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Review

# The improvement of probiotics efficacy by synergistically acting components of natural origin: a review*

Alojz Bomba[1]**, Zuzana Jonecová[2], Jana Koščová[2], Radomíra Nemcová[2], Soňa Gancarčíková[2], Dagmar Mudroňová[2], Ľubomíra Sciranková[2], Viktor Buleca[2], Gabriel Lazar[2], Ján Pošivák[2], Rudolf Kaštеľ[2] & Mária Mareková[1]

[1]*Institute of Experimental Medicine, Faculty of Medicine, Pavol Jozef Šafárik University, Trieda SNP 1, SK-04011 Košice, Slovakia; e-mail: bomba@medic.upjs.sk*
[2]*Institute of Gnotobiology and Prevention of Diseases in Young, University of Veterinary Medicine, Komenského 73, SK-04001 Košice, Slovakia*

**Abstract:** The protection of human health as well as the quality and safe food assurance becomes the priority of European research in the sphere of animal production. The negative experiences with the using of antibiotic growth promoters lead to subsequent reduction of their application. It is necessary to replace them by the growth promoters of natural origin, which are able to provide the comparable efficacy and will not contribute to the cumulative contamination of the environment. The probiotics represent an effective alternative to antibiotics and current research should be aimed at improving of their efficacy. This may be achieved by several methods. From the practical point of view, combination with synergistically acting components of natural origin seems to be the best way. Potentiated probiotics are defined as biopreparations containing production strains of microorganisms and synergistically acting components of natural origin which exert their intensified effect through effects on probiotic and gut microorganisms, the gut mucosa and the intestinal environment or immune system. A number of suitable components may be used for this purpose, such as prebiotics, non-specific substrates, plants and their extracts, metabolites of microorganisms and polyunsaturated fatty acids. In this report, the results of application of natural feed additives in animals are reviewed and their valuation for the enhancement of probiotic effectiveness is discussed.

**Key words:** probiotic; oligosaccharides; maltodextrin; plants; organic acids; polyunsaturated fatty acids.

## Introduction – the concept of potentiated probiotics

Soon after the introducing of the antibiotics for therapy of bacterial infections in humans and animals, the growth-promoting effect was observed and the antibiotics were used as growth promoting supplements to the feed of farm animals. The mode of action of antimicrobial growth promoters is still not exactly known, but hypotheses explaining their effect are based on a reduction of the growth of bacteria in the intestinal tract and consecutive protection of nutrients against bacterial destruction, the decrease of the production of toxins by intestinal bacteria and the reduction in the incidence of subclinical intestinal infections (Butaye et. al., 2003). The data that are available for different countries show that the use of antimicrobial agents for growth promotion normally equals or exceeds the usage of antimicrobial agents for therapy of farm animals (Aarestrup, 2000). Human health can either be affected directly through residues of antibiotic in food of animal origin, or indirectly through the selection of antibiotic resistance determinants that may spread to a human pathogen and limit the therapeutic potency of antibiotic. The legislators in European Parliament decided to prohibit the use of growth promoting antibiotics in animal feed from the beginning of the year 2006 in the view of reducing antibiotic resistance phenomena in human therapies.

Probiotics are biopreparations containing living microorganisms that optimize the colonization and composition of gut microflora in both animals and humans and have a stimulating effect on digestive processes and the immunity of the host (Fuller, 1992). They could be considered an effective alternative to the use of synthetic substances, e.g. antibiotics, in nutrition and medicine. The data concerning the efficacy of probiotics in practice are often contradictory (Simmering & Blaut, 2001). In order to enhance the efficacy of probiotics, we have to obtain the additional knowledge on the mechanisms mediating their effect in digestive tract (Stavric & Kornegay, 1995). The efficacy of probi-

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** Corresponding author

Table 1. Biological active feed additives of natural origin.

| Biological active substratum | The mechanisms of acting |
|---|---|
| Probiotics | Modulation of gastrointestinal microflora |
| Prebiotics | Stimulation of benefitial gastrointestinal microflora |
| Mannanoligosaccharides | Elimination of gut pathogens, stimulation of immune response |
| Non-specific substrates | Modulation of gastrointestinal microflora |
| Plant extracts | Antimicrobial activity, immunostimulatory effect |
| Organic acids | Antimicrobial activity, acidification of gut environment |
| Polyunsatured fatty acids | Immunostimulatory effect, modulation of bacterial adhesion and gut mucosa |

otics may be enhanced by the following methods: (i) the selection of more efficient strains of microorganisms; (ii) gene manipulation; (iii) the combination of a number of strains of microorganisms; and (iv) the combination of probiotics and synergistically acting components.

