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Probiotics in farm animal nutrition

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Probiotics applied in the form of feed additive are becoming popular as one of the alternatives to antibiotic growth stimulators that have been completely banned in the European Union countries since 1 January 2006 (the Regulation of the European Community no. 1831/2003, 2003). A growing interest in probiotics results from the proved positive effects of using them in nutrition of farm animals. It was demonstrated that they have a positive effect on limiting pathogen activity in the gastrointestinal tract, stimulate host immunity, improve the absorption of nutrients and finally improve animal performance. Currently, the above-mentioned functions are explained in the context of increasing knowledge on the role of microorganism ecology within the gastrointestinal tract or its segments, e.g. intestine.

The study presents a current review of knowledge on the classification of probiotics, their function in farm animal nutrition and safety of using them.

Probiotics: definition and classification

The history of the term "probiotics" started in 1965. It was used for the first time by Lilly and Stillwell (1965) who described the phenomenon observed in Protozoa, in which one microorganism produced an unknown substance that stimulated growth of the other one. The definition of this phenomenon was subjected to many changes until 2013, when the experts from the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) presented the following definition of probiotics: "probiotics are living microorganisms which bring health benefits to the host, when administered in the appropriate amounts". Such definition was accepted as effective by the International Scientific Association for Probiotics and Prebiotics (Hill et al., 2014).

The list of species and strains of microorganisms used as probiotics in animal feeds is long and new entries are still being added to it. The table 1 presents genera, species and strains of probiotic microorganisms used in animal nutrition according to the status approved by the FAO in 2016 and submitted in the work by Bajagai et al. (2016).

Table 1. Microorganisms used as probiotics in animal nutrition (acc. to Bajagai et al., 2016)

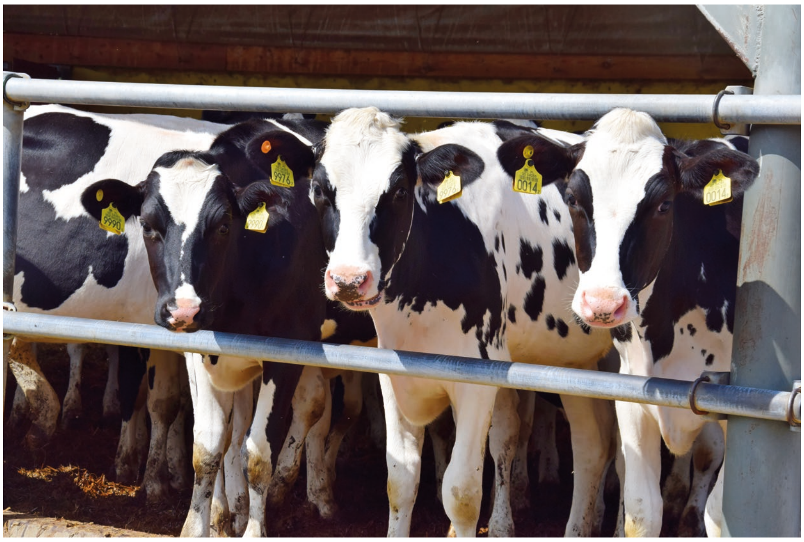
Genus	*Species*	*Strains*	*Genus*	*Species*	*Strains*
Aspergillus	*oryzae*		*Bifidobacterium*	*animalis*	503, DSM 16284
	niger			*bifidium*	
Bacillus	*amyloliquefaciens*	CECT 5940 H57		*bifidus*	
	toyonensis	BCT-7112		*thermophilus*	
	coagulans	ATCC 7050 ZJU0616		longum	
				pseudolongum	
	licheniformis	DSM 5749		lactis	
	megaterium		*Brevibacillus*	*laterosporus*	
	mesentricus		*Candida*	*pintolepesii*	
	polymyxa		*Clostridium*	*butyricum*	
	subtilis	588, CA #20, DSM 17299, PB6, ATCCPTA 6737, DSM 5750	*Lactobacillus*	*thermophilus*	
Escherichia	*coli*	Nissle 1917		*acidophilus*	
Enterococcus	*faecium*	589, NCIMB 11181, E1708, DSM 10663, NCIMB 10415, DSM 16211, DSM 3530, HJEF005		*brevis*	I 12, I 211, I 218, I 23, I 25
				bulgaricus	
				casei	CECT 4043
				delbrueckii subspecies bulgaricus	
				farciminis	
	faecalis			*fermentum*	JS
Lactococcus	*lactis*	CECT 539		*gallinarum*	I 16, I 26, LCB 12
Megasphaera	*elsdenii*			*jensenii*	
Pediococcus	*acidilactici*	DSM 16210		*paracasei*	
	parvulus			*plantarum*	
Prevotella	*bryantii*			*reuteri*	514, C 1, C10, C16, DSM 16350, DSM 16350
Propionibacterium	*shermanii*			*rhamnosus lactis salivarius*	DSM 16351, I 24
	freudenreichii			*sobrius*	
	acidipropionici		*Streptococcus*	*faecalis*	
	jensenii			*faecium*	
Saccharomyces	*bourlrdii*			*gallolyticus*	TDGB 406
	cerevisiae	KCTC No.7193		*salivarius subsp. thermophilus*	
	servisia			*bovis*	

