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Probiotics as alternatives to antibiotics in treating post-weaning diarrhoea in pigs: Review paper

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Abstract

The use of antibiotics to prevent post-weaning diarrhoea (PWD) in pigs has faced a setback owing to the associated antibiotic resistance in pigs and in the human populace that consumes the pork. In fact, antibiotic resistance that originates from the food chain is estimated to cause around 700,000 deaths globally each year. Consequently, scientists and researchers have suggested possible alternatives to antibiotics in pig diets. The chief of these has been the use of probiotics. The authors reviewed the literature on the use of probiotics as an alternative to antibiotics in treating PWD in pigs. It is clear that because of pathogenic *Escherichia coli* PWD continues to be a challenge to profitable swine production. The vast number of studies that was reviewed, point to the beneficial effects of probiotic supplementation on reducing the severity and incidence of PWD. However, some studies report inconsistencies to the general hypothesis. The majority of the microorganisms used as probiotics in the studies belong to the genera *Lactobacilli*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, probiotic *Escherichia coli*, and *Saccharomyces*. The review also revealed that the bacterial strains that are used as probiotics are given individually or as combinations of multiple strains, and at various dosages, yielding varied results in each case. Interestingly, the authors observed wide disparities in the onset of probiotic supplementation and duration of the treatment to attain the results. Hence there is a need to standardize supplementation strategies, including dosage, onset and duration of treatment for probiotics. Furthermore, many of the *in vivo* studies that revealed positive effects of probiotics on diarrhoea and other production parameters were carried out in more controlled environments. The authors therefore suggest that more field studies in more natural and commercial farm settings should be conducted to augment the literature in relation to the use of probiotics as alternatives to antibiotics in treating PWD.

Keywords: antimicrobial resistance, directly-fed microorganisms, post-weaning diarrhoea, swine diets
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Introduction

Post-weaning diarrhoea (PWD) has been documented as a major economic disease that affects swine farmers globally. PWD causes plenty of losses to the farmers through increased deaths, morbidities, lowered productivity of surviving pigs, and the costs of treatment protocols (Zhao & Kim, 2015). Generally, piglets are affected during the first two weeks post weaning. This is a stressful period that is characterized by separation from the sow, changes in diet, adaptation to new environments, mixing of litters, and small intestine histological changes. All these changes may affect the immune response, predisposing the piglets to gastrointestinal tract (GIT) dysfunction (Gresse *et al.*, 2017; Rhouma *et al.*, 2017). Moreover, it is widely accepted that dietary transition, coupled with environmental changes, as occurs during weaning, modifies the piglets' intestinal microbiome and this is believed to be connected with the development of observed diarrhoea and other enteric infections (Lallès *et al.*, 2007). PWD is associated with the colonization and proliferation of enterotoxigenic *Escherichia coli* (ETEC) strains in the pig intestine (Dubreuil, 2017; Pan *et al.*, 2017; Rhouma *et al.*, 2017). These strains function through producing enterotoxins that act on the small

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intestines and lead to the secretion of fluids and electrolytes, causing diarrhoea. To curb PWD and other opportunistic stressors and improve the growth of piglets, there has been widespread use of antibiotic feed additives (Pan *et al*., 2017; Reid & Friendship, 2002). However, the prolonged and irrational use of these antibiotics in the food chain has led to bacterial resistance in the animals and in consumers of their products. This resistance has led to the hindered administration of effective therapies, thereby increasing morbidities and mortalities (Daudelin *et al*., 2011; Yi *et al*., 2016). The resistance in humans stems from the fact that antibiotic molecules used in animals have a generic resemblance to those applied in humans (Liao & Nyachoti, 2017). Thus, the use of antibiotics has been banned in many regions of the world, such as the European Union in 2006 (Chen *et al*., 2005), or its use been limited, such as in the US, where the Food and Drug Administration (FDA) has given approval to very few antibiotics. Moreover, there is now a slowed production of new antibiotics on the market (Yi *et al*., 2016). This cascade of events around the use of antibiotics instigated researchers to come up with alternatives that could be applied in swine diets to prevent PWD, improve feed efficiency, promote growth, reduce odour, and ultimately confer health benefits to swine (Vondruskova *et al*., 2010). Thus, various naturally occurring materials have been suggested and applied in swine diets, and their effects have been investigated *in vitro* and *in vivo*. Alternative molecules that have been used include organic acids, probiotics, prebiotics, enzymes, medium chain fatty acids, essential oils, yeasts, zinc, and plant extracts. These have all proved to be effective replacements for antibiotics (Vondruskova *et al*., 2010; Omonijo *et al*., 2017). However, among all these alternatives, probiotics have been widely and extensively used as better replacements for the subtherapeutic antibiotic doses that are often used in curbing PWD. A summary of microorganisms that are commonly used as probiotics is shown in Table 1. In this article, the authors review and summarize the research outputs and literature pertaining to the use of probiotics in the prevention and treatment of PWD in swine.

