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THE NATURAL FEED ADDITIVES AS IMMUNOSTIMULANTS IN MONOGASTRIC ANIMAL NUTRITION – A REVIEW

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Abstract

Probiotics, prebiotics, and phytobiotics could be a possible solution as immunostimulants in monogastric animal nutrition. Beneficial effects of application thereof in animals are determined by many factors, e.g. the type of the probiotic strain, probiotic compounds, or plant species used as a supplement. A significant role is also played by the animal species, dosage, and the time and method of administration. The activity of these compounds is primarily focused on prevention of pathogen infections and, consequently, improvement of animal welfare. Probiotics compete with pathogenic bacteria by covering the intestinal epithelium mucosa, thereby interrupting pathogen colonization in the gastrointestinal tract. Supplementation with probiotics, prebiotics, and phytobiotics can also induce positive changes in the intestinal morphology, e.g. elongation of villi or deepening of intestinal crypts. In a majority of cases, they also modulate the immune response of the host. They mobilise the cellular components of the innate immune system (macrophages and heterophils), which defend the animal organism against gastrointestinal infection. Another possibility is the synthesis and release of pro-inflammatory cytokines that modulate adaptive immunity or stabilization of the intestinal microbiome. The main target of immunomodulatory feed additives is reduction of local inflammation, enhancement of the function of the immune system, a substantial impact on the health status of livestock animals, and improvement of their health status and production performance.

Key words: broilers, pigs, probiotics, prebiotics, phytobiotics

Animal productivity is subjected to various kinds of stresses due to intensive production pressure in the farming system, which adversely affects their performance. Monogastric animals, usually reared in intensive production systems, are exposed to a variety of infections, which reduce their production performance. The most com-

mon health-enhancing prophylaxis is nutritional strengthening of the gastrointestinal system in animals (Perry et al., 2013).

In monogastric animals, the gastrointestinal mucosa is a line of defence against environmental pathogens. To fight against infectious and potentially harmful agents, a complex system of the submucosal and mucosal lymphatic tissue (GALT – gut-associated lymphoid tissue) has developed in the intestines. GALT comprises over 75% of all lymphoid cells of the entire immune system. Approximately 80% of all immunoglobulins and 50% of lymphocytes are produced in the intestine. A characteristic feature of the GALT system is production of IgA antibodies, which are secreted on mucosal surfaces. Their main function involves capture of antigen and prevention of their passage through the mucosa into the organism. Antigens are captured by specialised antigen presenting cells (APC), which secrete appropriate cytokines, thereby determining development or mitigation of inflammation (Butler and Sinkora, 2013; Uddin et al., 2013).

Proper function of the mucosa-associated immune system relies on the presence of intestinal bacteria. By production of bacteriocins (e.g. lactic acid bacteria), the beneficial gastrointestinal microflora prevents the growth of potentially pathogenic bacteria and specific homeostasis prevails in the gastrointestinal tract. Intestinal bacteria produce or synthesise antibacterial compounds, thus enhancing the intestinal immune system (Madej and Bednarczyk, 2015; Asgari et al., 2016).

A way to strengthen the intestinal immune defence is the use of some growth promoters such as probiotics, prebiotics, and phytobiotics as feed additives. They have a positive effect on animal health status, mainly through enhanced host mucosa immunity and improved resistance to pathogenic bacterial colonization (Cheng et al., 2014).

Probiotics are defined as monocultures or mixed cultures of live microorganisms; when consumed, they exert a beneficial influence on animal health by quantitative and qualitative effects on the intestinal microflora or even modification of the immune system (FAO/WHO, 2001; Reid, 2016). The products available on the market contain *Bacilli*, *Saccharomyces, Streptococci*, *Lactobacilli*, and *Bifidobacteria* varieties. Probiotics act by competitive exclusion, reduce gut pH, and produce bacteriocins and lysozyme (Grashorn, 2010).

Prebiotics are non-digestible feed ingredients, which have a beneficial effect on the host by selective stimulation of the growth and activity of one or a limited number of bacteria in the colon. This has a positive impact on animal health status (Roberfroid, 2007). A supplement can be classified as a prebiotic if it fulfils three criteria, i.e. it cannot be hydrolyzed or absorbed in the stomach or small intestine, it has to be for *Bifidobacteria*, and its fermentation should yield beneficial effects in the gastrointestinal tract (Manning and Gibson, 2004). Prebiotics are typically non-digestible compounds, mainly polysaccharides and oligosaccharides, reducing pH in the gut and thus inhibiting colonization of pathogenic microorganisms, stimulating immunity, and neutralizing toxins (Nabizade, 2012). The group of prebiotics comprises mannanoligosaccharides (MOS), glucans, fructooligosacharides (FOS), yeast cell walls (YCW), inulin, and chitooligosaccharides (COS). Compounds such as FOS, MOS, and β-glucan are called immunosaccharides (Song et al., 2014).

Plants and their extracts are also well-known phytobiotics or phytogenics that are widely used in animal traditional and alternative medicine. These feed additives include herbs, spices and their derivative products as well as essential oils. Despite the large diversity of raw materials, a common trait of phytobiotics is their highly complex combinations of bioactive compounds. These products can also be called Plant Secondary Metabolites (PSM). Some of them have a nutritional value, but others have no nutrients and might even be anti-nutritional (Hashemi and Davoodi, 2011). The activity of phytobiotics is useful for e.g. stimulation of feed intake by animals, stabilization of the microbiota of their gastrointestinal tract, and enhancement of their resistance (Frankic et al., 2009; Yang et al., 2009; Huyghebaert et al., 2011; Iranparast et al., 2014; Kumar et al., 2014).

