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Influence of a combination of pre and probiotics in swine feeding: A review

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Abstract
Ever since the ban on the use of antibiotics was imposed, it has become imperative to find alternatives like organic acids, plant extracts, pre and probiotics. Prebiotics maintain gut health with beneficial bacteria by eliminating the pathogens as they become intestinal barrier against invading pathogens are said to be effective against the two most potent intestinal pathogens viz- *Salmonella* and *E. coli*. Prebiotics not only enhances the proliferation of epithelial cells but also show stimulatory effects on both exocrine and endocrine secretions of the pancreas. They produce SCFA which as an energy source for the colonocytes. FOS and multi strain probiotics with *Bifidobacterium longum, Lactobacillus acidophilus, Bacillus subtilis, Saccharomyces cerevisieae Aspergillus oryzae* improved FCR and weight gain. The main role of probiotics being a reduction of pH controlling pathogens, production of inhibitory substances like organic acids, stimulation of specific and non specific immunity and other related activities. It was reported by many researchers that probiotics improve feed efficiency, performance and milk fat composition. It is concluded that feed additives is more efficient when fed in combination rather than fed individually. The concept of synbiotic, a combination of pre- and probiotics components has been designated to focus on health enhancing foods and supplements used as functional food ingredients. It seems that synergistic effects of prebiotics and probiotics can be useful in stimulating beneficial bacteria and improving the health of the gut.

Keywords: Antibiotics, probiotic, prebiotics, performance, synbiotics, pathogens

Introduction
The gastrointestinal tract (GIT) of pigs is a very crucial region in pig where inwe can see the influence of the nervous, circulatory, endocrine and immune systems and so every care needs tobe taken to maintain the integrity of the GI tract which directly influences the health. Increasing interest in swine rearing has lead to obtain the market weight earlier for which early weaning at 3-4 weeks is practised as against the normal period of 6 weeks in order to maximize annual sow productivity. A complication of early weaning leads to post weaning diarrhoea, which causes retarded growth, increased mortality. In order to check the Post Weaning Diarrhoea (PWD) and improve the performance, prophylactic doses of antimicrobial feed additives like antibiotic growth promoters (AGP) are being added to weaner and grower diets. Addition of AGP showed increased weight gain by 3.3-8% and improves feed efficiency approximates by 3 percent. (Hillman, 2001) [21].

Since there were certain legal limitations on the use of antibiotic growth promoters, researchers had to work on alternatives to AGP like prebiotics, probiotics, enzymes, acidifiers, flavouring agents. Prebiotics, such as Mannan-oligosacaharides (MOS), Fruto-oligosacharides (FOS), are non-digestible feed ingredients that are fermented in the lower gut to select for beneficial bacteria. Two factors have to be considered important while maintaining the gut health viz- *intestinal mucosa* and *localized microflora*. The intestinal mucosa consists of villi utilized for the absorption of nutrients and the *lamina propria* at the base of the villi to prevent pathogen growth.

It was estimated that pig intestinal flora contains more than400 species of bacteria with a concentration 10^9 cfu/g of intestinal content, (Anadón *et al.*, 2006; Lee *et al.*, 2009**)** [2, 28] half of the bacteria are beneficial to the host – especially *Lactobacillus* and *bifidobacteria* while the remaining half are pathogenic. The microbial population in the large intestine is morein the stomach and small intestine due to the slower transit time of digest favouring rapid multiplication (Zimmermann *et al.*, 2001) [43]. The predominant bacterial species present in the stomach and small intestine of a healthy pig are *Enterobacteria*, *Streptococci* and *Lactobacilli*

in the stomach and small intestine. The predominant bacterial species in the large intestine are *Bacteroides, Prevotella, Eubacteria, Lactobacilli, Fusobacteria, Peptostreptococci. Bifidobacteria* which makes up less than 1% of the total population of bacteria in the pig gut (Jensen, 1999) [23]. The balance between beneficial and pathogenic bacteria is disturbed when pigs face stresses related to weaning, environment, diseases etc. (Cromwell, 2001) [15].