Post-weaning Diarrhoea in Piglets

Post-weaning diarrhoea that is caused by ETEC is a detrimental condition that often occurs in weaned pigs two weeks post separation from the sows and is characterized by watery faeces, sudden deaths, dehydration, and retarded growth in survivors (Lauridsen, 2017; Rhouma *et al*., 2017). Although ETEC is widely implicated in PWD, the condition is a multifactorial phenomenon whose exact cause has yet to be ascertained. In many scenarios, its occurrence has been associated with the interaction between the sow, piglet, pen environment, *E. coli*, and the overall farm management (Laine *et al*., 2008; Rhouma *et al*., 2017). Thus, the factors that influence the onset of PWD can be categorized broadly into predisposing, contributing, and determining factors. Among the predisposing factors are genetic predisposition, immunity, weaning weight, and age. Studies have indicated that low weaning weight and age, coupled with impaired pre-weaning health, contribute greatly to PWD (Laine *et al*., 2008; Rhouma *et al*., 2017). Additionally, early weaned piglets have been demonstrated to have impaired immune functions and this further predisposes them to diarrhoea development during the weaning period. Further, owing to the immaturity of the intestinal immunity and the loss of the passive immunity as a result of the lack of the IgA-rich sow's milk, piglets become susceptible to many opportunistic pathogens in their gut (Laine *et al*., 2008; Heo *et al*., 2013; Rhouma *et al*., 2017). The proliferation of strains of β-haemolytic ETEC (strains expressing F4 or F18 fimbriae) in the small intestines of piglets is widely associated with PWD and, as a crucial step in its pathogenesis, the interaction between the fimbria and receptor enables the pathogen to fully colonize the small intestines (Frydendahl *et al*., 2003; Fairbrother *et al*., 2005). Thus, fimbriae F4 and F18 are crucial to the colonization of small intestines by pathogenic strains that cause post-weaning diarrhoea. However, the presence or absence of intestinal receptors for F4/F18 is controlled genetically and determines whether the piglets are susceptible (Frydendahl *et al.*, 2003). Other factors that have been implicated in the predisposition of piglets to PWD and other microbial infections include litter size, parity of the sow, postpartum dysgalactia and other piglet associated conditions such as birth order and genotype (Hong *et al*., 2006).

Moreover, factors that occur immediately after weaning, including housing sanitation, the numbers of piglets per pen, feeding regimes and pig flow systems, contribute greatly to the scourge of PWD. As reviewed by Jayaraman & Nyachoti (2017), the sanitation status of the swine environment in commercial production has a direct bearing on pig health. In the same paper, it is reported that poor sanitary conditions in a pig farm are a precursor to low inflammation in weaned piglets. Poor sanitary and hygienic situations occur when the pens are not cleaned and disinfected prior to placement and during the rearing period, because of negligent stockmanship and unremoved leftover feed, among others.

Table 1 Microorganisms commonly used as probiotics in swine nutrition

Genus	Description	Species	Strain	Reference
Lactobacillus	Gram-positive, produce lactic acid as their end product of carbohydrate fermentation. Has a large number of species categorized as GRAS	*L. Plantarum*	ZJ316, DSMZ 8862/8866, LQ80, JDFMLP11	(Heo *et al*., 2018; Mizumachi *et al*., 2009; Pieper *et al*., 2011; Suo *et al*., 2012)
		L. fermentum	I5007	(Liu *et al*., 2014)
		L. casei	GG	(Roselli *et al*., 2005)
		L. acidophilus	C3, NCDC-15	(Dowarah, Verma*, et al*., 2017a; Giang *et al*., 2011)
		L. brevis	ATCC 8287, 1E1	(Gebert *et al*., 2011; Lähteinen *et al*., 2014)
		L. salivarius	UCC118	(Riboulet-Bisson *et al*., 2012)
		L. reuteri	BSA131, ATCC53608, NCIMB 30242,	(Hou *et al*., 2015)
Bacillus	Ubiquitous in the environment, endospore forming and hardy enough to survive in a variety of foods compared with other probiotic species	*B. subtlis*	LS 1–2, MA139, M-1, DSM 5750	(Additives & Feed, 2011; Guo *et al*., 2006; Lee *et al*., 2014; Wang *et al*., 2011)
		B. licheniformis	DSM 5749	(Additives & Feed, 2011)
		B. cereus	*toyoi*	(Papatsiros *et al*., 2011)
Bifidobacterium	Gram-positive, anaerobic ubiquitous inhabitants of the mammalian mouth, GI tract, and vagina. Being saccharolytic in nature, they produce acetic and lactic acid without CO_2 production except during gluconate degradation	*B. longum*	-	(Brown *et al*., 1997; Estrada *et al*., 2001)
		B. animalis	CSCC 1941, MB5	(Bird *et al*., 2009; Roselli *et al*., 2005)
		B. lactis	NCC2818	(Merrifield *et al*., 2013)
Probiotic Escherichia coli	Non-pathogenic in nature, a commensal isolate of E. coli and forms a basis for production of probiotics. Used in the treatment of intestinal disorders	*E. coli*	Nissle 1917 (O6:K5: H1)	(Duncker *et al*., 2006; D. Krause *et al*., 2010)
Enterococcus	Found in the mammalian GIT and on the skin. Belongs to the LAB group. Has antagonistic properties to harmful bacteria hence used as a probiotic	*Enterococcus faecium*	DSM 7134	(Lojanica *et al*., 2010)
Saccharomyces	Commonest symbiotic yeast inhabiting the respiratory, gastrointestinal tract and the vaginal mucosa. Also referred to as brewer's yeast. Probiotic properties of yeast include antagonizing other microorganisms such as moulds and bacteria	*Saccharomyces cerevisiae var. boulardii*	-	(Badia *et al*., 2012)
		Saccharomyces cerevisiae	CNCM I-4407 (SCC)	(Priori *et al*., 2015)

