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APPLICATION OF COMPLEX PROBIOTICS IN SWINE NUTRITION – A REVIEW

Wen Chao Liu[1#], Mao Ye[1#], Jia Hao Liao[1], Zhi Hui Zhao[1], In Ho Kim[2♦], Li Long An[1♦]

[1]Department of Animal Science, College of Agriculture, Guangdong Ocean University, Zhanjiang, Guangdong 524088, P. R. China
[2]Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam 31116, South Korea
[♦]Corresponding authors: inhokim@dankook.ac.kr; anlilong@126.com
[#]These authors contributed equally to this work

Abstract

The use of probiotics as alternatives to antibiotics for farm animals is gaining more and more interest during recent years. Probiotics are living microorganisms that provide a wide variety of health benefits to the host when ingested in adequate amounts. The bacterial strains most frequently used as probiotic agents are *Bacillus*, lactic acid bacteria, *Enterococcus* and *Saccharomyces cerevisiae*. It has been suggested that multi-strain probiotics might be more effective than mono-strain probiotics due to the additive and synergistic effects, and many previous studies demonstrated that dietary complex probiotics supplementation had growth promoting effects on pigs. However, the effect of complex probiotics in practice is not always consistent, the effect of probiotic could be affected by strain composition, dosage, feed formula, and the age of animals. In this review, we will give an overview on the current use of complex probiotics for weaning, growing and finishing pigs and sows.

Key words: complex probiotics, growing and finishing pigs, growth promotion, sows, weaning pigs

The gut of domestic animals is home to a dynamic microbial population that forms a complex ecosystem and has a symbiotic relationship with the host (Fouhse et al., 2016). The gut microbiota were established as playing key roles in disease prevention by developing and maintaining proper gut structure and immune function (Swanson, 2016). Disturbances in the gut microbial ecosystem during the rearing of animals can dramatically increase risk of respiratory diseases and diarrheas. During the past several decades, the antibiotics were used to balance the disturbances of gut microbiota, reduce the pathogen infection and decrease incidence of intestinal disease (Thacker, 2013). However, antibiotic resistance is a looming public health crisis, in particular, the antibiotic growth promoters (AGP) have been forbidden in the European Union, Korea, and Japan. As a result, there is an increasing interest concerning alternatives to AGP in livestock industry. Recently, an intensive amount of researches have focused on probiotics supplement in swine production (Fuller, 2012). The application of probiotics provides a potential alternative strategy to the use of AGP.

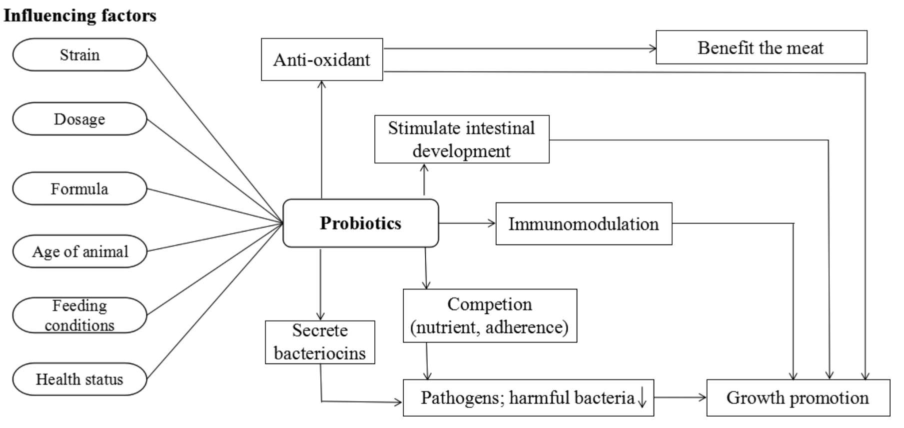

Figure 1. Overview on the mode of actions of probiotics

Probiotics are living microorganisms, and when consumed in adequate amounts can confer a health benefit to the host (FAO/WHO, 2002). Many claims relating to probiotic properties have been made, varying from the competitive exclusion of pathogenic bacteria, modulation of gut microflora, immunomodulation, improvement of intestinal development and antioxidant status, alleviation of weaning stress etc. (Figure 1). However, it has been previously suggested that multi-strain probiotics might be more effective than mono-strain probiotics due to the additive and synergistic effects (Chen et al., 2012). Nevertheless, the effect of multi-strain probiotics in practice is not always consistent, the properties of probiotics are strain-specific, and the efficacy of probiotic could be influenced by dosage, feed composition, and the age of animals. Therefore, in order to provide an overview of the previous reports using various strain composition probiotics, the current review aims to summarize and update the evidence on the application of complex probiotics in swine production.