Prebiotics

These are defined as a non-digestible food ingredient that beneficially affects the host by preferential stimulation ofthe growth ofone or a limited number of bacteria in the colon (Gibson and Roberfroid, 1995) [18]. Chang *et al.*, (2000) [14] and Xuan *et al.*, (2001) [42] have reported improved growth in nursery pigs supplemented with oligofructose (OF) where as others (Orban *et al.*, 1997) [34] did not find growth effect in young pigs which is attributed to the chemical structure. The main role of prebiotics is to maintain the gut with beneficial bacteria by eliminating the pathogens. They are shown to reduce the load of bacteria in the gut of swine and improve resistance to bacterial colonisation, and also enhance the intestinal barrier function against invading pathogens. Two major intestinal pathogens in swine were identified- *Salmonella* and *E. coli*. *Escherichia coli* pathogens are found only in the gastrointestinal tract (Andersen *et al.*, 2015) [4] and *Salmonella spp*. can be found in faeces and distal colonic content (Bahnson *et al.*, 2006) [6] of weaner and finisher pigs andin the gall bladder (Burns *et al.*, 2014) [11].

At the outset, let us first know how pathogens get attached to the intestinal mucosa.

Immediately following oral intake, bacteria that survive passage through the acidic stomach environment reach the small intestine in 2–3 h (Nguyen *et al.*, 2015) [33]. There, pathogens must first attach to the intestinal mucosa or intestinal epithelial cell surface to avoid washout by mucosal secretion and /or peristalsis (Kalita *et al.*, 2014) [25]. First, bacterial adhesins such as fimbriae, pilior surface antigensinteract with their receptor on host cell (Berry *et al.*, 2014) [9] Secondly, pathogens translocate the bacterial adhesin and their receptor in host cells which helps them in the initial attachment

Food is the major source of contamination of the GIT with pathogens especially *Salmonella* and *E coli*. However there are various strategies to reduce this contamination (Missotten *et al.* 2015) [31]. Feeding of coarse feed to swine leads a change in physicochemical conditions in the stomach. Coarsely ground feedmeals change the physicochemical conditions in the stomach with higher proliferation of anaerobic lactic acid producing bacteria and which produce lactic acid as their metabolite which inturn reduce the gastric pH and decreases the survival of *Salmonella* and *E. Coli* (Mikkelsen *et al.*, 2004) [30]. Coarse feed particles are not as efficiently digested as fine particles in the stomach and so they reach large intestines and get fermented leading to the production of SCFA which inhibit pathogens (Lebel *et al.*, 2016) [27] due to lowered pH. Besides organic acids in the diet, provision of low quantity protein and high amounts of fibre in the diet can reduce the pathogen load in the gut. The reason is explained here as the fibre level is increased, gut mucous secretions increase leading to the washing off the pathogens without being adhering to the mucosa (Heo *et al.*, 2015) [20] and low quality and lower digestibility protein when fed, will not be digested properly in the stomach and hence reach large intestines and get fermented releasing harmful metabolites like Ammonia which causes colonal epithelial irritation (Jha and Berrocoso, 2016) [24].

Now the prebiotics more broadly defined as any type of food ingredient that has a favourable direct and/or indirect impact on the beneficial GIT microbiota and the intestinal homoeostasis (Hutkins *et al.*, 2016) [22] and consequently inhibit pathogenic infections.

Suryanarayana *et al.*, (2013) [38] reported non-improvement in the digestibility of OM and CP when 24 weanling piglets fed with 1% Fructo oligosaccharides (FOS) alone wherein improvement was observed ($P<0.05$) in combination with a probiotic (*Sacchyromyces cerevisiae*). Similar trend was reported in FCR and weight gain when fed with pre and probiotic combo.

Prebiotics can inhibit pathogen adhesion via several mechanisms. These are a coating of the host epithelial surface, the promotion of beneficial bacteria and the down regulation of adhesion in pathogens. In a nut shell, prebiotics act in the following ways.

- They increase the proliferation of epithelial cells
- They have stimulatory effects on both exocrine and endocrine secretions of the pancreas
- They increase the production of SCFA in the lower tract and Butyric acid acts an energy source for the colonocytes and increase the barrier function of the colonic epithelial cells (Suryanarayana and Ramana, 2015) [36].
- They change the physiology of the epithelial cells and hence reduces the pathogen bacterial attachment to the gut.

Probiotics

Probiotics is a term coined to describe microbes used as a feed additives/ they are defined as live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the intestinal microflora. It was reported that probiotics act by-

1) reducing the pH, creating an unfavourable conditions for the gut pathogens; 2) attachment on the intestinal epithelial surfaces to prevent pathogen attachment; 3) competition for nutrients with pathogens; 4) production of inhibitory substances such as organic acids, hydrogen peroxide, and bacteriocins; and 5) stimulation of specific and nonspecific immunity such as IL and IgA. Commercial probiotics could be divided into three categories: *Bacillus* (Gram-positive spore forming bacteria), lactic acid – producing bacteria (*Lactobacillus, Bifidobacterium, Enterococcus*), and yeast (NRC, 2012).

