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THE EFFECTS AND MECHANISMS OF ACIDS ON THE HEALTH OF PIGLETS AND WEANERS – A REVIEW*

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Abstract

The rearing of piglets is the most difficult period in the pigs' production because of their poorly developed digestive system and the low activity of digestive enzymes. Problems in nutrition and stress cause some disorders in the functioning of the digestive system leading to diarrhea and the mortality of piglets. Starting in 2006 in the EU, a total ban on antibiotics in their use as growth promoters was introduced. Since then, new and safe feed additives have been sought in order to replace antibiotics. Organic and inorganic acids as well as their salts were recognized as effective and safe additives. Due to their properties, they can improve feed palatability and digestibility, reduce the buffer capacity of feed, impact the development and functioning of the pig's digestive system and improve the health and growth parameters. However, the effectiveness of acids is related to their qualitative and quantitative share in the feed additive. In this review, some strategies for using organic acids, their mixtures and also some new multi-component products will be discussed.

Key words: acidifiers, feed additives, organic acids, piglets

From an economic point of view, the production of suckling pigs should be as cheap as possible, while also ensuring the conditions that provide positive economic effects, that is, enabling profit to be made. The production of pork in modern industrial facilities mostly depends on the reproduction results because the number of suckling pigs per sow has a direct influence on the number of fattened pigs as well as on the quantity of meat (Mirilović et al., 2016). Therefore, the intensification of pig production requires reducing the rearing period up to 3–4 weeks, according to the European Union Directives (2001/93/EC; Journal of Laws of 2010 No. 56, item 344). The rearing of piglets is the costliest period in the pigs' production because it demands the best environmental conditions and high value feed components (Boas

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et al., 2016; Li, 2017). The biggest problem with piglets, however, is their poorly developed digestive system and the low activity of enzymes digesting raw materials of plant origin (Kiarie et al., 2016; Gresse et al., 2017). Therefore, the change in the nutrition system and stress associated with weaning piglets from sows commonly causes disorders in the functioning of the digestive system, an inadequate utilization of nutrients from the feed, the appearance of diarrhea, and an increase in the mortality of piglets, raising serious economic and public health concerns (Gresse et al., 2017). Rearing of piglets from the sow and especially changing the way of feeding from liquid mother's milk to solid feed only cause severe stress in piglets. In the first days after weaning, a low feed intake is observed. It has been proven that approximately 50% of piglets take their first feed within 24 hours of weaning and in 10% of piglets, the starvation period extends beyond 48 hours (Kil et al., 2011). During this time, the animals mainly use their reserves. Reduced feed intake is often correlated with intestinal villi atrophy, which, in turn, reduces the surface absorption of nutrients in the intestines (Abdelqader and Al-Fataftah, 2016). Conversion of liquid feed (milk) to solid feed (concentrate) and a slight secretion of hydrochloric acid increase the pH of the stomach and intestines up to 5.0. Acidic pH (2.0–3.5) is necessary to convert pepsinogen into pepsin. Above pH 6.0, this process is practically completely inhibited (Kil et al., 2011). In this environment, only half of the nutrients taken from the feed are digested. This condition can even persist for several days, which significantly lowers the weight gain of piglets (Suiryanrayna and Ramana, 2015). This requires the use of feed components characterized by a high concentration of nutrients and their high digestibility and availability. Undigested feed remains in the intestines where it is fermented by microorganisms, including pathogens such as *E. coli*, *Salmonella*, *Clostridium*, and *Lawsonia* (Chattopadhyay, 2014; Stensland et al., 2015; Sbardella et al., 2015). They are bacteria that reproduce the best in an environment that is pH 5.8–6.5 and they are the most often responsible for diarrhea in young animals (Trackova et al., 2014). Diarrhea during the post-weaning period is associated mainly with the presence of β-hemolytic strains of *E. coli* in the intestine. For many years, antibiotics have been used in feeds to reduce the health problems during the weaning period (Gresse et al., 2017; Li, 2017). Their activity was focused on both the reduction of microorganisms in the gastrointestinal tract and the stimulation of the growth of organisms (Pluske et al., 2018). The use of antibiotic growth promoters resulted in an increase in feed consumption by up to 7%, which also generated higher daily body weight gains (Kim et al., 2005). The negative side effect of using antibiotics in feed was the destruction of the beneficial bacteria due to the limited selectivity and, more importantly, the formation of strains of bacteria insensitive to one or even several antibiotics (cross-resistance) (Thacker, 2013; Li, 2017). The consumers' reluctance to consume antibiotic-containing animal products was the main reason for the withdrawal of feed antibiotics from animal nutrition in the EU (Regulation 1831/2003/EC). This ban led to the necessity to find alternative and safe preparations that would reduce the problems of the post-weaning period. Such preparations include, among others, organic and inorganic acids and their salts, probiotics, prebiotics, eubiotics, synbiotics, or phytobiotics (herbs, extracts, essential oils) (Suiryanrayna and Ramana, 2015; Gresse et al., 2017; Nowak et al., 2017;

Kiarie et al., 2018; Li et al., 2019; Nowak et al., 2019; Zhou et al., 2019). From these groups, short- and medium-chain organic acids (and also their salts and inorganic acids) are the most popular because of their bacteriostatic and bactericidal effects and very low cost.

The chemical characteristics and mode of action of acids and their salts

The effective and safe alternatives to antibiotic growth stimulants are organic acids and their salts as well as some inorganic acids. These compounds are the main active ingredients of acidifiers, which have been widely used in feeding pigs, cattle, rabbits, poultry, and fish for many years (Lević et al., 2007; Papatsiros et al., 2012; Chwen et al., 2013; Li et al., 2019).

The acids used as feed additives are naturally occurring compounds in living organisms as cellular metabolites and, therefore, they are well tolerated and have low toxicity (Kil et al., 2011; Suiryanrayna and Ramana, 2015). A particularly important role in animal nutrition is played by short-chain acids that contain up to 6 carbon atoms, such as formic, acetic, propionic, butyric, lactic, malic, tartaric, and citric as well as fumaric and sorbic acid. Benzoic acid, which is an aromatic carboxylic acid with the simplest chemical structure, is also of practical use in animal diets (Morel et al., 2019). The importance of using medium-chain acids with 6 to 12 carbon atoms in their chain, such as caprylic and capric acid, is also growing (Hanczakowska et al., 2016).

