

## **THE USEFULNESS OF PREBIOTICS AND PROBIOTICS IN MODERN POULTRY NUTRITION: A REVIEW\***

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### **Abstract**

**A probiotic is a culture of live microorganisms that can manipulate and maintain a beneficial microflora in the gut. Prebiotics are nondigestible feed ingredients that can positively affect the animal organism by stimulating the activity and growth of beneficial native bacteria in the gastrointestinal tract and eliminate the pathogenic ones. Some studies have shown their beneficial effects when they have been used separately or simultaneously in the form of synbiotics, to obtain enhanced mutual effect. These supplements were proposed with success as alternatives to antibiotic growth-promoting feed additives but further studies are needed to better understand their mode of action and effects. This review article presents growing interest in using these antibiotic alternatives, the potential mechanism of their action in the live organism, and discusses some recent data on the effects of these supplements in poultry nutrition.**

**Key words: probiotic, prebiotic, synbiotic, poultry, laying hens, broilers**

Besides quality attributes, special attention in recent years has been paid by the consumers to safety of animal products. Considering some evidence that the use of antibiotic growth promoters (AGP) may cause pathogen resistance (Phillips et al., 2004), the application of antibiotics as animal growth enhancers had already been prohibited in the European Union since 2006. Today's animal farming, especially the poultry industry have been greatly intensified with respect to both large number of animals and modern feeding systems. Concerns about the losses in animal performance and thus sustainability of production and its profitability coupled with this ban have led to an increase in research on the alternative supplements to AGP and strategies for food-producing animals.

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To date a number of products, including essential oils and plant extracts, spices, organic acids, probiotics and prebiotics have been recognized and proposed as antibiotic alternatives in farm animal nutrition. Although most of them have generated attention, extensive studies are primarily focused on prebiotics and probiotics. Probiotic means “for/in favour of life”. This term was introduced into the literature by Lilly and Stillwell (1965). It contrasts with the term antibiotic, which means “killing life”.

Today, the most accepted definition states that probiotics are mono or mixed cultures of live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002).

Unlike probiotics, prebiotics are not microorganisms – they are a sort of nourishment source for existing flora, allowing the natural colony of gut to grow naturally and replicate. Prebiotics were defined as non-digestible food (feed) ingredients that beneficially affect the host by selectively stimulating the growth and/or activities of one or a limited number of bacteria in the gut, thereby improving host health (Gibson and Roberfroid, 1995). However, more recent definitions stated that a prebiotic is a selectively fermented ingredient that allows specific changes, in both the composition and activity in the gastrointestinal microbiota which confers benefits to the host (FAO/WHO, 2002). The common point of these definitions is that prebiotics are characterized by a selective effect on the microbiota, and thus can improve the host health. Prebiotics include mainly oligosaccharides, sugar molecules of three to six chains, and soluble fibre. These carbohydrates are found naturally in fruit and vegetables (Charalampopolus and Rastall, 2009).

Nutritional supplements combining probiotics and prebiotics are referred to as synbiotics, which are a combination of “a probiotic and a prebiotic that beneficially affects the host by improving the survival and establishment of live microbial dietary supplements in the gastrointestinal tract” (Trachoo et al., 2008). The main importance of this form of synergism is that a probiotic alone, i.e. without a source of nourishment which can be represented by a prebiotic, cannot survive well in the digestive system (Bhupinder and Saloni, 2010). Synbiotics are gaining popularity and scientific credibility as functional food (feed) supplements at nutritional and therapeutic levels. It is believed that they can ensure a high level of viable probiotic cells once ingested (Trachoo et al., 2008). Some studies have shown the importance and benefits of this kind of synergy between probiotics and prebiotics and the effectiveness in helping young animals to achieve better growth performance (Patterson and Burkholder, 2003).

### **Aims of the use of probiotics, prebiotics and synbiotics as feed supplements** ***Pathogenic bacteria control***

Many studies have focused on the ability of probiotic additives to reduce and control pathogenic bacteria. Probiotics can provide antimicrobial substances that may be effective at the same level as antibiotics, especially in stress conditions, high temperature and abnormal intestinal pH. According to Charalampopolus and Rastall (2009) probiotics show high efficiency in reducing colonization of *Salmonella* and *Campylobacter*. Moreover, they can modulate immunological response

and suppress inflammatory immune reactions in the intestinal walls preventing tissue damage (Ferreira et al., 2011).