The antibacterial effect of each probiotic microorganism or its beneficial effect on the host may be mediated by one or a number of mechanisms that may be expressed to different degrees. They may operate through a nutritional and/or health or sanitary effect. This is the starting point for potentiating the efficacy of probiotics that may be realized by making some of the mechanisms more intensive. Microorganisms used in animal feed in the EU are mainly bacterial strains of Gram-positive bacteria belonging to the types *Bacillus*, *Enterococcus*, *Lactobacillus*, *Pediococcus*, *Streptococcus* and yeast strains belonging to the *Saccharomyces cerevisiae* species and *Kluyveromyces* (Anadon et al., 2006).

The combination of probiotics with synergistically acting components of natural origin seems to be the best way of enhancing their efficacy from the practical point of view. The most promissing synergistic components used in animal nutrition are listed in Table 1. By the above mentioned method, more effective probiotic preparations – potentiated probiotics – are developed. Potentiated probiotics are defined as biopreparations containing production strains of microorganisms and synergistically acting components of natural origin which exert their potentiated effect through the effects on probiotic and gut microorganisms, gut mucosa and intestinal environment or immune system. Therefore, our aim was to identify the most effective means to improve the efficacy of probiotics in animal nutrition. The results of application of natural feed additives in animals are reviewed and their valuation for the enhancement of probiotic effectiveness is discussed.

## Improvement of the probiotic effect of microorganisms by their combination with specific and non-specific substrates

The advanced growth of beneficial microbiota in gastrointestinal tract may be accomplished by the consumption of them as probiotic or by the stimulation of the growth of beneficial bacteria present in the gut by the specific substrates – prebiotic. A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson & Roberfroid, 1995). The advantage of prebiotics consists in their known chemical structure with marginal risk for the health and longer storage life. Recent data provide evidence that prebiotics, such as inulin/oligofructose modulate functions of immune system, especially in the area of the gut-associated lymphoid tissue (Watzl et al., 2005). A way of increasing the efficacy of probiotic preparations may be the combination of both probiotics and prebiotics as synbiotics, which provide an improved survival during the passage of the probiotic bacteria through the upper intestinal tract, enable the incorporation of probiotic strain into the community of endogenous bacteria, stimulate the growth and/or the activities of both the exogenous (probiotic) and endogenous bacteria (Roberfroid, 1998; Šuškovič et al., 2001). The most of the literature data concern the results of application of synbiotics in mice, rats or human volunteers, because of their effects not only on microbial and enzyme digestive activity (Tuohy et al., 2003), but also anti-infectious activity (Asahara et al., 2001), preventive effect on carcinoma of colon (LeLeu et al., 2005) and protection against endotoxin/bacterial translocation and liver damage (Marotta et. al., 2005). Immunosupporting enteral nutrition with synbiotics is an important tool to control superinflammation and infection, and might also reduce the multiorgan dysfunction syndrome and systemic inflammatory response syndrome (Bengmark, 2005).

The experiments with synbiotics applied in feed of farm animals are mostly oriented towards manipulation of gut microflora and immune response (Table 2). Prebiotics are specific substrates selectively fermented in the colon. It has been demonstrated that in order to enhance the efficacy of probiotics, non-specific substrates can be used as well as lactose and whey proteins (Pollmann et al., 1980; Mordenti, 1986). Due to the absence of endogenous lactase in poultry, lactose performs as prebiotic and the stimulating effect on bifidobacteria population and decrease of salmonella colonization of the caeca in chickens were observed (Chambers et. al., 1997). In a manner competitive exclusion, the mannans added to the diet attach to mannose-binding proteins on the cell surface of some strains of bacteria, thereby preventing these bacteria from colonizing the intesti-

Table 2. Summary of the effects of combination probiotic strains with specific and non specific substrates in animals.