Acetic acid, butyric acid, and propionic acids belong to the group of short-chain fatty acids that are also produced in the mammalian large intestine by bacteria of the genus *Clostridium*, *Eubacterium,* or *Fusobacterium*, by the fermentation of undigested carbohydrates. Butyric acid is the main source of energy for colonocytes, where it is used up to 90%. The presence of this acid has a positive effect on the development of the intestinal epithelium. Acetic acid is transported from the colon in the blood to the liver, where it is used up to 70%. It is a source of energy for hepatocytes, but it is also necessary for the formation of cholesterol and long-chain fatty acids. The remaining 30% of acetic acid is metabolized in muscle, heart, and kidney cells. Propionic acid is the main precursor of glucogenesis in the liver and is necessary for the formation of energy in the body (Hijova and Chmelarova, 2007; Besten et al., 2013; Canfora et al., 2015). The animals absorb fatty acids in the form of small molecules – triacylglycerols – medium-chain triglyceride (MCT). During the hydrolysis process, the participation of all the enzymes involved in fat metabolism, e.g. pancreatic lipase, is not necessary. After hydrolysis, medium-chain fatty acids are formed, which, together with the blood, reach the liver and undergo β-oxidation in the liver cells. The energy obtained during this process is used in various metabolic pathways and, therefore, triacylglycerols are a source of easily absorbed energy (Chwen et al., 2013; Zentek et al., 2013; Zhou et al., 2019).

The organic acids are characterized by acidifying properties and the measure of their strength is the ability of the acid to dissociate, i.e. dissociate hydrogen ions H^+ in solution. The index determining the strength of the acid is the negative decimal logarithm of the acid dissociation constant (pKa), which is reached for individual acids: formic acid – 3.75; acetic acid – 4.76; propionic acid – 4.88; butyric acid – 4.82; lactic acid – 3.83; fumaric acid – 3.02: citric – 3.13; benzoic acid – 4.19. A lower pKa

value means stronger acidifying properties of the acid and more hydrogen ions in the solution (Tachedjian et al., 2017).

In a cell in which the pH is about 7, the acid is dissociated, releasing hydrogen ions. It blocks the functions of enzymes and the system of transporting nutrients in the cells of the microorganism. The dissociating acid reduces the pH of the cytoplasm of the microorganism, as a result of which it forces it to be neutralized by the intracellular buffer system with the active participation of protons from the inside of the cell. Their removal requires a large amount of energy from the microorganism, the shortage of which stops the main life processes, including the course of the DNA replication that is necessary for the multiplication of bacteria (Kim et al., 2005). In case the microbial cell is not able to maintain its optimal pH, the organs and substances, such as enzymes, DNA, or cytoplasmic membranes, which are necessary to sustain the body's life processes, are destroyed and the cell dies (Hellweg et al., 2006). On the other hand, non-fully dissociated anionic acids pass through the membrane of bacterial cells into their interior, the pH of which is at level 7. In addition, carboxylic anions may have an inhibitory effect on the processes occurring in the cytoplasm or in the nucleus of pathogenic bacteria, e.g. the anion of formic acid inhibits the activity of enzymes and propionic anion affects membrane transport and is an inhibitor of the synthesis of certain amino acids (Gresse et al., 2017). The action of anions can cause changes in the synthesis of DNA and thus neutralize the multiplication of microorganisms. The acid concentration at which the growth and development of bacteria is limited is called "minimal inhibitory concentration" (MIC). MIC depends on the environmental pH and is a characteristic value of each acid. For example, MIC at pH 4.5 for *E. coli* is 0.15% for formic, 0.20% for propionic, and 0.40% for lactic acid, which is important for their effectiveness in the diet (Strauss and Hayler, 2001).

Moreover, the released hydrogen ions reduce the pH of the digestive system, which limits the effects of alkalosis in the stomach and creates optimal conditions for the reproduction of positive microflora. A very low pH is desirable for the development of beneficial intestinal microflora, mainly lactic acid bacteria, which develops in an acidic environment. The acidification of the digestive content to a pH of about 4.0 results in a greater secretion of digestive enzymes and also allows the transformation of the pro-enzyme of pepsinogen into pepsin, an enzyme digesting the protein.

Acidifiers are used in different ways. Thanks to their activity against microorganisms, they could inhibit the secondary contamination of food and water, preserve food and feed against pathogenic microflora (bacteria, fungi, and mold) as well as control the development of microorganisms in organisms by the inhibition of pathogenic bacteria and the reduction of diarrhea. Acids could also reduce the buffer capacity of feed, improve the smell and palatability of feed and feed intake, improve feed digestibility, affect the development of the pig's digestive system and, as a result, also improve the health of pigs and the growth parameters.

Activity against microorganisms

Feedstuffs and especially complete feed mixtures and water are a very good medium for the development of microorganisms (bacteria, fungi, mold). Microorgan-

isms multiply particularly quickly when the feed is stored in improper conditions (high humidity and temperature). Microorganisms lead to a reduction in the nutritional value of feed, as it breaks down the nutrients (Xu et al., 2016). In addition, some of them, as a result of metabolism, produce substances of a toxic nature, e.g. toxins and mycotoxins (Koyuncu et al., 2013). They are dangerous not only for animals but also for humans because, after absorption in the gastrointestinal tract, they can pass into animal products (Lückstädt, 2009). Water quality management is also very important for guaranteeing good animal performance. Contaminated drinking water is the most prominent risk factor for the spread of bacterial infections, such as *Campylobacter*, *E. coli*, etc. Therefore, acidified drinking water could play a crucial role in terms of biosecurity in preventing the spread of pathogenic organisms (Hossain and Nargis, 2016).

The antibacterial action of organic acids causes the acid to penetrate the cell membranes inside the microorganism and, in the undissociated form, enters the cytoplasm. The effectiveness of organic acids depends on the degree of dissociation and this depends on the length of the acid chain and level of their saturation (the number of multiple bonds). The acids may be used singly or in mixtures, on a solid support, which is an organic (e.g. bran) or mineral feed material (e.g. bentonite and silica). They can also be dosed in liquid form, where the carrier is usually glycerin. The total acid concentration in the feed should not exceed the recommended dose, as excessive amounts may cause the opposite effect, i.e. digestive disorders and deregulation of the acid-alkaline management of the body (Lückstädt, 2009).