Prebiotics containing xylose, fructose, galactose, mannose and glucose, earned much attention and appear to be particularly promising (Gibson and Roberfroid, 1995; Patterson and Burkholder, 2003). Some of them have proved the protection against *Salmonella* (Charalampopolus and Rastall, 2009) by providing binding sites for pathogenic bacteria flushing out of the digestive tract. Spring et al. (2000) screened some bacterial strains for their ability to agglutinate mannanoligosaccharides in yeast cell preparations (*Saccharomyces cerevisiae*). Five of seven strains of *E. coli* and 7 of 10 strains of *Salmonella typhimurium* and *Salmonella enteritidis* agglutinated MOS and *S. cerevisiae* cells.

### ***Improved health and production performance***

Many beneficial effects of probiotics were suggested, such as improved immune system, modification of gut microbiota, reduced inflammatory reactions, decreased ammonia and urea excretion, lower serum cholesterol, and improved mineral adsorption; on the other hand probiotics may have an indirect positive impact on performance parameters and production profitability (Ferreira et al., 2011).

A number of studies have shown improvements in growth performance, decreased mortality and morbidity or increased resistance to colonization by pathogens associated with feeding prebiotics. Numerical improvements in performance may be economically important on large-scale production farms (Patterson and Burkholder, 2003).

### ***Reduced antibiotic use in animal agriculture***

Pro-, pre- and synbiotics have been studied for their potential to replace antibiotics, as the latter contribute to the acquisition of resistance in the bacterial flora of livestock. The desired effects on animals were represented by maintaining high growth performance, particularly in poultry and swine, or diminution of methane production by ruminants (Charalampopolus and Rastall, 2009). The use of probiotics and prebiotics for long-term consumption and prophylactic approaches is much more safe as they do not cause side effects, such as antibiotic associated diarrhoea, sensitivity to UV radiation or liver damage, and do not stimulate antimicrobial resistance genes and allergic inflammatory response (Ferket, 2003; Lee and Salminen, 2009). In spite of promising results in preliminary studies, further research is needed to create an overall management strategy to match the performance efficacy comparable to that of antibiotics (Patterson and Burkholder, 2003).

### **Probiotics**

Probiotic is a culture of living microorganisms that are used as functional ingredients to manipulate and maintain good health by controlling gut microflora and increasing digestive enzyme activity. Probiotics were defined by Fuller (1992) as “live microbial food supplements which beneficially affect the host either directly or indirectly by improving its intestinal microbial balance”.

The probiotic microorganisms can have an effect only by surviving the digestive conditions, including bile acids, and these must facilitate their colonization of the

gastrointestinal tract without any harm to the host. However, only certain strains of microorganisms have these properties. Most probiotic active cultures are members of two bacterial genera: *Lactobacillus* and *Bifidobacterium*, or belong to yeasts, especially *Saccharomyces* (Charalampopolus and Rastall, 2009).

### ***Characteristics of ideal probiotics and their mode of actions***

Probiotics display several important ways of action, an antagonistic action towards pathogen bacteria by modification of gut pH, direct antimicrobial effect by secretion of products which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide, production of short chain fatty acids (SCFA) in the intestine, regulation of the immune system of the host, normalization of gut microbiota, and different metabolic effects (Vamanu and Vamanu, 2010; Ferreira et al., 2011). Another mode of action is competitive exclusion which represents colonization ability and adhering competition in the intestinal mucous membranes to prevent adhesion and invasion of pathogens and, which is a key performance parameter, inhibition of their colonization and replacement of already adhered ones; competing for available nutrient substances and growth factors (Patterson and Burkholder, 2003). A detailed list of ideal probiotics characteristics and their beneficial effects are presented in Table 1.