Various species of microorganisms are used as probiotics and all these were mostly isolated from GI tract, mouth and feces of animals and humans. The most common probiotic strains that are used commonly in animals are *Lactobacillus, Bifidobacterium, Bacillus spp, Streptococcus, Yeast and Saccharomyces cerevisiae*. They should be non pathogenic, gram positive, acid resistant, strain specific, anti E. coli, bile resistant, viable/stable, adhesion to intestinal mucosa, and contain a minimum 30 × 10 to the power of 9 colony forming unit per gram (Pal, 1999) [35].

Mode of action

It has been suggested that probiotics are strain specific, species and dose specific. There are four proposed

mechanisms by which probiotics may protect the host from the intestinal pathogens (Rolfe, 2000).

1) It was reported that probiotics produce several substances like organic acids, hydrogen peroxide and bacteriocins which inhibit the growth of pathogens in the gut. All lactic acid bacteria produce organic acid.
2) Probiotics compete with pathogens especially with *E.Coli* for gut epithelial site attachment and hence they are eliminated from the gut by competitive exclusion
3) Probiotics may prevent the utilization of nutrients by pathogenic bacteria.
4) It was reported that probiotics can protect against intestinal disease by stimulation of specific and nonspecific immunity.

The microbial population in the large intestine is more numerous than in the stomach and small intestine mainly due to the slower transit time of digesta in the large intestine, and this condition favours the microbes to multiply rapidly. The predominant bacterial species present in the stomach and small intestine of a healthy pig are *Enterobacteria*, *Streptococci* and *Lactobacilli* in the stomach and small intestine. The predominant bacterial species in the large intestine are *Bacteroides, Prevotella, Eubacteria, Lactobacilli, Fusobacteria, Peptostreptococci. Bifidobacteria* makes up less than 1% of the total population of bacteria in the pig gut (Jensen, 1999) [23]. The balance between beneficial and pathogenic bacteria is disturbed when pigs face stresses related to weaning, environment, diseases etc. (Cromwell, 2001) [15]. It was also reported that feeding with probiotics in sows increase feed consumption during late pregnancy stages or lactation, improving body condition at the end of lactation. The basic mechanism in improving the body condition after parturition is that it minimises the energy mobilization during lactation. Addition of probiotics after weaning reduces the energy mobilization at lactation. It was also reported that a reduction in weaning-estrus interval (Kritas *et al.*, 2015; Hayakawa *et al.*, 2016) [26, 19]. It was established that milk components such as oligosaccharides, fat and proteins (Bian *et al.*, 2016; Alexopoulos *et al.* (2004) [10, 26] are also affected by probiotic treatments. A reduction of clinical signs of uterus and/or udder disease, together with fewer clinical signs of diarrhoea in piglets which is due to a reduction in gut pathogens (Apic *et al.*, 2014; Baker *et al.*, 2013; Kritas *et al.*, 2015) [5, 8, 26]. The effect of probiotics on pregnant and lactating sows was proved positive for both the dam and the off springs. However the effect depends on the mode, dose and length of supplementation (Kritas *et al.*, 2015) [26]. In weaned piglets probiotics improve weaning outcome especially from the 1st week to the market weight (de Lange *et al.*, 2010) [16]. Probiotics in weaned piglets prevent diarrhoea,re-establish microbial balance, stimulating immunity, increasing the intestinal barrier function. It was reported that probiotics make higher biovailability of nutrients, improves gut health by relieving weaning stress, preventing diarrhoea, improving the intestinal beneficial microbiota balance. Certain behavioural trait changes like increasing the eating habit was also observed with probiotics especially with *Bacillus licheniformis* (Barba- Vidal *et al.*, 2017) [7]. It was reported that probioticslike *Lactobacillus acidophilus, Sacchyromyces cerevisiae* enriched with Selenium (Gan *et al.*, 2014) [17] when fed to weaned piglets revealed positive results with growth performance, anti oxidant status, immune function and also prevent heat stress (Xiang-hong *et al.*, 2011) [41].

Probiotics can be more effective under poor sanitary conditions with pigs having any sub clinical infections instead of supplementing under good environmental conditions (Kenny *et al.* 2011). Probiotics have some demerits in utilizing the dietary energy for their own metabolism and reduces the availability of energy to the pigs. Meat quality could also be improved with probiotics. These have been described to affect meat color, marbling and firmness scores (Černauskienė *et al.*, 2011) [13], potentially increasing the organoleptic properties of the meat. In addition, probiotics were reported to reduce the incidence of Zoonotic diseases like *Salmonella spp*. (Barba Vidal *et al.*, 2017; Upadhaya *et al.*, 2017) [7, 39].