In the feed, organic acids can effectively reduce the microorganism content. Mroczek et al. (2005) studied the microbiological stability of a PP-grower type mixture during 3 months of storage under typical farm conditions. The experimental samples differed with regard to the type and amount of the acidifying additive (fumaric acid, sodium formate, Schaumacid, Agricid F, Agro-Cid, and Orego-Cid). The acidifying additives that were used improved the microbial stability of the feeds, especially with regard to the development of *Clostridium* bacteria. No *E. coli* were found in these samples. The research showed that when organic acids are added in the correct amount, they can significantly change the pH of the feed, while their salts have practically no effect on its acidity (Bonos et al., 2010). The dose of acidifying preparation may also vary depending on the moisture and temperature of the complete mixtures production as well as the susceptibility of bacteria. The anion-proton effect on the bacterial cell has an inhibitory effect on the growth of both gastric and intestinal bacteria. At a pH below 5, the growth of such microorganisms as *Clostridium perfringens*, *E. coli,* and *Salmonella* is limited (Stensland et al., 2015). Low pH provides effective protection against pathogenic microorganisms in the large intestine and ileum. Gram-negative bacteria are particularly sensitive to acids with less than eight carbon atoms per molecule, while Gram-positive bacteria are sensitive to longer-chain acids. Microorganisms such as *Lactobacillus* competing in the gastrointestinal tract with pathogenic bacteria and not allowing their excessive development remain undamaged and their growth may even be further stimulated (Hellweg et al., 2006). The addition of organic acid as an acidifier for animal feed affects the creation of an effective barrier to pathogenic microorganisms and entails the creation of

optimal conditions for the digestion of feed and absorption of nutrients (Mroz, 2005). Butyric, caproic, lactic, and malic acids have prebiotic properties, i.e. conducive to the development of the symbiotic, saprophytic microflora of the gastrointestinal tract (Gresse et al., 2017). Kasprowicz-Potocka et al. (2009) studied the effectiveness of three acidifying preparations (sodium formate, benzoic acid, fumaric acid) in an amount of 10 g/kg of pigs diet and found that all of the applied acidifiers had an impact on intestinal microbiota composition and the number and activity of bacteria in the given segments of the gastrointestinal tract. Supplementation of formic acid or sodium formate allows for protection against *Salmonella* that are resistant to acidic conditions and high temperature in the environment, which contribute to an improvement in feed intake and the prevention of piglets' diarrhea (Walles et al., 2010; Koyuncu et al., 2013). Ahmed et al. (2014) found that the supplementation of piglet diets with 0.5% citric acid reduces the number of *Salmonella typhimurium* bacteria in the feces. This microorganism causes the inflammation of the small and large intestine. Rasschaert et al. (2016) offered 4 different combinations of preparation against *Salmonella* to pigs. It was butyric acid; a combination of short-chain organic acids (mixture of free acids and salts); natural extracts blend of two commercial products consisting of medium-chain fatty acids, lactic acid, and oregano. Only the blend based on the medium-chain fatty acids was able to significantly reduce *Salmonella* prevalence both on the farm and at the slaughterhouse. With this combined supplement, the *Salmonella* reduction in the feces at slaughter age, in the fecal content at slaughter and the lymph nodes was 50, 36, and 67%, respectively, compared with the control animals. In addition, Leonard et al. (2017) found that dietary supplementation with organic acids reduced the shedding and transmission of *Salmonella*. In the present study, three commercially-available organic acid-based products (sodium butyrate, benzoic acid, and formic-citric acid) were used in growing pigs' (about 15 kg) diets. A significant reduction in fecal *Salmonella* concentration was observed after supplementation with sodium butyrate and a formic--citric acid blend. The average daily weight gain was significantly increased in all of the groups fed the supplemented feed when compared to the positive control group.

The supplementation of formic acid to feed for piglets in the post-weaning period also caused a reduction in the number of *E. coli* (Kirsch, 2011; Stensland et al., 2015). Zentek et al. (2013) found that the addition of formic acid and lactic acid to feed resulted in the reduction of cells containing enterotoxin STb (heat-stable), which is produced by *E. coli*. This substance is characterized by a very strong acidifying activity but does not damage the dispensers due to corrosion. The acidifiers used in feeding piglets reduce the number of pathogenic bacteria as a result of competition for colonization because they cause the multiplication of symbiotic microorganisms. Jendza et al. (2018) studied the effect of different dosing (0, 3, 6, or 9 g/kg) of sodium diformate 2 weeks prior to the first cuts on animal performance in a commercial swine barn. The results indicate that the acidification of finisher feed in the weeks immediately pre-slaughter can reduce the microbial load of pigs. Acidification with 3 and 6 g/kg tended to reduce the fecal aerobic plate count by 1.2 and 1.1 $\log_{10}$ cfu/g, respectively (P=0.07). Total coliforms, *E. coli,* and *Enterobacteriaceae* counts

were decreased by 1.2 to 1.6, 1.3 to 1.7, and 1.6 $\log_{10}$ cfu/g, respectively, relative to the control ($P<0.05$).

Reduction of the buffer capacity of feed

The intensification of production led to the diets for the animals to be characterized by a high content of protein and minerals, which maximizes the production results; however, on the other hand, it generates high buffer capacity. The animal protein used in pig feed (fish meal, whey) is more digestible, but it has over 15 times greater buffer capacity in relation to grains such as wheat, which reduces the level of free hydrochloric acid in the stomach (Lückstädt, 2009). The buffering process consists in binding digestive acids by feed components (e.g. calcium or protein) and increasing the pH value in the stomach, which creates favorable conditions for the development of pathogens, mainly *E. coli*, *Salmonella,* and anaerobes. These microorganisms are responsible for the formation of the majority of inflammation of the stomach and intestines, diarrhea, and also for reducing the use of nutrients (Bosi et al., 1999). At high pH, pepsin activation and the secretion of pancreatic enzymes are also limited, which reduces digestion. Therefore, apart from the hygienic effect, the acids are also responsible for reducing the buffering properties of the feed, which has a beneficial effect on its digestion (Lückstädt, 2009). Of the six organic acids studied by Li et al. (2016), acetic acid had the strongest buffering capacity at the pH and buffering capacities of lactic acid, citric acid, succinic acid, fumaric acid, and pyruvic acid were 30, 50, 77, 15, and 9% as much as acetic acid, under the condition of the same mass concentration. Torija et al. (2003) found that all of the organic acids used in this study (tartaric, malic, citric, and succinic acids) showed a buffering capacity, but not all of the acids had the same capacity.