The application of complex probiotics in weaning pigs

The weaning period in pigs represents a time of gastrointestinal and immunological instability, which is so-called weaning stress. It results in a critical period of low voluntary feed intake and an increased susceptibility to infection (Lallès et al., 2007). Numerous previous studies demonstrated that the complex probiotics could alleviate weaning stress and promote intestinal health.

Improves growth performance

The most common probiotics for pigs are yeast (*Saccharomyces cerevisiae*), *Lactobacillus* spp. and *Bacillus* spp., therefore, the most commonly used complex probiotics are lactobacilli complex, *Bacillus* complex and other combinations (Table 1). Giang et al. (2010 a) reported that pigs fed diets with *Lactobacillus* complex (*Enterococcus faecium 6H2*, 3×10^8 cfu/g, *Lactobacillus acidophilus C3*, 4×10^6 cfu/g, and *Lactobacillus plantarum 1K8*, 2×10^6 cfu/g) had higher average daily gain (ADG), average daily feed intake (ADFI), and lower feed/gain ratio during early weaning period (d 0-14). Ahmed et al. (2014) reported that inclusion of *Bacillus* complex, Bioplus 2B® (*B. subtilis* and *B. licheniformis*, 3.2×10^6 cfu/g), increased the ADG and ADFI throughout the experiment period (d 0–28 after weaning). Dong et al. (2014) suggested that dietary complex probiotics (*L. plantarum* and *B. subtilis*, 4.3×10^9 and 1.0×10^8 cfu/g) had positive effects on reducing the feed/gain ratios during d 0–14 and d 0–35 after weaning. Zhao and Kim (2015) reported that dietary lactobacilli complex (*L. reuteri* and *L. plantarum*, 1×10^6 cfu/g complex) supplementation improved the ADG during d 0–28 in weaning pigs. In addition, Cai et al. (2015) demonstrated that dietary *Bacillus*-based probiotics supplementation (*B. subtilis* and *B. amyloliquefaciens*) at 1.5×10^5 cfu/g concentration improved ADG during d 0–14 after weaning, and showed positive effects on increasing gain/feed ratios during d 0–42. Kim et al. (2017) reported that dietary complex probiotics (*L. acidophilus* K31, 1.2×10^6 cfu/g, *B. subtilis* K42, 1.5×10^7 cfu/g, and *S. cerevisiae* K47, 3.0×10^4 cfu/g) supplementation improved the gain/feed ratios during d 0–35 in weaning pigs. However, on the contrary, Huang et al. (2004) reported that dietary lactobacilli mixture (2.0×10^8 cfu/g) supplementation (*L. gasseri*, *L. fermentum, L. reuteri* and *L. acidophilus*) had no significant effect on ADG and feed/gain ratio during d 0–14 and d 15–21 in weaning pigs. Mair et al. (2010) suggested that addition of 1.0×10^9 cfu/g complex probiotics (*Enterococcus faecium*, *L. salivarius*, *L. reuteri* and *Bifidobacterium thermophilum*) to post-weaning pigs diets had no effects on ADG, ADFI and feed conversion ratio (FCR) throughout the 28-day experiment period. In another study carried out by Lähteinen et al. (2015), they also demonstrated that feeding weaning pigs with multispecies lactobacillus formulation (*L. amylovorus*; *L. mucosae*; *L. salivarius*; *L. reuterii* and *L. johnsonii*) at the level of 1.7×10^9 cfu/g did not influence the body weight (BW) and ADG through a 21-day feeding trial.

In summary of these previous findings, it is indicated that the effects of complex probiotics in weaning pigs are not always consistent, and the efficacy of complex probiotics could be affected by strain composition and inclusion levels. Moreover, according to the results of previous reports, it should be noted that the beneficial effects of probiotics on growth performance were always observed in the early period after weaning. It may be due to the dramatic changes in gut after weaning, and the gut needs time to adapt to the new situation, when the most critical phase after weaning is passed, a normal intestinal function has been re-established (Heo et al., 2013). As such, compared to later period after weaning, the efficiency of probiotics should be expected to be higher when the pigs are confronted with stress during the early days (d 0–14) after weaning. Therefore, the effects of complex probiotics are also associated with the age of weaning pigs.