In this review, we will describe the concept of probiotics, prebiotics, and phytobiotics used as immunostimulants in monogastric animal nutrition and the observed effects on animal performance.

Probiotics

The role of probiotic bacterial strains, primarily *Lactobacillus*, is to maintain the equilibrium in the intestinal micropopulation, which prevents the spread of pathogenic microorganisms. This effect results from increased concentration of lactic acid and volatile fatty acids (VFA). *Lactobacillus* bacteria were found to increase the proportion of mucous membrane proteins involved in energy metabolism and their availability in the animal jejunum. Elevated concentrations of intestinal VFA and lactic acid bacteria (LAB) reduce the occurrence of diarrhoea. Furthermore, LAB fermentation leads to production of greater amounts of lactic and acetic acids in the ileum and colon, which have indirect effect on the concentration of propionic and butyric acids. Their presence reduces the intestinal pH, thereby inhibiting the proliferation of pathogenic bacteria (Lerner, 2015; Broderick and Duong, 2016; Wang et al., 2016).

Supplementation of feed mixes for animals with probiotics containing *Lactobacillus*, *Bacillus* spp., and *Bifidobacterium* spp. can increase the height of small intestine villi. Inflammations are less prevalent as well. Colonization of probiotic intestinal bacteria can contribute to various mucosal immune responses, including increased expression of TLR2, TLR9, and nucleotide-binding oligomerization domain (NOD) as well as enhanced cytokine secretion and increased amounts of immunoglobulin (Ig)-A producing cells. *Lactobacillus* bacteria can increase the number of intraepithelial lymphocytes (IELs) and IgA-producing cells in the intestinal tract. This leads to development of resistance of the intestinal mucosa, which is also favourably influenced by increased secretion of interleukin (IL-6) (Salah et al., 2012; Rajput et al., 2013; Jayaraman et al., 2013; Ruiz et al., 2015).

In poultry, probiotics are able to promote intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), increasing digestive capacity, preventing enteric pathogens from colonizing the intestine, lowering the pH, and immunomodulatory effects (Nikpiran et al., 2013) (Table 1). Many studies have confirmed the positive effect of probiotics on animal health and pro-

ductivity. In their investigations, Zhang et al. (2016) indicated that *Escherichia coli* challenge lowered the body weight (BW) and average daily gain (ADG), but dietary supplementation of *Clostridium butyricum* reversed these observations, promoted the immune response, and improved the intestinal barrier function and digestive enzyme activities in broiler chickens challenged with *Escherichia coli* (Table 1). Similar results were obtained by Higgins et al. (2008). Bai et al. (2013) reported that diets supplemented with a yeast product and a *Lactobacillus*-based probiotic increased intestinal immunity in chickens (Table 1). Furthermore, there is growing evidence suggesting that probiotics might increase regulation of local mucosal cell-mediated immune responses, enhance dendritic cell-induced T cell hyporesponsiveness and Toll-like receptor (TLR) signalling, and promote epithelial barrier integrity in avian and mammalian species (Ng et al., 2009). Furthermore, administration of probiotics results in secretion of cytokines and changes in lymphoid cells in the chicken gut, which may lead to enhanced immunity to *Eimeria acervulina* (Jacobs and Parsons, 2009). Additionally, in broiler cecal tonsil cells, it was shown that *Lactobacillus acidophilus* was more effective in inducing T-helper-1 cytokines while *Lactobacillus salivarius* induced more potent anti-inflammatory response (Brisbin et al., 2010).

Table 1. Studies using probiotics and prebiotics as immunostimulants in monogastric animals

Animals/time on treatment	Dietary information and experimental treatment	Major findings	Reference
1	2	3	4
1-d-old male Arbor Acres broiler chickens/ 6-wk trial	Three treatment groups: control group – basal diet, probiotic group – diet containing 0.1% *Enterococcus faecium* (NCIMB 11181) E1708 2×10^{12} CFU/kg, prebiotic group – diet containing prebiotic preparation (0.1% inac. *Saccharomyces cerevisiae*, MOS, propionic acid, formic acid, calcium formiate, calcium propionate, citric acid, diatomaceous earth)	↑ BW* and ↓ FCR* in the prebiotic group compared to the control group, ↑ SRBC* antibody titre in the probiotic group compared to the control and prebiotic groups	Nikpiran et al., 2013
1-d-old male Cobb broiler chickens/ 28-d trial	Four treatment groups: negative control group – basal diet without *Escherichia coli* K88 challenge, positive control group – basal diet and challenged with *E. coli* K88, probiotic group – diet containing 2×10^{7} CFU *Clostridium butyricum*/kg of diet and challenged with *E. coli* K88, antibiotic group – diet containing 20 mg colistin sulfate/kg of diet and challenged with *E. coli* K88	↑ BW* and ADG* in the probiotic group compared to positive control group chickens, ↑ jejunal mucosa TNF-α* in the probiotic group compared to control groups, ↑ jejunal mucosa IL-4* in the probiotic group on d 14 post-challenge compared to the positive control group	Zhang et al., 2016

Table 1– contd.

