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MODULATING GASTROINTESTINAL MICROFLORA OF PIGS THROUGH NUTRITION USING FEED ADDITIVES

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Abstract

The benefits of feeding pigs with feed additives such as pre- and probiotics, acidifiers, herbs, enzymes, macro- and micronutrients and zootechnical additives include higher daily weight gains, better feed conversion, increased intake of feed by animals as a result of improved palatability, and improvements in health and welfare, as reflected in the lower frequency of diarrhoea and limited mortality of piglets. It is therefore necessary to continue research on the development of better and better feed additives that ensure optimum composition of the gastrointestinal microflora in piglets while preventing diarrhoea and its negative consequences. The use of advances in molecular biology and the search for new methods that are more accurate than culture-dependent techniques are critical to success in more complete identification of the gastrointestinal microflora and its influence on normal body function. The possibility of observing changes within the populations of intestinal microorganisms, strictly correlated to the ingested food, offers hope for improvement of the health of farm animals and of production parameters by modifying the composition of intestinal bacteria.

Key words: gut microbiota, pig, feed additives

Piglet mortality in the first weeks of life and immediately after weaning, i.e. during the most critical periods of growth, is often caused by diarrhoea and enteritis resulting from an upset balance of the gastrointestinal flora. The optimum composition of the gastrointestinal microflora is an important factor having beneficial effects on the health and rearing performance of piglets. Various efforts are made in pig nutrition to maintain a balance between beneficial bacteria (*Lactobacillus*, *Enterococcus*, *Bifidobacterium*) and potentially pathogenic bacteria (*Clostridium perfringens*, *Escherichia coli*, *Salmonella sp*.), which should form 90 and 10% of the gastrointestinal microflora, respectively (Swords et al., 1993; Taras et al., 2006). All factors (stress, weaning of piglets, dietary changes or unbalanced rations, low feed digestibility, improper gastrointestinal pH, etc.) that change the quantity and quality of these bacterial populations, tipping the balance in favour of pathogenic bacteria, compromise animal health and productivity. The digestive tract of pigs is colonized by several hundred species of bacteria, the numbers of which range from 10^3 to 10^{12} bacteria per g of digesta in different segments, i.e. the stomach and small and large intestine (Ewing and Cole, 1994; Jansen et al., 1999; Du Toit et al., 2003).

Microflora composition is strictly related to the system of feeding, which influences the pH of intestinal contents. The acid pH contributes to the growth of beneficial microflora, while the neutral or alkaline pH contributes to the development of pathogenic flora (Gaskins, 2003). Some preparations used as feed additives, by having beneficial effects on the activity of gastrointestinal microorganisms, may limit the losses sustained by breeders. Over the last 50 years, the most important role in maintaining the balance of the gastrointestinal microflora was played by feed antibiotics whose pathogen-destroying properties made it possible to eliminate chronic intestinal infections, thus improving the health and performance of animals. Antibiotics ensured better feed conversion (2–5%), a more rapid rate of growth (2–8%), higher resistance to disease, and lower mortality (Freitag et al., 1998; Close, 2000). Antibiotic growth promoters that have been removed from feed formulations since 1 January 2006, began to be replaced with alternative products such as probiotics, prebiotics, synbiotics, acidifiers, herbs, feed enzymes and immunostimulants. Some of these preparations influence gastrointestinal microflora of the pigs, thus modulating its qualitative and quantitative composition. A direct effect on the gastrointestinal flora is exerted by prebiotic preparations. The other additives act by altering gastrointestinal milieu conditions (acidifiers), supporting selective growth of bacteria (prebiotics) or containing biologically active agents that destroy undesirable bacteria (herbs).

The use of modern analytical methods such as FISH (fluorescent *in situ* hybridization), PCR (polymerase chain reaction), DGGE (denaturing gradient gel electrophoresis) and TGGE (temperature gradient gel electrophoresis) enables highly accurate identification of the gastrointestinal microflora.

Determining the effect of diet (feed additive) on modulating the composition of pig gastrointestinal flora will enable the optimal formulation of rations to create the ideal environment of the digestive tract that ensures high digestibility of nutrients, normal function of the digestive tract, and good health of the animals. Here we describe new diagnostic techniques used to determine the composition of gastrointestinal microflora in mammals and present the results of studies on the application of feed additives that stimulate gastrointestinal microflora in pigs.

Intestinal microflora

A mature intestinal flora includes various species of bacteria and fungi serving many beneficial functions essential for normal digestive processes and nutrient absorption in the host's body. The digestive tract of a suckling animal is sterile at birth, but becomes rapidly colonized with microorganisms that form the initial microflora, which is later modified until it becomes mature and stable (Rolfe, 1996; Zoetendal et al., 2001). Bacteria colonize the intestinal lumen, the mucous layer and the mucous membrane area. The total number of species resident in the digestive tract is still unknown. It is estimated to be 500 in the hindgut of pigs (van Kessel et al., 2004) and from 500 to 10,000 in humans (Hooper and Gordon, 2001). The total population of bacterial cells is 10^{14}, which is ten times the number of all cells in the human body (Pickard et al., 2004), whereas the number of genes encoded by intestinal microorganisms is 2–4 million, which is about 100 times the number found in the host's genome (Hooper and Gordon, 2001).

Obligatory anaerobic bacteria predominate in the gastrointestinal ecosystem, but the composition of intestinal flora varies strictly according to the colonization site and its physiological conditions such as passage rate, presence of enzymes, hydrochloric acid and bile, availability of nutrients or oxygen presence. Bacteria are divided into two groups: autochthonous (endogenous) bacteria are permanent residents of the digestive tract that proliferate at such a rate that prevents a dramatic decrease in their numbers when digesta are removed from the intestine; allochthonous bacteria are transient bacteria introduced into the body through the diet (Zabielski, 2007).