Table 1. Effects of complex probiotics on growth performance in weaning pigs

Composition	Supplied dose (cfu/g of feed)	Period (after weaning)	Treatment effects (%, difference to control)			Reference
			ADG	ADFI	G:F	
Enterococcus faecium 6H2, Lactobacillus acidophilus C3, Lactobacillus plantarum 1K8	3×10^8 4×10^6 2×10^6	d 0–35	+9.23**	+1.12ns	+6.17**	Giang et al. (2010 a)
Lactobacillus acidophilus, Bacillus subtilis, Saccharomyces cerevisiae	4.0×10^8 4.8×10^9 1.0×10^4	d 0–28	+12.08**	+6.09*	+5.80**	Choi et al. (2011 a)
Lactobacillus plantarum GF103, Bacillus subtilis B27	4.3×10^9 1.0×10^8	d 0–35	+10.50ns	–15.39**	+23.23**	Dong et al. (2014)
Bacillus subtilis, Bacillus licheniformis	3.2×10^7 3.2×10^7	d 0–28	+16.05**	–1.90ns	+15.50*	Ahmed et al. (2014)
Bacillus subtilis, Bacillus amyloliquefaciens	1.5×10^5 1.5×10^5	d 0–42	+1.67ns	+0.33ns	+2.03*	Cai et al. (2015)
Lactobacillus reuteri, Lactobacillus plantarum	1×10^6 1×10^6	d 0–28	+8.75*	–0.82ns	+9.65ns	Zhao and Kim (2015)
Lactobacillus amylovorus, Lactobacillus mucosae, Lactobacillus salivarius, Lactobacillus reuterii, Lactobacillus johnsonii	1.7×10^9	d 0–21	+4.76ns	-	-	Lähteinen et al. (2015)
Lactobacillus acidophilus, Bacillus subtilis, Saccharomyces cerevisiae	4.0×10^8 4.8×10^9 1.0×10^4	d 0–28	+5.96**	+3.20ns	+3.27**	Choi et al. (2016)
Lactobacillus acidophilus K31, Bacillus subtilis K 42, Saccharomyces cerevisiae K47	1.2×10^6 1.5×10^7 3.0×10^4	d 0–35	+4.35ns	+1.81ns	+6.22*	Kim et al. (2017)
Bacillus subtilis, Bacillus amyloliquefaciens	1.5×10^5 1.5×10^5	d 0–43	–0.43ns	–6.51ns	+5.25*	Jaworski et al. (2017)

Note: ns – no significance; * – $P<0.05$; ** – $P<0.01$.

Promotes nutrient digestibility and intestinal development

In addition to growth promoting properties, it has been well documented that the probiotics also exert positive effect on nutrient digestibility. For instance, many previous studies proved that dietary complex probiotics supplementation could enhance the apparent total tract digestibility (ATTD) of dry matter of weaning pigs (Giang et al., 2010 b; Choi et al., 2011 a; Giang et al., 2012; Ahmed et al., 2014; Choi et al., 2016). Also, other studies reported that dietary complex probiotics supplementation stimulated the ATTD of nitrogen or gross energy in weaning pigs (Choi et al., 2011a; Cai et al., 2015; Zhao and Kim, 2015). The probiotics are known to be related to competitive exclusion against pathogenic microorganisms on the gastrointestinal tract epithelium of pigs, and they improve host microbial balance and gut health, consequently promoting the nutrient digestibility (Fuller, 2012; Zhao and Kim, 2015). Regarding the lactobacilli complex probiotics, the increased number of lactobacilli in the gut would potentially increase the activity of useful enzymes such as β-galactosidase, which will add to the beneficial effects on nutrient utilization (Fuller, 2012). In addition, *Bacillus* can also produce some useful enzymes (α-amylase, arabinase, levansucrase, cellulase, maltase, dextranase, alkaline protease, neutral protease and β-glucanase) in the animal gut, and *Saccharomyces* is able to produce antimicrobial substances and enhance gut function (Hentges, 1992), which can explain the improved nutrient digestibility in weaning pigs by feeding probiotics.

Villus height and crypt depth were indirect indicators of the maturity and functional capacity of enterocytes, and longer villi provided an increased absorptive area in the small intestine (Hampson, 1986). Weaning leads to villus atrophy due to the increase of apoptosis and the decrease of replacement of enterocytes within the crypts. Previous study has demonstrated that villus height was decreased in weaning transition with a consequent impairment of nutrient utilization and absorption (Boudry et al., 2004). Probiotics have been proved to contribute to the gut health of weaning piglets by enhancing the intestinal development. Cai et al. (2015) reported that dietary supplementation of *B. subtilis*-based multi-strain probiotics (1.5×10^5 cfu/g) led to longer villi of duodenum and jejunum in weaning pigs (Table 2). Also, Choi et al. (2016) found that inclusion of *L. acidophilus*, *B. subtilis* and *S. cerevisiae* complex (4.0×10^8; 4.8×10^9; 1.0×10^4 cfu/g respectively) in weaning diet improved the villus length of duodenum, jejunum and ileum. The enhanced gut morphology could also account for significant improvement in the nutrient digestibility of pigs fed complex probiotics. However, failure to observe any positive effects of complex probiotics on intestinal morphology has also been reported (Walsh et al., 2007; Choi et al., 2011 b). The inconsistency may be attributed to the strains and the health status of the piglets.