1	2	3	4
1-d-old male Cobb broiler chickens/ 6-wk trial	Four treatment groups: negative control group – basal diet, positive control group – basal diet supplemented with an antibiotic (100 mg of chlortetracycline/kg of diet), probiotic groups – diets containing 0.1%, or 0.2% probiotic (containing 1×10^7 CFU/g of *Lactobacillus fermentum* and 2×10^6 CFU/g of *Saccharomyces cerevisiae*)	↑ ADG* and ↓ FCR* in the probiotic groups compared to the negative control group during the starter period, ↑ mRNA expression levels of TLR2* and TLR4* at d 21, and ↑ mRNA expression TLR2* at 42 d, in the foregut probiotic groups compared to control groups, ↑ proportions of CD_3+*, CD_4+*, and CD_8+ T lymphocytes* in the intestine of broilers at 21 and 42 d in probiotic groups compared to control groups	Bai et al., 2013
Newborn piglets (Taihu × Landrace) barrows/ 28-d trial	Four treatment groups: control group – without administration of probiotics, probiotic groups – piglets were orally administered 1 ml of *Bacillus subtilis* RJGP16 preparation (5×10^9 CFU/ml), or *Lactobacillus salivarius* B1 preparation (5×10^9 CFU/ml), or both probiotics (2.5×10^9 CFU/ml RJGP16 and 2.5×10^9 CFU/ml B1*)*	↑ gene expression of IL-6** in the duodenum and ileum, and ↑ gene expression of pBD-2** in the duodenum, ↑ expression and release of TLR-2**, and the ↑ number of IgA producing cells** in the duodenum and ileum with co-administration of the *B. subtilis* and *L. salivarius* compared to the control	Deng et al., 2013
7-wk-old piglets (Finnish Landrace, Finnish Yorkshire, and crossbred)/3-wk trial	Two treatment groups: control group – without administration of probiotics, probiotic group – piglets were orally administered 1 ml of *Lactobacillus brevis* ATCC 8287 cells (1×10^{10}) daily	→ BW and morphology of the intestinal mucosa, and ↓ TGF-β1* in the ileum, and ↑ IL-6* in the cecum by the probiotic treatment	Lähteinen et al., 2014
28-d-old barrows (Large White × × Landrace)/10-d trial	Four treatment groups: negative control group – without administration of probiotics, probiotic group – piglets were orally administered 20 ml of *Lactobacillus fermentum* (10^8 CFU/ml), *E. coli* group – piglets were challenged on the first day with 20 ml of *Escherichia* coli K88ac (10^8 CFU/ml), probiotic and *E. coli* group – piglets were challenged on the first day with 20 ml of *E. coli* K88ac (10^8 CFU/ml) and orally administered 20 ml of *L. fermentum* (10^8 CFU/ml)	↑ ADG **, ↓ FCR* , and ↑ CD_4+ T** lymphocyte percentage in the serum and ↑ TNF-α** and IFN-γ** in the ileum by supplementation with *L. fermentum*, ↑ TNF-α** in the jejunum and IFN-γ** in the ileum, duodenum, ileum in *E. coli* challenged pigs	Wang et al., 2009

Table 1 – contd.

1	2	3	4
Pregnant sows (Large White × Yorkshire)/ from d 86 of gestation until d 20 of lactation; 7-d-old piglets/28-d trial	Two treatment groups of sows: control group – basal diet, prebiotic group – diet containing 400 mg/kg MOS Two treatment groups of piglets: control group – basal diet, prebiotic group – diet containing 800 mg/kg MOS	↑ weaning BW* and pre-weaning ↑ADG** of piglets from sows supplemented with MOS compared to piglets from control sows, ↑ serum concentrations of IgA**, IgG**, C3**, LYZ ** at weaning, and ↑C4* of piglets on d 35 of age from sows supplemented with MOS compared to control, ↑ pre- and post-weaning ADG**, ↑serum concentrations of IgA**, IgG** at weaning, and ↑C3**, C4**, LYZ** of piglets on d 35 of age of piglets from the group supplemented with dietary MOS compared to control	Duan et al., 2016
1-d-old Ross 308 broiler chickens/49-d trial	Four treatment groups: control group – basal diet (plus anticoccidial vaccine), control group – basal diet (plus coccidiostat), prebiotic group – diet with 0.05% of yeast hulls (a concentrate of yeast hulls obtained via autolysis of *Saccharomyces cerevisiae*) plus anticoccidial vaccination, prebiotic group – diet with 0.05% of yeast hulls plus coccidiostat	↑ ADG* and ↓ FCR*, ↑ local mucosal IgA secretions**, ↑ tracheal IgA*, ↓ parasite excretion in faeces** by supplementation with *S. cerevisiae*	Gómez--Verduzco et al., 2009
2-wk-old piglets (Polish Landrace × Pietrain)/70-d trial	Four treatment groups: control group – basal diet, MOS group – basal diet supplemented with a yeast cell wall preparation (3 g Bio-Mos on kg diet), FOS group – basal diet with couch grass (5 g meal per kg diet)	↓ losses in the period of 1-28 days* ↑ BW on d 84 of age*, → FCR, → WBC and leucogram by supplementation with MOS or FOS	Grela et al., 2006
1-d-old Cobb 500 male broiler chickens/6-wk trial	Four treatment groups: control group – basal diet, inulin groups – basal diet supplemented with 5, 10, or 15 g/kg of inulin	→ BWG and FCR, ↑ feed intake**,↑ IgA in cecal** and mucin mRNA expression in jejunum tissue** at d 21, ↓ IL-6** and IFN -γ* in ileum tissue by inulin supplementation	Huang et al., 2015

Table 1 – contd.