The stomach and the initial part of the small intestine contain a relatively small number of microorganisms. The main factors that limit their proliferation are unfavourable physiological conditions such as low pH and rapid digesta flow. The dominant groups are lactic acid bacteria of the genera *Lactobacillus* and *Streptococcus* (Jensen, 2001). Less prevalent are unicellular bacteria such as enterobacteria, *Clostridium*, *Eubacterium* and *Bifidobacterium* (Conway, 1994; Melin, 2001).

More favourable living conditions for microorganisms are found further into the digestive tract, i.e. in the ileum. Slightly higher pH and slower passage rate are conducive to the growth of microorganism populations, resulting in greater biodiversity. The ileal bacteria include *Lactobacillus*, *Streptococcus*, *Clostridium*, enterobacteria, *Bacillus* and *Bacteroides* (Conway, 1994; Jensen, 2001; Hill et al., 2005).

The richest biocenosis is characteristic of the large intestine, which contains the most numerous populations of microorganisms that grow in favourable environmental conditions. The presence of undigested nutrients, the slow transit of intestinal digesta, neutral pH and the low redox potential provide conditions for the formation of stable and numerous populations (Jensen and Jorgensen, 1994). Several hundred species of microorganisms make up 10^{11}–10^{12} cells/g digesta (Ewing and Cole, 1994). Among these, the most numerous group is formed by *Bacteroides sp.*, which account for 30% of the microflora found in this part of the intestine (Salyers, 1984). These obligatory anaerobes serve many positive functions in the host's body, but they may also induce opportunistic infections. Their main role is to produce some vitamins, amino acids, compounds that inhibit pathogenic microorganisms, and agents promoting the proliferation of *Bifidobacteria*. Essential to the physiological microflora of the large intestine are also bacteria of the genera *Eubacterium*, *Bifidobacterium*, *Ruminococcus* and *Clostridium* (Zabielski, 2007; Zawadzki, 2008).

Bacteria are not the only group of microorganisms resident in the digestive tract. A special situation is visible especially in the ruminant stomach which provides a good niche for the growth of symbiotic fungi and even protozoa. The first group includes obligatory anaerobes of the genera *Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Neocallimastix*, *Orpinomyceces* and *Piromyces* that use lignified portions of the feed. Yeast fungi of the genus Candida are also found in the intestine. Their populations are small at equilibrium, but if the beneficial bacterial flora is depleted, they may grow into invasive form, leading to candidiasis (Zawadzki, 2008).

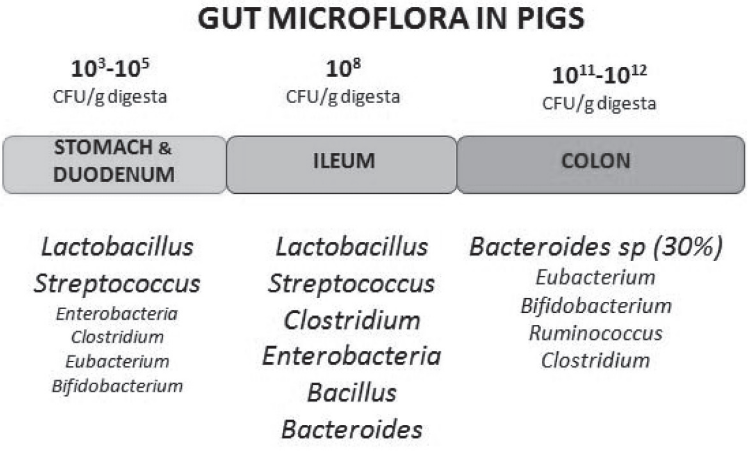

Figure 1. The scheme of the bacteria species living in the pig's gut

Function of intestinal flora

Intestinal microflora is a highly complex ecosystem of relationships not only between the symbiont and the host, but also between individual microorganisms. In a healthy body, its qualitative and quantitative composition is stable but subject to subtle changes. Maintaining the balance between the dominant autochthonous flora and the presence of pathogenic microorganisms is critical for the normal function of the digestive tract and, as a result, for the host's health.

Research on the function of microorganisms living in symbiosis with their host was initiated by Louis Pasteur, who already established over a hundred years ago that they are essential for normal function of the human body (Schottelius, 1902). Today we know that some of their tasks are to digest nutrients, supply essential products (e.g. vitamins, mainly K, B_{12}, thiamine, riboflavin and pyridoxine) or regulate mineral metabolism. They are an important factor responsible for normal morphology of the intestine, stimulation of the host's immune response, as well as protection against colonization by pathogenic microorganisms.

Autochthonous microflora helps the maturing digestive tract to reach normal length and thickness, ensuring the appropriate structure of the intestinal villi and crypt depth in a young organism (Wostman et al., 1983). Intestinal bacteria are also responsible for the normal morphology of the intestinal wall and epithelium. All these processes make it possible to increase the absorption area, which enhances feed conversion and increases weight gains in animals. Valuable research material is offered by gnotobiotic (GB) animals whose digestive tract is completely sterile as a result of special breeding procedures and maintenance of aseptic conditions. Comparative analysis made it possible to determine the role of microorganisms in the normal course of physiological processes taking place in the intestine. Germ-free (GF) rodents that showed considerable abnormalities in the development of the digestive tract, resistance and biochemical processes, were affected with abnormal growth of the villi, alterations in the shape of enterocytes (Falk et al., 1998), shallow crypts, and reduced number of their cells (Alam et al., 1994).