Table 2. Effects of *Bacillus subtilis* and *Bacillus amyloliquefaciens* complex on nutrient digestibility and villus height of small intestine in weaning pigs[1]

Items	CON	TRT	SEM	P-value
Apparent total tract digestibility (%)				
Dry matter	72.62	73.73	0.64	0.171
Nitrogen	69.75 b	72.10 a	0.85	0.038
Gross energy	73.36	73.89	0.65	0.560
Villus height (µm)				
Duodenum	462.6 b	548.3 a	17.93	0.002
Jejunum	455.5 b	543.3 a	13.82	0.001
Ileum	471.8	520.4	28.61	0.167

Source: Cai et al. (2015). J. Anim. Sci., 93: 4336–4342.

[1]Abbreviations: CON, basal diets (antibiotics-free); TRT, supplied 1.5×10^5 cfu/g complex probiotics; SEM, standard error of means.

a, b – means in the same row with different letters differ ($P<0.05$).

Modulates the gut microbial balance

Probiotics are known to benefit the host intestinal balance by creating gut micro-ecological conditions, and suppressing harmful microorganisms and favoring beneficial microorganisms (Fuller, 2012). Specifically, lactobacilli can reduce the harmful effects of pathogens by producing organic acids, hydrogen peroxide and antimicrobial substances (Lidbeck and Nord, 1993). *Bacillus* and *Saccharomyces* can also produce antimicrobial substances and exert antagonistic effects against several bacterial pathogens including *E. coli.* (Spriet et al., 1987; Czerucka and Rampal, 2002). The release of antimicrobial substances, such as bacteriocins, which inhibit the growth of pathogenic bacteria, or production of enzymes were able to hydrolyze bacterial toxins (Buts, 2004). Some probiotics produce nutrients and growth factors which are stimulatory to beneficial microorganisms of the intestinal microbiota (Fuller, 2012). Also, most probiotic strains can competitively exclude pathogenic bacteria through their higher affinity for nutrients or adhesion sites in the gut (Chaucheyras-Durand and Durand, 2010). Finally, some probiotics like *Saccharomyces cerevisiae* can metabolize or aid in the detoxification of certain inhibitory compounds such as amines or nitrates or scavenge for oxygen, which is of great importance in gut anaerobic ecosystems (Chaucheyras-Durand et al., 2008). These mechanisms can explain the effects of probiotics in modulating the intestinal microbial balance. Likewise, Choi et al. (2011 b) reported that a complex probiotics preparation (*L. acidophilus* 4.0×10^8 cfu/g, *B. subtilis* 4.8×10^9 cfu/g and *S. cerevisiae* 1.0×10^7 cfu/g) were effective in reducing the coliform and *Clostridium* and improving the *Lactobacillus* spp. population in the ileum and *Bifidobacterium* spp. population in the cecum in weaning pigs. Ahmed et al. (2014) demonstrated that dietary *Lactobacillus*-based multi-strain probiotics (3.2×10^7 cfu/g) supplementation could decrease fecal *S. typhimurium* and *E. coli* counts, whereas increase fecal *Lactobacillus* spp. concentration in weaning pigs at 21 and 28 d of age. Choi et al. (2016) reported that dietary multi-species probiotics (*L. acidophilus* 4.0×10^8, *B. subtilis* 4.8×10^9 and *S. cerevisiae* 1.0×10^4 cfu/g) improved cecal *Lactobacillus* spp. populations, but reduced the cecal *E. coli* counts in weaning pigs (d 28). Similar effects of complex probiotics were also reported by Kim et al. (2017). However, the strains composition might have different effectiveness in modulation of gut microbial balance, therefore, the effects of various strain combinations should be validated from study to study. In general, it has to be kept in mind that the microbial results of most previous studies were based on cultural plate count methods. The modern molecular biological methods, such as PCR-DGGE, q-PCR, FISH and metagenomic sequencing are required for further studies on the effects of complex probiotics on intestinal microbial communities of piglets, and to explore the underlying mechanism.