In view of such a large number of genera, species and strains of microorganisms with a beneficial effect on host's organism, various classification rules were accepted. In the review presented by

Bajagai et al. (2016), a division of the applied probiotics depending on the following criteria was proposed:

1. taxonomic belonging to:
   a. bacteria – the majority of probiotics are bacteria from genera *Lactobacillus, Bifidobacterium, Bacillus* and *Enterococcus* (Domain: Bacteria),
   b. non-bacterial, fungal organisms – *Aspergillus oryzae, Candida pintolopesii, Saccharomyces bourlardii* and *Saccharomyce cerevisiae* (Kingdom: Fungi);
2. the ability to form spores;
   a. spore-forming species, such as, for example *Bacillus subtilis, Bacillus amyloliquefaciens*,
   b. non-spore-forming species, e.g. *Lactobacillus* and *Bifidobacterium*;
3. composition:
   a. singlespecies, e.g.:
   – Bro-bio-fair (*Saccharomyces servisia*),
   – Anta Pro EF (*Enterococcus faecium*),
   b. multispecies, e.g.:
   – Poultry Star® (contains *Enterococcus faecium, Lactobacillus reuteri, L. salivarius* and *Pediococcus acidilactici*),
   – PrimaLac (contains *Lactobacillus* spp., *Enterococcus faecium* and *Bifidobacterium thermophilum*),
   – Microguard (contains various species of *Lactobacillus, Bacillus, Streptococcus, Bifidobacterium* and *Saccharomyces*);
4. environment of origin:
   a. autochthonous – they occur as residents of the gastrointestinal tract (e.g. *Lactobacillus* and *Bifidobacterium*),
   b. allochthonous – they do not naturally occur in the gastrointestinal tract of animals (e.g. yeast).

Probiotics are also classified according to the mode of action, based on which they function. The diagram below presents the basic modes of action of the commonly used probiotics, characteristic only for the particular species or characteristic only for the particular strains (diagram 1). None of the individual probiotics has the effect in accordance to all of the mechanisms described above, but many mechanisms are often represented by a single strain.



Fot. A. Wojciechowski

Diagram 1. Classification of modes of action: widespread among commonly used probiotic genera, observed among most strains of a probiotic, and present in only a few strains of a given species (diagram elaborated based on Hill et al., 2014).