Improvement of smell and taste, feed palatability, and feed intake

In addition, acids also affect the smell and taste of feed. Ions formed as a result of the dissociation of acids stimulate the membrane cavity associated with transitory potential receptors TRPA1 in the oral cavity, responsible inter alia for the sensation of burning and pain, which, in turn, transmit sensory signals to the brain via the trigeminal nerve. The acidic taste associated with the presence of acids is discovered by sensory cells embedded in the taste buds of the tongue, from which the taste stimulus is transmitted to the brain via the cranial nerves responsible for the innervations of the tongue. Organic acids (citric, malic, tartaric, etc.) may be used as flavor additives masking the negative effect of some feed materials (extractive rapeseed meal, some legume seeds), affecting the palatability of mixtures with their content, which also increases the digestibility of fiber, suberin, and sugars (Lückstädt, 2009). In the experiments on pigs, it has been proven that the sour taste desired for pigs is most often effective by way of the use of citric or tartaric acid (Ahmed et al., 2014). These acids are mild and do not cause a burning sensation. On the other hand, acetic, formic, and propionic acids in high doses may cause somatic pain and affect the deterioration of feed intake (Wang et al., 2011). The supplementation of a diet with acetic acid up to 150 mmol/kg or lactic acid up to 200 mmol/kg feed does not cause the deterioration of its taste and feed intake (Rudbäck, 2013). The use of the correct

amount of acids can improve the taste and smell of feed and water, which may increase their intake by piglets.

Improvement of feed digestibility

The effective activity of proteolytic enzymes requires an acidic environment below 4 (Lückstädt and Mellor, 2011). In addition, the secretion of pancreatic enzymes is limited at a higher pH, which leads to the deterioration of the digestion of protein and other nutrients. As a result of the use of acids, lowering the pH in the stomach and intestine stimulates the secretion of pancreatic juice and positively affects the intestinal epithelium morphology (Lückstadt and Kühlmann, 2013; Giannenas et al., 2016; Long et al., 2018). The research of Kim et al. (2005) confirmed that the addition of 2.5% lactic acid to piglet feed during the weaning period increased the volume and content of pancreatic juices and the activity of trypsin and chymotrypsin in the gastrointestinal tract. It was also found that the lower pH in the gut increases the solubility of phosphorus and phytates, which promotes the absorption of phosphorus in the small intestine (Lević et al., 2008). Oliveira et al. (2017) found that the addition of the acidifier (citric, phosphoric, and fumaric acids) to the weaner pigs' diets improved the digestibility of dry matter, NDF, and Ca. Supplementation of fumaric acid increases the absorption of calcium, phosphorus, and zinc (Kirchgessner and Roth, 1982). In addition, the level of toxic ammonia and biogenic amines in the small intestine is also reduced, which results in the better absorption of proteins, micro- and macronutrients as well as fats (Lević et al., 2007).

Moreover, organic acids affect the metabolism as a source of energy and stimulate the growth of animals (Bosi et al., 1999). Pigs are able to use formic acid with energy efficiency similar to that obtained during the metabolism of glucose. It was determined that the total energy value of the metabolized formic acid is 5.8 kJ/g (Long et al., 2018). The research shows that propionic acid can have up to five times more energy than wheat (Lückstädt, 2009). Some metabolic reactions can be also stimulated by the addition of acids, which increases the use of proteins and amino acids (Bosi et al., 1999). Luise et al. (2017) evaluated the influence of two doses of formic acid supplementation for six weeks to weaner pigs. The long-term effects of formic acid were tested for growth performance and microbiota composition. Experimental diets contained 1.4 g/kg or 6.4 g/kg. During the first three weeks only, formic acid supplementation, at any of the above-mentioned dosages, tended to increase the average daily feed intake and decrease the F:G ratio. At the end of the trial, formic acid supplementation only had a minor effect on the microbiota composition in the jejunum. Formic acid intake did not affect the thickness of the gastric mucosa, number of parietal cells, or stomach weight.

Development of the pig's digestive system

Another benefit of using acids is to stimulate the growth of digestive tract cells. Better developed villi allow for more efficient absorption and better utilization of nutrients from feed (Abdelqader and Al-Fataftah, 2016). Many reports in the literature show that the disability in the development of intestinal villi is caused by the colonization of pathogenic bacteria in the gastrointestinal tract that causes

the apoptosis and exfoliation of enterocytes. The presence of lipopolysaccharides in the cell membrane of Gram-negative bacteria, apart from the disappearance of intestinal villi, can also cause inflammation, submucosal edema, necrosis, and hemorrhage in piglets. Cells located in the intestinal epithelium, which are part of the intestinal barrier, prevent the passage of bacteria and pathogens through the intestinal epithelium. These are: goblet cells that produce mucus that is a physical barrier to bacteria, Paneth cells that contain lysozyme, IgA and defensin-related compounds involved in the phagocytosis of microorganisms, and M cells that capture bacterial antigens that are then presented to T lymphocytes. Therefore, chronic inflammation may have a detrimental effect on the proper functioning of the intestinal barrier.