Intestinal microflora has a beneficial effect on the physiological and biochemical processes taking place in the digestive tract. GB animals were observed to be affected by slowed intestinal peristalsis and digestion, altered redox potential, more alkaline pH of the intestine, decreased enzyme activity for β-glucuronidase in the large intestine, and increased activity for alkaline phosphatase and disaccharidase (Berg, 1996).

The fermentation processes carried out by the colon microflora (mainly *Bifidobacterium* and *Lactobacillus* bacteria; Fooks and Gibson, 2002) generate short-chain fatty acids (SCFA), including acetic acid, propionic acid and butyric acid, which is most readily metabolized by intestinal mucosa cells, providing them with energy and improving the absorption of mineral substances (Steer, 2000). SCFA are vital to many biological functions. The main purpose of these acids is to exert a positive influence on colon mucosa and to regulate cholesterol and triglyceride synthesis in the liver. As a result of biochemical and morphological disturbances in gnotobiotic mice, their daily requirement for energy increased by as much as 30% (Wostman et al., 1983).

One of the major functions of gastrointestinal microflora is to protect the intestine from the overgrowth of other, harmful bacteria. By competing for the site, cell receptors and nutrients (Stewart, 1999) and by secreting toxic substances (Brook, 1999), autochthonous flora generates unfavourable environmental conditions that act as a barrier to pathogenic microorganisms. Bacteria of the genera *Lactococcus lactis*, *Bacillus subtilis*, *Pediococcus*, *Leuconostoc*, *Lactobacillus*, *Carnobacterium*, *Enterococcus*, *Streptococcus*, *Staphylococcus*, and *Escherichia* produce protein bacteriocins which are bactericidal or bacteriostatic metabolites (Gwiazdowska and Trojanowska, 2005), acting antagonistically to many pathogenic species such as *Klebsiella*, *Salmonella*, *Shigella* or *Staphylococcus*.

Potentially pathogenic bacteria are present in small amounts in the digestive tract. Severe stress conditions, a sudden change of diet, intake of (especially broad-spectrum) antibiotics or excessive bacterial invasion upsets balance of the intestinal microflora, which is conducive to the proliferation of pathogenic microorganisms and expression of their toxicity (Zabielski, 2007). The elevated numbers of *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Aeromonas*, *Clostridium* and *Escherichia* bacteria (Zabielski, 2007) may induce gastrointestinal disorders, which are a particular threat to young animals. One of the most common gastrointestinal diseases of piglets is colibacillosis, which occurs during the first month of life (Nemcova et al., 1999) and causes considerable economic losses, especially considering the ban on antibiotic growth promoters.

Competition for environmental resources is not the only form of protection against pathogenic organisms. Another mechanism is activation of the intestinal mucosa-associated immune system (GALT) and stimulation of the secretion of antibodies, mainly IgA. Produced by B lymphocytes, these antibodies are secreted onto the surface of mucosa and neutralize harmful antigens (Gołąb et al., 2004). The GALT system comprises Peyer's patches, solitary lymphoid nodules, palatine and pharyngeal tonsils, T and B lymphocytes distributed in the lamina propria of the gut, macrophages, dendritic cells, and intraepithelial lymphocytes localized in the mucosal layer. Microorganisms stimulate the immune system to resist pathogens and to block allergic reactions by inhibiting proallergic Th2 lymphocytes. Many immune defects have been observed in animals lacking symbiotic bacteria. Germ-free mice have poorly developed Peyer's patches (Gordon, 1959) and few B lymphocytes (Cukrowska et al., 2001). However, most of these changes may be easily reversed by the administration of normal gut microflora. The immune system may be stimulated and its function improved by colonization of the gut with just one species (Mazmanian et al., 2005).

Intestinal microflora has also been implicated in the initiation of colon carcinogenesis due to the secretion by some bacteria of toxic metabolites and fecal enzymes responsible for the generation of carcinogenic substances. Overgrowth of these bacteria may pose a threat to human health and life. Also normal intestinal motility shortens contact time between the oncogenic agent and the mucosa.

In summary, intestinal bacteria perform a number of functions in the animal body. They affect many processes, starting from their influence on the normal development of the alimentary tract, digestive, biochemical and fermentation functions, to vitamin synthesis, and maintenance of normal pH and coordination of the immune system. The multitude of these functions leads one to continue studies to gain a more thorough understanding of the composition and activity of intestinal microorganisms and, as a result, to use them for modifying different physiological functions.

New methods for determination of intestinal flora

Microorganism populations resident in the digestive tract show a much higher degree of differentiation than was previously thought. Previous methods for evaluation of intestinal microorganisms were based on selective enrichment of bacterial cells. The main problems associated with this technique are because some species cannot be cultured on media (it is estimated that as much as 10–40% of intestinal bacteria are not amenable to *in vitro* culture; Zoetendal et al., 1998; Suau et al., 1999) and results may be distorted by the presence of strains that easily proliferate under *in vitro* conditions. Also additional analyses that classify bacteria into appropriate groups based on physiological and biochemical observations are not entirely reliable (Leser, 2002). Another disadvantage of the classical culture techniques that drives scientists to search for new methods of determining gut biocenosis is that they are time and labour intensive (Dutta et al., 2001).