Effects of Prebiotics and Probiotics

In general, feeding probiotic and prebiotic combo to the animals should meet many specifications. It should have a beneficial effect on the host and should stimulate the growth of a specific type of beneficial live microbials suppressing the growth of other bacteria. However formulation of a combo depends on type of feed supplied, physiological condition and state of the animal, health status, age and other related factors. Most of the studies confirmed the combination of *Bifidobacteria* and *Lactobacilli* with FOS was proved promising.

How does the combination work?

The combination beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GI tract, by selectively (Gibson and Roberfroid, 1995) [18] stimulating the growth and/or activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare. Brestensky *et al.* (2016) [12] reported lower (P<0.05) concentrations of Propionic, Butyric, Valeric and caproic acids in the jejunal digest of growing pigs when fed a high fat diet with *Lactobacillus plantarum*, Inulin and horse chestnut. However in the caecum, the concentration of Butyric and Lactic acids were greater.

In a trial with weaned piglets Shim *et al.* (2005) [37] which were fed with FOS and multi strain probiotics with *Bifidobacterium longum, Lactobacillus acidophilus, Bacillus subtilis, Saccharomyces cerevisieae, Aspergillus oryzae* reported improved (P<0.05) feed efficiency and weight gain.In an experiment with 24 weanling Large White Yorkshire X Desi cross bred male pigs with 75% exotic inheritance Suryanarayana *et al.*, (2013) [38] reported that synergy of probiotic (*Sacchyromyces cerevisiae*) and prebiotic (Fructo oligosaccharide) enhanced nutrient digestibility (OM,CP) and stimulated the growth of benefactor micro organisms –Lactobacillus with concurrent depression in the growth of potentially harmful pathogens like *Coliforms* and *Salmonella* symboliozing better gut health of the host. The results were not promising when probiotic and prebiotic were fed individually.

Nemkova *et al.* (1999) [32] reported increased faecal count of *Lactobacillus* and *Bifidobacteria* in piglets when fed with *Lactobacillus paracasei* and FOS with a corresponding decrease in *Escherichia coli, Enterobacteriaceae* and *Clostridium* genus bacteria. Lee *et al.* (2009) [28] studied the effect of a combination of a probiotic derived from anaerobic bacteria and prebiotic (MOS) on growth, digestibility of nutrients, emission of harmful gases and

composition of intestinal microbiota of 150 weaned piglets and reported improved digestion of nutrients, reduced output of harmful gases and prevented bacterial infections during weaning period.

Vicente *et al.* (2007) [40] studied the effect of a synbiotic containing *Lactobacillus* spp.in combination with lactosein 320 turkeys infected with *Salmonella* and observed a positive effect on feed conversion and body weight gain. Li *et al.* (2008) [29] studied the effect of supplementing FOS and *Bacillus subtilis* bacteria to 720 broiler chickens. They reported improvement of the average daily growth and of the feed conversion ratio, as well as reduced incidence of diarrhoea and mortality of animals in comparison to animals treated with aureomycin for the control group.

Conclusion

In spite of various technologies that are developed to enhance the utilization of nutrients by the animals, competitive world aspires newer technologies in the use of feed additives. Probiotics and prebiotics play a promising role either alone or in combination in terms of feed efficiency, weight gain, nutrient utilization and other related aspects sparing no nutrients to pathogens and helping for the elimination of these by the process of competitive exclusion for the attachment sites. But before testing the probiotic and prebiotic combo, it should have a beneficial effect on the host and should stimulate the growth of a specific type of beneficial live microbials suppressing the growth of other bacteria and formulation depends on type of feed supplied physiological condition and state of the animal, health status, age and other related factors. The most suitable combination is *Bifidobacteria* and *Lactobacilli* with FOS and results were promising. It was reported by many researchers that a combination of the feed additives- may be enzymes, probiotics, prebiotics, acidifiers etc proved the best instead of feeding them individually. The reasons were attributed to the combined effect of the individual feed additives, host specificity for each feed additives viz- one additive may be effective under certain conditions and the other may differ under the same conditions and so the combination in the form of synergism proves economical. It should be noted that the use of feed a of action of probiotic organisms, prebiotics, as well as their combinations in synbiotics, require further research as many factors like age of the animal, physiological condition, health status, dose and other related factors.

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