Immunomodulation

Probiotic bacteria are also associated with the enhanced immune system of animals. Stimulation of unspecific immune functions is considered as one of the main modes of action of probiotics (Fuller, 2012). The immune system of weaning pigs is poorly developed, and the young pigs are susceptible to diseases (Lallès et al., 2007). The immunoglobulins (IgM, IgG and IgA) act as an important part of the immune response to bind with specific antigens. Various immunoglobulin isotypes can offer a conception about the complex humoral immune response (Lefranc and Lefranc, 2001). Several previous studies reported that the complex probiotics were capable to act as an immunomodulators by enhancing the serum immunoglobulin levels in weaning pigs. For instance, Dong et al. (2014) demonstrated that *L. plantarum* and *B. subtilis* in combination improved the serum IgA during the first 2 weeks after weaning. Ahmed et al. (2014) showed that serum IgG values were significantly increased in the *Bacillus*-based probiotics (Bioplus 2B®) treated group in *E. coli* KCTC2571 challenged piglets. Moreover, it has been suggested that administration of lactobacilli-based multi-strain probiotics could also alter the cytokine gene expression in intestinal mucosa of piglets, and these alterations resulted in both pro- and anti-inflammatory responses, including up-regulation on *IL-4* and interferon α (*INF-α*) expression in cecum and down-regulation on *IL-8* and tumor necrosis factor (*TNF*) expression in colon (Lähteinen et al., 2015).

Probiotics play a role in defining and maintaining the delicate balance between necessary and excessive defense mechanisms including innate and adaptive immune responses (Oelschlaeger, 2010). Points of interaction with the immune regulation for probiotics include bacteria direct interaction with intestinal epithelial cells, or following internalization by M cells through interaction with dendritic cells and follicle-associated epithelial cells, initiating responses mediated by macrophages and T and B lymphocytes (Chaucheyras-Durand and Durand, 2010). According to previous studies, these functions were achieved with probiotics-derived components, for instance, a *Lactobacillus rhamnosus* GG-derived soluble protein, p40, was shown to reduce *TNF-α*, *IL-6*, *IFN-γ* gene expression of intestinal epithelial cell (Yan et al., 2011). In another report, two active compounds produced by *Lactobacillus reuteri* RC-14, cyclic dipeptides cyclo (L-Tyr-LPro) and cyclo (L-Phe-L-Pro), were shown to inhibit the staphylococcal quorum-sensing system agr and decrease the expression of toxic shock syndrome toxin-1 in *Staphylococcus aureus* MN8, a pathogen in menstrual toxic shock syndrome (Li et al., 2011). Therefore, regulation of gene and protein expression and signaling pathways by probiotic-derived compounds in the host cells might be the major mechanisms underlying probiotic action leading to immunomodulation.

The application of complex probiotics in growing and finishing pigs

In general, supplementing swine diets with probiotics has given more positive and consistent effects in weaned piglets than in growing or finishing pigs, which may be due to their inducing better digestibility of feed, improved immunity, and increased resistance to intestinal disorders than young pigs. However, some positive influences of complex probiotics on meat quality and fecal noxious gas emission have been documented. Moreover, it has been suggested that the effects of complex probiotics could be affected by energy and nutrient density of feed (Chen et al., 2005; Chen et al., 2006; Wang et al., 2009; Meng et al., 2010).

Impacts on growth performance

Chen et al. (2005) reported that dietary 0.2% complex probiotics (*L. acidophilus* 1.0×10^7 cfu/g, *S. cerevisae* 4.3×10^6 cfu/g and *B. subtilis* 2.0×10^6 cfu/g) improved the