1	2	3	4
10 to 12-d-old piglets (PIC hybrid line × Penarlan P76)/84-d trial	Five treatment groups: control group – basal diet, inulin groups – basal diet supplemented with 20 g of aqueous or of aqueous-alcoholic extracts of inulin, or 40 g of dried artichoke tubers or of dried chicory root	↑ ADG* and ↑ FCR* in group supplemented aqueous-alcoholic extracts of inulin, dried artichoke tubers, and dried chicory root compared to the control, ↑ IgA and IgG concentrations * in the group supplemented extracts of inulin compared to the control	Grela et al., 2014

ADG, average daily gain; BW, body weight; C, complement; CD_3+, chicken T cells; CD_4+, chicken T helper lymphocytes; CD_8+, chicken cytotoxic T lymphocytes; CFU, colony-forming units; FCR, feed conversion ratio; IFN, interferon; IL, interleukin; LYZ, lysozyme; pBD porcine beta-defensins; SRBC, sheep red blood cell; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumour necrosis factor; WBC, white blood cell counts; ↑, increase; →, no significant differences; ↓, decrease; *$P<0.05$; **$P<0.01$.

Probiotics are applied for stimulation of immune response in pigs (Table 1). In the investigations conducted by Deng et al. (2013), supplementation of *Bacillus subtilis* and *Lactobacillus salivarius* in neonatal piglets stimulated intense mucosal immunity in the duodenum and ileum. Moreover, different species of probiotics may have different immunomodulatory activities attributed to their ability to induce cytokine production, which leads to modulation of innate and adaptive immune responses (Brisbin et al., 2010). Lähteinen et al. (2014) applied *Lactobacillus brevis* in recently weaned piglets and detected expression of certain cytokines in the intestinal mucosa. Wang et al. (2009) used a *Lactobacillus fermentum* strain and reported similar results in weaned pigs with or without *E. coli* challenge. Probiotics enhanced T cell differentiation and induced ileum cytokine expression.

Prebiotics

Prebiotic compounds are represented by agarooligosaccharides (AOS), arabinoxylans, cyclodextrins, FOS, inulin, isomaltose, lactose (for poultry), lactulose, MOS oligofructose, raffinose, and stachyose, xylooligosaccharides (XOS), β-galactooligosaccharides (GOS) (Patterson, 2005).

MOS and FOS are the major prebiotics used in poultry and pig nutrition. MOS and FOS have been applied to increase farm animal body weight gain, feed efficiency, energy utilization, and gut microbiota population as well as decreased serum cholesterol levels (Grela et al., 2001, 2006; Nabizade, 2012; Fallah and Rezaei, 2013; Lindberg, 2014; Duan et al., 2016).

MOS, indigestible to non-ruminant animals, derived from the cell wall of *Saccharomyces cerevisiae* yeast have been shown to improve animal performance and health through several mechanisms such as prevention of pathogen binding to the gastrointestinal tract (GIT), alteration of GIT microbial populations, and enhancement of immune functions (Che, 2010). There are several mechanisms responsible

for the beneficial effects of MOS. They have also been linked with improved gut health and promotion of mucosal immunity reflected in increased numbers of goblet cells and villus length, increased populations of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria* in the guts of monogastric animals, and reduced populations of *Escherichia coli* and *Salmonella* (Baurhoo et al., 2009; Brümmer et al., 2010; Lindberg, 2014; Andrés-Barranco et al., 2015; Spring et al., 2015). Various studies indicate that *Lactobacilli* and *Bifidobacteria* can increase the synthesis and secretion of mucin in the gut (Smirnov et al., 2005). The immunomodulating effects of yeast cell wall polysaccharides are associated with the ability to stimulate cytokine production by macrophages as well as an improved humoral immune parameter (immunoglobulin) and innate (non-specific) immune factors (lysozyme and complement) (Gómez-Verduzco et al., 2009; Spring et al., 2015; Duan et al., 2016). In the investigations conducted by Che et al. (2012), inclusion of MOS in the diet of growing pigs exerted a beneficial effect on growth efficiency and concentrations of antibodies and inflammatory mediators in pigs that were experimentally challenged with porcine reproductive and respiratory syndrome virus (PRRSV).

On the other hand, prebiotics have been indicated to enhance the immune response of broilers and pigs, resulting in rapid clearance of pathogens from the gut (Kim et al., 2011; Werner et al., 2014). With the immune-enhancing effect of prebiotics, this may be due to a direct interaction between prebiotics and gut immune cells as well as due to an indirect action of prebiotics via preferential colonization of beneficial microbes and microbial products that interact with immune cells. Administration of slight amounts of MOS (0.05%) to feed mixes increased mucosal IgA secretions and humoral and cell-mediated immune responses of neonatal chicks (Table 1) (Gómez-Verduzco et al., 2009).

FOS cause an increase in the growth of *Lactobacillus plantarum*. The highest antibacterial activity is exhibited by *Lactobacillus plantarum* strain LE5 against *Listeria monocytogenes* and *Enteroccocus faecalis*, whereas *Lactobacillus plantarum* strain LE27 has additional strong antibacterial activity against *Escherichia coli* and *Salmonella enteritidis* (Munoz et al., 2012). Prebiotics are also degraded by other gut commensal microbiota such as *Faecalibacterium prausnitzii* and the end product of fermentation is butyrate (Ramirez-Farias et al., 2009). On the other hand, by reducing the pathogen load in the gut, prebiotics modify immune parameters in the host by modulating the composition and functionality of microbiota (Roberfroid et al., 2010).