The introduction of molecular methods (temperature gradient gel electrophoresis, TGGE; denaturing gradient gel electrophoresis, DGGE; polymerase chain reaction, PCR; and fluorescence *in situ* hybridization, FISH) has revolutionized the techniques of qualitative and quantitative determination of microflora composition (Langendijk et al., 1995; Franks et al., 1998; Millar et al., 1996; Zoetendal et al., 1996) and enabled intestinal microorganisms to be determined more accurately and reliably. Temperature gradient gel electrophoresis and denaturing gradient gel electrophoresis allow polyacrylamide gel separation of the amplified 16S rDNA gene (Raszka et al., 2009). The 16S rDNA gene sequence is highly conserved and is present in large amounts in bacterial cells, as a result of which it may be a marker of a particular species (Woese, 1987; Amann et al., 1990). Both techniques depend for their action on the different denaturation sites of fragments of nucleic acids with different base sequences. 16S rDNA molecules migrate to higher temperatures or to higher concentrations of urea with formamide that induce the melting of nucleic acids (Muyzer et al., 1993; Nakatsu et al., 2000). DNA fragments with different composition of nitrogenous bases denature and stop at specific positions in the gel because of different chemical stability (Reisner et al., 1992). This method makes it possible to differentiate fragments having the same length but different sequences (Krawczyk, 2007). Being rapid, reliable and relatively inexpensive (Muyzer, 1999), these techniques are often used to study the diversity of bacteria both in the environment and in the digestive tract. The major limitations are that only dominant species are detected (MacNaughton et al., 1999), the migration rate of fragments containing different nitrogenous bases is similar (Gelsomino et al., 1999) and the fact that certain species of bacteria may have a large number of 16S rRNA genes (Maarit-Niemi et al., 2001).

Easy to perform, genotyping techniques based on PCR amplification of DNA are increasingly used to determine bacterial profiles. Examination of the biological diversity of t-RFLP is based on analysis of restriction rDNA fragment length polymorphism. For this purpose, a species-specific DNA region with a known nucleotide sequence is multiplied. The difference in relation to the classical RFLP method is that one of primers is fluorescently labelled at the 5'end (Liu et al., 1997). Next, the products obtained are digested at specific positions with restriction enzymes and then separated electrophoretically. This process enables labelled DNA fragments to be detected. The t-RFLP method is characterized by high sensitivity, repeatability and possibility of testing many samples at the same time. However, as with most PCR techniques, the limited choice of primers precludes complete analysis (Osborn et al., 2000). Nevertheless, it is used in practice and works well when analysing the bacterial composition of the environmental samples (Liu et al., 1997; Clement et al., 1998; Dunbar et al., 2000; Blackwood et al., 2003) of gastrointestinal microflora in different species of animals: pigs (Leser et al., 2000; Khan et al., 2001; Högberg et al., 2004), chickens (Gong et al., 2003), rats (Kaplan et al., 2001) and also humans (Gong et al., 2003; Nagashima et al., 2003; Ott et al., 2004).

Another variant of the PCR technique is real time PCR. Fluorescently labelled nucleotides or DNA-binding probes are added to the reaction mixture to read the matrix used in the reaction. Pigments emit light after binding double-stranded DNA. The increase in PCR products is paralleled by the increase in fluorescence, which enables strains to be detected in a given sample. Real time PCR requires small amounts of DNA, differentiates species even within one strain and being a highly sensitive method performs well as a diagnostic tool (Ke et al., 2000; Klaschik et al., 2003). During quantitative analysis of gut microflora, the results tend to be inflated. This is most probably due to the presence of dead bacteria, which would remain unobserved in traditional culture-dependent methods (Rigottier-Gois et al., 2003).

Some of the imperfections of the procedures described above can be avoided using FISH analysis. Current research confirms that it can be successfully used to diagnose human gut microflora (Harmsen et al., 1999; Hold et al., 2003; Takada et al., 2004), to identify environmental bacteria, especially those originating from the marine environment (Ramsing et al., 1996; Glöckner et al., 1996; Alfreider et al., 1996; Lemke et al., 1997; Jürgens et al., 1999) and to detect tissue pathogens (Boye et al., 1998; Trebesius et al., 1998; Jensen et al., 2000). The typing of microorganisms is possible due to the fusion of fluorescent probe, i.e. a short DNA fragment with a complementary, target fragment in the analysed sample. In this way it is possible to perform qualitative analysis. The improved multi-colour FISH technique makes it possible to detect as many as several species of bacteria in a single reaction (Takada et al., 2004). Using a confocal scanning fluorescent microscope and dedicated software it is also possible to quantify the microflora accurately. The most important advantages of FISH are that it is accurate, rapid and easy to perform. Being the only molecular method used in microflora analysis, it eliminates the need for a purification of the sample and amplification of DNA fragments, thus avoiding the errors associated with PCR (Wintzingerode et al., 1997). The combination of fluorescence *in situ* hybridization with flow cytometry to evaluate gut biocenosis considerably accelerates the analysis and also makes the method more sensitive compared to traditional technique that uses microscope analysis (Wallner et al., 1997). The main disadvantage of FISH is that the probes differently penetrate bacterial cells with different cell wall properties. Most often, the number of gram-positive bacteria is understated because of the more complex nature of the cell wall structure (Langendijk et al., 1995; Jansen et al., 1999).

Feed additives contributing to intestinal microbial balance in piglets

One of the important areas of research in pig nutrition is to develop alternative natural feed additives to antibiotics that ensure optimum composition of the gastrointestinal microflora in suckling and weaned piglets while preventing diarrhoea and its negative consequences for the health and productivity of piglets. To exert a specific effect on the metabolic activity of the intestine, to create conditions for the development of beneficial bacterial flora, or to inhibit the growth of pathogenic bacteria, different feed additives (including probiotics, prebiotics, acidifiers, herbs and enzymes) have been long used in dietary rations with various degrees of success.

Probiotics

The term probiotic is derived from the Greek "pro bios" and means "for life". It was first used by Lilly and Stillwell (1965) to describe substances produced by microorganisms which stimulate the body in humans and animals. Over the years, with advances in probiotics research, their definition was repeatedly modified and expanded to include new elements (Parker, 1974; Fuller, 1989; Schrezenmeir and de Vrese, 2001; Holzapfel and Schillinger, 2002).