Table 1. Characteristics of ideal probiotics and their desirable properties (adapted from Simmering and Blaut, 2001 after Patterson and Burkholder, 2003)

Probiotics	
Properties	Positive influences
Belong to host origin	Change intestinal microbiota
Non pathogenic	Induce immune system
Resist processing and storage	Decrease inflammatory reactions
Endure gastric acid and bile	Prevent pathogen proliferation
Epithelium or mucus attaching capability	Enhance animal performance
Persist in the intestinal tract	Reduce carcass contamination
Produce repressive compounds	Decrease ammonia and urea excretion
Immune response regulation	
Modify microbial activities	

### ***Most used probiotic genera***

A variety of microbial species have been used as probiotics with nearly 20 known species, which beneficially affect the host by improving its intestinal microbial balance (Bhupinder and Saloni, 2010). Various types of probiotic bacteria include species of *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, other bacteria and a kind of yeast species, and indefinable mixed cultures. *Lactobacillus* and *Bifidobacterium* species have been used above all in humans, whereas species of *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast have been the most predominant organisms used in livestock (Ferreira et al., 2011). However, recently, enhanced performance was noticed when feeding *Lactobacillus* to livestock (Vicente et al., 2007; Vicente et al., 2008).

These bacteria species are often grouped according to their common metabolic, morphological and physiological characteristics rather than a phylogenetic class. This entails bacteria known as lactic acid bacteria (LAB). LAB includes species of *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus* and *Leuconostoc* genera. Recent taxonomic adjustments have proposed several new genera and the rest of the group is now represented by the following: *Aerococcus*, *Alloiooccus*, *Carnobacterium*, *Dolosigranulum*, *Enterococcus*, *Globicatella*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*. *Lactobacilli*, *Carnobacteria* and some *Weissella* are rods while the rest of genera are cocci (Jin et al., 2009). Classification of LAB genera was based on morphology, mode of glucose fermentation, growth at certain temperatures, and range of sugar utilization. These bacteria are gram-positive, nonsporulating, non-respiring cocci or rods, which do, through fermentation of carbohydrates, produce lactic acid as their major end product (Kolida and Gibson, 2011). Practically, the major part of the antimicrobial activity demonstrated by probiotics can be assigned to the production of lactic acid (Makras et al., 2006). The remaining unlisted probiotic microorganisms belong to the non-lactic acid bacteria like *Bifidobacterium* species or *Saccharomyces* yeast.

## **Prebiotics**

### ***Most used prebiotics***

Various types of oligosaccharides, including inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), soya-oligosaccharides (SOS), xylo-oligosaccharides (XOS), pyrodextrins, isomalto-oligosaccharides (IMO) and lactulose, are commonly considered as prebiotics. But the majority of studies completed to date point to inulin, FOS and GOS (Macfarlane et al., 2008). An array of prebiotics exists and the most reported in a large number of studies and with the most consistent evidence accumulated for the effects of prebiotics have been non digestible oligosaccharides (NDOs), which results from various origin and chemical properties (Chen and Chen, 2004; Macfarlane et al., 2008; Zduńczyk et al., 2011). The regulatory regimes for NDOs have been under active review in many countries in recent years. Fructooligosaccharides, and the polyfructan inulin, galactooligosaccharides, lactulose and polydextrose are recognized as the established prebiotics, whereas lactosucrose, xylo-, isomalto-, and soybean-oligosaccharides are categorized as emerging prebiotics (Piva, 1998). To date, only three oligosaccharides: fructans (inulin and fructooligosaccharides), galactooligosaccharides and lactulose have accomplished prebiotic status in the European Union (Kolida and Gibson, 2011).

Animal feeding and *in vitro* trials data showing possible bifidogenic effects have been indicated for gluco- and galactomannan oligosaccharides, alpha-glucosaccharides, pectic-oligosaccharides, gentiooligosaccharides, and oligosaccharides from agarose (Cao et al., 2005; Macfarlane et al., 2008; Zduńczyk et al., 2011). Chicory root inulin-derived fructooligosaccharides, xylooligosaccharides and wheat bran-derived arabinoxylooligosaccharides (AXOS) were shown to have ample applications (Xu et al., 2003). Lactulose, mannitol, maltodextrin, raffinose, and sorbitol are also prebiotics with established health benefits (Vamanu and Vamanu, 2010).

In animals, prebiotics have a long history. Many ancient farmers guided animals to specific pastures in order to obtain the desired prebiotic from the pasture (Charalampopolus and Rastall, 2009). In recent years prebiotics have been commonly utilized in *in vivo* feeding experiments with a range of companion and livestock animals, such as poultry, cattle, pigs and horses, to investigate the effects on gut microflora, immunomodulation of the host, suppressive effects on the enteric and systemic infections by pathogens, nutrient digestibility, performance indices, quality of products of animal origin and general welfare of animals (Charalampopolus and Rastall, 2009). Gibson and Roberfroid (1995) originally classified prebiotics, as defined previously; this definition, however, was based on prebiotics use in humans and their use in animals and especially in poultry and ruminants may be more complicated.

