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Role of probiotic in the performance of young piglets: A review

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Abstract
A balanced microbiota is an indispensable constituent of a healthy gut. The word "probiotic" was derived from the Greek meaning "for life" or "in favour of life". Probiotics, the live microorganisms which, when administered in adequate amounts, confer good health benefits onto the host, are a category of feed additives that can be used to replenish the gut microbial population while recuperating the host immune system. Besides their antitoxin and diarrhoea reduction effects, dietary supplementation of probiotics can improve gut health, nutrient digestibilities and, therefore, benefit nutrient utilization and growth performance of pigs. Thus, this review paper aims to provide some beneficial role of probiotics in the performance of young piglets.

Keywords: Probitic, piglets, growth performance

Introduction
Over recent years probiotics have been successfully used to stimulate animal growth and to increase the intensity of animal productivity. Probiotics are utilized for prophylaxis and treatment of mixed gastro-intestinal infections, digestive disorders arising from sudden changes of diet, irregularity of the feeding process and technological stress, and for the stimulation of non-specific immunity. They are also used for faster restoration of microbiocenosis of intestines after antibiotic treatment the replacement of antibiotics in the fodder for young animals, the acceleration of animal's adaptation to high-energy diet and non-nitrogenous substances, the improvement of the efficiency of feed utilization and the productivity of animals.
The concept behind probiotics was introduced in the early 20th century at the Pasteur institute by Russian scientist and Nobel laureate Elie Metchnikoff, was the first conceptualize "probiotics". Metchnikoff is regarded as the god father of probiotic. Metchnikoff, in 1907 suggested that it would be possible to modify the gut flora and to replace harmful microbes with useful microbes. Probiotics are microorganisms that are believed to provide health benefits when consumed. They are considered to be generally safe, but they may cause bacteria-host interactions and unwanted side effects in certain cases. In 1953, Werner Kollath came up with the term "Probiotic," which is derived from the Greek word pro (for) and bios (life) or 'in support of life. The term probiotic was introduced by Lilly and Stillwell [1] to describe substances produced by one microorganism that stimulated the growth of other microorganisms. The World Health Organization's (WHO) 2001 definition of probiotics is "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host". Commonly used bacterial probiotics include Lactobacillus species, Bifidobacterium species, Escherichia coli, Streptococcus species, Lactococcus lactis and some Enterococcus species. Currently, the only probiotic yeast used is the nonpathogenic Saccharomyces boulardii.

Probiotics Classification
There are many different microorganisms used as probiotics. They are grouped based on the similarity of qualities, such as physical characteristics, metabolic needs, and metabolic end products. The common microorganisms are:
1. Lactobacillus spp**:** acidophilus, plantarum, rhamnosus, paracasei, fermentum, reuteri, johnsonii, brevis, casei, lactis, delbrueckii gasseri.

2. Bifidobacterium spp**:** breve, infantis, longum, bifidum, thermophilum, adolescentis, animalis, lactis.
3. Bacillus spp**:** coagulans.
4. Streptococcus spp**:** thermophilus.
5. Enterococcus spp**:** faecium.
6. Saccharomyces spp**:** cerevisiae.

Characteristics of good probiotics

Fuller (1989) [2] listed the following as features of a good probiotic:

1. It should be a strain, which is capable of exerting a beneficial effect on the host animal, for example increased growth or resistance to disease.
2. It should be non-pathogenic and non-toxic.
3. It should be present as viable cells, preferably in large numbers.
4. It should be capable of surviving and metabolizing in the gut environment for example, it should be resistant to low pH and organic acids.
5. It should be stable and capable of remaining viable for periods under storage and field conditions.

Mechanisms of Probiotic Function

The following are the major mechanisms of action of probiotics on the host

1. Enhancement of the epithelial barrier
2. Increased adhesion to Intestinal Mucosa:
3. Concomitant inhibition of pathogen adhesion
4. Competitive exclusion of pathogenic microorganisms
5. Production of anti-microorganism substances
6. Modulation of the immune system
7. Quorum sensing signaling

1) Enhancement of the epithelial barrier

Probiotic (Lactobacillus plantarum MB452) will enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. So, as a result there will be no way for the pathogenic bacteria for colonozation. Normaly Probiotics influences the components of epithelial barrier function by two process 1) either by decreasing apoptosis of intestinal cells or 2) increased mucin production. Firstly for e.g. Lactobacillus rhamnosus GG was able to prevent cytokine-induced apoptosis in intestinal epithelial cell models by inhibiting tumor necrosis factor (TNF). Besides Lactobacillus rhamnosus GG also shown to prevent inflammation and programmed cell death of the lining intestinal epithelial cells and shown to exert mitogenic effects and enhancing mucosal regeneration. Secondly Lactobacillus, species have been shown to increase mucin production by goblet cells thus blocking pathogenic E. coli invasion and adherence. Besides the Protein Kinase C (PKC) can reconstruct the tight junction complex and repair the barrier function.