GRAS: Generally regarded as safe, GIT: Gastrointestinal tract, LAB: Lactic acid bacteria

Poor hygiene allows the proliferation of pathogenic microorganisms in the piglets' environment, which terminally find their way into the gut via the faecal-oral pathway. Post-weaning feeding and feeding regimes have been implicated in PWD, and the sudden change from sow milk to a solid ration, as occurs during weaning, has been demonstrated to reduce villus height while increasing crypt depths in the ileum, leading to diarrhoea (Dong & Pluske, 2007). Since the piglets are not accustomed to the solid feed that is offered after weaning, there is a reduction in voluntary feed intake, which affects overall nutrient capture and adsorption (Lalles *et al.*, 2007). The scope of this paper does not dwell on the details of feeding in relation to diarrhoea since this has been discussed extensively in other reviews (Dong & Pluske, 2007; Lalles *et al.*, 2007). However, studies have indicated that farms that fed piglets twice a day with restricted amounts of feed suffered a higher prevalence of PWD compared with farms that provided feed ad libitum to the piglets (Laine *et al.*, 2008; Rhouma *et al.*, 2017).

Escherichia coli is the most abundant aerobic coliform species in the normal colon. However, it has the potential to be an enteric pathogen and cause diarrhoea in the host. The ETEC proliferation in the gut that usually occurs post weaning in pigs is highly responsible for the common intestinal disorder that is designated post-weaning colibacillosis (Wellock *et al.*, 2007). ETEC elicits hypersecretory diarrhoea once their colonies reach enough numbers in the gut, and this lasts for up to 14 days. These *E. coli* pathogens act through attaching to the small intestinal microvilli and producing enterotoxins that act locally to disrupt the normal functioning of enterocytes. This leads to hypersecretion of water and electrolytes, coupled with impaired absorption and thus the observed severe watery diarrhoea (Nagy & Fekete, 1999; Wellock *et al.*, 2007). A flow of events that lead to diarrhoea and death is shown in Figure 1 (Fairbrother *et al.*, 2005). Studies have revealed four *E. coli* toxins that interact with the intestines of the weaners. LT enterotoxin (heat labile), STI and STII (heat stable), and Shiga-like exotoxin type 2 variant (SLTIIv) are involved in oedema disease (Madec *et al.*, 2000). *E. coli* pathogenic strains colonize the gut using fimbriae adhesion factors to produce the exotoxins that are responsible for disease occurrence. The most common fimbriae used by pathogenic *E. coli* in weaned pigs include F4 (K88) and F18. However, other fimbriae such as F5 (K99), F6 (987P) and F41 are detectable and often associated with E. coli that causes neonatal diarrhoea (Frydendahl, 2002; Nagy & Fekete, 1999; Fairbrother *et al.*, 2005). Infection of the piglets with ETEC is evidenced by signs that include the loss of body condition, depression, dehydration, lowered feed intake, hampered weight gain, loss of weight, and, ultimately, death (Madec *et al.*, 2000).