### ***Characteristics of ideal prebiotics and their mode of actions***

Prebiotics beneficially interact with animal's physiology by selectively stimulating favourable microbiota in the intestinal system. This may have valuable effects in reducing the incidence of enteric pathogens. However, from the available published evidence, the exact mechanism involved in prebiotics to reduce pathogenic infections is still unclear. Competitive exclusion of pathogens by increasing numbers of microbiota that are associated with a healthy host can produce a variety of bacteriocins that have a detrimental effect on the pathogen by promotion of macrophages, stimulation of antibody production, and antitumour effects (Vamanu and Vamanu, 2010).

Contrary to these microbiota-dependent theories, it may be that prebiotics are able to directly affect the pathogen or host in a microbiota-independent manner. It has been suggested that the principal mechanisms of prebiotics is immunomodulation, that includes selective growth of lactic acid-producing bacteria, resulting in an increased concentration of SCFA like acetate, propionate, and especially butyrate which is the preferred energy source of colonocytes and stimulates gut integrity. High fermentation activity and high concentration of the SCFA is correlated with a lower pH, which is associated with a suppression of pathogens and increased solubility of certain nutrients (Józefiak et al., 2004). This increase of SCFA with immune cells, direct contact in the digestive tract and the change in mucin production contribute to lower incidence of bacteria moving across the gut barrier (Lee and Salminen, 2009). This phenomenon may inhibit some pathogenic bacteria and reduce colonization of some species like *Salmonella* and *Campylobacter* (Charalampopolus and Rastall, 2009).

Other beneficial effects of probiotic addition can be reflected in increased intestinal enzymes secretion, diminution of ammonia and phenol products, and promotion of resistance to pathogenic bacteria proliferation in the gut (Yusrizal and Chen, 2003 b). The advantage of prebiotics compared to probiotics is that they promote growth of useful bacteria which are ubiquitous in the host organism and are capable to survive in all environmental conditions.

Fructooligosaccharides selectively promote the growth of beneficial bacteria by acting as a source of nutrients. FOSs can be found in a variety of foods, including

onions, garlic, banana and asparagus (Charalampopolus and Rastall, 2009). FOS is neither hydrolyzed nor absorbed in the upper gastrointestinal tract and acts as a food source for host-beneficial bacteria, which competitively excludes pathogenic bacteria.

Mannan oligosaccharides provide alternate binding sites for pathogenic bacteria. These are typically derived from yeast (*Saccharomyces cerevisiae*) outer cell wall components. According to Ferket (2003) supplementary dietary MOS improves animal resistance to enteric disease and promotes growth by six different means: (1) restricts colonization of enteric pathogens by inhibiting bacterial adhesion to gut lining; (2) improves the brush border mucin barrier; (3) changes microflora fermentation to favour nutrient availability for the host; (4) improves immunity; (5) enhances the unity of the gut lining; and (6) brings down enterocyte turnover rate. In Table 2 are presented the most important characteristics of prebiotics and their positive effects on animal organism.

Table 2. Characteristics of ideal prebiotics and their desirable properties (adapted from Simmering and Blaut, 2001 after Patterson and Burkholder, 2003)

Prebiotics	
Properties	Positive influences
Not hydrolyzed or absorbed	Higher SCFA production
Selectively stimulate growth of one or a limited number of beneficial bacteria	Better biomass and stool bulking
Beneficially modify the intestinal microbiota activities	Enhanced vitamin B synthesis
Positively modulate host defence system	Positively affected mineral absorption
	Cancer prevention
	Decrease in blood cholesterol level
	Diminution in ammonia and urea excretion
	Lower excreta content of skatole, indole, phenol, etc

### ***Action of prebiotics in animal organism***

The principal effect of prebiotics is to stimulate the resident microbiota of host to proliferate, to stop harmful bacteria, and to share health benefits to the host. The supplementation of poultry and pig diets with oligosaccharides is generally associated with stimulation of microbiota proliferation. Specifically, in pigs, GOS has been associated with increasing the numbers of bifidobacteria in both *in vivo* and *in vitro* evaluations (Charalampopolus and Rastall, 2009). Prebiotic oligosaccharides supplementation of the diets, such as FOS, may have an effect on improvements in the gut microbial population, including a reduction in *Salmonella* colonization. This suggests that supplemental dietary FOS may be a viable option in both *Salmonella* control and antibiotic free programmes.