The positive effect of benzoic acid on the growth of intestinal villi was demonstrated. The villus height, after two weeks of using the acidifier, increased on average by 20%, while the depth of the crypts decreased by 5–10% (Diao et al., 2014). The best effects were observed for butyric acid, where the height of the villus, the surface absorption of nutrients as well as the concentration of gastrin in the colon and jejunum generally increase. Sodium butyrate is more commonly used than pure acid due to the less intense aroma of rancid butter. Supplementation to a ton of feed 500 g or 1,000 g of sodium butyrate positively affects the production results and causes a significant elongation of the intestinal villi compared to the control group (Zheng-Shun et al., 2012; Nowak et al., 2019). Supplementation of sodium butyrate to feed causes the increase of occludin activity, or the protein responsible for intestinal permeability. As a result, the intestinal epithelium becomes more integral and all the epithelial damage is regenerated faster (Ma et al., 2015). The use of sodium butyrate reduces the percentage of TNF-α tumor necrosis factor associated with the inflammatory process by 69%. As a result of its activity, there were also a smaller number of clostridium microorganisms in the colon, which reduced the number of recorded cases of diarrhea in weaned piglets (Huang et al., 2015). Supplementation of sodium butyrate increases the innate immunity and may be useful in neutralizing infections caused by antibiotic-resistant microorganisms (Xiong et al., 2015; Walia et al., 2016; Casanova-Higes et al., 2017). Other experiments show that sodium butyrate affects the activity of endogenous defense peptides (antimicrobial), which contributes to the neutralization of *E. coli* with the serotype O157: H7, causing hemolytic-uremic syndrome. Among them, some peptides are distinguished that interrupt bacterial cell membranes, such as protegrins and β-defensins pBD-2, pBD-3, and pEP2C. Supplementation of 2 g of sodium butyrate per kg of complete mixture for piglets infected with this bacterium decreased the number of these pathogens in the feces. As a result, the clinical symptoms of the hemolytic-uremic syndrome were not so noticeable and the effects of intestinal inflammation had a less adverse effect on the health of piglets (Zeng et al., 2013). Fang et al. (2014) found that the addition of sodium butyrate to the feed significantly increased the efficiency of nitrogen utilization. It could also regulate and enhance the immune function of piglets by increasing the serum IgG concentration and IgA+ cell count in jejunum. These results suggest that sodium butyrate may reduce some of the adverse effects of weaning stress and play an important role in maintaining the integrity of intestinal mucosa.

A positive effect on the development of intestinal villi was also noted after use in medium-chain fatty acids in piglet feed. In the experiments of Chwen et al. (2013), supplemented milk, with the addition of MCT triglycerides in sucking piglets, influenced the height of the duodenum villi by about 480 μm after 6 hours from the use of the supplement compared to the control group piglets that were fed with milk alone. On the other hand, the depth of crypts significantly decreased only after 6 days by approx. 30 μm and after 8 days by approx. 100 μm compared to the control group. In the research of Hanczakowska et al. (2013), the use of capric acid in the feed resulted in an increase in the dry matter digestibility coefficient and the total protein. The experiments also proved that the mixture of capric, formic, and propionic acid positively influenced the growth of the intestinal villi. These compounds are a very important factor in the processes of remodeling and the development of the intestinal epithelium (Tonel et al., 2010). In the research of Nowak et al. (2019), the villi height and villi height to crypt depth ratio was significantly higher in pigs offered a feed acidifier (phosphoric, citric, fumaric, tartaric, and malic acids) and sodium butyrate together with monochloroacetic acid (MCA) or acidifier. The addition of MCA resulted in an increase in the absorption area of nutrients and an increase in body weight after 6 and 8 days. It has also been proven that MCA supplementation in piglets' pre-starter improves the absorption of nutrients (Hong et al., 2012; Li et al., 2015).

Improvement of health and growth parameters

All of the above-mentioned actions led to the improvement of pigs' health as well as the production results. The use of organic acids in feeding pigs reduces the incidence of gastrointestinal diseases, such as diarrhea or edema disease (Pluske et al., 2018). Ahmed et al. (2014) found that the use of citric acid significantly increased the level of IgG immunoglobulins that may result in improved health as a result of increased humoral immunity. Fang et al. (2014) found that the addition of sodium butyrate to the feed significantly decreased the diarrhea incidence of weaned piglets. In addition, supplementation of formic acid or sodium formate prevented piglets' diarrhea (Walles et al., 2010; Zheng-Shun et al., 2012; Koyuncu et al., 2013). Luise et al. (2017) found that the long-term effects of formic acid tended to increase the average daily feed intake and decrease the F:G ratio. Mesonero et al. (2016) used a blend of free and buffered organic acids in the water, together with a reduced medication program, and observed improved growth performance during the first month after weaning compared to the control with full medication and a combination between organic acids and full medication. MCA additive in a starter diet also increased daily gains (Hong et al., 2012; Li et al., 2015).

Synergy effect

The variety of mechanisms of action of various acids causes them to perform different functions in piglet feeds. Therefore, it is often appropriate to use the mixtures of organic and inorganic acids in order to enhance the desired effect. On the other hand, the undesirable qualities of acids, such as unpleasant odor, low stability, or corrosivity, can also be reduced or neutralized by the proper balancing of the mixture

(Piva et al., 2007). The main principle of balancing multicomponent acidifiers is to achieve a synergy effect of their action, resulting in such a mixture being more effective in action than its individual components and should act actively along the entire length of the gastrointestinal tract. The literature shows that the most beneficial effects on intestinal microflora are exerted by mixtures containing both short-chain and medium-chain organic acids (Zentek et al., 2011, 2013; Hanczakowska et al., 2016). In the research of Nowak et al. (2019), the villi height and villi height to crypt depth ratio was significantly higher in pigs offered in feed sodium butyrate together with MCA or acidifier. Thomas et al. (2018) adding medium chain fatty acids (MCFA), lactic acid, and monolaurin blends to the diet of nursery pigs found improved average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (F/G) compared with pigs fed a control diet. Long et al. (2018) evaluated the effect of two mixed organic acids on the performance, serum immunity, intestinal morphology, and microbiota of weaned pigs (about 9 kg) in comparison with antibiotic growth promoters (AGP – zinc bacitracin, colistin sulfate, and olaquindox). The first preparation consisted of a synergistic blend of free and buffered short-chain fatty acids (mainly formic acid, acetic acid, and propionic acid) combined with medium-chain fatty acids and the second – a synergistic blend of a phenolic compound, slow release C12, target release butyrate and sorbic acid, MCFA, and organic acids). The results of the research indicated that both blends can be used to replace AGP because of the positive effects on performance, serum immunity, intestinal morphology, and microbiota in the weaned piglets. Both preparations reduced ($P<0.05$) the incidence of diarrhea and fecal *E. coli* counts. The apparent total tract digestibility of total carbohydrates, dry matter, neutral detergent fiber, acid detergent fiber, fat, and phosphorus were improved. The concentration of IgG, growth hormone, and total antioxidant capacity in serum tended to be higher and the amount of hydroxyl radicals in serum was lower ($P<0.05$) in pigs supplemented with the second preparation (Stensland et al., 2015; Giannenas et al., 2016; Omonijo et al., 2018; Li et al., 2019). The other acid mixtures and their effect on the performance results, digestibility of nutrients, health of pigs, and microflora structures are shown in Table 1. The analyzed literature data show that the best results could be achieved when both short- and medium-chain fatty acids are present in the acid mixture (Hanczakowska et al., 2013; Kuang et al., 2015; Devi et al., 2016; Long et al., 2018; Thomas et al., 2018; Li et al., 2019; Yang et al., 2019). It seems that the previous publications concentrated on the correct proportions of particular acidifier constituents in order to achieve the best performance results.