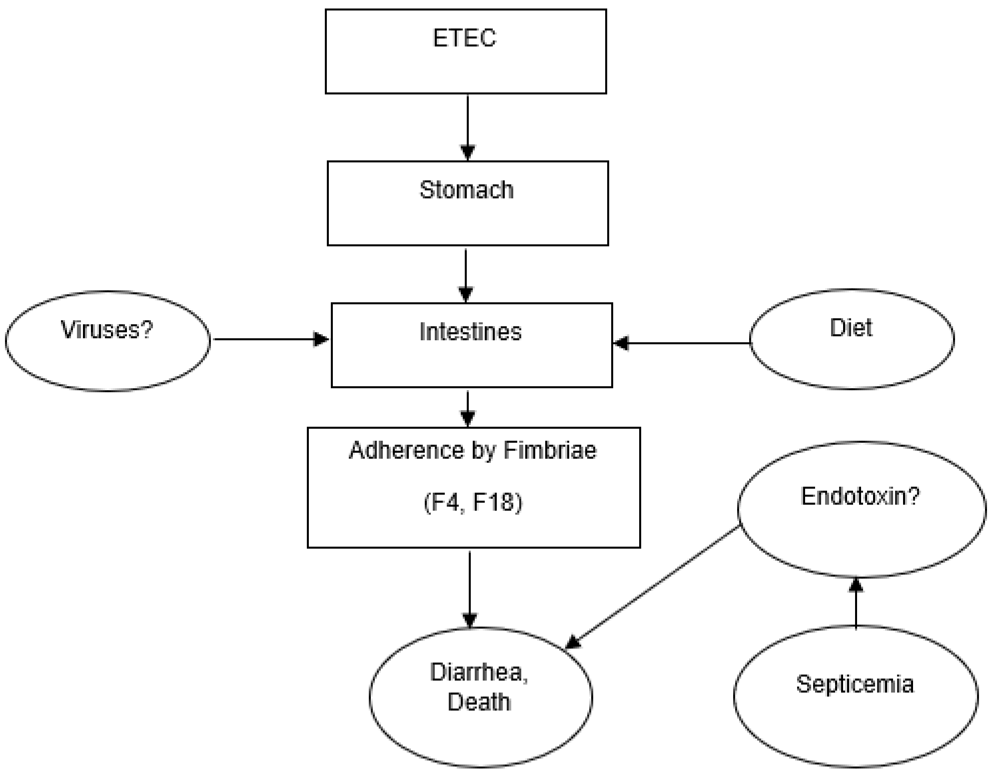

Figure 1 Pathogenesis of diarrhoea due to enterotoxigenic *Escherichia coli*

Probiotics Use in Prevention and Control of Post Weaning Diarrhoea

After the ban on the use of antibiotics in animal feed – which was executed in January 2006 in the EU owing to feared and observed emergence of antibiotic-resistant bacteria (ARBs), coupled with increasing demands from consumers for safe and health foods of animal origin – newer strategies to curb PWD and promote growth have been suggested and tried. Hence, as an alternative to antibiotics, the use of probiotics has gained interest among farmers and researchers in the past couple of years (Dowarah, Verma, *et al.*, 2017b). Probiotics have been defined by FAO as live microorganisms that confer health benefits to the host once ingested in the correct amounts (FAO, 2016). However, in recent years, owing to increasing interest and research on probiotics, the definition has been extended to incorporate microbial and fermentation products. Therefore, probiotics have been defined as concoctions of known viable microbes in adequate numbers that modify the host microflora to confer health benefits (Callaway *et al.*, 2008). These probiotics are in a sense feed additives and are used to modulate gut microbiota of the host while replenishing the intestinal immune system. A number of probiotics, individually or in combination, have been used to prevent and control post-weaning diarrhoea with positive results. (A summary of some is shown in Table 2.) Probiotic preparations that have been used in livestock are always a single species of a microorganism or a mixture of species, for example lactic acid bacteria (LAB), yeasts, *Bifidobacterium*, *Enterococcus* and *Bacillus* (Callaway *et al.*, 2008). Table 3 documents some of the commercial probiotic products on the market that are used by farmers in swine and other livestock.

In a field study, the supplementation of a probiotic combination of *Bacillus licheniformis*-DSM 5749 and *B. subtilis*-DSM 5750 (1:1) spores to diets of weaners, growers and finishing pigs in a commercial farm revealed lowered incidences of diarrhoea because of *E. coli* in all the groups, and improved other parameters, including feed conversion efficiency, weight gain and carcass quality (Alexopoulos *et al.*, 2004). They demonstrated that the probiotic effects were much more pronounced when medium and high doses were used (400 g/ton of feed equivalent to 1.28×10^6 viable spores per gram of feed and 600 g/ton = 1.92×10^6 spores per gram, respectively). A study by Zeyner & Boldt (2006) revealed that daily supplementation of piglets from birth to weaning twice a day with *Enterococcus faecium* DSM 10663 NCIMB 10415 (EcF) at a dosage of 1.26×10^9 colony forming units (CFU) orally through drenching reduced the percentage of piglets that suffered from diarrhoea and improved their daily weight gain. On the other hand, it was shown in the same study that a glucose-based solution with additional $2.9 - 5.8 \times 10^8$ CFU of EcF did not have any therapeutic effects once diarrhoea was present. However, the reduction in diarrhoea scores and the percentage of viable piglets that developed diarrhoea post EcF supplementation was convincing enough to conclude that the probiotic stabilized the gut environment, which later translated into improved daily weight gain. Bacteria *Enterococcus faecium* have been demonstrated to prevent adhesion of the ETEC K88 strain to the intestinal mucous membrane of piglets and its ability to regulate intestinal microbial balance through increasing digestive enzyme activity, improving digestion, feed digestibility and nutrient utilization results into lowered morbidity, mortality and increased performance of farm animals (Vondruskova *et al.*, 2010). In another study, a *Lactobacilli* complex containing *Lactobacillus gasseri*, *L. reuteri*, *L. acidophilus and L. fermentum* (strains not specified in the reference) that were isolated from the digestive tract of a health weaner piglet were demonstrated to reduce the diarrhoea index (66%) and incidence (69.1%) in weaned piglets challenged with *E. coli* solution (serovars K99, K88 and 987P at the ratio of 1 : 1 : 1). In the study, three-way crossbred piglets (Duroc × Landrace × Yorkshire) weaned at 28 days were fed a basal diet formulated according to the NRC (1998) requirements and the lactobacilli complex as a liquid supplement via water at the inclusion of 0.1% (v/v). The *E. coli* challenge was after seven days of probiotic consumption, while diarrhoea scores were recorded a week later. It was observed that the control group registered the first diarrhoea incidence one day post challenge, while in the probiotic group this was seen four days later. This therefore pointed to the possibility of lactobacilli preventing *E. coli* induced diarrhoea before challenge (Huang *et al.*, 2004). Moreover, in the same paper, the probiotic complex significantly (P <0.01) reduced the counts of *E. coli* and other aerobic bacteria, while increasing lactobacilli and anaerobic counts. Probiotic *Enterococcus faecium* NCIMB 10415 and *Bacillus cereus* var. toyoi of different ecological origins were studied to assess their impact on swine health and performance. It was established that both molecules reduced the incidence of PWD significantly (Taras *et al.*, 2007). Furthermore, the inclusion of these probiotics in the sow feed was associated with their detection in the piglets' faecal matter even before piglets could access the supplemented diets, which indicated the possibility of vertical transfer via contact with the sow's faeces. In the same study, the ability to reduce the relative magnitude of post-weaning diarrhoea by E. faecium NCIMB 10415 was independent of its concentration in the diet and commencement time for supplementation.