2) Increased adhesion to Intestinal Mucosa

A fraction of ingested probiotics are able to interact with intestinal epithelial cells (IECs) and dendritic cells (DCs), depending on the presence of a dynamic mucus layer. Probiotics can occasionally encounter DCs through two routes: DCs residing in the lamina propria sample luminal bacterial antigens by passing their dendrites between IECs into the gut lumen, and DCs can also interact directly with bacteria that have gained access to the dome region of the gutassociated lymphoid tissue (GALT) through specialized epithelial cells, termed microfold or M cells. The interaction of the host cells with microorganism-associated molecular patterns (MAMPs) that are present on the surface macromolecules of probiotic bacteria will induce a certain molecular response. The host pattern recognition receptors (PRRs) that can perceive probiotic signals include Toll-like receptors (TLRs) and the C type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN). Some molecular responses of IECs depend on the subtype of cell, for example, Paneth cells produce defensins and goblet cells produce mucus. Important responses of DCs against probiotics include the production of cytokines, major histocompatibility complex molecules for antigen presentation, and co-stimulatory molecules that polarize T cells into T helper or CD4+CD25+ regulatory T cells in the mesenteric lymph nodes (MLNs) or subepithelial dome of the GALT. IFNγ, interferon-γ; IL, interleukin; TGFb; transforming growth factor-β.

3) Concomitant inhibition of pathogen adhesion

In this mechanism firstly the mucin produce by the goblet cell will cause Qualitative alterations in intestinal mucins that prevent pathogen binding. Secondly probiotic will induce the epithelial cell for the release of antimicrobial proteins (AMPs) like α- and β-defensins, cathelicidins, C-type lectins and ribonucleases from epithelial cells. These AMPs provide first line of chemical defense. These AMPs provide first line of chemical defense. For eg Surface layer proteins purified from L. helveticus R0052 inhibited enterohemorrhagic Escherichia coli O157:H7 adherence and the subsequent rise in permeability, without altering the growth of the pathogen. On the other hand S. boulardii secretes a heat-labile factor which also shown to be responsible for the decreased bacterial adherence.

4) Competitive exclusion of pathogenic microorganisms

There will be Creation of a hostile micro ecology (specific adhesiveness between probiotics and mucous) and preventing from colonizing a given environment because of the prior presence of other organisms that are better able to establish and maintain themselves in that environment. Probiotics share the same receptor sites on host cells with pathogens. This property makes them able to exclude pathogens from host intestine. And as a result there will be Elimination of available bacterial receptor sites. There will be also competitive depletion of essential nutrients between the probiotic and pathogen. Probiotics share the same receptor sites on host cells with pathogens. This property makes them able to exclude pathogens from host intestine, urogenital tract and other host sites. Competitive exclusion by adhered probiotics, by competition for receptor sites and by displacement of adhered pathogens. Non competitive exclusion by induction of secretion of antimicrobial components from host cell and by regulation of epithelial barrier function. Probiotic bacteria can also modify environment less suitable for pathogens – formation of lactic acid etc and cause exclusion of Pathogenic Microorganism.

5) Production of anti-microorganism substances

In normal condition, Defensins (hBD protein) and cathelicidins are the antimicrobial peptides expressed constitutively by the intestinal epithelial cells and display antimicrobial activity against a wide variety of bacteria, fungi and some viruses. Probiotics either by inducing host cells to produce peptides or by directly releasing peptides interfere with pathogens, and prevent epithelial invasion. Certain probiotic strains like E. coli strain DSM 17252 G2 and several Lactobacilli species have shown to express certain defensins Probiotics release of a variety of antimicrobial factors like defensins, bacteriocins, hydrogen peroxide, nitric oxide, and short chain fatty acids (SCFA), such as lactic and acetic acids, which reduce the pH of the lumen. SCFA can disrupt the outer membranes of gram-negative pathogens causing inhibition of pathogen growth. Bacteriocins (By Gram positive probiotics) can either permeabilize the inner membrane of gram-negative bacteria, leading to disruption and formation of pores. Microcins (produced by gram negative bacteria), on the other hand, can target the inner membrane, enzymes that are involved in DNA or RNA structure and synthesis, or protein synthesis enzymes.