COMMON MODES OF ACTION AMONG PROBIOTICS

Modification of gastrointestinal tract microbiome
1. Resistance to colonization by pathogens
2. Normalization of disturbed microflora
3. Competitive exclusion of pathogens

Modification of the function of the gastrointestinal tract wall

4. Regulation of intestinal transit
5. Increased rate of enterocyte development

FREQUENT MODES OF ACTION CHARACTERISTIC FOR THE SPECIES
1. Synthesis of vitamins
2. Increase in synthesis of antimicrobial factors
3. Increase in enzymatic activity
4. Regulation of metabolism of bile salts
5. Enhancement of protective intestinal barrier
6. Neutralization of cancerogenic factors

RARE MODES OF ACTION SPECIFIC FOR THE STRAIN

Production of specific bioactive substances causing the following effects:
1. Neurological
2. Immunological
3. Endocrine
4. Other

Probiotic functions

In view of a rich variety of probiotic organisms as well as their modes of action on host organism, there is no uniform interpretation of their function. However, it is recognized that they realize their health-promoting functions in the lumen and/or wall of the gastrointestinal tract. Apart from nutrients and beneficial microorganisms, the lumen of the gastrointestinal tract also contains pathogens, toxic and harmful substances that include food antigens as well, whereas the mucous membrane of the gastrointestinal tract plays a role of selectively permeable barrier between the intestinal lumen and the internal environment of the host.

Modification of gastrointestinal tract microbiome

Lumen of the gastrointestinal tract is the habitat of microbiome that includes all the microrganisms living in it, not excluding the pathogenic ones. Chemical ingredients taken with food provide the environment specific for the certain microbiome that together with the taken food, products of its digestion and products of organism's own metabolism create environmental conditions for the reduction or increase in the number of specific bacteria. When probiotics are introduced apart from nutrients, we use the possibility of modulation of the dynamics of changes in the population of microorganisms in order to determine the majority of beneficial microorganisms over the harmful ones (Choct, 2009). It was demonstrated that the process of reduction in the number of pathogenic microorganisms has many levels, results from the synergy of production of anti-microbial substances, elimination of pathogens via competition and more effective adhesion (sticking) of probiotic microbes to the epithelium (Shim et al., 2012). All of these stages were observed during the process of modification of gastrointestinal tract microflora with the use of probiotic bacteria of genera *Lactobacillus* and *Bifidobacteria*, directed against the coli group bacteria, especially *Escherichia coli* (Forte et al., 2016). It was found that in the mechanism of this modification the basic role is played by the synthesis of bacteriocins with an antagonistic effect towards pathogenic strains as well as by the synthesis of enzymes participating in the processes associated with an increase of content acidity above the level facilitating the development of pathogens (fermentation to lactic acid, short-chain fatty acids, hydrogen peroxide production) (Kawai et al., 2004; Mookiah et al., 2014; Yirga, 2015). Similar mechanisms modulating intestinal homeostasis were observed in spore-forming probiotics of genus *Bacillus* (Yirga, 2015; Elshaghabee et al., 2017).

In conclusion, it can be stated that the mechanism of favourable, health-promoting effect of probiotics within the lumen of the gastrointestinal tract includes an increase in the population of beneficial microorganisms by the production of substances with bacteriostatic properties, targeted only to the beneficial microorganisms, with the concurrent synthesis of quantitatively increasing substances with bactericidal properties, directed specifically against pathogens. The analysis of the effects of probiotics on gastrointestinal tract microbiome conducted in recent years indicated that both the dynamics of this process and the final effect are specific for the species and host's age.

Modification of the function of the gastrointestinal tract wall

Mucous membrane of the gastrointestinal tract wall plays a protective role of the physiological stability of the organism against the harmful external factors, mainly by the modulation of the activity of the immune system and control of the transfer of beneficial and harmful substances between the intestinal lumen and the internal environment of the host's body. In response to the antigens present in the intestinal content, it has the effect on the mechanisms of induction of anatomical activities of the structures of the immune system of intestinal mucous membranes (small intestine, caecum and colon) (GALT; *gut-associated lymphoid tissue*), stimulation of the secretion of immunoglobulins, mainly of the A class (Górska et al., 2009), and also the activation of the specific responses of macrophages and lymphocytes as well as natural cytotoxic cells, releasing cytokines in a manner specific for the strain, dose of the microorganism and also host species (Ashraf and Shah, 2014). The mechanism of probiotic regulation of immune functions also includes the communication between microorganisms and epithelial cells, regulating the adhesion of microorganisms to the epithelial cells, intercellular communication of the wall tissues regulating intercellular adhesion, signalling and transporting processes of epithelial cells (Peterson and Artis, 2014; Ortega et al., 2017).