MOS have been used to modulate the resident gut flora of the host and subsequently reduce the incidence of pathogen colonization by binding and eliminating pathogens from the intestinal tract and stimulation of the immune system (Spring et al., 2000; Fernandez et al., 2002). Concerning the prebiotic use to reduce pathogens

in livestock, the majority of studies have been performed in poultry, with positively influenced growth and improved host intestinal health (Spring et al., 2000). According to Macfarlane et al. (2008), supplementation with GOS increases the growth of certain gastrointestinal bacteria, especially the LAB, bifidobacteria, and/or their fermentation products. Fermentation products such as SCFA increase after prebiotic supplementation as a result of oligosaccharide fermentation by resident microbiota. SCFA production is an important physiological process of colonic microorganisms and may be useful in improving gastrointestinal health by reducing the occurrence of diarrhoea through modulating the microbiota (Macfarlane et al., 2008).

Macfarlane et al. (2008) reviewed the importance of the colonic microbiota for improvement of host's immune system, and underlined that lactobacilli and bifidobacteria have been linked to an increase of sIgA levels and phagocyte number. *In vitro* and *in vivo* studies have been conducted to show the relation between prebiotics and immune system (Fernandez et al., 2002). Apparently, prebiotics have not only an immune-stimulatory effect on the host but can act as adjuvants to boost vaccine induced immune responses.

Other prebiotic effects have been investigated for the effect on lipid metabolism, mineral absorption and reduction of fatty acid synthesis (Macfarlane et al., 2008).

#### ***Use of lactose derived from whey as a prebiotic***

Whey products are consumed in varying amounts by humans and a wide variety of animal species, including swine, young ruminants (calves), dogs and cats, poultry and aquaculture. Lactose (disaccharide sugar formed from galactose and glucose) derived from whey products is highly functional and growing interest in its prebiotic effects is currently observed (Szczurek, 2008). Purified natural lactose or whey powder, a product with high (70–80%) lactose content, are obtained in a series of steps of liquid whey processing with membrane filtration technology (Bednarski, 2001). Unlike mammals, birds lack the enzyme lactase ( $\beta$ -galactosidase) required to digest lactose.

Studies with above whey products in poultry diets reviewed by Szczurek (2008) have shown some inhibiting effects of lactose on *Salmonella* and other pathogenic bacteria in the digestive tract of broiler chickens by production of SCFA and lactic acid from lactose as a substrate for host bacteria enzymes, with deep reduction in cecal pH. Whey lactose may also act in favour of enhancing immunity, improving survival rates, and stimulating growth of beneficial intestinal bacteria (Majewska et al., 2009). However, data from some experiments have shown that the high lactose inclusion in the diet may decrease performance of birds grown to market age and cause incidence of diarrhoea (Kermanshahi and Rostami, 2006). Thus, there is a need for further comparative studies with broilers to determine the optimum dietary level of lactose and/or dried whey with high lactose content.

#### **Synbiotics**

Synbiotics refer to nutritional supplements combining probiotics and prebiotics in a synergistic form. The principal reason for using a synbiotic is the conception that a probiotic, without its prebiotic substrate does not survive well in the diges-

tive system. Without the necessary source of nutrients for the probiotic, it will have a more important intolerance for oxygen, low pH, and temperature. As prebiotics furnish better conditions for probiotics to expand, the colonies of these “good” bacteria are maintained. Studies have shown that by using the benefits of both prebiotics and probiotics, the number of desirable bacteria in the digestive system increases, and as a result the positive effects on health status can be observed. Such positive influences of synbiotics are obtained in two ways: (1) by improving the viability of probiotics and (2) by delivering specific health benefits (Bhupinder and Saloni, 2010).

In the last few years, studies on synbiotics have started to appear, with the main role on applications against diseases (Kolida and Gibson, 2011). The intake of a synbiotic leads to a regulation of the gut metabolic activities with a maintenance of the gut biostructure. Particularly, the significant increase of SCFA, carbon disulfide, ketones, and methyl acetate indicated possible health promoting effects of the synbiotic feed supplements.