6) Modulation of the immune system

Interaction of probiotic bacteria with epithelial cells (E) or M cells (M) or the Dendritic cells (DC) results in the internalization of the bacteria or its components.This interaction Stimulates the release of IL-6 by epithelial cells and stimulates macrophages (MQ) and dendritic cells to produce TNF-alpha and IFN-g. Mast cells (MAC) or also stimulated to produce the cytokine IL-4, which together with IL-6 and TGF-b induce the Tindependent switch from IgM to IgA on the surface of B lymphocytes (BL), thereby enhancing the production of IgA. IL-6 favours the clonal expansion of IgA B lymphocytes. There is also an associated increase in the production of antibodies such as IgM, IgG and reduced secretion of IgE. Th1 cells produce pro-inflammatory cytokines such as IFNγ, TNFα and IL-2, which stimulate the phagocytosis and destruction of microbial pathogens and induce macrophages, natural killer cells and cytotoxic T-lymphocytes to kill viruses and tumors.

7) Quorum sensing signaling

Bacteria communicate with each other as well as with their surrounding environment through chemical signalling molecules called auto-inducers. This phenomenon is called quorum sensing. The use of this cell-to-cell signaling mechanism facilitates the regulation of important traits of enteric microbes that allow them to successfully colonize and/or start infection in their host. Medellin-Pena *et al*. demonstrated that *Lactobacillus acidophilus* secretes a molecule that inhibits the quorum sensing signalling or directly interact with bacterial transcription of *E. coli* O157 gene, involved in colonization and thus, bacterial toxicity is opposed.

Effect on different performance parameters of young piglets

1) Control of post-weaning diarrhea:

At the time of weaning, entero-pathogens takes upper hand as weaning of the piglets reduce digestibility of high protein diet which results increase production of BCFA and ammonia nitrogen (NH3eN) leading to more incidence of diarrhea [3]. The BCFA and NH3eN are the main toxic metabolites for intestinal mucosa and tagger of post weaning diarrhea in piglets [4]. E. coli are the major enteropathogen of postweaning diarrhea and causes 26% cases of neonatal diarrhea . Addition of lactobacilli as probiotic in the diet results beneficial fermentation resulting increased concentration of short chin fatty acids and lactic acid in GIT. These may reduce pH of the gut which in turn decrease growth of opportunistic enteropathogensas they need alkaline medium to proper growth and multiplications.

2) Improved performance

In swine, the use of probiotics improves intestinal well-being which leads to improve performance. Higher growth rate and improved feed efficiency ratio results in improve profitability due to greater output and reduction in overhead costs [5]. However, age and weight at weaning are closely related to postweaning growth rate. The administration of probiotics, soon after birth could be effective as probiotic bacteria by enhancing intestinal barrier function which restrict colonization of pathogenic bacteria to intestinal mucosa. This is evident with better absorption of nutrients and immunoglobulins of the colostrum, enabling better sustainability of the piglet, and minor loss of piglets at its first days of life. Supplementation of lactobacilli has resulted in improved growth and feed efficiency in nursery) and weaning [6] piglets. A healthy intestinal tract has a dominance of LAB, however, this equilibrium within the intestinal tract is troubled when the animal is in stressful condition like castration, weaning, high temperature and humidity and change of feed. This could be improved by continuous feeding of lactobacilli, which encourages rapid growth of other beneficial bacteria and reduce the growth of pathogenic bacteria by competitive exclusion. Feeding of probiotics (Lactobacillus spp.) to weaning piglets resulted in an increased growth rate due to high feed intake and better feed conversion ratio. Supplementation of complex probiotics, including yeast (S. cerevisiae) and Lactobacillus spp. have been reported to improve growth performance of weaned piglets [7]
.

3) Effect in intestinal microflora and gut health:

Microflora in the digestive system plays a very important role in the defense mechanism of the body. The major intestinal microflora of pig are Lactobacilli, Bifidobacteria, Streptococci, Bacteriodes, Clostridiums perfringes and E. coli may vary with age. One of the important ability of stable microflora in gastrointestinal tract is colonization resistance. About 4e6 weeks is needed to establish a stable microflora in the GIT [8]. However, supplementation of Lactobacilli in neonatal piglets helps in early development of stable gut microbflora, stimulation of immune system and prevents diarrhea. When piglets are weaned, the intestinal microflora of piglets is altered due to dietary and environmental change after weaning of piglets. The entero-pathogenic E. coli are markedly increased in the anterior small intestine resulting post weaning diarrhea. Oral administration of Lactobacillus fermentum I5007 in formula-fed piglets improved intestinal health and reduced the number of potential entero-pathogens like E. coli and Clostridia in neonatal piglets. Addition of complex lactobacilli previously isolated from GIT of piglet (Lactobacillus gasseri, L. reuteri, L. acidophilus, L. fermentum, L. johnsonii and Lactobacillus mucosae) increased number of lactobacilli and bifidobacterium, also reduced E. coli and aerobic bacteria counts in jejunum, ileum, cecum and colon mucosa [9]