Gastrointestinal tract epithelium plays a role of a transport barrier controlling absorption and secretion of many substances. This function is destroyed as a result of an inflammatory state evoked by infection. It was demonstrated that some probiotics prevent or minimize transport dysfunctions of the epithelium by interrupting the proinflammatory signalling cascade and by the reduction in secretion of chlorides that participate in maintaining electrolyte balance of the epithelial cells affected by the disease (Barrett, 2017). The use of probiotics in strengthening the protective function of the epithelium in various gastrointestinal tract diseases requires a precise identification of their effectiveness depending on the applied strain, type of the disease as well as age and species of the host. Results of the current studies indicate that there are new mechanisms specific for some probiotic strains. Selective movement of a substance through the epithelial tissue and the individual epithelial cells takes place with the participation of gap junctions that form both a barrier and a system of connections between the cells and the extracellular spaces. It was demonstrated that some probiotics increase the expression of proteins forming the gap junctions in the gastrointestinal tract epithelium, improving the effectiveness of its transport function (Qin et al., 2005).

In conclusion, it may be stated that the mechanisms of beneficial, health-promoting effect of probiotics on the function of the gastrointestinal tract wall within the small intestine, caecum and colon include activation of its immune system (GALT; synthesis of substances inhibiting colonisation by pathogens) and improvement of transport functions (selective transport; functioning of the intercellular communications) or maintaining them during the course of various gastrointestinal tract diseases.

Is the final effect always an increase in the performance of farm animals?

A description of the mechanisms of action of probiotics on host's organism presented in this study may suggest their unambiguously positive effect on the performance of farm animals. In recent years the number of studies dedicated to assessment of the effect of probiotics on performance of farm animals increased, but the results are not unambiguous. Many study results confirm a positive effect of the introduction of probiotics to feed on the improvement in production indicators such as weight gains, feed intake and the effectiveness of its use, but, at the same time, results of many studies do not confirm these relations. Table 2 presents the results of the selected studies from recent years, characterizing the divergence in the assessment of the effectiveness of the effect of probiotics on improvement in performance of farm animals.

Table 2. Effect of probiotics on performance of farm animals

Species	*Production group*	*Microorganisms*	*Trait*	*Feed intake*	*Feed conversion*	*Literature source*
Cattle	*calves*		*increased body weight*			
		B. amyloliquefaciens	+[1]	+	n-b	Le i in., 2016
		L. acidophilus	NS	NS	NS	Abu-Tarboush i in., 1996
	heifers	*S. cerevisiae*	+			Ghazanfar i in., 2015
	cows		*pH of ruminal contents*			
		S. cerevisiae	+	n-b	n-b	Desnoyers i in., 2009
Trzoda – *Pigs*	prosięta *piglets*	*Bacillustoyonensis*	+	+	-	Kantas i in., 2015
		Bi. longum (AH1206)	NS	NS	n-b	Herfel i in., 2013
	tuczniki *fatteners*	*B. subtilis* *C. butyricum*	+	NS	+	Meng i in., 2010
Drób *Poultry*	brojlery *broilers*		wzrost masy ciała *increased body weight*			
		B. subtilis	+	+	NS	Afsharmanesh i Sadaghi, 2014
		B. amyloliquefaciens	+	+	-	Ahmed i in., 2014
		B. coagulans	NS	NS	-	Hung i in., 2012
		L. acidophilus, B. subtilis DSM 17299, C. butyricum	+	NS	NS	Zhang i Kim, 2014
	kury nieśne *laying hens*		wzrost produkcji jaj *increased egg production*			
		L. acidophilus D2/CSL	+	n-b	-	Gallazzi i in., 2009
		E. faecium	NS	n-b	NS	Capcarova i in., 2010

[1] + — statystycznie istotny wzrost, - — statystycznie istotny spadek, NS — różnice statystycznie nieistotne, n-b — nie badany.
[1] + — statistically significant increase, - — statistically significant decrease, NS — statistically non-significant differences, n-b — not studied.