The latest definition defines probiotics as preparations containing live and/or dead microorganisms and their metabolites, which stabilize microorganism balance and enzymatic activity in the digestive tract, thus having a beneficial effect on the growth and development of animals (Grela, 2004).

A probiotic preparation may contain a specially selected, natural strain of intestinal bacteria or a mixture of microorganisms and their metabolites. Probiotic manufacturers make use of lactic acid bacteria, in particular the strains *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus lactis* as well as *Bifidobacterium bifidum*, *Pediococcus acidilacti*, *Enterococcus faecium*, *Bacillus subtilis* and *Bacillus toyoi* (Fuller, 1999; Alexopoulos et al., 2004). Some species of yeast (*Saccharomyces cerevisiae*, *Saccharomyces boulardi*) or mycelium of *Aspergillus oryzae* and *Aspergillus niger* are also used. In addition, probiotic preparations may be enriched with elements (Fe, Cu, Co), vitamins (A, E, D_3, B_{12}) and immunoglobulins. Probiotics for pigs are made from microorganisms that are isolated from the lumen of the pig digestive tract.

The main function of bacteria found in probiotic preparations is to maintain the quantitative and qualitative microbial balance in the digestive tract by reducing the pH of intestinal contents and thus preventing the growth of pathogenic microflora.

Probiotics have the following beneficial effects on the animal body:

– reducing the pH of intestinal contents through the production of organic acids (lactic, acetic, propionic and butyric) by probiotic bacteria,

– normalizing beneficial gut microflora and preventing the development of pathogenic microflora,

– improving digestion and nutrient absorption,

– lowering the level of toxic metabolic products in the digestive tract and in blood, which reduces the incidence of diarrhoea,

– producing natural antibiotic substances known as bacteriocins, which have bactericidal or bacteriostatic effects on pathogenic microorganisms, and producing enzymes and B and K vitamins,

– increasing resistance to bacterial infections and improving health,

– increasing the activity of some intestinal enzymes (lactase, saccharase, maltase) and thus increasing feed digestibility,

– stimulating the body's general immunity and local immunity within gastrointestinal mucosa,

– reducing the level of triglycerides and cholesterol in blood and tissues (Tahara and Kanatani, 1996; Nowachowicz et al., 1999; Hadami et al., 2002; Bocourt et al., 2004; Grela, 2004).

Probiotics help to increase rearing performance of piglets by improving their health (limiting the incidence of diarrhoea and oedema disease), increasing resistance to stress, reducing mortality, increasing body weight gains and improving feed conversion per kg gain (Nowachowicz et al., 1999; Turner et al., 2002; Hadami et al., 2002; Herzig et al., 2003; Grela, 2004; Mikołajczak et al., 2004).

When used as feed additives, probiotic preparations should be resistant to influences of temperature, pressure, water and heavy metals during both treatment and storage. Because they must remain active for at least 4 months, treatments that extend bacterial activity (such as encapsulation) are often used. Probiotics are registered for periods of 1 year and after this time they need to be prolonged. Because the mode of action of some bacterial strains may change, probiotics are registered for periods of 1 year depending on the period of use and environmental conditions. Probiotic preparations are available as powder, suspension, gel, pellets or paste, their amount per dose and mode of administration depending, among others, on animal's age. In piglets probiotics are administered orally, once or twice after birth, to prevent gut colonization by pathogenic bacteria, and after the transition to solid feed, as a regular dietary supplement (Grela, 2004).

Over the recent years, probiotics have been shown to be useful in piglet nutrition although with variable results, especially with regard to production parameters such as growth rate and feed conversion ratio (Rekiel and Kulisiewicz, 1996; Kyriakis et al., 1999; Simon et al., 2001; Zimmermann et al., 2001; Herzig et al., 2003). The most frequent outcome is a reduction in the incidence of diarrhoea (Simon et al., 2001; Hadami et al., 2002), a shorter duration of diarrhoea, and limitation of piglet mortality in the pre- and post-weaning period (Rekiel and Kulisiewicz, 1996; Mikołajczak et al., 2004; Grela, 2004). This prophylactic action of probiotics largely depends on the type and amount of lactic acid bacteria strains, their dose, mode and period of administration to piglets, health status, and the sanitary conditions of pig farms (Close, 2000). Another observed effect is a reduced risk of oedema disease in weaned piglets (Zeyner and Boldt, 2006). As regards production traits, sometimes higher weight gains and better feed conversion were found in piglets fed diets supplemented with probiotics (Rekiel and Kulisiewicz, 1996; Nowachowicz et al., 1999; Simon et al., 2001; Turner et al., 2002; Zimmermann et al., 2001; Herzig et al., 2003; Živković et al., 2003; Bocourt et al., 2004; Estienne et al., 2005). Analysis of the results of many experiments, in which various probiotic preparations were administered to piglets and weaners, showed changes in daily weight gains (from about –8% to +24%), daily feed intake (from –9% to +26%) and feed conversion ratio (from –7% to +3%) (Rekiel and Kulisiewicz, 1996; Nowachowicz et al., 1999; Simon et al., 2001; Turner et al., 2002; Herzig et al., 2003; Bocourt et al., 2004; Estienne et al., 2005).