Current and future technology of applying acids in animal production

Due to the fact that acids are easily dissociated in the stomach and acidification is desired in the intestines, new solutions have been introduced in the production of acidifiers consisting of their buffering, coating, and microencapsulation.

Buffering acids, e.g. using ammonia, causes the product to be less aggressive in relation to the digestive system and devices for mixing and feeding as well as safer for people who have to dispense them (Liu et al., 2018).

Table 1. The characteristic of the effect of blended acids and their salts on pigs' health status, performance, digestibility and microflora content

Literature	Composition /Doses	Performance results	Digestibility effect	Health status	Microflora effect
1	2	3	4	5	6
Hanczakowska et al., 2013	Propionic acid, formic acid, caprylic and capric acids. Doses: 0.5% propionic and formic acids and 0.2% of caprylic and/or capric acids.	Piglets receiving capric acid had the highest body weight gains ($P<0.05$).	Piglets receiving MCFA had higher protein and fiber digestibility ($P\leq0.05$) than piglets receiving SCFA. Villi of the mucosal epithelium were the highest ($P\leq0.05$) in piglets receiving SCFA with capric acid.	NA	There was no difference in intestinal microflora except for *Clostridium perfringens*, the population of which was reduced by SCFA.
Ahmed et al., 2014	0.5% pure citric acid or 0.4% acidifier blend (17.2% formic acid, 4.1% propionic acid, 10.2% lactic acid, 9.5% phosphoric acid, SiO_2 34.0%).	FCR was improved by citric acid additive ($P<0.05$).	NA	Serum IgG concentration was increased by citric acid additive ($P<0.05$), whereas IgM and IgA were not significantly affected by any of the dietary treatments ($P<0.05$).	All dietary treatments showed significant reduction in fecal counts of *Salmonella* and *E. coli* ($P<0.05$). Significant elevation in fecal *Lactobacillus* spp. counts and increased *Bacillus* spp. counts were found in groups with organic acids additive ($P<0.05$).
Kuang et al., 2015	Blended calcium formate, calcium lactate, citric acid and MCFA at 340, 160, 70 and 130 g/kg, respectively). Doses: 3 g/kg diet.	Feed intake and growth of pigs were improved by acidifier ($P<0.001$) and FCR was lower ($P<0.01$).	Higher apparent ileal digestibility coefficients of majority of AA were found ($P<0.05$).	Lower plasma TNF-α, but higher plasma immunoglobulin-G concentrations ($P<0.05$) were found.	Higher ileal and rectal *Lactobacillus* content ($P<0.05$) was found.
Li et al., 2015	Blends of capric and caprilic acids. Doses: 0.7%, 1.4%, 2.1%.	Acids improved daily gains and FCR ($P<0.05$).	Apparent total tract digestibility coefficients of ether extract and dry matter were improved ($P<0.05$).	NA	NA

Upadhaya et al., 2014, 2016; Devi et al., 2016	17% fumaric acid, 13% citric acid, 10% malic acid, 1.2% MCFA capric and caprylic acid. Doses: Fatteners 0.1%; 0.2%, 0.4%. Sows and piglets 0.1%; 0.2%.	ADG were improved (P<0.001).	Improved dry matter, nitrogen and energy digestibility (P<0.001) – for fatteners and sows.	Immunoglobulin G concentration was higher in lactating sows and sucking piglets.	*Escherichia coli* counts decreased, *Lactobacillus* counts increased (P<0.05) for fatteners and sows. The *E. coli* counts decreased in weaning piglets.
Boas et al., 2016	0.5% of a mixture of organic acids (21% lactic acid, 18% formic acid and 10% citric acid); 0.1% of sodium butyrate; 0.5% of a mixture of organic acids and 0.1% sodium butyrate. Microencapsulated sodium butyrate at a concentration of 30% was used.	No effect (P>0.05) on animal performance was found.	No effect (P>0.05) on apparent ileal digestibility of nutrients was found.	NA	NA
Wang et al., 2016	34% calcium formate, 16% calcium lactate, 7% citric acid and 13% MCFA. Doses: 0.3 or 5.0 g/kg diet.	Dietary treatments had no effect on growth performance of piglets.	Higher apparent ileal digestibility coefficients of calcium dry matter, and overall gross energy were found.	Lower plasma TNFα, higher IgG levels and lower diarrhea number were found.	Higher ileal *Lactobacillus*, with lower total bacteria content were found.
Long et al., 2018; Li et al., 2019	MOA1 – synergetic compound of free and buffered SCFAs (formic, acetic, propionic acid) combined with MCFA (C8:0 and C10:0). MOA2 phenolic blend, contain monounsaturated fatty acids and MCFA (C10:0 and C12:0), target release butyrate and sorbic acid. MOA1 – 3000 mg/kg; MOA2 – 2000 mg/kg.	Daily gains and FCR were improved (P<0.05).	Apparent total tract digestibility coefficients of gross energy, dry matter and organic matter were improved (P<0.05).	OA1 and OA2 reduced (P<0.05) the incidence of diarrhea.	Pigs fed MOA1 showed higher concentration of *Lactobacillus* and lower concentration of *Escherichia coli* in feces. OA1 and OA2 reduced (P<0.05) fecal *E. coli* counts.

Table 1 – contd.

