

A new look at probiotics in cattle breeding

Agnieszka Galus-Barchan¹, Iwona Radkowska², Agata Szewczyk²

¹University of Agriculture in Kraków, Department of Microbiology, al. Mickiewicza 24/28, 30-059 Kraków;
agnieszka.galus-barchan@urk.edu.pl

²National Research Institute of Animal Production, Department of Cattle Breeding,
32-083 Balice near Kraków

A name “probiotic” originates from the Greek words “pro bios” which mean “for life”. This term was used for the first time by Lilly and Stillwell in 1965 and meant “substances secreted by certain microorganisms and stimulating growth of the other ones”. In 1974, Parker assessed probiotics as “organisms and substances that contribute to microbiological balance of intestines”. In turn, in 1989, Fuller defined them as “living food additives that have a positive effect on host organism by improvement of the balance of the intestinal microflora”. Charteris et al. (1997) defined probiotics as “microorganisms that after the consumption may have a beneficial influence in prevention and treatment of pathological conditions”, whereas Salminen et al. (1998) described them as “food containing living bacteria that are beneficial for health”. According to the current definition of FAO and WHO from 2002, probiotics are determined as “living strains of specific microorganisms that modulate the bacterial balance of the intestinal flora and have a beneficial effect on consumer’s health when administered in the proper amounts” (FAO, 2002).

Probiotic microorganisms play a very important role in the protection of organism against harmful microorganisms and their task is to strengthen the immune system of the host. A prebiotic is a non-digestible food ingredient that brings benefits to the host by the selective stimulation of one bacteria or a group of bacteria in the colon. A complex of a probiotic with a prebiotic added as a nutritive and carrier substance for bacteria is called a synbiotic. Due to a synergistic action, this combination has a stronger effect on animal organism. Most commonly used prebiotics include oligosaccharides, inulin, lactose, galactose derivatives or cereal bran. Probiotic bacteria stimulated in this way have optimal conditions for proliferation and development. Bacteria genera that are most often used in probiotic formulations are: *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Bacillus* and *Streptococcus*. Some yeast strains of *Saccharomyces* genus are applied as well. It was demonstrated that probiotics are effective in various clinical conditions – diarrhea, necrotizing enterocolitis, diarrhea associated with antibiotics, recurrent colitis evoked by *Clostridium sp.*, *Helicobacter pylori* infections, inflammatory bowel disease, cancer, infections of the female genital tract and surgical infections (Gupta and Garg, 2009; Mazurkiewicz et al., 2015). Intestinal flora is a peculiar ecosystem. It is involved in maintaining homeostasis in host organism. Composition of this ecosystem, quantitative and qualitative proportions depend on the environment in which the organism lives, physiological conditions (e.g. pregnancy, childbirth), taken medications and, primarily, on stress level and the maintained diet. Microbiome consisting of anaerobic bacteria, aerobic bacteria, yeasts and viruses together with mucus layer, intestinal epithelium, cells of the circulatory system, lymphatic system, immune system and nervous system form the intestinal barrier. The intestinal barrier ensures a selective transport through the intestinal walls, being permeable to nutrients and blocking adhesion and penetration of pathogens inside the intestinal wall. Microbiome living in mucus layer is the most important element of the intestinal barrier. Thanks to changes in its composition, it is also the most variable factor conditioning health or disease of the organism (Węgrzyn et al., 2017). Intestinal microorganisms crea-

te a complex group consisting of approx. 100 trillion bacteria. A pattern of intestinal microorganisms is a unique (individual) feature of each organism; this group has not been completely characterized yet and little is known about their biodiversity, because the majority of bacteria – that are unculturable microorganisms – cannot be detected with the use of traditional microbiological methods (Nowak et al., 2010). Currently, in animal breeding there is a growing interest in products that have physiological and nutritional functions, prevent diseases, improve animal health condition, contribute to an improvement in their immunity, better feed use and thus to obtain better production efficiency.