### **Use of probiotics, prebiotics and synbiotics in poultry nutrition**

Due to the ban in Europe many alternatives to AGP have been evaluated in poultry production with mixed results. Most of the experiments conducted with pro-, pre- and synbiotics have focused on improving the microbial health, performance, and decreasing carcass contamination of young meat birds.

#### ***Reported effects in broiler chickens***

Broiler chicks display very fast growth rates, attaining more than 60 times their hatching weight at 6 weeks of age. This high growth rhythm is reached thanks to genetic selection, improved housing techniques, sanitary and veterinary care, and extremely balanced high-energy diets.

The effect of probiotics on broilers and layer hens were thoroughly reviewed by Fuller (1992), and more recently by Kabir et al. (2004) and Kabir (2009), who confirmed the existence of some effects. However, after a number of years, there is still insufficient evidence regarding the efficacy of probiotics in poultry other than for the competitive exclusion of pathogens. Many of the studies conducted today still remain poorly designed, with either limited number of animals or insufficient statistical analysis. However, there seems to be a unique point of view about the efficiency of probiotics in poultry when they are kept under suboptimal conditions (Fuller, 1992).

One of the most successful probiotic bacteria used in poultry are *Bacillus subtilis* (Lee and Salminen, 2009). Apart from improving the growth performance, *B. subtilis* is also efficient in inhibiting the growth of pathogens in the digestive tract of chickens, which can lead to a considerable economic loss. Dietary supplementation with *B. subtilis* could improve also the performance, body weight, and immune response. Lee et al. (2010) investigated the effect of *Bacillus* on *Eimeria maxima* infection in broiler chickens, and found that *Bacillus subtilis* reduced the clinical signs of experimental avian coccidiosis and increased various parameters of immunity in broiler chickens. A *Lactobacillus* probiotic was shown to increase the quantity of lactic acid producing bacteria and decrease the gut lesion score of broilers infected

with coccidiosis, and *Salmonella* (Vicente et al., 2008). In the experiment of Brzóška et al. (2012) the beneficial effect of *Lactobacillus* Spp. or *Lactococcus lactis* bacteria on chicken liveability was observed, but the probiotics used had no effect on body weight gain and feed conversion.

Prebiotic research on poultry has been performed since 1990 and, as a result, a large database of research is accessible in this area. Prebiotics in broiler diets have been shown to increase lactobacilli counts in the gastrointestinal tract (Xu et al., 2003; Yusrizal and Chen, 2003 b; Baurhoo et al., 2007). Also, increased bifidobacteria and decreased clostridia have been reported in some studies that investigated the microbial effects of prebiotic supplementation (Sims et al., 2004; Cao et al., 2005). Some authors reported decreased *Salmonella* and coliforms (Fernandez et al., 2002; Spring et al., 2000), while others observed a decrease in *E. coli* (Xu et al., 2003; Zduńczyk et al., 2005; Baurhoo et al., 2007). Some other pathogenic bacteria like streptococci, staphylococci, bacilli, and yeast, have also been reported to decrease with prebiotic supplementation (Samarasinghe et al., 2003; Cao et al., 2005).

Prebiotic supplementation of poultry diets modifies fermentation profiles. Increased butyrate concentrations were reported (Zduńczyk et al., 2004; Yang et al., 2008). Supplementation with FOS decreases cecal indole and phenol concentrations (Cao et al., 2005). Total SCFA and lactic acid concentrations more often increased with intestinal pH decrease when prebiotics were supplemented (Yang et al., 2008; Zduńczyk et al., 2005). Regarding intestinal morphology, increased intestinal villus height was reported when prebiotics were included in the broiler diet (Xu et al., 2003; Baurhoo et al., 2007). Other changes of intestinal characteristics have been observed, including increased gut length (Yusrizal and Chen, 2003 a). Some authors studied also the effect of prebiotics on mineral utilization and bone quality in broilers, but the results were not positive (Świątkiewicz and Arczewska-Włosek, 2011).

Performance parameters in broilers have been evaluated with prebiotic supplementation. Body weight was reported to increase in the majority of studies (Yusrizal and Chen, 2003 a; Sims et al., 2004; Zduńczyk et al., 2005). In parallel, body weight gain, feed conversion and carcass weight were improved (Samarasinghe et al., 2003; Xu et al., 2003; Yusrizal and Chen, 2003 a; Sims et al., 2004; Józefiak et al., 2008; Yang et al., 2008). Feed intake and feed:gain ratios (F:G) generally decreased with supplementation of fructans and MOS (Baurhoo et al., 2007; Samarasinghe et al., 2003; Xu et al., 2003; Yusrizal and Chen, 2003 a). Also, MOS and inulin supplementation increased carcass weight and abdominal fat weight (Samarasinghe et al., 2003; Yusrizal and Chen, 2003 a).