ADG in growing pigs. Chen et al. (2006) suggested that the complex probiotics at a supplemental level of 0.2% (*B. subtilis*, 1.0×10^7 cfu/g; *B. coagulans*, 2.0×10^6 cfu/g and *L. acidophilus*, 5.0×10^6 cfu/g) could increase the ADG of finishing pigs. Meng et al. (2010) found the ADG and gain-to-feed ratio (G:F) of growing-finishing pigs were improved by dietary 0.2% probiotics mixture (*B. subtilis*, 1.0×10^{10} cfu/g and *Clostridium butyricum* 1.0×10^9 cfu/g) throughout a 10-week experimental period. At the same time, they suggested that energy and nutrient density of diets influenced the effects of complex probiotics on the gastrointestinal (GI) tract and on subsequent pig performance, and the use of probiotics in higher energy and nutrient density diets was more favorable than in lower energy and nutrient density diets. Similarly, Yan and Kim (2013) also found that the effects of complex probiotics affected energy and nutrient density of diets in growing pigs. In addition, Jørgensen et al. (2016) demonstrated dietary *B. licheniformis* (DSM 5749) and *B. subtilis* (DSM 5750) complex (3.2×10^9 cfu/g of probiotics product) at a concentration of 400 mg/kg improved the ADG while reduced the feed-to-gain ratio (F:G) at 70-120 d of age, also, the data of their study indicated an interactive effect of the probiotics and energy density diets. Balasubramanian et al. (2016) proved that *Bacillus*-based probiotic (*B. coagulance* 1×10^9 cfu/g, *B. licheniformis* 5×10^8 cfu/g, and *B. subtilis* 1×10^9 cfu/g) exerted beneficial effects on ADG and G:F overall growing-finishing period (25–110 kg). However, inconsistent results have been also reported. For example, Munoz et al. (2007) suggested that dietary supplementation of 0.05% *B. subtilis* and *B. licheniformis* complex (BioPlus 2B®) in finishing pigs diets improved the average daily feed intake (ADFI), but had no effects on ADG and G:F. Wang et al. (2009) reported that addition of various levels (0, 0.05, 0.10, 0.20%) of BioPlus 2B® had no obvious impacts on ADG and G:F of growing pigs. Giang et al. (2011) also reported that *Bacillus* combined with *Saccharomyces* or *Bacillus*, *Saccharomyces* and Lactic acid bacteria complex did not alter the growth performance during finisher period (50–90 kg). The variation in the results of these studies can be ascribed to several factors, including the age of the pigs, the dose and strains of complex probiotics, and the feed formula. In addition, probiotic administration strategies can also impact the effects of the probiotics (Giang et al., 2011; Jørgensen et al., 2016). Importantly, the increased resistance against gastrointestinal infections was a key mechanism behind the growth enhancing effect of probiotics in animals. In fact, as the pigs became older, the digestive system and immunity were developed, thus increasing the resistance to intestinal disorders and infections. As such, the lack of growth promotion was probably due to a good level of hygiene in swine house. Therefore, it can be concluded that the feeding environment and health status are important factors in determining the effectiveness of probiotics in growing or finishing pigs.

Improves meat quality

Previous studies provided evidences that the complex probiotics could improve the meat quality by modifying meat color and reducing drip loss and thiobarbituric acid reactive substances (TBARS) values. For instance, Kim et al. (2008) reported that dietary 0.1% complex probiotics (*Phaffia rhodozyma* 1.0×10^8 cfu/g, *S. cerevisiae* 1.0×10^8 cfu/g, *L. crispatus* 1.0×10^8 cfu/g, *Enterococcus faecium* 1.0×10^8

cfu/g, *L. plantarum* 1.0 × 10^8 cfu/g) supplementation in finishing pigs diets reduced drip loss and increased meat redness (a*). Ko and Yang (2008) demonstrated that inclusion of 0.5% and 1.0% green tea probiotics containing *L. acidophilus* 3.2×10^8 cfu/g, *L. plantarum* 2.2×10^8 cfu/g, *B. subtilis* 4.5×10^9 cfu/g and *S. cerevisiae* 5.2×10^8 cfu/g significantly reduced the TBARS value of loin meat. Meng et al. (2010) also suggested that 0.2% complex probiotics (*B. subtilis*, 1.0 × 10^{10} cfu/g and *Clostridium butyricum* 1.0 × 10^9 cfu/g) improved the sensory color and meat color (redness, a*). Additionally, according to Balasubramanian et al. (2016), the sensory color could be increased, whereas the drip loss of right loin muscle could be reduced by feeding *Bacillus*-based probiotic mixture (*B. coagulance* 1 × 10^9 cfu/g, *B. licheniformis* 5 × 10^8 cfu/g, and *B. subtilis* 1 × 10^9 cfu/g). Meat color is the single most important sensory attribute affecting consumer purchasing decisions of red meats, because they associate a red color with freshness (Morrissey et al., 1994). Changes in a* (redness) and b* (yellowness) values over a period of time describe meat color deterioration from red to brown, and reflect the myoglobin concentration and its redox state in meat (Mancini and Hunt, 2005). Drip loss is commonly assessed as indicative of meat quality, and the TBARS is a frequently used method for measurement of lipid oxidation, the lower TBARS value, the less oxidation has taken place (Yang et al., 2006). Moreover, it has been suggested that some *Bacillus* and *Lactobacillus* strains could produce antioxidants and had antioxidant effect. Therefore, it is assumed that the beneficial effects of complex probiotics on meat quality, which were observed from these previous studies, are possibly due to the antioxidant properties of probiotics.