As it results from the data presented in table 2, probiotics are used as additives to cattle feeds, pig feeds and poultry feeds. It seems that they are particularly effective in young animals, which is confirmed by the improvement in production indicators in calves, piglets and broilers. The influence of probiotics on the performance of older farm animals is not explicitly beneficial, however, high hopes are still associated with their health-promoting functions. In 2017, the world market of additives to animal feeds amounted to more than USD 17 billion, and an increase of more than 8% is predicted up to 2024 (Probiotics Market, 2018).

Safety of using probiotics in farm animal nutrition

According to the applicable legislation, in Europe it is required to precede the approval of a probiotic product with results of studies confirming the safety of use of this product in human nutrition and farm animal nutrition. Performance of these studies according to the procedure contained in the study prepared by the European Food Safety Authority (EFSA) entitled ”Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance” (EFSA, 2012) and in accordance with the guidelines to obtain the status of Qualified Presumption of Safety (QPS) presented by the EFSA Scientific Committee (EFSA, 2007) is effective.

The majority of information on safety of use of probiotics is based on the knowledge concerning the safety of using bacteria of genera *Lactobacillus* and *Bifidobacterium.* On one hand, there is an opinion that they are the safest microorganisms used as probiotics and safety of using them has been confirmed both by the historically documented facts of applying them in the production of traditional food, and their presence in human microbiome (Huse et al., 2012) and animal microbiome (Yeoman and White, 2014). On the other hand, some study results indicating that in relation to human health the use of some strains is burdened with risk, raise doubts. In 2016, 8 cases of liver abscesses in humans, causatively associated with bacteremia resulting from the consumption of large amounts of dairy products containing lactic acid bacilli, were presented (Sherid et al., 2016). The described cases concerned patients particularly susceptible to infections, e.g. after liver transplant, in advanced age and/or burdened with diabetes. In relation to the danger posed to human health resulting from the use of bacteria of genera *Lactobacillus* and *Bifidobacterium* in animal nutrition, no confirmation was found in current subject literature. Thirty-seven *Lactobacillus* species and 5 *Bifidobacterium* species were placed on the QPS list by EFSA – as procedurally confirmed, safe in the use in farm animal nutrition (Panel on Biological Hazards, EFSA BIOHAZ, 2013; Panel on Additives and Products or Substances used in Animal Feed, EFSA FEEDAP, 2016).

Spore-forming bacteria, especially of genus *Bacillus*, are becoming more popular as probiotics used in animal nutrition. This is caused by their resistance to high temperatures as well as by easier manufacturing, storage and transport of feeds. However, the representatives of this bacteria genus are able to produce enterotoxins and endotoxins that are harmful to humans and animals, including the emetic toxin (Elshaghabee et al., 2017). Therefore, it may be concluded that the use of each strain and species of genus *Bacillus* should be controlled. EFSA approved the QPS status for 13 species of genus *Bacillus* (EFSA BIOHAZ, 2013).

The largest number of studies examining the health risk of using probiotic strains were conducted on representatives of *Enterococcus faecium,* commonly used in products for animals. An interest in representatives of genus *Enterococcus* results from the fact that some of them are recognized as common, drug-resistant hospital pathogens (Łysakowska et al., 2009). For example, when analyzing the drug-resistance of strains *Enterococcus* contained in commercial products used in cattle and pig nutrition in the United States, Amachawadi et al. (2018) demonstrated that 15 out of 22 studied strains *Enterococcus* being a component of probiotics are resistant to medically important antimicrobial agents. Due to a very common danger of infections and proved high virulence of these bacteria, EFSA did not approve the QPS status for none of the representatives of genus *Enterococcus*, requiring confirmation of safety of use in each case of its application (EFSA BIOHAZ, 2013).