Inulin is another health-enhancing feed additive in monogastric animal nutrition. It has a multidirectional impact on the alimentary tract microbiome. In the gastrointestinal tract, inulin is a hydrolysis and fermentation substrate for the beneficial intestinal microbiome and increases the abundance of bacteria, primarily from the genus *Bifidobacterium* (Gibson, 1998) and some *Lactobacillus* species (Han et al., 2014). It has been shown that this long-chain fructan is useful for improvement of serum lipid profiles (Grela et al., 2014; Sobolewska et al., 2014), stimulation of the animal immune system (Grela et al., 2014; Vos et al., 2007), intensification of productivity (Grela et al., 2014; Samolińska and Grela, 2017), and increasing the bioavailability of minerals, including zinc, iron, and copper, which exert an effect on the function of

the immune system (Yasuda et al., 2006; Samolińska and Grela, 2017). This fructan has a positive impact on the immune system by stimulation of the production of cytokines, mononuclear cells, and phagocytising macrophages and by induction of immunoglobulin synthesis, especially IgA (Macfarlane and Cummings, 1999; Watzl et al., 2005; Vos et al., 2007). This has been confirmed by the research conducted by Huang et al. (2015), where the results indicate that dietary inulin at the levels of 5–10 g/kg may enhance intestinal immune function of younger broiler chicken when the intestinal function is not fully developed. Such a beneficial effect of supplementation with inulin mixtures on the production and resistance indices in piglets were reported by Grela et al. (2014) as well (Table 1).

Phytobiotics

Phytobiotics are plant-derived natural bioactive compounds used in animal nutrition as alternatives to antibiotic growth promoters and added to the feed to enhance the performance in animals (Windisch et al., 2008; Puvača et al., 2013). Phytobiotics include herbs, botanicals, essential oils, and oleoresins. The active compounds of phytobiotics are terpenoids (mono- and sesquiterpenes, steroids, etc.), alkaloids (alcohols, aldehydes, ketones, esters, ethers, lactones, etc.), glycosides, and phenolics (tannins). There are many variations in the composition of phytobiotics due to the biological factors (plant species, growing location, and harvest conditions), manufacturing (extraction, distillation, and stabilization), and storage conditions (light, temperature, oxygen tension, and time) (Huyghebaert et al., 2011). The mechanisms of the action of herbs have been identified to include alteration of the gastrointestinal functions with consequent effects on herb absorption, induction, and inhibition of metabolic enzymes and transport proteins, and alteration of renal excretion of herbs and their metabolites (Fasinu et al., 2012).

On the other hand, the possible mechanisms of the growth-promoting herb action in the animal include changes in the intestinal microbiota, increased nutrient digestibility and absorption, enhanced nitrogen absorption, improved immune response, and antioxidant activity. Application of phytobiotics in animals can contribute to morphological and histological modifications of the gastrointestinal tract. Elongation of villi and deepening of intestinal crypts has been observed, as well as activation of toll-like receptors, luminal capture by dendritic cells, or stimulation of epithelial cells and release of proinflammatory cytokines in the mucosa (Kumar et al., 2014). Terpenes and phenols represent the most biologically active components. The mechanism of the action of these compounds consists in causing damage to the glycolipid walls of bacterial cells, which leads to leakage and reduction of cytoplasmic compositions (Iranparast et al., 2014); therefore, plant herbs possess strong antimicrobial activity especially against Gram (−) and Gram (+) bacteria (Bakht et al., 2013; Al-Mariri and Safi, 2014).

Phytogenic effects have been proven in poultry and pigs for feed palatability and quality, growth promotion (improved weight gain and feed conversion ratio), gut function, endogenous enzyme secretion and nutrient digestibility (improved growth), gut microbiota (improved growth, reduced mortality), and immune func-

tion (improved health) (Yang et al., 2009; Grela et al., 2013; Liu et al., 2013, 2014; Yazdi et al., 2014; Kiczorowska et al., 2016 a, b).

The immune system generally benefits from herbs and spices rich in vitamin C, carotenoids, and flavonoids. Herbs containing molecules that possess immunostimulatory properties are echinacea (*Echinacea* Moench), liquorice (*Glycyrrhiza* L.), and garlic (*Allium sativum* L.). In addition, these plants can improve the activity of lymphocytes, macrophages, and natural killer (NK) cells; they increase phagocytosis or stimulate interferon synthesis (Frankic et al., 2009).

Garlic is one of the phytobiotics frequently used in animal nutrition. It is successfully applied in poultry production. The plant exhibits very potent antiviral, bactericidal, antifungal, and antiparasitic properties (Daka, 2013; Gautam and Garg, 2013). It is effective in the fight against fungal infections of the skin and mucous membranes of the gastrointestinal and respiratory tracts and mobilizes the immune system in birds (Pourali et al., 2010; Toghyani et al., 2011). Many investigations in this field indicate that supplementation of feed mixes with 0.5–3% of garlic can be used for enhancement of intestinal health status, increased energy and nutrient utilization, and stimulation of broiler growth (Mahmood et al., 2009; Elagib et al., 2013). It was found in the investigations carried out by Hanieh et al. (2010) that addition of garlic or onion to broiler chicken mixes has potential to enhance the immune functions (Table 2). Similar production and health effects have been reported in the case of swine nutrition. Upon supplementation of fattener mix with garlic, Grela et al. (2013) noted not only improved animal health status but also higher quality of produced meat. Blood test results of sows fed garlic-supplemented mixes prove that the additive can be a valuable alternative to antibiotic growth promoters in complete mixes for growing pigs (Czech et al., 2009). Liu et al. (2013, 2014) found that feeding weaned pigs with plant extracts (capsicum oleoresin, garlic botanical, or turmeric oleoresin) induced enhanced expression of genes associated with immune responses and reduced diarrhoea and inflammation caused by *E. coli* infection (Table 2).