Table 2 Probiotic combinations shown to have positive effects on the reduction of post-weaning diarrhoea

Probiotics	Breed	Age	Weight (kg)	Dosage	Duration -days	Effect on diarrhoea	Ref
Bifidobacterium lactis HN019	-	21 days	5–7	10^8 CFU/mL 10ml/piglet/day	21	Lowered severity	(Shu *et al.*, 2001)
Bifidobacterium longum subsp. *infantis* CECT 720 + *Bifidobacterium animalis* subsp. *lactis* BPL6	LW×LL	25 - 31 days	7.7 ± 0.28	10^9 CFU/2 mL	16	Decreased	(Barba-Vidal *et al.*, 2017)
Lactobacillus murinus DPC6002 *and* DPC6003*, Lactobacillus pentosus* DPC6004*, Lactobacillus salivarius* DPC6005*, and Pediococcus pentosaceous* DPC6006	LW×LL	Weaners	-	$4×10^{10}$ CFU/day	30	Reduced incidence and severity	(Casey *et al.*, 2007)
Lactobacillus acidophilus NCDC-15 *Pediococcus acidilactici* strain FT28	Indian Local×LL	28 days	-	200 g/pig/day	180	Lowered scores (*P* <0.05)	(Dowarah, Verma, Agarwal *et al.*, 2017)
LAB complex (*Enterococcus faecium 6H2*, *Lactobacillus acidophilus C3*, *Pediococcus pentosaceus D7*, *L. plantarum 1K8* and *L. plantarum 3K2*)	LL×Y	21 - 23 days	6.6 ± 0.5	600 mg/kg	35	Less affected in first 2 weeks	(Giang *et al.*, 2010)
LAB complex *(Enterococcus faecium* 6H2, *Lactobacillus acidophilus* C3, *Pediococcus pentosaceus* D7, and *L. fermentum* NC1) + B. *subtilis* H4 and *Saccharomyces boulardii* Sb.	LL×Y	26 - 28 days	7.7 ± 0.9	B. subtilis;4-$8×10^{11}$, S. bourlardii; 3-$9×10^{10}$, E. faecium; 4-$8×10^9$, L. acidophilus; 3-$7×10^9$, P. pentosaceous 1.3-$8.5×10^9$, L. fermentum; 5-$7×10^9$ CFU/mL Each day 2 mL/kg of basal diet	35	Lower incidence	(Giang *et al.*, 2012)
L. reuteri and *L. plantarum* complex[a]	LL×Y×D	28 days	7.90 ± 0.92	0.1%($1 × 10^9$ CFU/kg)	28	Lower score (*P* <0.05)	(Zhao & Kim, 2015)
Lactobacillus rhamnosus GG (ATCC 53103)	D×LL×LW	18 days	5.3 ± 0.43	$1 × 10^{10}$ CFU/mL	14	Lower *P* <0.05	(Zhang *et al.*, 2010)

[a]Strains not specified in the reference, LL: Landrace, Y: Yorkshire, LW: Large White, D: Duroc, CFU: Colony forming unit

Table 3 Commercially available probiotics commonly used in pigs and other livestock

Probiotic name	Composition	Target species	Dosage in pigs	Indication
World Labs®	*Lactobacillus casei* *Bacillus subtilis* *Saccharomyces cerevisiae* *Aspergillus oryzae* *Streptomyces grieus*	Pigs, poultry, cattle	1 kg/ton of feed (0.1%)	Improve FCR, weight gain, promote growth and improve environmental livestock farm Increase milk production in cattle
BioPlus 2B®	*Bacillus subtilis* DSM 5750 and *Bacillus licheniformis* DSM 5749	Pigs, poultry, calves, rabbits	1.3×10^9 CFU/kg feed 6.5×10^8 CFU/mL water	Improve ADG, feed efficiency, growth promotions, Decrease in piglet diarrhoea
MICROGUARD®	*Bacillus Licheniformis* DSM5749 *Bacillus Megaterum* *Bacillus Mesentricus* *Bacillus polymyxa* *Saccharomyces bourlrdii* *Bacillus subtilis*	Pigs and poultry	Starter/Sow: 100 g/ton of feed Grower/Finisher: 50 g/ ton of feed	Optimizing gut integrity, improving immunity, odour reduction, Increasing absorption in the intestines (brush border)
PrimaLac®	*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium thermophilum*, *and Enterococcus faecium*	Pigs, cattle, horses, poultry	1.0×10^8 CFU/g	Maintaining an optimal microbial balance of commensal bacteria against pathogenic microbes
PORCBOOST® EB	*Bacillus subtilis*	Suckling and weaned piglets	-	Reduction of *E. coli* induced diarrhoea
Protexin®	*L. debrueckii* subsp. *bulgaricus*, *L. acidophilus*, *L. plantarum*, *L. rhamnosus*, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Streptococcus thermophilus*	Most farm animals including pigs	Pigs: 0.5 - 0.6kg/ton of feed Piglets: 1.4 kg/ton of feed	Improve growth, feed utilization, reduced intestinal dysfunction,
ENVIVA MPI®	*Lactobacillus rhamnosus and Lactobacillus farciminis*	Pigs	1 kg/ton of feed	Support gut health improve body weight gain
B. *infantis* IM1®	*Bifidobacterium longum* subsp. *infantis* CECT 7210	Pigs, Human	10^9 CFU supplied in 2 mL solution	Enhancement of gut health and intestinal immunity