As it results from the doubts described above, probiotics may be responsible for many health hazards, also including life-threatening infections. The current knowledge is not sufficient to state that a group of probiotics is 100% safe and the safety of use requires an individual assessment of each strain and combination of strains composing the probiotic. Health hazards are associated not only with the individual characteristics of bacteria species and/or strains, but also with a phenomenon of resistance to antimicrobial agents (most often antibiotics), the ability to gain such resistance and to increase the acquired resistance. According to the definition: “resistance to antimicrobial agents is defined as the ability of microorganisms, such as bacteria, to increase their resistance to antimicrobial agents to which they were sensitive before" (Communication from the European Commission, 2017). On one hand, resistance is associated with the presence of genes encoding various factors, such as aggregation substance (asa), gelatinase (gelE), endocarditis antigen (efaA), cytolysin operon (cylA, cylLs, cylLl, cylM, cylB), surface protein (esp), collagen-binding protein (ace) and hyaluronidase (hyl) (Łysakowska et al., 2009). Resistance is identified based on the presence of certain genes in genetic material of bacteria. However, on the other hand, it is also associated with a consequence of genetic mutations targeted to obtain the feature of resistance that acquired once is then passed to the descendant strains, giving them a characteristic of increased survivability in altered conditions via the natural selection. This process of natural selection is enhanced by humans by the inappropriate (excessive) use of antimicrobial agents in medicine and veterinary medicine, improper management in the sectors of health, animal production, food production, agriculture and aquaculture, facilitating the transfer of antibiotic residues and resistant microorganisms to food, soil and water. The above-mentioned process causes a gradual decrease in the effectiveness of antimicrobial agents that finally are becoming ineffective and useless.

Summary

It is expected that since two decades the increasing knowledge and social awareness of the impact of probiotics used as a feed additive for farm animals, combined with the growing demand for high quality food products of animal origin, contribute to the increased use of probiotics in the feed industry. These changes are accompanied by rising consumer interest in health and livestock welfare issues, and in the safety of products obtained from them. In response to the increasing interest in the application of probiotics in livestock nutrition, the definitions were clarified as was the classification in accordance with the taxonomic affiliation of the organisms recognized as probiotics. The classification according to the mode of action on the host continues to be the subject of research. This is due to the diversity of the modes of action, both reciprocal and on the host organism, which are still not completely understood. In response to the need of assessing the risk of probiotic microorganisms used in food and/or feed production, strict and formal regulations are applied in human and animal safety studies. These activities allow us to assume that the marketed probiotic microorganisms present no danger to human and animal health.

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PROBIOTICS IN FARM ANIMAL NUTRITION

Abstract

It is expected that the increasing knowledge and social awareness of the impact of probiotics used as a feed additive for farm animals, combined with the growing demand for high quality food products of animal origin, contribute to the increased use of probiotics in the feed industry. These changes are accompanied by rising consumer interest in health and livestock welfare issues, and in the safety of products obtained from them.

In response to the increasing interest in the application of probiotics in livestock nutrition, the definitions were clarified as was the classification in accordance with the taxonomic affiliation of the organisms recognized as probiotics. The classification according to the mode of action on the host continues to be the subject of research. This is due to the diversity of the modes of action, both reciprocal and on the host mutual, which are still not completely understood. In response to the need of assessing the risk of probiotic microorganisms used in food and/or feed production, strict and forma regulations are applied in human and animal safety studies. These activities allow us to assumed that the marketed probiotic microorganisms present no danger to human and animal health.

Key words: probiotics, livestock feeding, safety