Role of probiotics

In the gastrointestinal tract of humans and animals, mucous membrane is a barrier separating the internal environment from the external one. Intestinal epithelium together with mucus constitute the first defense line against colonization by the pathogenic microorganisms. Bacteria living in the gastrointestinal tract have a double function: they stimulate the mucous membrane and defense mechanisms in order to trigger immune response and maintain homeostasis (Heller, 2001; O'Hara and Shanahan, 2007). Any kind of stress, both physiological and psychological, weakens the immune system that results in intestinal dysfunction, increases the permeability of the intestinal barrier and predisposes for gastrointestinal tract colonization by pathogenic microorganisms (Gareau et al., 2009). Loss of integrity leads to a gradual increase in the intestinal permeability that causes a transformation from physiological condition to pathological inflammatory state which is characteristic for intestinal diseases (Lambert, 2009). Probiotics available on the market are pure cultures of one or more bacteria strains that are naturally present in the intestines. Microorganisms with probiotic effect on animals primarily include lactic acid producing bacteria of genus *Lactobacillus* (*L. acidophilus*, *L. amylovorus*, *L. brevis*, *L. casei*, *L. crispatus*, *L. farmicinis*, *L. fermentum*, *L. murinus*, *L. plantarum*, *L. reuteri*, *L. ramosus*, *L. salivarius*), genus *Bifidobacterium* (*B. animals*, *B. lactis*, *B. longum*, *B. pseudolongorum*, *B. termophilium*), Gram-positive cocci of genus *Streptococcus* (*S. infantarius*, *S. salivarius*, *S. termophilus*), of genus *Enterococcus* (*E. faecium*, *E. faecalis*), of genus *Pediococcus* (*P. acidilactici*, *P. pentosaceus*), of genus *Lactococcus* (*L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis*), of genus *Leuconostoc* (*L. citreum*, *L. lactis*, *L. mesenteroides*), *Propionibacterium freundenreichii*, Gram-positive rod-shaped bacteria of genus *Bacillus* (*B. cereus*, *B. licheniformis*, *B. subtilis*), yeasts of genus *Saccharomyces* (*S. cerevisiae*, *S. pastorianus*), of genus *Kluyveromyces* (*K. fragilis*, *K. marxianus*) and fungi of genus *Aspergillus* (*A. orizae*, *A. niger*) (Mizak et al., 2012).

Mechanisms of action of probiotics are multidirectional and have not been fully known yet. A competition for receptors or the place of bacterial adhesion to the large intestinal epithelial cells is one of them (Guarner and Malagelada, 2003; Ley et al., 2008; Lambert, 2009). Probiotics also compete with pathogens for indispensable nutritional ingredients, increase the secretion of mucins – glycoproteins that seal the intestinal epithelium and alter the structure of receptors for bacterial toxins. Balance of the intestinal ecosystem is a basis for the appropriate functioning of the organism. Knowledge of these issues is currently used to manipulate the intestinal biome by transplanting fecal microflora from a healthy donor to the patient's gastrointestinal tract to increase the effectiveness of therapy of *Clostridium difficile* infections. The impact of the transfer on the improvement of the intestinal biome structure, the improvement of intestinal barrier function, and thus the increase in immunity and health improvement are also observed. After the transfer, an increase in the population of the desired bacteria, a decrease in the number of bacteria acting adversely, a reduction in the occurrence of diarrhea and an increase in the average daily gain in recipients is observed in the animals (Weingarden and Vaughn, 2017; Hu et al., 2017).

According to the data from the literature (Schrezenmeir and de Vrese, 2001; Saulnier et al., 2009; Oelschlaeger, 2010), the impact of probiotics on maintaining homeostasis in the animal organism consists in:

- maintaining or restoring balance in the natural gastrointestinal microflora,
- inhibition of adhesion of other bacteria to the intestinal epithelium,
- protection against the growth of pathogenic microorganisms (viruses, bacteria and fungi) in the gastrointestinal tract,
- lowering the pH of the intestinal content thanks to organic acids produced by probiotic bacteria,
- inhibition of bacterial fermentation leading to the formation of intestinal gases,
- improvement of digestion and absorption of nutrients by increasing the pool of digestive enzymes,
- improvement of digestibility of the consumed feed by stimulating the activity of some intestinal enzymes – lactase, sucrase, maltase, reducing the level of toxic metabolites in the gastrointestinal tract and blood,
- production of natural antibiotic substances – bacteriocins, which have bactericidal or bacteriostatic effect on pathogenic microorganisms,
- synthesis of vitamins, mainly from group B and vitamin K, and digestive enzymes (e.g. α -galactosidase),
- reduction of triglyceride and cholesterol level in blood and tissues,
- stimulation of the immune system by the activation of gut-associated lymphoid tissue (GALT),
- modulation of the immune system activity by reducing the activity of pro-allergic Th2 lymphocytes (allergy prevention),
- reduction in the level of procancerogens and cancerogens in the gastrointestinal tract (Sanders et al., 2007; Dominguez-Bello and Blaser, 2008; Mizak et al., 2012).