The popular turmeric (*Curcuma longa* L.) spice is also commonly used as a feed additive in animal production. Its activity of intestinal inflammation alleviation and potent antiviral, antibacterial, and antifungal activity are successfully used in large-scale production, as they improve animal health status and stimulate greater production efficiency. Good production results, i.e. improved FCR, were obtained upon supplementation of broiler feed mixes with 0.9% of turmeric (Ahmadi, 2010) and 0.5% of the additive in the case of laying hen feed (Radwan et al., 2008). As shown in the study conducted by Liu et al. (2014), supplementation of piglet feed with 10 g of turmeric oleoresin/kg resulted in a significant increase in the expression of 327 genes, including those related to immune responses. Turmeric oleoresin upregulated the expression of genes related to the recruitment of neutrophils, the complement system and its regulatory proteins, chemokines, cytokines, and antigen processing and presentation. These results support the usefulness of turmeric supplementation to enhance the gut mucosal immunity of weaned pigs (Table 2).

Table 2. Studies using phytobiotics as immunostimulants in monogastric animals

Animals/time on treatment	Dietary information	Major findings	Reference
1	2	3	4
One-wk-old White Leghorn broiler chickens/9-wk trial	Four treatment groups: control group – basal diet, garlic groups – basal diet supplemented with 10 g or 30 g garlic, onion groups – basal diet supplemented with 10 g or 30 g onion, Chickens were immunized with NDV, SRBC and BA	→BW, ↑ ND*, SRBC* and BA* antibody production in immunized chicken supplemented with 10 g phytobiotics/ kg diet compared to the control, ↓ $CD4^{+}$* and ↑ $CD4^{-}CD8^{-}$lymphocyte ratios* in groups with 30 g inclusion of phytobiotics compared to the control, ↑ weight of the lymphoid organ* in groups supplemented with garlic compared to the control, → WBC by phytobiotic treatment	Hanieh et al., 2010
21-d-old weaned pigs (G performer × Fertilium 25)/11-d trial	Eight treatment groups: groups with or without an F-18 *Escherichia coli* challenge, groups supplemented or not with 10 mg/kg of capsicum oleoresin, garlic botanical, or turmeric oleoresin	↑ ADG*, and ↓ ileal macrophages* and ↑ neutrophils*(d 0 to 5), and ↓ diarrhoea score* and ↓frequency of diarrhoea* in groups supplemented plant extracts, compared with the control, → growth performance, ↓ diarrhoea score* and ↓ frequency of diarrhoea*, ↓ haptoglobin*, WBC*, ↓ ileal macrophages* and ↑ neutrophils* in the challenged groups, feeding plant extracts compared with the control	Liu et al., 2013
21-d-old weaned pigs (G performer × Fertilium 25)/9-d trial	Four treatment groups: control group – basal diet, plant extracts groups – basal diet supplemented with 10 mg/kg of capsicum oleoresin, garlic botanical, or turmeric oleoresin	↑ expression of genes related to immune response* in groups supplemented plant extracts, compared with the control	Liu et al., 2014
1-d-old broiler chickens	Four treatment groups: control group – without supplementing drinking water, ginger groups – drinking water supplemented with 30, 40 and 50 ml/l of ginger extract	↑ ADG*, → antibody titre against IBD and ND in supplemented groups, compared with the control	Arshad et al., 2012
Pregnant sows (Landrace × Yorkshire)/ from d 30 before their expected farrowing date until d 28 postpartum	Three treatment groups: control group – basal diet, ginger groups – basal diet supplemented with 0.25% or 0.5% g ginger extract	↑ IgG* concentrations in the plasma of sows and piglets and in colostrum at 0.5% ginger extract supplementation compared to the control, ↑ BW* of piglets (0 d postpartum) from ginger extract-fed sows compared to the control	Lee et al., 2013

Table 2 – contd.