Mitigation of fecal noxious gas emission

The noxious gases such as ammonia (NH_3), hydrogen sulfide (H_2S) and total mercaptan are major aerial pollutants originating from livestock operations, and swine is one of the principal contributors among farm animals (Eriksen et al., 2010). Moreover, airborne pollutants in swine production can increase the susceptibility to common and important respiratory diseases, and have to be considered in terms of environmental risk assessments, and the manure is the main source of airborne pollutants in the farm (Jongbloed and Lenis, 1998). It has been suggested that improving the nutrient utilization, altering the intestinal microbiota ecosystem, and reducing the pH of manure were the effective strategies to decrease the levels of pollutants from animal manure (Ferket et al., 2002). Probiotics can benefit the gut microbiota ecosystem and improve the gut health status, thus mitigating the emission of noxious gas from animal manure (Table 3). Chen et al. (2005) proved that dietary 0.2% complex probiotic (*L. acidophilus*, *S. cerevisae* and *B. subtilis*) decreased the fecal NH_3-N concentration by 10% in growing pigs. Chen et al. (2006) suggested that addition of *Bacillus*-based probiotic to finishing pig diets reduced the fecal NH_3-N and butyric acid content by 17.1% and 24.4%, respectively. According to Wang et al. (2009), probiotic supplements containing *B. subtilis* and *B. licheniformis* spores (BioPlus 2B®) reduce NH_3 emissions by about 50% with inclusion rates ranging from 0.05% to 0.2% in growing pigs, meanwhile, they suggested that *B. subtilis* generates subtilin, which may reduce urease generating microbiota in the gastrointestinal lumen thereby attenuating NH_3 releases. Chu et al. (2011) reported that dietary

0.2% multi-strain probiotics (*Aspargillus spp.*, *Saccharomyces spp.* and *Lactobacillus spp.*) significantly decreased the fecal amine and propionate content in growing pigs. Liu et al. (2018) found that complex probiotics (*B. subtilis* and *S. cerevisae*) supplementation in growing pigs diets at 6.0×10^7 cfu/g concentration reduced fecal NH_3, H_2S and total mercaptans emission. However, some studies failed to show beneficial effects on fecal gas emission (Han and Shin, 2005; Balasubramanian et al., 2016). Therefore, further investigations are needed to confirm the beneficial effects of complex probiotics on fecal noxious gas emission, and to illustrate the underlying mechanism of these effects.

Table 3. Positive effects of complex probiotics on fecal noxious gas emission in growing and finishing pigs

Composition	Growth stage	Positive effect on noxious gas emission	Reference
Lactobacillus acidophilus, Saccharomyces cerevisae, Bacillus subtilis	Growing pigs	NH_3-N↓10%	Chen et al. (2005)
Bacillus subtilis, Bacillus coagulans, Lactobacillus acidophilus	Finishing pigs	NH_3-N↓17.1%; butyric acid↓24.4%	Chen et al. (2006)
Bacillus subtilis, Bacillus licheniformis	Growing pigs	NH_3↓50%; H_2S↓38.4%	Wang et al. (2009)
Aspargillus spp., Saccharomyces spp., Lactobacillus spp.	Growing pigs	Amine↓28.9%; propionate↓21.7%	Chu et al. (2011)
Bacillus subtilis, Bacillus coagulans, Bacillus licheniformis	Finishing pigs	H_2S↓27.3%*	Balasubramanian et al. (2016)
Bacillus subtilis, Saccharomyces cerevisae	Growing pigs	NH_3↓7.9%; H_2S↓12.5%; total mercaptans↓10.7%	Liu et al. (2018)

Note: *A tendency effect: P=0.062.

The application of complex probiotics in gestating and lactating sows

Sows are subjected to many stressors during their breeding life, such as repeat services, gestation, farrowing, changes of housing, lactation and weaning, and these stressors can greatly influence the balance of the intestinal microbiota, thus affecting the reproductive performance of sows (Stamati et al., 2006). Considering the beneficial effects on intestinal microbial balance, dietary administration of probiotics may relieve the stress of gestation and lactation sows (Chaucheyras-Durand and Durand, 2010). On the other hand, nutrient utilization and absorption during gestation and lactation had a significant impact on the number of stillborn piglets, the number of nursing pigs born alive, and the BW of suckling pigs at birth and weaning (Alexopoulos et al., 2004; Liu et al., 2017). The improvement of nutrient utilization by feeding probiotics in sows can also benefit the milk yield and litter performance, which would improve the overall pig production (Stamati et al., 2006). Furthermore, epidemiological studies suggest that the administration of probiotics to mothers can affect the health of their infants, including their immune system and development (Fanaro et al., 2003; Schultz et al., 2004). Earlier study proved that administration of probiotic supplement to the sows during gestation and lactation had a positive effect on gut flora and function of piglets, which has an enormous impact on the subsequent performance, suggesting that the colonization of the gut microbial in neonatal piglets could be influenced by the gut flora of sows (Mori et al., 2011).