Probiotics are currently called “life promoters” and are considered as factors stimulating growth of the organism and the proper functioning of the gastrointestinal system. They are applied as growth stimulators in many animal species. Probiotics may also replace antibiotics in feeds. The effects of using probiotic formulations in animal breeding are as follows: a reduction in the consumption of feed necessary to produce animal body weight (through a more complete use of feed ingredients), an improvement in animal health, an increase in resistance to stress and a reduction of body recovery time after the disease, receiving the so-called safe food and a reduction in the costs of production (Mizak et al., 2012).

Probiotics in cattle

Probiotics are used in the nutrition of ruminants both in slaughter cattle and in adult fattened bulls or dairy cows. It was proved that the administration of probiotics to young animals accelerates the development of proventriculus in them, restricts the occurrence of intestinal inflammatory states and increases body weight gains. The effectiveness of using probiotic formulations in calf feeding is assessed mainly on the basis of weight gains, feed utilization and improvement of their health. The administration of probiotics has a beneficial effect on the increase in body weight gains during the first two weeks of their use by the reduction of diarrhea and the number of days with diarrhea. A trend towards lower mortality in calves fed with a milk replacer with the addition of a probiotic was observed as well. Most often in the eighth week of life, the calves receiving the probiotic are characterized by higher weight gains and better utilization of ingredients from the feed (Timmerman et al., 2005). In authors' own studies that have not been published yet (conducted in the National Research Institute of Animal Production) it was observed that the calves receiving the probiotic up to the 60th day of life, showed an improvement in health and immunity. The probiotic had a beneficial effect on morpholo-

gical composition of blood and the number of pathogenic bacteria in the feces as well as increased weight gains in animals.

The most frequently used feed additives in cattle are lyophilized living cultures of yeasts of *Saccharomyces cerevisiae* species that are activated in the rumen environment. Their use mainly results in better feed utilization and body weight gain in animals. However, the presence of yeasts mainly affects the metabolic activity of lactic bacteria that remain in symbiosis with them. Good bacterial growth depends on group B vitamins produced by yeasts. Yeasts also have the effect on a reduction in ammonia concentration, a better decomposition of cellulose, a reduction in the amount of soluble sugars and methanol. Together with an increase in the population of microorganisms, ammonia concentration in rumen decreases (Lipiński, 1998). Microorganisms also contribute to the rapid increase in the amount of microbial protein derived from cellulolytic bacteria, which are stimulated to increase the population that uses structural carbohydrates for the production of volatile fatty acids, which in turn contribute to an increase in fat content in milk (Śliżewska et al., 2006).

Recently, more and more attention in cattle breeding has been directed not only at maximizing the production results, but also at maintaining animal welfare by keeping them in good condition and providing them with proper nutrition, beneficial to the gastrointestinal tract and metabolism. An important role is also attributed to maintaining the proper functioning of the immune system. It is crucial especially in the newborns whose gastrointestinal tract is sterile in the first hours of life. In general, animals are born without their own antibodies or are only provided with maternal immunoglobulins that are able to cross the placenta. Prior to the production of its own antibodies, the gastrointestinal tract of a young animal is inhabited by microorganisms from the external environment that may be harmful to the defenseless organism (Fooks et al., 1999). The probiotics that form a natural "biofilm" (adhesion capacity) in the intestinal mucosa, acting as a protective barrier against potentially pathogenic factors and thus increasing the body's immunity, are of particular importance. Probiotic immunological stimulation manifests itself in the enhanced production of immunoglobulins (antibodies), increased activity of macrophages (phagocytic cells) and lymphocytes (specific immunity) and stimulation of the production of γ -interferon (immune proteins). Components of the cell wall of lactic acid fermentation bacteria stimulate the growth of activity of macrophages, which are responsible for a rapid destruction of pathogenic microorganisms (Salisbury et al., 2002). The use of antibiotics and other bactericides affects the intestinal microbiome of cows and calves by modeling the quantitative and qualitative composition of bacteria and reducing the number of desired microorganisms. Condition of the microflora of cows and calves varies between farms and individuals. Intestinal flora of cows is usually more diverse, with predominance of bacteria belonging to genera *Bacillus* and *Bacteroidetes*. In calves, a greater number of *Clostridium* and *Actinobacterium* is often observed (Weese and Jelinski, 2017).