4) Improved immune status

The probiotics have the capacity to modulate the immune system of animal by enhancing the systematic antibody response to soluble antigens in the serum. The immune-modulatory effects can even be achieved by dead probiotic bacteria or just probiotics derived components like peptidoglycan fragments or DNA. Dietary supplementation with probiotics enhanced humoral and cell mediated immune responses with increased the serum concentration of IgM [10] and IgG [11] growing pigs. Probiotic supplementation in sow also increased IgG level in colostrum and plasma of piglets. Since, probiotics facilitate the suppression of lymphocyte proliferation and cytokine production by T cells which down regulating the expression of proinflammatory cytokines such as tumor necrosis factor-a. L. fermentum enhanced T-cell differentiation, induced cytokine expression in the ileum of E. coli challenged piglets with increased pro-inflammatory cytokines and percentage of CD4þ lymphocyte subset in blood [12]. However, it is difficult to confirm that probiotics contribute significantly to the immune system of the host. The main reason behind this caveat is that probiotics differ from antibiotics in that they are not intended to eradicate invasive pathogens in the gastrointestinal tract. Therefore, such observed improvements or positive effects are always hindered due to the animal's immune status and the various applied situations.

5) Antioxidant status

An abnormality in the antioxidant defense system can increase the susceptibility of pigs to stress, resulting in decreased performance and reduced immune function. As a result of incomplete reduction of oxygen, the reactive oxygen are formed which includes superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen. A physiological concentration of reactive oxygen species (ROS) is required for normal cell function, energy production, phagocytosis and intercellular signaling regulation. Early weaning of piglets cause oxidative stress by producing excessive quantities of ROS which not only damage proteins, lipids and DNA but also decline intestinal antioxidant enzyme activities under NF-kB, p65 and Nrf2/Keap1 signals. The probiotic cell have defenses mechanisms against the damaging effects of ROS by involving both in enzymatic (superoxide dismutase and catalase) and non-enzymatic components. The lactic acid bacteria diminish the activity of ROS through the production of superoxide dismutase that converts superoxide radicals to oxygen and hydrogen peroxide [13]. Some of the species of LAB may also produce catalase, which can destroy hydrogen peroxide at a very high rate that blocks formation of peroxyl radicals while some lactobacilli produced non-enzymatic antioxidants such as glutathione and thioredoxin to reduce reactive oxygen intermediates. Supplementation of Lactobacilli sp. increased serum concentration of superoxide dismutase, glutathione peroxidase and catalase in suckling and weaning piglets whereas, total antioxidant capacity, hepatic catalase, muscle superoxide dismutase improved in grower-finisher pigs [14].

6) Effect on intestinal morphology

The gastrointestinal tract is the main digestive and absorptive organ in animal. The GIT permits the uptake of dietary substances into systemic circulation and it also excludes pathogenic compounds simultaneously. There is a reduction in villous height (villous atrophy) and crypt depth at weaning. As weaning leads to temporary starvation which resulted villous atrophy, reduces mucosal protein content and digestive enzymes activity. Hence, improve feed intake immediately after weaning reduced the histological changes of small intestinal morphology [15]. As feeding of probiotic increased daily feed intake thus it had positive effect on development of intestinal epithelium. However, the effects of probiotic on villus height may change depending upon species of microorganism. Longer villi (V) height, deeper crypt (C) depth and smaller V: C ratio was observed in pigs supplementation with L. acidophilus (Rodrigues *et al*., 2007), Lactobacillus plantarum and P. acidolactici. However, no change in the crypt depth was observed with E. faecium [16].

7) Nutrient digestibility

Maxwell *et al*. (1983) observed improvements of DM and N digestibility when pigs fed diet with probiotics include different bacteria strains. Pigs fed diet Bacillus+Sacchromyces had higher digestibility of CF and pigs fed diet Bacillus+Saccharomyces+Lactic acid bacteria complex had higher digestibility of CP, CF and OM compared with pigs fed the basal diet. Nitrogen retention was not affected by treatment. However, there was a tendency to higher nitrogen retention on diet BSL compared with the control. Regulating intestinal microbial balance by increasing the activity of microbial digestive enzymes, it improves digestion, feed digestibility and nutrient utilization [17].

Conclusion

In conclusion, Although different probiotics strains, even of the same species may have different metabolic effects; which in turn affect growth performances, microbial count and blood parameters of piglets differently. Various studies and application of probiotics in swine husbandry clearly indicate that various spp. have a great potential as an alternative of antibiotics. More attention should be paid for utilizing effects of different probiotic preparations and corresponding feeding strategy in pigs.

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