Most of these are commercial products and the strains have not been specified in the references (Alexopoulos *et al.*, 2004; Barba-Vidal *et al.*, 2017; FAO, 2016)
FCR: Feed conversion ratio, ADG: Average daily gain, CFU: Colony forming units

A probiotic product that contained viable spores of *Bacillus licheniformis* was tested for its efficacy on PWD in a low health status farm after 28 days. The probiotic was administered at two inclusion levels, namely 10^6 and 10^7 viable spores of *B. licheniformis* per gram of feed. In the same study, one group of piglets was offered feed supplemented with 10^6 viable spores of *Bacillus toyoi*. The results showed that all groups that received probiotic supplements had reduced severity and incidence of diarrhoea, coupled with lower morbidity and mortality compared with the controls (Kyriakis *et al.*, 1999). The group of piglets that received higher doses of *B. licheniformis* (10^7 viable spores per gram of feed) was associated with better performance. Supplementation of weaned barrow diets with *Lactobacillus rhamnosus* GG ATCC 53103 after experimental infection with *Escherichia coli* K88 reduced diarrhoea incidences, lowered faecal coliform counts, and increased *lactobacilli* counts. The barrows were a cross of Duroc, Landrace and Yorkshire, weighing 5.3 ± 0.43 kg, and weaned at 18 days. In addition to the standard weaner diet, which comprises mainly 22.3% crude protein and 14.0 MJ dietary energy/kg, the piglets were orally administered 10 mL of 10^{10} CFU LGG probiotic solution and seven days later were challenged with ETEC to induce diarrhoea. It was shown that LGG led to increased concentrations of secretory immunoglobulin A in the jejunum and ileum, and high titers of the tumour necrosis factor TNF-α alongside the reduced diarrhoea scores (Zhang *et al.*, 2010). Another study showed that inclusion of a direct fed probiotic complex of *Lactobacillus reuteri* and *Lactobacillus plantarum,* added at 0.1% (1 × 10^9 CFU/kg), elevated the numbers of *lactobacillus* in faeces, reduced diarrhoea, malodour emission, and *Escherichia coli* shading in weaners (Zhao & Kim, 2015). In a pig model of intestinal infection with porcine enterotoxigenic *Escherichia coli* Abbotstown (EcA), it was revealed that pre-treatment with probiotic *Escherichia coli* strain Nissle 1917 completely eliminated signs of secretory diarrhoea in infected piglets (Schroeder *et al.*, 2006). Non-avirulent *Escherichia coli* was also shown to reduce PWD and conserve a low intestinal coliform diversity in piglets that were environmentally exposed to three strains of pathogenic *E. coli*, namely E. coli (O147; K89, STb), E. coli O141 (K85, STb, VT2) and E. coli O149 (K91, K88, STa, STb, LT) (Melin & Wallgren, 2002). A symbiotic combination of raw potato starch and probiotic *E. coli* strains, UM-2 and UM-7, led to reduced diarrhoea incidence, increased gut microbial diversity and conferred beneficial effects on growth performance in weaned piglets that were experimentally challenged with pathogenic *E. coli* K88 (Krause *et al.*, 2010). Furthermore, liquid metabolic combinations of five strains of *Lactobacillus fermentum* (TL_1, RG_{11}, RI_{11}, RG_{14} and RS_5) were fed to weaned piglets, After five weeks, the results showed reduced incidences of diarrhoea and increased counts of faecal LAB, irrespective of the combinations (0.3% metabolite of TL_1, RG_{11} and RI_{11} or TL_1, RG_{14} and RS_5 strains, or RG_{11}, RG_{14} and RI_{11} strains) (Thu *et al.*, 2011). However, they reported no significant difference (P >0.05) in average daily gain between the treatment and control piglets. In a comparative study, dietary probiotic containing strains of *Bacillus licheniformis*- 1.5 × 10^{10} CFU/g and 0.3 × 10^{10} CFU/g *Saccharomyces cerevisiae*, and an antibiotic (zinc bacitracin 10%, 50 mg/kg of colistin sulphate 10%, and 100 mg/kg olaquindox 5%) were used independently in two experiments to test their effect on attenuation of intestinal damage and nutrient digestibility after enterotoxigenic *E. coli* K88 challenge in weaned piglets. The results showed that both probiotics and antibiotics decreased (P <0.05) diarrhoea, and improved average daily intake and average daily gain in the weaned piglets. Hence, they showed that probiotics could be potential alternatives to in-feed antibiotics (Pan *et al.*, 2017).