Probiotics and dangers

The introduction of a ban on the use of antibiotics as feed additives was a consequence of the rapid increase in bacterial resistance to these compounds (Balcazar et al., 2006). Studies have shown that probiotic bacteria, especially the most widespread ones of the genus *Lactobacillus* or *Bifidobacterium*, are natural carriers of antibiotic resistance genes. These genes may be transferred to other cells as a result of horizontal gene transfer. Such an exchange may occur within one species or between the species. Resistance genes are encoded by bacterial plasmids, so as a result of conjugation they may be transferred to pathogenic bacteria such as, for example, *Escherichia coli* or *Salmonella enterica*. The ability of probiotic microorganisms to neutralize bacteria increases the amount of nucleic acids that are released after the breakdown of microbial cells in the intestines, and thus creates favorable conditions for cell transformation. Such material may be absorbed by the microorganisms, including the pathogenic ones, and incorporated into their own genomes. Both bacteria and the other microorganisms coexist together in the environment in the appropriate proportions. There is a number of relations between them. Probiotic strains can effectively regulate the microbiological composition of the occupied environment, and therefore affect the numerous relations between microorganisms, however, more

further studies are needed to control the culture without harming the environment (Augustyniak and Nawrotek, 2014).

Summary

There is no question that gut microflora and its modification with probiotic preparations and products can prevent intestinal diseases and improve the host's health in general. There is also no doubt that some saccharides, by stimulating the growth of probiotic bacteria, can play an important role in the functioning of the digestive tract. Many studies have shown that probiotic use may be an alternative to improving the performance and health of animals without resorting to antibiotic use. Probiotics have been proven efficient in veterinary prophylaxis, conducive to the welfare of animals, and having no harmful effects. Although researchers have high hopes for large-scale use of lactic bacteria, it must be remembered that nature likes to defend itself and act against science. There are many scientific experiments ahead to eliminate errors, so as to cause no harm to animals and their environment in the future.

References

- Augustyniak A., Nawrotek P. (2014). Probiotyki w żywieniu zwierząt hodowlanych – zastosowanie, działanie, zagrożenia. *Prz. Hod.*, 1: 20-22.
- Balcazar J.L., Decamp O., Vendrell D., De Blas I., Ruiz-Zarzuola I. (2006). Health and nutritional properties of probiotics in fish and shellfish. *Microb. Ecol. Health. Dis.*, 18: 65–70.
- Charteris W.P., Kelly P.M., Morelli L., Collins J.K. (1997). Selective detection, enumeration and identification of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in mixed bacterial populations. *Int. J. Food Microbiol.*, 35: 1-27.
- Dominguez-Bello M.G., Blaser M.J. (2008). Do you have a probiotic in your future? *Microbes Infect.*, 10: 1072-1076.
- FAO (2002). Guidelines for the Evaluation of Probiotics in Food, Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Kanada, 30 kwietnia i 1 maja 2002 (http://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf).
- Fooks L.J., Fuller R., Gibson G.R. (1999). Prebiotic, probiotics and human gut microbiology. *Int. Dairy J.*, 9: 53-61.
- Fuller R. (1989). Probiotics in man and animals. *J. Appl. Bacteriol.*, 66: 365-78.
- Gareau M.G., Wine E., Sherman P.M. (2009). Early life stress induces both acute and chronic colonic barrier dysfunction. *NeoRev.*, 10: 191-197.
- Guarner F., Malagelada J.R. (2003). Gut flora in health and disease. *Lancet*, 361: 512-519.
- Gupta V., Garg R. (2009). Probiotics. *Indian J. Med. Microbiol.*, 27 (3): 202-209.
- Heller K.J. (2001). Probiotic bacteria in fermented foods: product characteristics and starter organisms. *Americ. J. Clin. Nutr.*, 73: 374S–379S.
- Hu L., Geng S., Li Y., Cheng S., Fu X., Yue X., Han X. (2017). Exogenous fecal microbiota transplantation from local adult pigs to crossbred newborn piglets. *Front Microbiol.*, 8: 2663.
- Lambert G.P. (2009). Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. *J. Anim. Sci.*, 87: 101-108.
- Ley R.E., Hamady M., Lozupone C., Turnbaugh P.J., Ramey R.R., Bircher J.S., Schlegel M.L., Tucker T.A., Schrenzel M.D., Knight R., Gordon J.I. (2008). Evolution of mammals and their gut microbes. *Science*, 320: 1647-1651.
- Lilly D.M., Stillwell R.H. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science*, 147 (3659): 747-748.
- Lipiński K. (1998). Mechanizm działania probiotyków paszowych. *Trzoda Chlew.*, 1: 65-67.
- Mazurkiewicz J., Mleko S., Tomczyńska-Mleko M., Stój A., Sobota A., Solarska E. (2015). Zdolność różnych zakwasów piekarskich do degradacji trichotecenów z grupy B w cieście żytnim. W: *Bezpieczeństwo zdrowotne żywności. Aspekty mikrobiologiczne, chemiczne i ocena towaroznawcza*. Wyd. Nauk. PTTŻ, Kraków, ss. 193-203.
- Mizak L., Gryko R., Kwiątek M., Parasion S. (2012). Probiotyki w żywieniu zwierząt. *Życie Wet.*, 87 (9): 736-742.
- Nowak A., Śliżewska K., Libudzisz Z. (2010). Probiotyki – historia i mechanizmy działania. *Żywność. Nauka. Technologia. Jakość*, 4 (71): 5-19.
- Oelschlaeger T.A. (2010). Mechanisms of probiotic actions – a review. *Int. J. Med. Microbiol.*, 300: 57-62.