With respect to the application of complex probiotics in sows, Alexopoulos et al. (2004) reported that dietary BioPlus 2B® (*B. licheniformis* and *B. subtilis* complex) at 400 g/ton of feed to sows during gestation and lactation (the interval from 2 weeks prior to the farrowing up to weaning), which improved certain blood and milk composition parameters, suckling piglet health and performance, as well as subsequent reproductive performance of the sows. Another study which was carried out by Link et al. (2007), suggested that dietary BioPlus 2B® supplementation at a level of 400 g/ton from 2 weeks before farrowing until weaning increased the blood total lipids and cholesterol of lactating sows at d 15 after parturition. Silva et al. (2010) demonstrated that the use of complex probiotics (*Bifidobacterium bifidum* 3.33×10^6 cfu/g, *Enterococcus faecium* 1.66×10^6 cfu/g, *L. acidophilus* 3.33×10^6 cfu/g, *L. plantarum* 1.66×10^5 cfu/g) in the diet of sows in late gestation and during lactation improved the piglets growth performance and intestinal development, and decreased the incidence of diarrhea. The improved gut development of suckling pigs was probably due to the probiotics supplementation which promoted the intestinal microbial balance of sows, and enhanced sows metabolism, thereby improving the colostrum and milk composition (Scharek et al., 2007; Kim et al., 2013). Milenković et al. (2011) indicated that supplementation of 0.2% complex probiotics (*L. acidophilus*, *B. subtilis* and yeast *S. cerevisiae*) during 2 weeks before farrowing and overall lactation period increased the piglets BW at weaning and ADG during suckling. Mori et al. (2011) reported that administration of multispecies microbial supplements (10^7 and 10^8 cfu/g) to pregnant sows changes the fecal SCFAs composition and gut microbiota in their offsprings. Bula et al. (2012) suggested that inclusion of 400 g/ton of feed BioPlus 2B® in late gestation and lactation (from two weeks before farrowing to weaning) diets reduced the sows BW loss during lactation, improved the number of weaned piglets and weaned litter weight. Baker et al. (2013) found the use of 2 strains of *Bacillus subtilis* mixture (3.75×10^5 total cfu/g of feed) in 6 weeks before and throughout the lactation period, could increase the piglet numbers of total born and born alive, and improve the initial litter weight and litter weaning weight, as well as increase the probiotics counts, and suppress the harmful bacteria concentration in the piglets small intestine at d 3 and 10 after birth. According to Link et al. (2016), dietary supplementation of 400 ppm BioPlus 2B® from 2 weeks before farrowing to weaning improved the weaning BW and reduced the diarrhea score for nursing piglets. Additionally, Hayakawa et al. (2016) suggested that addition of *Bacillus mesentericus* 1×10^8 cfu/g, *Clostridium butyricum* 1×10^8 cfu/g and *Enterococcus faecalis* 1×10^9 cfu/g mixture to late gestation (3 weeks before farrowing) and lactation diets increased the sows feed intake and litter weight at birth. Finally, it should be noted that, although numerous previous studies showed beneficial effects of complex probiotics on performance of sows and their offsprings, the underlying mode of action is not fully understood yet. Therefore, more details are needed to study and reveal the mode of action of these effects, thus providing new insight into animal nutrition, as well as human fertility.

Conclusions and implications

In conclusion, the available data from previous studies suggested that the use of complex probiotics could improve the growth performance in weaning, growing and finishing pigs, as well as the reproductive performance of sows. The beneficial effects of complex probiotics were related to various modes of action, including competitive exclusion of pathogenic bacteria, modulation of gut microbiota, immunomodulation, anti-oxidation. However, the effects of complex probiotics in practice is not always consistent, the efficacy of complex probiotics could be influenced by strain composition, dosage, formula, feeding environment, nutritional level of feed, the age and health status of animals. Therefore, more studies on the efficacy of complex preparations in pigs are required, also, randomized, double-blind, case-controlled, placebo-controlled studies, as well as further studies on optimal supplementation stages and doses, are needed. More importantly, to ensure the bioactivity of probiotics during feed processing, coating techniques such as microencapsulation should be developed to maintain bacterial stability.

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