Although a number of published studies point to the positive effects of probiotic supplementation on the reduction of PWD and improving the production performance indices in pigs, few no-response studies are published, and in most cases these are probably not published at all (Dubreuil, 2017). Trevisi *et al.* (2011) reported contrasting results to those by Zhang *et al.* (2010) on the effects of *Lactobacillus rhamnosus* GG ATCC 53103 in weaned piglets. Trevisi and colleagues showed that dietary supplementation of weaner diets with LGG had neither preventative nor control properties on the adverse effects of enterotoxigenic *E. coli* O149: F4ac. In detail, LGG supplementation after E. coli challenge did not reduce the incidence of diarrhoea or the number of E. coli shade in faeces. There was no significant difference in total lactic acid bacteria, enterobacteria and yeast compared with the control, while ETEC numbers tended to increase with LGG. In another study by Zhou *et al.* (2015), F4 receptor − negative (F4R−) crossbred piglets (Landrace × Yorkshire × Duroc) were orally administered with low and high doses of the probiotic combination, *Bacillus licheniformis* (DSM 5749) and *Bacillus subtilis* (DSM 5750)-BLS-mix, for seven days preceding F4 (K88) − positive ETEC/VTEC/EPEC challenge. The results showed no difference in diarrhoea incidence among the treatments groups. However, the probiotic mixture ameliorated the enteritis symptoms. Furthermore, their data indicated that BLS-mix increases the generation of $CD4^+$ IL-10 T cells during active inflammation of the intestinal tract owing to pathogenic bacteria, but this might actually prohibit clearance of the pathogen. Also, although consumption of BLS-mix induced IL-10 producing Tr1 cells, this alone cannot account for protection of weaned piglets from $F4^+$ ETEC/VTEC/EPEC infection. Additionally, neither low nor high dose BLS-mix had a significant influence on daily feed intake and average daily gain in comparison with the control. For seven days Maneewan *et al.* (2011) hand-fed three-way crossbred piglets 10 mL of Bacillus subtilis MP9 and MP10 (10^{11} CFU/mL) per day. The results of their study revealed no significant (P >0.05) difference in diarrhoea scores between the probiotic and control groups, except for shortened diarrhoea periods in the MP9 and MP10 groups. In a study to investigate the effect of crowding stress and ETEC $K88^+$ challenge in nursery piglets, researchers showed that reduction in space allowance and ETEC challenge caused detrimental effects to some immunological and performance parameters, and increased E. coli numbers, and that probiotic supplementation with *E. coli* UM-2 and UM-7 had few positive effects in ameliorating these parameters (Nyachoti *et al.*, 2014).

Mechanisms of Action of Probiotics

Several researchers and reviewers have documented probable mechanisms through which probiotics confer their effects to the host (Boirivant & Strober, 2007; Vondruskova *et al.*, 2010; Brown, 2011; Cho *et al.*, 2011; Bermudez-Brito *et al.*, 2012; Bajaj *et al.*, 2015). The ability of probiotics to effect their mechanisms on the swine gut is highly dependent on whether they can tolerate the gastric and bile secretions as they descend along the upper intestinal tract (Bajaj *et al.*, 2015). The caecum and colon are target sites for probiotic activity since they harbour diverse and dense populations of microorganisms and thus, once ingested, probiotics modulate the balance and activity of the gut microbiome (Chaucheyras-Durand & Durand, 2009). Probiotics are believed to improve the host health through increasing the abundance of commensal microflora in the gut (Chaucheyras-Durand & Durand, 2009). Although the exact modes of action of probiotics on the host gut are still unclear (Wohlgemuth *et al.*, 2010), probable mechanisms have been suggested and demonstrated, such as modulation of gut microbiota, effects on nutrient digestibility and absorption, modulation of the host immune system, secretion of antimicrobial compounds and reduction of diarrhoea (Chaucheyras-Durand & Durand, 2009; Brown, 2011; Bajaj *et al.*, 2015; Do *et al.*, 2017; Sánchez *et al.*, 2017). Some of these probiotic mechanisms may be strain specific (Boirivant & Strober, 2007). However, the authors summarized some important aspects from the literature pertaining to probiotic modulation of the swine immune system in the next subchapter, because they are of interest and value to the current study.

Probiotics Modulation of the Immune System

Germ-free animals can grow adequately without the contribution of the gut microbiota. However, in the absence of microbial colonization, these animals remain functionally immature in certain bodily systems, for example the mucosal and systemic immune system, the development of the secondary lymphoid tissues and response and susceptibility to pathogenic microorganisms (Roselli *et al.*, 2017). This phenomenon thus provides a basis for demonstrating the relationship between gut microbiota colonization (with symbiotic bacteria) and their diversification with the maturation of the immune system. The gut has a larger endowment of lymphocytes than any other organ in the body, and is thus regarded as the largest body immune organ (Brown, 2011). The intestinal enterocytes provide a protective barrier against passive loss of nutrients and also block the entry of pathogens into the body system, revealing how the innate and adaptive immune systems in the intestines are closely integrated with other intestinal functions such as absorption (Bron *et al.*, 2011; Brown, 2011). In addition, the GIT lumen and the upper part of its mucous layer harbour a large population of microorganisms whose composition affects the epithelial barrier and immune system functionality. Probiotic bacteria modulate the immune system directly through adjusting the secretion of immunoglobulins or cytokines, increasing the activity of macrophages or natural killer cells, or through indirect mechanisms such as enhancing the gut epithelial barrier, and altering the mucus secretion or through competitive exclusion of other pathogenic bacteria (La Fata *et al.*, 2017). Immunoglobulins A and G are among those whose production and circulation can be stimulated by probiotic bacteria (Vondruskova *et al.*, 2010; Bajaj *et al.*, 2015). Probiotics elevate the function of phagocytic action and increase the phagocytic receptor expression in the neutrophils of an individual. *Lactobacillus plantarum* elevates antibody production against pathogenic *Escherichia coli* while *Bifidobacterium longum* and other LAB increase the numbers of Immunoglobulin A (IgA) (Scharek *et al.*, 2005). Several studies have documented the influence of probiotic supplementation in pigs on the functionality and response of the immune system (see Table 4).