- O'Hara A.M, Shanahan F. (2007). Mechanisms of action of probiotics in intestinal diseases. *Sci. World J.*, 7: 31-46.
- Parker R.B. (1974). Probiotics, the other half of the antibiotic story. *Anim. Nutr. Health*, 29: 4-8.
- Salisbury J.G., Nicholls T.J., Lammerding A.M., Turnidge J., Nunn M.J. (2002). A risk analysis framework for the long-term management of antibiotic resistance in food-producing animals. *Int. J. Antimicrob. Agents*, 20: 153-164.
- Salminen S., Wright A. von, Morelli L., Marteau P., Brassart D., Vos W.M. de, Fondén R., Saxelin M., Collins K., Mogensen G., Birkeland S.E., Mattila-Sandholm T. (1998). Demonstration of safety of probiotics – A review. *Int. J. Food Microbiol.*, 44: 93-106.
- Sanders M.E., Gibson G., Harsharnjit S.G., Guarner F. (2007). Probiotics: their potential to impact human health. *CAST Issue Paper*, 36: 1-20.
- Saulnier D.M., Spinler J.K., Gibson G.R., Versalovic J. (2009). Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. *Curr. Opin. Biotechnol.*, 20: 135-141.
- Schrezenmeir J., Vrese M. de (2001). Probiotics, prebiotics and synbiotics-approaching a definition. *Am. J. Clin. Nutr.*, 73: 361-364.
- Śliżewska K., Biernasiak J., Libudzis Z. (2006). Probiotyki jako alternatywa dla antybiotyków. *Zesz. Nauk. Politechniki Łódzkiej*, 984 (70): 79-91.
- Timmerman H.M., Mulder L., Everts H., Espen D.C. van, Wal E. van der, Klaassen G., Rouwers S.M.G., Hartemink R., Rombouts F.M., Beynen A.C. (2005). Health and growth of veal calves fed milk replacers with or without probiotics. *J. Dairy Sci.*, 88: 2154-2165.
- Weingarden A.R., Vaughn B.P. (2017). Intestinal microbiota, fecal microbiota transplantation and inflammatory bowel disease. *Gut Microbes*, 4, 8 (3): 238-252.
- Weese J.S., Jelinski M. (2017). Assessment of the fecal microbiota in beef calves. *J. Vet. Int. Med.*, 31 (1): 176-185.
- Węgrzyn D., Adamek K., Łoniewska B. (2017). Budowa bariery jelitowej. *Pomeranian J. Life Sci.*, 63 (3): 6-9.

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Summary

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Key words: probiotics, livestock, health



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