Probiotics can be used not only on a preventive basis but also in sick animals and in those treated with antibiotics and chemotherapeutic drugs, because they speed convalescence by bringing the natural gut microflora back into balance. The existing body of research suggests that the mode of action of probiotics on the animal body, especially its digestive tract, is complex, diversified, and not completely understood. The effects of probiotic preparations are similar to the effects of feed antibiotics because both reduce the number of pathogenic bacteria, but probiotics have a different mode of action compared to antibiotics (Zimmermann, et al., 2001). The effects of probiotics can be made more efficient when they are administered together with other feed additives such as prebiotics or acidifiers (Grela, 2004).

To gain a more complete understanding of the way probiotics influence pig bodies, it is necessary to carry out further studies to find microorganisms with better probiotic characteristics. This may be of great importance to the development of new principles for prevention of intestinal diseases in piglet rearing without the use of antibiotic growth promoters.

Prebiotics

Prebiotics are feed additives containing substances classified as non-digestible oligosaccharides (NDOs). They selectively stimulate the growth of natural beneficial microflora in the digestive tract of animals and limit the colonization of intestinal wall mucosa by pathogenic bacteria (Pettigrew, 2000). The most common prebiotics used in animal nutrition are mannan- (MOS), fructo- (FOS) and transgalacto-oligosaccharides (TOS) (Grela, 2006). Their main characteristic is that they have beneficial effects on intestinal microflora. At present, mannan-oligosaccharides appear to be the most promising alternative to antibiotic growth promoters, especially in piglets.

The beneficial effects of oligosaccharides on piglet health are because they compete with pathogenic microorganisms; promote the growth of beneficial gut microflora; inhibit the intestinal colonization by pathogenic bacteria; adsorb microorganisms and their toxins on their surface; stimulate the immune system; and influence body metabolism. Lactic acid bacteria and *Bifidobacteria*, which are desired in the digestive tract, feed on sugars from prebiotics. On the other hand, pathogenic bacteria (*E. coli*, *Salmonella*) and many gram-negative bacteria do not have such abilities and are eliminated from the gut microflora by beneficial bacterial flora that multiplies more intensively.

The mannan-oligosaccharide (MOS), obtained from the walls of *Saccharomyces cerevisiae* yeast and added to piglet diets, often (but not always) has a beneficial effect on growth rate, feed intake and feed conversion while limiting the incidence of diarrhoea (Pettigrew, 2000; Zimmermann et al., 2001; Turner et al., 2002; Rozeboom et al., 2005). Meanwhile, LeMieux et al. (2003) and Davis et al. (2002) showed that mannan-oligosaccharide (Bio-Mos) added to the feed improves the rate of growth but the results are dependent on dietary levels of copper (Cu) and zinc (Zn). The effects of prebiotics on the health and production traits of piglets show considerable variation. The efficiency of prebiotics in piglet rearing may be improved by their combined administration with probiotics as synbiotics (Piva et al., 2005). Also, mannan-oligosaccharide and herbs (garlic) may produce better results when given to piglets together rather than individually (Lipiński et al., 2010).

Acidifying additives

Acidifiers are organic and inorganic acids and their salts. They include formic, propionic, lactic, acetic, butyric, malic, citric, fumaric, orthophosphoric and sorbic acids and their salts, i.e. calcium formate, potassium diformate, calcium propionate, and calcium citrate. They are available in the form of preparations containing one acid or a mixture of several acids and their salts in dry or liquid form.

Acidifiers have a strong bactericidal action. They penetrate the bacterial cell, and inhibit DNA synthesis and proliferative capacity. Intestinal flora is sensitive to pH and its decrease to 5.5 by acidifiers causes changes to bacterial composition, e.g. inhibition of the growth of pathogenic *Salmonella* or *E. coli*.

Feeds are acidified primarily to reduce the pH of intestinal contents and of the initial portion of digestive tract, thus providing unfavourable conditions for the growth of pathogenic bacterial flora, which proliferates most readily in the environment with pH 6.0 to 7.5, and the least readily when pH ranges between 4.0 and 5.5. The decrease in pH is particularly important in piglets whose stomachs fail to produce sufficient amounts of hydrochloric acid to maintain the optimum pH of about 3.5 during the first weeks of life (Ravindran and Kornegay, 1993). Another factor that has a detrimental effect on the pH of the digestive tract of piglets is their transition to solid feed, characterized by high buffering capacity. This may increase the pH of intestinal contents and create conditions favouring the growth of pathogenic microflora, thus causing inflammation of the stomach and intestines, which adversely affects nutrient absorption and induces diarrhoea. Organic acids improve digestion of proteins and amino acids in the small intestine and absorption of dietary minerals and nutrients (Mróz, 2001). They may also find application as additives that improve the palatability of feeds containing ingredients such as rapeseed meal or legume seeds. The addition of acidifiers to feeds improves rearing performance (health and productivity) in piglets, although this effect shows considerable variation (Rekiel and Kulisiewicz, 1996; Batorska and Mieńkowska-Stępniewska, 2000). Reduced incidence and duration of diarrhoea and lower mortality were reported in piglets in addition to increased feed intake, increased daily weight gains of the piglets from +1.5% to +7.9%, and improved feed conversion with feed intake per kg weight gain being lower by –0.5% to –5.2% (Simon et al., 2001). Variation in the results of using acidifiers in piglet nutrition depends on the health status, body condition and age of animals, the amount and type of acidifiers added to the ration, and the type and composition of the feed. The growth-stimulating effect of acidifiers is most evident within the first weeks after weaning, when the digestive tract of piglets is still undeveloped and largely susceptible to infections.