| Probiotic | Prebiotic | Animal | The primary effect observed | Reference |
|---|---|---|---|---|
| Probiotic | Oligofructose | Piglets | Higher average daily weight gains, decrease of coliform bacteria, increase of bifidobacteria | Shim et al. (2005) |
| *Enterococcus faecium* | Inulin | Pigs | Improvement of the survival of probiotic strain through upper intestinal tract, positive effect on gut microflora | Bohmer et. al. (2005) |
| *Lactobacillus paracasei* | Fructo-oligosaccharides | Pigs | Increase of total anaerobes, lactobacilli and bifidobacteria, decrease of clostridia and *E. coli* | Nemcová et al. (1999) |
| *Lactobacillus casei* | Dextran | Chickens | Increase of humoral immune response to mixed inactivated vaccines against Newcastle disease | Ogawa et al. (2006a) |
| *Lactobacillus casei* | Dextran | Chickens | Increase of humoral immune response | Ogawa et al. (2006b) |
| Lactic acid bacteria | Mannan-oligosaccharides | Pigs | Higher growth and feed conversion | Kumprecht & Zobač (1998) |
| *Lactobacillus casei* | Maltodextrin | Pigs | Decrease of *Escherichia coli O8: K88* adhesion at jejunal wall | Bomba et al. (1999) |

nal tract by interfering with the binding of carbohydrate residues on epithelial cell surfaces (Spring et al., 2000). Mannans may also alter the immune response due to the presence of mannose receptors on many cells of the immune system (Davis et al., 2004). Supplementation of mannans in the diets of weanling pigs increased the daily weight gains and improved efficiency of feed, and intermittently affected components of the young pigs immune function both systemically and enterically. The use of mannan-oligosaccharides seems to be less expensive and enhancement of serum immunological traits was recorded after feeding of brewers dried yeast as a source of mannan-oligosaccharides in weanling piglets (White et al., 2002). Samarasinghe et al. (2003) compared the weight gains in chickens with 0.2% mannan-oligosacharides in the diet with the effect of virginiamycin, the antibiotic growth promoter, used in feed of poultry and the effect was approximately the same (8% vs. 8.8%).

In various disorders of the gastro-intestinal tract, there occur conditions which encourage the translocation of pathogens from the colon into the anterior part of the digestive tract. There is a need for protecting the digestive-tract mucosa throughout its length, so that the adhesion of pathogenic microorganisms can be prevented. The combined application of *Lactobacillus paracasei*, maltodextrin and fructo-oligosaccharides in pigs proved to be more effective in inhibiting the counts of *E. coli O8:K88* adhering to the mucosa of the jejunum and colon in comparison with the groups receiving *Lactobacillus paracasei* with maltodextrin, *Lactobacillus paracasei* with fructo-oligosaccharides and *Lactobacillus paracasei* alone (Nemcová et al., 2006). The promising results were obtained in farm experiment with administration the same combination (*Lactobacillus paracasei*, maltodextrin and fructo-oligosaccharides) to the feed of 4,000 piglets (Gancarčíková et al., 2002). The therapy costs were reduced by 78.5%, mortality of the piglets by 7% and in piglets with low birth weight the mortality decreased even by 26% in comparison with the results of the same farm one year before, when the feed with commercial antibiotic growth promoter was used.

## Enhancement of the probiotic effect of microorganisms by their combination with plants, organic acids and polyunsaturated fatty acids

Plants and their extracts have been used in natural medicine for many years. The bioactive plant products are essentially the secondary metabolites and they represent complex mixtures of various compounds, such as terpens, phenols, glycosides, aldehydes, esters and alcohols. The antibacterial effects of essential oils have been well discussed in the literature (Valsaraj et al., 1997; Ali-Shtayeh et al., 1998; Mourey & Canillac, 2002). The antiviral properties, antifungal, antioxidant, anti-inflammatory effects and the effects against insects were observed. Antibacterial activity of essential oils depends on their composition (some of them contain more than sixty compounds), concentration in feed and structural configuration of functional groups of volatile oils in plants (Russo et al., 1998). A number of mechanisms by which essential oils exert their antimicrobial activity have been postulated, mainly the inactivation of intracellular enzymes and increase of permeability or disrupting the cell wall structures (Greathead, 2003). Antibacterial effect of thyme (Azaz et al., 2004), oregano, sage, clove, cinnamon, garlic, fennel, tea (Elgayyar et al., 2001; Carson et al., 2006) and juniper (Pepeljnjak et al., 2005) were demonstrated *in vitro*. The results of feed supplementation by plant extracts in animals are listed in Table 3. The effect of plant extracts on parasitic diseases, especially coccidiosis in poultry seems to be very important from the viewpoint of veterinary medicine. The inhibitory effect of essential oil from oregano on coccidia *Eime-*