1	2	3	4
1-d-old Lohman male broiler chickens/6-wk trial	Four treatment groups: control group – basal diet without vaccines, basal diet + vaccine group – basal diet with vaccination against ND, IB, and IBD, basal diet + medicinal plants group – basal diet supplemented with 2.0% crushed anise, nigella seeds, and thyme leaves mixture (1:1:1), basal diet + medicinal plants + vaccine group – basal diet supplemented with medicinal plants and vaccinated against ND, IB, and IBD	↑ BW*, ADG*, ↓ FCR* from d 1-21 and ↓ mortality* from d 1-42 in the basal diet + medicinal plants group, ↑ BW*, ADG*, ↓ FCR* from d 1-21 and from d 1-42, and ↓ mortality* from d 1-42 in the basal diet + medicinal plants + vaccines group, ↑ Antibody titre against ND*, IB*, and IBD* at d 21 and 42* in the basal diet + medicinal plants group, and the basal diet + medicinal plants + vaccines group	Al-Beitawi et al., 2010
1-d-old Ross broiler chickens/6-wk trial	Four treatment groups: control group – basal diet, echinacea groups – basal diet supplemented with 0.1% or 0.5% echinacea root powder with short (1 wk) and long (6 wk) term application	↑ FCR*,↑ WBC*, number of lymphocytes* and heterophils*, and antibody titres: ND* and avian influenza diseases* in echinacea groups with long term application	Dehkordi et al., 2011
32 to 54-wk-old white layers (LSL)/24-wk trial; fattening pigs/ 19-d trial	Five treatment groups of laying hens: control group – without administration of echinacea juice, echinacea groups – vaccinated against ND, and IB laying hens were administered 0.25 ml/kg of $BW^{0.75}$ ethanolic formulation of echinacea juice or fermented juice, with 2 or 5 days of application, Three treatment groups of fattening pigs: control group – without administration of echinacea juice, echinacea groups – were periodically (5 d) administered 2.5 ml/kg feed of ethanolic formulation of echinacea juice or fermented juice.	→performance of the hens by echinacea treatment, ↑ number of lymphocytes* in the hen group receiving ethanolic echinacea juice with 5 days of application, ↑ ND-, and IB-antibody titres* in the hen group receiving fermented juice for 2 days, ↑ WBC* and number of lymphocytes* in the pig group receiving ethanolic echinacea juice, ↑ phagocytosis rate** in the pig by echinacea treatment	Böhmer et al., 2009
1-d-old Ross 308 broiler chickens/6-wk trial	Four treatment groups: control group – basal diet, resin groups – basal diet supplemented with three levels of *Boswellia serrata* resin (1.5, 2.0, and 2.5%)	↑ FCR* and improved structure of the jejunal wall* in groups supplemented with 2.0, and 2.5% resin, ↑ *Lactobacillus** and *Enterococcus** in groups supplemented with 2.5% resin	Kiczorowska et al., 2016 a

Table 2 – contd.

1	2	3	4
1-d-old Ross 308 broiler chickens/6-wk trial	Four treatment groups: control group – basal diet, resin groups – basal diet supplemented with three levels of *Boswellia serrata* resin (3, 4 and 5%)	↑ FCR*, jejunum and duodenum length, improved structure of the jejunal wall* in groups supplemented with 3, and 4% resin, ↑ *Lactobacillus** and *Enterococcus**, and *Bifidobacterium* sp. in groups supplemented with 3, 4 and 5% resin ↓*Escherichia coli** and *Clostridium perfringers** in groups supplemented with 4 and 5% resin	Kiczorowska et al., 2016 b
1-d-old male Arbor Acres broiler chickens/6-wk trial	Two treatment groups: control group – basal diet, alfalfa group – basal diet supplemented with 0.06% extract from alfalfa	→ BW, FCR,↑ thymus, spleen and the bursa weight*, ↑ proliferation of T* and B lymphocytes*,↑serum ND* hemagglutination inhibition antibody titre* by the extract alfalfa treatment	Dong et al., 2007

ADG, average daily gain; BA, *Brucella abortus*; BW, body weight; CD_4+, chicken T helper lymphocytes; CD_8+, chicken cytotoxic T lymphocytes; FCR, feed conversion ratio; Hb, haemoglobin; IB, infectious bronchitis; IBD, infectious bursal disease; ND, Newcastle disease; RBC, red blood cell counts; SRBC, sheep red blood cell; TNF, tumour necrosis factor; WBC, white; ↑, increase; →, no significant differences; ↓, decrease; *P<0.05; **P<0.01.

Similarly, ginger rhizomes (*Zingiber officinalis*) used in broiler diet have been reported to stimulate growth performance and health. The application of ginger and cinnamon at a level of 0.8% of feed mixes significantly improves the health parameters in birds: it reduces the serum level of low-density cholesterol lipoproteins and increases the haemoglobin level and red blood cell counts (RBC) (Ademola et al., 2009). The authors suggest that this type of nutrition may be an effective alternative to virginiamycin with respect to the feed efficiency and health parameters. Similar results presenting improved immunity and growth performance of commercial broiler chicks were reported by Arshad et al. (2012) in their study based on application of ginger extracts in drinking water (Table 2). Inclusion of ginger in sow nutrition also produced favourable results. Upon addition of ginger extracts to sow mixes, Lee et al. (2013) observed enhanced immune function of piglets by improving the level of immunoglobulin in the sow colostrum (Table 2).

Furthermore, thyme (*Thymus vulgaris*), nigella (*Nigella sativa*), or anise (*Pimpinella anisum*), which are commonly used as spices, increase poultry productivity and resistance (Table 2) (Al-Beitawi et al., 2010). This was confirmed in the investigations of broiler chickens conducted by Yazdi et al. (2014), in which addition of anise seeds at a level of 10 g/kg of diet increased the antibody titre against avian influenza virus. A positive effect of supplementation of mixtures for laying hens with black cumin was observed by Khan et al. (2013). The additive increased hen productivity, egg weight, and egg shell quality. Moreover, there was a reduced low-density lipo-

protein (LDL) cholesterol concentration in the serum and yolk and enhanced birds' resistance to Newcastle disease virus.

The purple coneflower (*Echinacea purpurea*), i.e. one of the most important and popular herbs with an immunostimulating effect, is used both in human medicine and as a feed additive in animal production. In their investigations of this phytobiotic, Dehkordi et al. (2011) reported results suggesting that feeding with *Echinacea purpurea*, particularly for a long time, may improve performance and enhance immunity response in chicken broilers (Table 2). Böhmer et al. (2009) also noted that repeated short-time application of *Echinacea* juice has immune stimulating effects in laying hens and fattening pigs (Table 2). A study carried out by Kuhn et al. (2005) revealed an immune stimulating effect of *Echinacea* on sows and their offspring. In one-day-old piglets, the concentration of immunoglobulins G and A (IgG and IgA) was significantly higher than in the control. The investigations showed a decreasing trend in the health-enhancing effect of the *Echinacea* addition up to day 70 of piglets' life, but this did not affect weight gain and carcass quality.