Herbs

Herbs have multiple effects on animal bodies depending on the type and concentration of biologically active substances such as tannins, essential oils, organic acids, terpenes, flavonoids, alkaloids, glycosides, saponins and phytosterols. They have antibacterial, antiviral, anti-inflammatory, antioxidant, appetite-stimulating, digestant, antidiarrhoeal, and hormone- and immune-stimulating effects (Grela, 2004; Grela et al., 1998). Herbs can concurrently affect the function of specific organs such as liver, pancreas and stomach, increasing their endocrine function. They also influence the taste and aroma of feed and its intake by animals (Close, 2000). Herbs are also a source of nutrients such as carbohydrates, protein, fats, minerals and vitamins. Above all, herbs are expected to improve factors potentially affecting the health and productivity of animals as well as meat quality in pigs (Oetting et al., 2006; Janz et al., 2007). The efficiency of herbal preparations depends on numerous factors, including botanic composition and proportion in the diet, which ranges widely from

0.2 to 4% of the feed. They are used singly or as preparations containing a mixture of herbs, the latter being more effective. They can also be added to feeds in the form of herb extracts. This group includes oil and water extracts from plants containing biologically active substances, many of which act as plant growth promoters. They exhibit stable and beneficial effects on productive and health parameters of animals provided they are standardized for the level and activity of the ingredients. The effect of herbal preparations on the health and productivity of pigs most often reflects the action of many biologically active substances that show diverse pharmacological activities.

Pigs are eager to eat feeds containing garlic, juniper fruits, comfrey root, dandelion, nettle, yarrow, St John's wort, knotgrass, peppermint leaves and couch grass rhizome. They are not very willing to consume calamus rhizome, creeping thyme and wormwood. An important property of herbs is that they improve the palatability of feeds. Herbs stimulate gustatory and olfactory receptors in pigs, making them more willing to eat feed. Stimulation of the appetite plays a significant role, especially during the period when piglets are adjusted to solid feed and weaned from their mothers (Rekiel, 1998).

Plants that hold promise as substitutes for antibiotic growth promoters include garlic and oregano, the active substances of which show strong bacteriostatic and bactericidal activities. When supplemented to feeds, these herbs have positive effects on the health and productivity of piglets. They considerably reduce the amount of pathogenic bacteria in the digestive tract and limit the incidence of diarrhoea and mortality while improving daily weight gains and feed conversion (Jost, 1996; Radford et al., 2002). Because of its value as spice and its taste properties, garlic also stimulates digestion and metabolism, and its characteristic odour attracts the animals' interest in feed, improves their appetite and feed intake, and increases the secretion of saliva, stomach acids and bile.

Some but not all studies demonstrated beneficial effects of herbs in the form of mixtures, extracts and infusions on rearing performance of piglets (Jost, 1996; Rekiel, 1998; Greiner et al., 2001; Radford et al., 2002; Grela et al., 2003; Grela, 2004; Oetting et al., 2006; Rząsa et al., 2007). The beneficial effects of using herbal supplements in feeds include the attainment of higher body weight gains in piglets (by +0.2% to +7.6%), the increased intake of feed as a result of its improved flavour and taste, better feed conversion (lower feed intake per kg weight gain) by –0.1% to –4.9%, reduced incidence and duration of diarrhoea, and lower mortality (Jost, 1996; Kyriakis et al., 1998; Rekiel, 1998; Rząsa et al., 2007).

It was also found that infusions of herb mixtures, used on a preventive basis in piglets, reduced the incidence of diarrhoea and mortality, relieved gastrointestinal problems, stimulated appetite, feed intake and feed conversion, increased the growth rate of piglets and weaners, and made it possible to rear unthrifty piglets (Rekiel, 1998). Meanwhile, Grela and Semeniuk (2006) found a positive effect of the combined supplementation of garlic and BioMos mannan-oligosaccharide on limiting the incidence of diarrhoea and mortality and on improving body weight gains.

In summing up the results of nutritional studies on the feed additives discussed above, it is concluded that their separate use in piglet rearing does not always produce desirable results, which depend on many factors. For this reason, more and more researchers believe that using a single feed additive offers no alternative to antibiotic growth promoters. To achieve consistent and repeatable results similar to those offered by antibiotics in pig nutrition, it is necessary to administer two or three natural feed additives together according to the age and health status of animals, sanitary conditions in the pig buildings, and the housing system (Grela, 2006). One example of such a complex supplement are synbiotics, i.e. combinations of probiotics and prebiotics.

Macro- and micronutrients

In recent years, the gastrointestinal microflora of piglets has been identified as one of the major factors affecting the health and performance of growing pigs (Gaskins, 2003). In studies with rats and ruminants, some essential minerals such as Ca and P proved to be important modulators of microbial fermentation (Durand and Komisarczuk, 1988; Bovee-Oudenhoven et al., 1997). *In vitro* studies using rumen bacteria showed that phosphorus is necessary for carbohydrate fermentation (Komisarczuk et al., 1987). However, little is known about what amount of phosphorus in the large intestine of pigs is needed for carbohydrate fermentation. The rations used in the feeding of pigs contain various amounts of carbohydrates that differ in the origin, composition and level, thus determining their availability to bacteria and the course of fermentation. Out of environmental concern, the release of phosphorus into the environment has become limited by reducing the phoshorus content of diets and by supplementing or adding preparations of microbiological phytase that releases phosphorus from phytates found in cereals (Knowlton et al., 2004; Veum et al., 2007). However, limiting the dietary levels of phosphorus may affect carbohydrate fermentation. Therefore, it seems important to use appropriate levels of phosphorus and phytase supplements in the diets so as to fully meet the phosphorus requirement of animals and thus ensure optimum carbohydrate fermentation in the digestive tract of pigs (Metzler-Zebeli et al., 2010). It must be remembered that the phosphorus and calcium content in the body is strictly related, which should be taken into account when formulating dietary rations.