Table 3. Summary of the results of dietary application of plants or their extracts in farm animals.

| Plant | Animal | The effect observed | Reference |
|---|---|---|---|
| Oregano | Pigs | Control of the post-weaning diarrhoea syndrome | Kyriakis et al. (1998) |
| *Coleus froskohlii* Briq. Roots | Pigs, rabbits | Action against an *Escherichia coli* toxin-induced secretory response in ileal loops | Yadava et al. (1995) |
| Oregano | Pigs | Improvement of the growth in growth-retarded pigs non-specific immunostimulatory effects | Walter & Bilkei (2004) |
| Curcuma | Pigs | Stimulation of antibodies and cytokines production | Ilsley et al. (2005) |
| Mix of essential oils | Chickens | Reduction of *Clostridia perfringens* counts in gut, higher daily weight gains | Losa & Wiliams (2000) |
| Oregano | Pigs | Reduction of post weaning diarrhoea and mortality due to inhibition of intestinal *E. coli* | Mellor (2000) |
| Oregano | Pigs | Reduction of post weaning diarrhoea, reduction of *E. coli* in faeces, increase of weight gains and feed conversion | Gill (1999) |
| Oregano | Pigs | Increase of daily weight gains and utilisation of feed | Bilkei & Gertenbach (2001) |
| Aloe | Chickens | increase of interleukin IL-6, control of *Salmonella galinarum* infection | Waihenya et al. (2002) |
| Oregano, cinnamon, pepper | Pigs | Modifying the gastrointestinal ecosystem, stomach contents and stomach emptying rate | Manzanilla et al. (2004) |
| Oregano, cinnamon, pepper | Poultry | Increase of daily weight gains and feed conversion | Jamroz & Kamel (2002) |
| Oregano, cinnamon, pepper | Chickens | Increase of digestibility of feed | Hernandez et al. (2004) |
| Sage, thyme, rosemary | Chickens | Increase of digestibility of feed | Hernandez et al. (2004) |
| NEBSUI (commercial mix) | Pigs | Reduction of dysentery, growth stimulating effect | Wheeler & Wilson (1997) |
| GREENLINE | Poultry | Reduction of *Enterobacteriaceae* and staphylococci stimulation of lactobacilli | Gajewska et al. (2002) |
| XTRACT | Pigs | Stimulation of digestive enzymes – amylase, lipase, trypsin | Manzanilla et al. (2002) |

*ria tenella* in chickens was observed (Giannenas et al., 2003). The evaluation of plant extracts in term of replacement of antibiotic growth promoters must take into account the number of experiments in animals, in which the immune system response and performance indicators were appreciated (Table 3). The disadvantages of plants extracts result from their nonstandard composition and the fact that they consist of many efficient components and some of them can exhibit unfavorable responses after their absorption.