1	2	3	4	5	
Thomas et al., 2018	1.0% MCFA (C6, C8 and C10, 1:1:1 ratio), or a 1.0% inclusion of 4 different blends of MCFA, lactic acid and monolaurin-based additives. The 4 blends consisted of 50% C6, 20% lactic acid and increasing amounts of monolaurin (0, 10, 20 and 30%) at the expense of C12 (30, 20, 10 and 0%).	The addition of a 1.0% 1:1:1 MCFA blend resulted in improved daily gains, feed intake and F/G compared with pigs fed control diet ($P<0.05$).	NA	NA	NA
Morel et al., 2019	Benzoic acid (4.95 g/kg diet), sodium butyrate (1.47 g/ kg diet) and sodium butyrate (0.9 g/kg diet) coated with benzoic acid (2.1g/kg diet).	No effect on the growth performances ($P>0.05$) was detected.	No effect on energy digestibility ($P>0.05$) was found.	NA	NA
Yang et al., 2019	0.2% unprotected organic acid – blended: 17% fumaric acid, 13% citric acid, 10% malic acid, 1.2% MCFA (capric and caprylic acids), or 0.1% or 0.2% POA.	Feeding POA diets to weanling pigs increased ($P<0.05$) ADG.	POA increased ($P<0.05$) the apparent total tract digestibility coefficients of dry matter.	0.2% POA to weanling pigs has the potential to reduce diarrhea incidence.	POA increased ($P<0.05$) the fecal *Lactobacillus* counts, whereas the *Escherichia coli* and *Salmonella* counts were reduced (linear effect, $P<0.05$).

MCFA – medium chain fatty acids; NA – not analysed; SCFA – short chain fatty acid; FCR – feed conversion ratio; ADG – average daily gain; MOA – mixed organic acids; OA – organic acids; POA – protected organic acids; AA – amino acids.

It is very important that this process does not adversely affect the activity and effectiveness of acids in various sections of the gastrointestinal tract of piglets. In turn, the process of encapsulation consists in placing the acid on a fatty matrix, resistant to changes in pH, which is digested only by intestinal lipases. The microencapsulated blend of organic acids allowed the slow release of the active ingredients and prevented the immediate disappearance of compounds on exiting the stomach. As a result, the acids are released only in the intestines, where they cause a reduction in the number of pathogenic microorganisms.

The available literature suggests that the supplementation of protected acids significantly improves the production results, digestibility of nutrients, and reduces the formation of biogenic amines in the large intestine as well as the release of ammonia and hydrogen sulfide from feces (Devi et al., 2016). Xu et al. (2018) evaluated the effects of organic acid preparation (consisting of benzoic acid, calcium formate, and fumaric acid, coated with palm oil and silicon dioxide) and essential oil (EO) used individually or in combination on performance, fecal microbiota, intestinal morphology, and digestive enzymes of weaned pigs. Dietary organic acids strongly improved the gastrointestinal tract environment, including nutrient digestion, microbes, and digestive enzymes. In the experiments of Upadhaya et al. (2016), the supplementation of the pigs' feed with 0.2% of the encapsulated acidifier, which consisted of a mixture of organic acids and medium-chain fatty acids, resulted in the growth of beneficial lactic acid bacteria. *Lactobacillus* produces lactic acid during fermentation, as a result of which the pH in the gastrointestinal tract is lowered, thereby preventing the growth of pathogenic bacteria. Supplementation of the encapsulated acidifier also resulted in a greater ADG and G/F ratio than pigs fed the control diet as well as higher *Lactobacillus* counts in the feces (linear, $P=0.01$). Changes in the composition of the intestinal microflora were also observed in an experiment conducted by Diao et al. (2014) in which 5,000 mg/kg encapsulated benzoic acid was added to feed for piglets aged 24 days. Two weeks of supplementation with this acid caused a decrease in pH in the colon and, after 6 weeks, also in the ileum and cecum. In addition, the number of lactic bacteria from the genus *Bifidobacterium* and *Bacillus* has increased. On the other hand, there was a decrease in the number of *E. coli* and *Enterobacteriaceae* and a decrease in the content of ammonia in the caecum contents. Zhao et al. (2016) found that the inclusion of 0.2% microencapsulated organic acids (MO) in the diet can increase body weight, ADG, the apparent total tract digestibility of dry matter, and fecal *Lactobacillus* counts in weanling pigs, whereas 0.05% MO can increase G/F in finishing pigs. Yang et al. (2019) evaluated the effect of the dietary inclusion of protected organic acids (POA) on growth performance and fecal microflora in weanling pigs compared with unprotected organic acids. They found that the dietary inclusion of POA has the potential to improve growth performance, while balancing the microbial counts in weanling pigs without any adverse effects on pigs' health. Increased fecal *Lactobacillus* and decreased *E. coli* and *Salmonella* counts were observed in diets wherein POA was fed to weanling pigs ($P<0.05$).

Table 2. The combinations of eubiotics and their effect on pigs' health status, performance, digestibility and microflora content

Literature	Composition/ Doses	Performance results	Digestibility effect	Health effect	Microflora effect
Jansons et al., 2011	Blends of herbs (*Thymus vulgaris* L., *Urtica dioica* L., *Quercus robur* L., *Melissa officinalis* L.) and organic acids: formic, acetic, citric and phosphoric acid and calcium. Organic acid mixture doses: 0.3%, 0.4% and 0.6%; herbs 0.5% and 1.0%.	Feed additive improved gains of pigs and FI ($P<0.05$) and FCR ($P<0.05$).	NA	NA	NA
Sbardella et al., 2015	Formaldehyde-propionic acid blend in doses: 0.0, 1.0, 2.0 and 3.0g of blend kg^{-1} of feed.	No effects ($P>0.05$) on pigs performance, FI and feed utilization were found.	NA	NA	Increasing dietary levels of blend reduced quadratically ($P<0.01$) total *Enterobacteriaceae* counts.
Stensland et al., 2015	OACP in doses 1.5 g/kg for pigs infected with enterotoxigenic *E. coli.*	No effects ($P>0.05$) on pigs performance, FI and feed utilization were found.	NA	No effects ($P>0.05$) on diarrhea index was found.	No effect ($P>0.05$) on bacteria count was found.
Giannenas et al., 2016	Blends of: probiotic Cylactin® at 35 mg/kg (containing 2 × 1010 CFU/gr *Enterococcus faecium*), or the mannan-oligosaccharide Biomos® at 1 g/kg, or the organic acid Vevovital® at 5 g/kg (containing 100% benzoic acid).	Improved growth performance of fattening pigs was found ($P<0.05$).	Jejunal villus height increased ($P<0.05$).	No differences were noted in the goblet cell numbers.	Higher inclusion of the *E. faecium* was found.
Hanczakowska et al., 2016	0.3% of caprylic or 0.3% of capric acids and Cylactin® (*E. faecium*) in the amount of 0.35×10^9 CFU per kg feed.	Improved daily gains ($P<0.05$), FCR and FI ($P>0.05$) were found.	Capric acid reduced the total length of the intestines. Mixtures improved villus width ($P<0.05$).	NA	Supplements had strong antibacterial activity: acids against *E. coli* and probiotic against *C. perfringens* ($P<0.05$).