Chemical elements such as zinc and copper are usually added to piglet feed to influence beneficial bacterial flora in the digestive tract and to improve health. Pharmacological doses of ZnO were given to piglets to improve rearing performance (Case and Carlson, 2002) and to prevent the incidence of diarrhoea after weaning (Poulsen, 1998; Rekiel et al., 2009), which is indicative of the modulating effect of zinc on gut microflora. Large doses of zinc increase the biological diversity of colibacteria and improve the stability of microorganisms in the digestive tract of piglets (Katouli et al., 1999). High doses of ZnO (2500 ppm) supplemented to piglets reduced the total number of anaerobic bacteria and lactic acid bacteria in the stomach and small intestine while increasing the growth of *E. coli* and enterococci in the entire gastrointestinal tract (Höjberg et al., 2005). Similar pharmacological activity to ZnO is shown by copper sulphate ($CuSO_4$), which improves feed conversion and increases weight gains in animals (Dove, 1995; Hill et al., 2000). Improvements in daily gains of piglets were due to the antibacterial action of copper (Fuller et al., 1960). Other studies showed that copper limits the population of lactic acid bacteria and colibacteria throughout the digestive tract (Höjberg et al., 2005). It is likely, however, that in addition to possible benefits resulting from the antibacterial action of copper sulphate and zinc oxide, good productive results may be also caused by overall body condition (Pérez et al., 2011), as exemplified by the study of Zhou et al. (1994), who reported higher weight gains in pigs receiving intravenous doses of copper.

Other additives

Feed raw materials of plant origin, including grains of wheat, barley, rye, triticale and oats contain considerable proportions of non-starch polysaccharides (NSP) such as pentosans (xylans and arabans), ß-glucan and smaller amounts of other hexosans (mannans and galactans). These carbohydrates are characterized by the presence of chemical bonds that are not digested by monogastric animals and birds because their digestive juices lack proper enzymes. In order to reduce the negative effects of NSP, feed mixtures containing high amounts of ground cereals are supplemented with proper feed enzymes: xylanase, ß-glucanase, cellulase and pectinase. The beneficial activity of these enzymes consists in lowering digesta viscosity, reducing the amount of substrates available to intestinal microorganisms, improving nutrient conversion, and increasing metabolizable energy of the feed. These effects were reported in a study with broiler chickens (Wu et al., 2004; Cowieson et al., 2010), which suggests that the use of enzymes in pig diets will produce similar results and cause changes to the microbial profile.

Research on the use of some natural and synthetic aluminosilicates (zeolite, vermiculite, perlite, saponite and kaolin) and aluminosilicates modified by copper ions in animal nutrition has been underway for many years. Aluminosilicates have beneficial physicochemical properties from the viewpoint of digestive physiology of the animal gastrointestinal tract: they form bases with water, reduce the surface tension of intestinal contents and emulsify fats, thus supporting liver function. This is particularly important when feeding fat-enriched diets to pigs. Another advantage of aluminosilicates is that they are not digested in the digestive tract, their therapeutic action involves the mechanical binding of bacterial toxins and mycotoxins (Knezevich and Tadic, 1994; Magnoli et al., 2008; Trckova et al., 2009), they reduce the emission of toxic gases from litter (Rudzik, 1998), limit bioaccumulation of toxic metals (Yu et al., 2008), and enrich the diet with several macro- and micronutrients (Yablonska, 2003).

The results of nutritional studies with pigs (Korniewicz et al., 2006; Trckova et al., 2009) and poultry (Bailey et al., 1998) confirm the absorbing action of aluminosilicates in relation to mycotoxins and bacterial enterotoxins by inhibiting the growth of pathogenic bacteria and limiting the incidence of diarrhoea. Furthermore, few reports point to their positive effects on proper intestinal function, regeneration of intestinal mucosa, improved nutrient digestibility, and increased body immunity (Xia et al., 2005; Alexopoulos et al., 2007; Trckova et al., 2009).

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DOROTA BEDERSKA-ŁOJEWSKA, MAREK PIESZKA

Modulowanie mikroflory przewodu pokarmowego świni na drodze żywieniowej poprzez dodatki paszowe

STRESZCZENIE

Do korzyści płynących ze stosowania w żywieniu prosiąt takich dodatków paszowych, jak pre- i probiotyki, zakwaszacze, zioła, enzymy, makro- i mikro-pierwiastki oraz dodatki zootechniczne zaliczyć można osiąganie wyższych przyrostów dziennych, lepsze wykorzystanie oraz zwiększone pobranie paszy przez zwierzęta na skutek polepszenia jej smakowitości, a także poprawę statusu zdrowotnego i dobrostanu, co przejawia się m.in. zmniejszeniem częstości występowania biegunek i ograniczeniem liczby upadków prosiąt.

Konieczne zatem jest kontynuowanie badań nad opracowaniem coraz doskonalszych dodatków paszowych, zapewniających utrzymanie optymalnego składu mikroflory przewodu pokarmowego prosiąt oraz skutecznie zapobiegających biegunkom i ich negatywnym konsekwencjom.

Wykorzystywanie osiągnięć biologii molekularnej i poszukiwanie nowych metod wykazujących większą dokładność niż techniki kulturozależne jest niezwykle istotne w próbach pełniejszego opracowania składu mikroflory przewodu pokarmowego oraz wpływu, jaki wywiera na prawidłowe funkcjonowanie organizmu. Możliwość obserwacji zmian w obrębie populacji mikroorganizmów jelitowych, ściśle skorelowanych z otrzymywanym pokarmem, stwarza nadzieję na poprawę stanu zdrowia zwierząt hodowlanych oraz polepszenie parametrów produkcyjnych poprzez modyfikację składu bakterii jelitowych.