In animal production, nutritionists and breeders commonly use herbal medicines that have already been tested in alternative human medicine. Examples of such plants comprise *Boswellia serrata* and *Commiphora mukul*, trees from the family *Burseraceae* growing in Africa and Asia. They are an important element of ayurvedic medicine. Their resin contains a number of bioactive compounds with a wide range of activity, i.e. anti-inflammatory, antiseptic, analgesic, antibacterial, hypolipidemic, hypocholesterolaemic, immunomodulatory, and antiproliferative action (Van Vuuren, 2008; Singh et al., 2015; Kiczorowska et al., 2016 a, b; Al-Yasiry and Kiczorowska, 2016). Literature provides very few reports on the use of these plants as additives for increased productivity and animal health. In a study conducted by Iranparast et al. (2014), supplementation of broiler diets with *Commiphora mukul* resin had significant effects on daily weight gain, feed conversion, and feed intake during the growth period (22–42 days). The addition of the phytobiotic had a significant effect on the total cholesterol level, total antibody titres, and immunoglobulin Y (IgY). However, there was no significant effect of the supplementation on immunoglobulin M (IgM) production. In turn, supplementation of broiler mixes with *Boswellia serrata* resin stimulated production performance in broiler chickens and exerted a beneficial effect on intestinal microflora and morphology (Kiczorowska et al., 2016 a, b) (Table 2), which was confirmed in other studies (Tabatabaei, 2016).

Similarly, the alfalfa (*Medicago sativa*) representing the legume family exhibits a multidirectional impact on the organism, i.e. it supports cleansing, detoxification, and nutrient intake and absorption and stimulates the immune system, which is associated with its rich chemical composition. It contains such valuable phytochemical compounds as alpha-carotene, beta-carotene, beta-sitosterol, chlorophyll, coumarin, cryptoxanthin, daidzein, fumaric acid, genistein, limonene, lutein, saponins, stigmasterol, and zeaxanthin (Balch and Balch, 2000; Avato et al., 2006). The available literature shows that the effect of alfalfa supplementation on animal resistance indicators has been confirmed in several studies. Dong et al. (2007) reported that addition of alfalfa extract to broiler chickens mixes improved the immune response (both humoral immunity and cell-mediated immunity) without an adverse effect on the

performance (Table 2). A similar effect of alfalfa supplementation on the swine immune system was reported by Wang (2007). Similarly, the investigations conducted by Pietrzak and Grela (2015) confirmed the positive effect of alfalfa extract on the increase in total white blood cell counts (WBC) and lymphocyte count in fatteners. The researchers suggest that this is associated with the presence of saponins, which may stimulate the immune system to produce an array of antigen-specific and non-specific immune response (Chavali and Campbell, 1987). As shown by Maharaj et al. (1986), one mode of saponin action includes increased permeability of the intestinal mucosa, allowing increased uptake of viral antigens.

The use of fungal additives in animal production yields an immune stimulating effect. Addition of champignon (*Agaricus bisporus*) at 5% in broiler diets at 49 days of age increased chicken body weight (Willis et al., 2013). Already 3% of the additive in feed led to increased antibody titre against Newcastle disease (ND) and enhanced antibody titre against sheep red blood cell (SRBC) (Kavyani et al., 2012). Addition of the *Flammulina velutipes* (enokitake) mushroom to swine nutrition improved their productivity and enhanced immune response, which was related to an increase in the amounts of beneficial *Bifidobacterium* and *Lactobacillius* bacteria in the intestines and reduction of pathogenic bacteria (*Clostridium,* enterobacteria) (Jiang, 2015).

The differences in the results may be attributed to numerous factors, e.g. the type and part of plant used, harvest time, phytogenic additive preparation methods, and herbal extraction methods (Yang et al., 2009).

Conclusion

Alternative growth promoters like probiotic, prebiotic, and phytobiotic additives are immune enhancers. By increasing the growth of beneficial microbes or by reduction and removal of potential pathogens, the alternatives to feed additives can possibly improve the health and performance of monogastric animals. Particularly good effects in stabilization of the intestinal microflora are achieved by supplementation of feed rations for monogastric animals with probiotic *Lactobacillus* and *Bifidobacterium* strains, while phytobiotics such as garlic, turmeric, echinacea, and *Boswellia serrata* resin exhibit potent antibacterial activity. However, their effects on gut microbiota interact with digestive physiology and thus growth in many complex ways, which can be further influenced or even determined by many other factors such as compatibility between the diet and alternative hygiene standards and animal husbandry practices. Immunomodulatory feed additives, i.e. *Lactobacillus* and *Saccharomyces* strains, MOS and inulin prebiotics, and ginger, anise, nigella, and thyme phytobiotics mobilize humoral and cell-mediated immune response. Enhancement of the beneficial intestinal microbiome and alleviation of mucosal inflammation result in improvement of the morphological structure of the gastrointestinal tract and strengthening of the intestinal barrier. Good results are mainly achieved by supplementation with MOS and phytobiotics: botanical garlic, turmeric, and *Boswellia serrata* resin. In this context, probiotics, prebiotics, and phytobiotics are a powerful strategy for manipulating the microbial composition and immune responses of the host.

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