Among other functions of the gut, the epithelium creates a physical barrier between the external environment and the host's immune system, which makes its functionality and integrity paramount in aiding permeability to nutrients and other important micro molecules, in addition to protecting the host from aberrant pathogens. Multi-protein complexes, which are referred to as tight junctions (TJs), are responsible for maintaining the integrity of the gut epithelium (Lee, 2015). Compounds that have been demonstrated to regulate the expression of TJs include probiotics. These have been shown to regulate the expression and localization of the tight TJs (La Fata *et al.*, 2017). Various probiotics strains, including *Escherichia coli Nissle* 1917, *Lactobacillus rhamnosus GG*, *Lactobacillus casei* DN-114001 and *Lactobacillus plantarum* MB452, have been shown to improve the gut epithelial barrier function through modulation mechanisms on the TJs (Parassol *et al.*, 2005; Ukena *et al.*, 2007; Johnson-Henry *et al.*, 2008; Anderson *et al.*, 2010). These probiotics modulate the TJs via upregulation of the Zona occludens (1,2,3), and other tight junction-associated proteins. Up-regulation of ZO-1 is believed to stabilize the TJs and therefore improve the barrier function of the gut epithelium (La Fata *et al.*, 2017).

Table 4 Influence of probiotics on the immune system in swine

Probiotics	Animal	Dosage	Immune system parameter	Effect	Ref
Enterococcus faecium SF 68	Gestating sows	1.6 × 10 CFU/kg	Total serum IgG at 5 wks	Same as control	(Scharek *et al*., 2005)
	Lactating sows	1.2 × 10 CFU/kg	Total serum IgG at 8 wks	Reduced	
	Suckling piglets	1.7 × 10^{8} CFU/kg	$CD4^{+}/CD8^{+}$ in payers' patches	Same as control	
	Weaners	2 × 10^{8} CFU/kg	Cytotoxic T-cells ($CD8^{+}$) in jejunum	Reduced	
Lactobacillus/Pediococcus (*Lactobacillus murinus* DPC6002 and DPC6003, *Lactobacillus pentosus* DPC6004, *L. salivarius* DPC6005, and *P. pentosaceus* DPC6006)	Weaners	100 mL skimmed milk containing 5 × 10^{7} CFU/mL daily	$CD4^{+}/CD8^{+}$	Increased	(Walsh *et al*., 2008)
			Ileal IL-8 mRNA expression	Increased	
Lactobacillus casei (No. 1.570) and *Enterococcus faecalis* (No. 1.2024)	Suckling piglets	Incremental doses (1,2,3,4 mL)/wk of probiotic containing 1 × 10^{9} CFU/mL	Plasma IgA	Increased	(Liu *et al*., 2017)
			Jejunal tumour necrosis factor-α	Decreased	
Bacillus subtilis C-34	Sows	1 × 10^{9} endospores/g of concentrate	IgG	Increased	(Ayala *et al*., 2016)
L. amylovorus GRL 1112+ *L. mucosae* GRL 1167+ *L. salivarius* GRL 1169+ *L. johnsonii* GRL 1171+ *L. reuteri* GRL 1168+ *L. reuteri* GRL 1170	Piglets	1 × 10^{10} cells/mL	IL-4 and IFN-α in caecum	Up regulated	(Lähteinen *et al*., 2015)
			IL-8 and TNF in colon	Down regulated	
			TGF-β1 expression in jejunum, ileum and colon	Down regulated	
L. plantarum B2984	Weaner pigs	1 × 10^{10} CFU/piglet/day	Serum IgM, IgG, IgA	Enhanced	(Naqid *et al*., 2015)
Lactobacillus reuteri ZJ625, *Lactobacillus reuteri* VB4, *Lactobacillus salivarius* ZJ614, *and Streptococcus salivarius* NBRC13956	Weaner piglets	10 mL containing 4.45 × 10^{9} CFU/mL of probiotic strains	IgG	Increased	(Dlamini *et al*., 2017)
Lactobacillus reuteri 15007	New born piglets	6 × 10^{9} CFU/g	mRNA expression of IL-1β in ileum	Reduced	(Hou *et al*., 2015)
			T-cell differentiation	Enhanced	
			Serum specific anti-OVA IgG level	Increased	
L. reuteri X-1	Weaned piglets	1 × 10^{8} CFU/g	Serum IgG and IgM	Decreased	
L. reuteri 5007	Growers	1.02 × 10^{8} CFU/g	Antioxidant capacity	Increased	

CFU: Colony forming unit, IgG: Immunoglobulin G, IgM: Immunoglobulin M, TNF: Tumour necrosis factor, IL: Interleukin, IFN: Interferon, wks: weeks

Conclusion

It is evident that wide and increasing lists of probiotics have been studied and tested for their ability to prevent PWD in pigs, and consequently are fronted as potential alternatives to antibiotics. The majority of the microorganisms used as probiotics in the studies belong to – but are not limited to – the genera *Lactobacilli*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, probiotic *Escherichia coli* and *Saccharomyces*. These probiotic strains can be given as single concoctions or in combination. Generally, probiotic feeding may be a better alternative to treating E. coli induced diarrhoea than antibiotic feed additives. However, there is a need to standardize supplementation protocols, including dosage, onset and duration of treatment for each probiotic strain. The authors suggest that more field studies in more natural and commercial farm settings should be conducted to augment the literature in relation to the use of probiotics as alternatives to antibiotics in treating PWD.

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Authors' Contribution

All authors contributed equally to sourcing the materials and writing the final copy of the manuscript.

Conflict of Interest declaration

The authors declare no conflict of interest.

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