Liu et al., 2017	Blends of organic acids and essential oils in doses 0.2% into feed of pigs infected by *Escherichia coli*.	Improved daily gains, FI ($P<0.05$) and FCR ($P>0.05$) were found.	NA	NA	NA
Thuy et al., 2017	Blends organic acid (fumaric acid, lactic acid, calcium formate and phosphoric acid), probiotic (*Bacillus subtilis*, *Lactobacillus* spp. and *Saccharomyces cerevisiae)* and β-glucan in doses 2 g/kg.	Increased daily gains were found ($P<0.05$).	NA	Positive effects on incidence of diarrhea and fecal score.	In the 2 weeks post-weaning, the piglets supplemented with organic acids or with probiotic had lower concentrations of *E. coli* in feces.
Wang et al., 2018	Blends of multi-enzyme complexes, essential oils and benzoic acid.	No effect ($P>0.05$) on pigs performance was found.	NA	NA	Dietary treatments had no clear effect on serum cytokines or fecal microbiota.
Xu et al., 2018	Organic acids blend: benzoic acid 50%, calcium formate 3%, fumaric acid 1%, coating with: palm oil and silicon dioxide and essential oil (is the blend of flavourings (thymol 25%, carvacrol 25%). OA – 1.5 g/kg, EO – 30 mg/kg.	OA and OA+EO mixtures improved daily gains and FCR ($P<0.05$).	OA and OA+EO mixtures reduced some nutrients digestibility ($P<0.05$).	No effect ($P>0.05$) on diarrhea index was found. No influence on plasma immunity indices and gut morphology was found.	Increased number of lactobacilli was found ($P<0.05$).
Nowak et al., 2019	Blended component: MCT (caprylic-caprinic acid) – 0.3%, acids mixture (phosphoric, citric, fumaric, tartaric and malic acids – 0.2%, sodium butyrate – 0.3%); phytobiotics 0.2%, yeast 1%, probiotic 0.12%.	There were no significant differences in gain, FI or FCR ($P>0.05$).	NA	NA	No effect on microbial count in caecal digesta was found ($P<0.05$).

NA – not analysed; FI – feed intake; FCR – feed conversion ratio; OACP – mixture of an organic acid blend, cinnamaldehyde and permeabilizing complex; CFU – colony-forming unit; MCFA – medium chain fatty acids; OA – organic acid; EO – essential oil; MCT – medium chain triglyceride.

Multicomponent preparations

In the current literature as well as in practice, we can find proposals of different combinations of feed additives, containing organic or inorganic acids. These products are also called "eubiotics" (Greek "*Eubiosis"*), which refers to a healthy balance of microbiota in the gastrointestinal tract. Using more than one eubiotic substance (multi-eubiotic) as a feed additive could be more efficient than using them separately. According to Nowak et al. (2019), the strategy of mixtures of eubiotics in piglets' diets should be their complementary action in the gastrointestinal tract, including the development of microflora, development of intestine villi, pH regulation, and diarrhea incident reduction. Many authors (Table 2) used different active substances in the eubiotic mixture, such as: probiotics, prebiotics, phytobiotics, acids, enzymes, etc. (Jansons et al., 2011; Liu et al., 2018; Thuy, 2017; Wang et al., 2018; Xu et al., 2018). Phytobiotics, especially spices, herbs, and essential oils, are commonly used in animal nutrition as flavoring additives as well as for their antiseptic or medicinal properties. Phytogenic substances could have an effect on digestive enzymes, which can lead to an intensive growth of animals. Probiotics and prebiotics affect the development of normal microbiota in the gastrointestinal tract, reduce the pH of the intestinal contents, and enhance the digestion of feed, prevent or stop the spread of pathogenic microorganisms, improve the resistance of the organism, and positively affect the integrity and proper functioning of the gastrointestinal tissue. Liu et al. (2017), using blends of organic acids and essential oils, found improved gains and feed utilization in piglets challenged with *E. coli*. Thuy (2017), on the other hand, offered blended organic acids, probiotic and prebiotic, which improved pigs' growth. However, Wang et al. (2018) found no effects of mixtures containing enzymes, essential oils, and benzoic acid on pigs' growth and health parameters. It seems clear that the efficacy of natural additives, however, is highly dependent on the type, composition, and form of the preparation. Moreover, it suggested that many tests on animals must be performed before using mixtures in practice.

Summary

From an economic point of view, acidifiers are necessary in feeding piglets, especially when antibiotics are banned. The dosing of acidifying preparations allows for the neutralization of the main problems associated with the weaning period, which is currently the most critical stage in pig production. In conclusion, the reduction of the pH of the digestive system content results in an increase in the activity of secretion of digestive enzymes and activation of proenzymes, which facilitates the digestion of proteins and other nutrients. As a consequence, there is less retention of undigested feedstuff in the intestines, and the multiplication of pathogenic microflora is limited. In addition, the process of the dissociation of acids in microbial cells prevents the proliferation of bacteria of the genus *E. coli* and *Salmonella*, which are the most common causes of diarrhea and mortality of piglets. The effect of feed additives in the digestive system of piglets is even better when they are subjected to the process of coating. On the other hand, the use of multicomponent formulations with a suitable acid composition will result in a stronger effect.

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