Organic acids, and in particular the combination of lactic acid with other acids, can serve as an alternative to in-feed antibiotics for newly weaned piglets and their effect appears primarily be mediated through the effects on gut microbiota (Jensen, 1998; Namkung et al., 2004). The dietary organic acids can influence the intestinal microbiota by changing the intestinal environment to one less favorable to the growth of pathogenic bacteria or by a direct bactericidal effect on some pathogens (Martin et al., 2002). The weanling piglets do not produce required amount of hydrochlorid acid for maintenance of optimal pH for effective digestion of proteins in gastric content (approx. 3.5), as well as for supporting of beneficial microbiota and suppression of pathogenic bacteria. The supplementation of the diet of weanling piglets by organic acids is recommended as effective prevention of post-weaning syndrome in piglets (Blanchard & Wright, 2000). The experimental application of 6 organic acids (1% propionic acid, 1.6% lactic acid, 1.2% formic acid, 1.5% citric acid, 1.5% fumaric acid and 1.2% malic acid) reduced incidence and severity of diarrhoea and increased weight gains and feed conversion, but the best results were achieved with dietary application of lactic acid (Tsiloyiannis, 2001). The higher concentrations of organic acids retrograde the palatability of the feed and increase corrosion of materials in stalls (Partanen & Mroz, 1999). The salts of organic acids are characterized by the same effect on growth of animals, but they are less aggressive. They are suitable for application in feed mixtures or drinking water and the environment contamination is very low. The organic acids and their salts are used in poultry, too, because of their inhibition effect on microbial activity in cranial parts of gastrointestinal tract. The addition of organic acids into the drinking water of chickens seems to be effective in prevention of *Campylobacter* spp. infection (Chaveerach et al., 2004). An alternative to the use of organic acids in combination with probiotics in pig diets is the use of fermented feed. Under certain conditions probiotic strains may be used as the sole fermenting agent in milk. However, in many cases, the use of a support culture is preferable. The combination of the probiotic culture and the support culture enhances the acidification rate (Saxelin et al., 1999). Fermented liquid feed is characterized by a high count of lactic acid bacteria and yeast, and a high concentration of lactic acid. Perdigón et al. (1991) and Nader de Macias et al. (1993) described the immunostimulant effect (lymphokines and macrophages) of fermented milk responsible for the elimination of the pathogens from the liver and spleen in the *E. coli* and *Listeria*-challenged mice.

The administration of the milk fermented with *Lactobacillus acidophilus* LA-2 caused a remarkable decrease (71.9% on the average) in faecal mutagenicity and increased *Lactobacillus* spp. and *Bifidobacterium* spp. populations in the human intestine (Hosoda et al., 1996).

Recently, polyunsaturated fatty acids (PUFA) have been used as diet supplements influencing productive health, immune system and prevention of diseases. Two groups of PUFA are distinguished: n-3 PUFA and n-6 PUFA (Calder, 1998). N-3 PUFA are noted for anti-inflammatory and anti-proliferative effect on the cells of immune system, while n-6 PUFA via the arachidonic acid effect are inflammatory and activate the immune system (Révajová et al., 2001). Polyenic fatty acids are organic components of membranes of each cell and they are responsible for the fluidity of the cells and their physical and chemical properties. The organism of mammals receives n-3 and n-6 PUFA exclusively from the feed and therefore they are considered to be essential. The deficiency of essential fatty acids causes malfunction of their most important feature, i.e. the ability to create and maintain the physiological status of the cellular membranes and synthesis of eicozanoids – biologically active substances of lipid pattern (oxygenous derivates of PUFA), which regulate important physiological attributions in the organisms (Vaško & Kašteľ, 2004). The PUFA from the feed influence gastrointestinal microflora – they inhibit or support the growth of bacteria, they modulate the properties of the membranes of the cells, such as thickness or the characters of lipoid compounds, and thereby affect the sites of adherence of bacteria. Ringo & Gatesoupe (1998) found positive impact of PUFA on gut colonization by lactic acid producing bacteria in fishes. Kankaanpaa and co-workers (2001, 2004) observed reducing effect of n-6 PUFA on adhesion of lactic acid bacteria, whereas n-3 PUFA increased this activity. Linolenic acid in low concentration supported growth of *Lactobacillus casei*. After experimental application of PUFA, 12% higher concentration of lactobacilli colonizing mucosa of jejunum in gnotobiotic piglets in comparison with control group was observed (Bomba et al., 2003). It is suggested that PUFA could modify the adhesion sites for gastrointestinal microorganisms by changing the fatty acid composition of the membranes of intestinal epithelial cells (Ringo & Gatesoupe, 1998). The ability of probiotics to adhere to mucosal surfaces may be essential for their health promoting effects. The improvement of the colonization of the intestinal mucosa by probiotic microorganisms could result in the potentiation of the inhibitive effect of probiotics upon the adhesion of pathogens.

## Conclusions

Probiotics represent an effective alternative to antibiotics, although it is very difficult to achieve the same or comparable effectiveness of traditional growth promoters. Future research should be aimed at the possibilities of improving the efficacy of probiotics by their combination with components of natural origin which should beneficially influence the probiotic and indigenous microorganisms, the mucosa and environment of the intestine or stimulate the immune system. The use of potentiated probiotics may result in more effective modulation of gastrointestinal ecosystem for improvement of animal growth and health.

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