or even suspension in water can be considered. For instance, Gebert *et al.* (2011) supplemented a milk replacer with a *Lactobacillus* probiotic strain and saw positive effects on pre-weaning animals.

Besides, early dosing of probiotics in the pre-weaning period should be considered. Gut microbiota plays a critical role in the adaptation from a neonatal-immature gut to a functional adult system, resistant to adverse ecological shifts at challenges such as weaning (Lewis *et al.* 2012). Hence, providing probiotics at this point could potentially permit the establishment of early and life-long health benefits (Kenny *et al.*, 2011). Sows should be given more importance here, as many studies have shown how introducing probiotics in the sow diet is an effective way to modify the gut ecosystem and the health of piglets (Alexopoulos *et al.*, 2004; Bohmer *et al.*, 2006; Apic *et al.*, 2014; Siepert *et al.*, 2014; Kritas *et al.*, 2015; Scharek-Tedin *et al.*, 2015). Alternatively, the introduction of probiotic strategies via 'creep feed' is increasingly being studied (Alexopoulos *et al.*, 2004; Shim *et al.*, 2005; Giang *et al.*, 2010).

Nevertheless, results of these experiments are largely variable, probably due to the fact that piglets usually ingest small or null quantities of them (Pajor *et al.*, 1991).

## Conclusions

A systematic approach should be undertaken when designing a probiotic intervention to identify potential risk factors of the target animals, the suitability of a specific probiotic strain and the appropriateness of the dosing method. This process is difficult in pig production where a collectivity is being treated. More research is needed to further characterize the mechanisms of action of probiotics and their interaction in different gut health situations. We are, nowadays, able to make science-based prescriptions of probiotics in a limited amount of situations. However, eventually, when sufficient evidence is built up, we will be able to make reliable recommendations for every particular situation. Once at this point, probiotics will be used much more efficiently and the swine industry will be able to obtain the most by investing in these products.

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## Declaration of interest

Authors declare no conflict of interest.

## Ethics statement

None.

## Software and data repository resources

None.

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