Synbiotics are relatively recent among additives used in poultry nutrition. Studies have suggested that performance can be further enhanced when using both prebiotics and probiotics. Investigations demonstrated that synbiotics are much more efficient when used in combinations than singly (Ušćebrka et al., 2005). As an example, Fukata et al. (1999) found in broilers that a probiotic and FOS each reduced intestinal *Salmonella enteritidis* colonization when used singly, but their combination was more effective.

### **Reported effects in laying hens**

Some studies have shown that pro- and prebiotics would enhance the performance of egg laying birds by using them during stress periods, at the first three to four weeks of life, and immediately prior to and after the move from pullet house to layer house (Radu-Rusu et al., 2010; Zarei et al., 2011). It has been suggested that the continuous feeding of a probiotic will improve layer performance, feed cost/dozen eggs and feed cost/kg eggs compared to the control and control plus antibiotic treatments.

Zarei et al. (2011) fed laying hens on diets supplemented with some commercial pro-, pre- and synbiotics, and reported increased egg mass and weight, and egg shell weight and thickness.

Chen et al. (2005 b) demonstrated some changes in digestive system, mainly by an elongation of both small and large intestine in laying hens receiving FOS supplementation. As a consequence, increased egg production and improved feed efficiency were observed. Furthermore, FOS supplementation increased egg shell strength by skeletal and plasma calcium levels augmentation (Chen and Chen, 2004) and reduced yolk cholesterol concentrations without affecting yolk weight (Chen et al., 2005 a). Significantly decreased concentration of yolk cholesterol was also reported when inulin was added to the diet for layers (Shang et al., 2010). The results of some studies with hens have also shown that such prebiotic fructans as inulin or oligofructose may positively affect mineral utilization and in this way, improve eggshell and bone quality (Świątkiewicz et al., 2010 a, b; Świątkiewicz and Arczewska-Włosek, 2012).

As mentioned previously, prebiotics may have some protective effects against *Salmonella*, and it would be a viable option for maintaining a healthy microbial population in fasted egg layers by using prebiotics or even a synbiotic combination, especially during stress, because the number of bifidobacteria and lactobacilli populations in these periods would decrease in the gut of stressed birds.

### **Reported effects in turkeys**

Some of probiotic effects on turkeys can be summarized in a significant increase of body weight gain, improved feed efficiency and decreased gut colonization by pathogens (Vicente et al., 2007). Improved physiological and health status with prebiotics has also been reported in turkeys. As mentioned before, Zduńczyk et al. (2005) demonstrated that a FOS inclusion in the feed led to decreasing cecal pH and increasing cecal production of SCFA, especially butyrate.

MOS are of particular interest to the turkey industry, because taking into account their rapid growth rate and longer growth period, turkeys are relatively sensitive to gut colonization by harmful bacteria species. Much of the research on MOS has focused on their ability to improve the overall performance of poults like increasing weight gain and reduce the incidence and severity of coccidiosis and necrotic enteritis in turkeys. Research has shown reduced clostridia levels in MOS fed turkeys (Ferket et al., 2003). MOS may be an alternative component to an antibiotic free management in broilers and turkeys as *Clostridium perfringens* is generally associated with necrotic enteritis.

Vicente et al. (2007) evaluated the effect of a lactobacillus culture as a probiotic jointly with dietary lactose as a prebiotic in turkey poult challenged with *Salmonella enteridis*. An improvement of body weight and feed conversion ratio in challenged birds was observed while no differences in unchallenged poult were noted. However, Buteikis et al. (2008) found that the addition of dietary lactose or a compound of lactose with probiotic negatively alter the growth rate of turkeys, but had a positive effect on mortality of turkeys. This suggested that dietary lactose with appropriate probiotic organisms may act differently on poult performance.

### **Conditions and prospects for the use of these dietary supplements in poultry**

Live microorganisms, together with enzymes and feed additives of biological origin were added to the list of feed additives regulated by the European Union in the 1980s due to the emerging market trends.

The term “probiotics” has been declined on the grounds of being too generic. In 2002, under the framework of establishing the European Food Safety Authority, a new draft regulation would group microorganisms as “zootechnical additives,” defined as agents producing beneficial effect on gut microflora. This proposal was adopted in 2003, when the European Commission passed a new regulation (EC) No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition.

The microorganisms included in probiotic preparations should be generally recognized as safe (GRAS) to be used in Europe. This status can be achieved by a positive safety evaluation by qualified, independent experts, with respect to the target species. It should be resistant to bile, stimulate immune system, have reduced intestinal permeability, produce lactic acid, and be able to survive in both acidic environment of the stomach and alkaline environment of the duodenum. It is very important that a probiotic remain viable during processing and transit through the gastrointestinal tract (Simmering and Blaut, 2001). These ranges of test evaluations may vary from one country to another and are often very complicated and difficult, which can limit the number and the approval of these kinds of products, especially in the case of multispecies or symbiotic combinations which may have better advantages compared with the single use, but because of the rigorous regulations, feed additives available on the market contain generally only one, or exceptionally two strains (Applegate et al., 2010).

These additives must satisfy several criteria with regards to their identity, characteristics, and conditions for use of the additive; their safety of use in animals, humans, and environment such as the lack of pathogenicity and production of antibiotics and antibiotic resistance; and their efficacy on animals or categories of the target animal species such as improved zootechnical performance, reduction of morbidity and mortality. It is not easy to select and introduce the optimal quantities at the optimal conditions in an adequate way.

Authorization of feed additives in Europe is granted by The European Food Safety Authority (EFSA), which evaluates the data delivered on safety, efficacy, and toxicology of the feed additive. Once the commission is satisfied with the data, it prepares a draft regulation to grant authorization, following the procedure involv-

ing Member States within the Standing Committee on the Food Chain and Animal Health–Animal Nutrition. Authorizations are given for specific animal species, particular conditions of use and for 10-year periods. These are important procedures to ensure safety of probiotics and prebiotics used as feed additives that eventually contribute to their efficacy. Approved feed additives are published in the Community Register of Feed Additives (EFSA, 2005; Lee and Salminen, 2009).

## Conclusion

Probiotics, prebiotics and synbiotics are gaining importance because they seem to exert their nutritional benefits in various animal species and their concept is more and more comprehensible. Poultry studies have demonstrated clear benefits in performance and health status of birds. They affect the intestinal microbiota and immune system to decrease colonization by pathogens in some conditions.

As with growth promoting antibiotics, environment and stress may have an impact on the efficiency of prebiotics and probiotics. These feed supplements can present immense potential as alternatives for antibiotics to totally eliminate antibiotic use, because they do not cause microbial resistance. The beneficial consequences of these supplements may generally translate into improved health and performance parameters. More studies are now focusing on their symbiotic effects that apparently can be more efficient than a separate use.

This represents an important perspective of applied biotechnological research that must be better understood to reveal more mechanisms through which they could positively impact poultry, but these will depend largely on the regulatory developments in the area that can bring new products for poultry and livestock, which are currently limited because of time and generated expenses of safety testing.

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### **Przydatność prebiotyków i probiotyków w nowoczesnym żywieniu drobiu – przegląd**

#### STRESZCZENIE

Probiotyki to kultury żywych mikroorganizmów, które modyfikują i utrzymują korzystną mikroflorę przewodu pokarmowego. Prebiotyki to niestrawne składniki paszy korzystnie wpływające na organizmy zwierząt poprzez stymulowanie aktywności i wzrostu korzystnych bakterii naturalnie występujących w przewodzie pokarmowym i eliminowanie bakterii patogennych. Niektóre badania wykazały ich korzystne efekty przy stosowaniu pojedynczym lub równocześnie w postaci synbiotyków, w celu nasilenia ich wzajemnego działania. Dodatki te z powodzeniem stosowano jako alternatywę dla antybiotykowych stymulatorów wzrostu w paszy, jednak konieczne są dalsze badania w celu lepszego zrozumienia sposobu ich działania i skutków. Niniejszy artykuł przeglądowy prezentuje rosnące zainteresowanie użyciem tych zamienników antybiotyków i potencjalny mechanizm ich działania w żywych organizmach, omawia także najnowsze dane dotyczące wpływu tych dodatków w żywieniu drobiu.