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PROBIOTICS, PREBIOTICS AND SNYBIOTICS IN POULTRY – MODE OF ACTION, LIMITATION, AND ACHIEVEMENTS

PROBIOTYKI, PREBIOTYKI I SYNBIOTYKI - SPOSÓB DZIAŁANIA, OGRANICZENIA I OSIĄGNIĘCIA

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ABSTRACT
The withdrawal of antibiotic growth promoters from poultry industry have forced farmers to seek alternatives for the posing a risk factors of cross-resistance acquisition by harmful bacteria. A particular nuisance became salmonellosis and campylobacteriosis forcing to the elimination of whole poultry flocks as well as causing dangerous zoonotic diseases in humans. An excellent replacement for antibiotics have become the pro-, pre-and synbiotic substances which have a beneficial effect on the host organism through the development intensification of healthy intestinal microbial strains and the elimination of pathogenic strains. Such preparations may be administered both in the water spray as well as in feed. Excellent and promising method appears to be their injection directly into the egg air chamber in the 12th day of incubation. However, further studies are required to determine the appropriate doses as well as combinations of bioactive substances and to determine the optimal way for their delivery.

Keywords: bioactive substances, campylobacteriosis, in ovo injection, poultry, salmonellosis

STRESZCZENIE
Wycofanie antybiotykowych stymulatorów wzrostu z przemysłu drobiarskiego zmusiło hodowców do poszukiwań alternatyw dla czynników stwarzających ryzyko nabycia oporności krzyżowej przez szkodliwe bakterie. Szczególnym utrapieniem stały się salmonellozy i campylobakteriozy zmuszające do eliminacji całych stad drobiu a także powodujące niebezpieczne zoonozy wśród ludzi. Doskonałym zamiennikiem antybiotyków stały się substancje pro-, pre- i synbiotyczne mające korzystny wpływ na organizm gospodarza poprzez potęgowanie rozwoju prozdrowotnych szczepów mikroorganizmów jelitowych i jednoczesnej eliminacji szczepów patogennych. Preparaty takie można podawać zarówno w wodzie, w sprayu jak i w paszy. Doskonałą i obiecującą metodą okazuje się także ich iniekcja wprost do komory powietrznej jaja w 12 dobie inkubacji. Jednak wymagane są dalsze badania w kierunku określenia odpowiednich dawek jak i również kombinacji substancji bioaktywnych oraz ustalenia optymalnego sposobu ich dostarczania.

Słowa kluczowe: drób, iniekcja in ovo, campylobakteriozy, salmonellozy, substancje bioaktywne

DETAILED ABSTRACT
Stosowanie preparatów probiotycznych niewątpliwie wpływa korzystnie na układ pokarmowy ptaków i hamuje rozwój patogenów. Układ pokarmowy stwarza szczelną i selektywną barierę dla patogenów. Naturalna mikroflora przewodu pokarmowego odgrywa bardzo ważną rolę w procesach trawienia, wchłaniania oraz w zdrowotności zwierząt, gdyż tworzy integralną całość z układem immunologicznym (GALT – Gut Associated Lympholid Tissue). W pierwszych dniach życia przewód pokarmowy ptaków rozwija się bardzo intensywnie. Zmiana budowy błony śluzowej jelita oraz ilości receptorów powierzchniowych może wpływać na kolonizację układu pokarmowego przez bakterie. Skład mikroflory jelitowej kształtuje się jeszcze w czasie ostatnich dni inkubacji. W wolu, dwunastnicy i jelicie cienkim dominują bakterie grupy coli, enterokoki oraz bakterie fermentacji mlekowej. Po pierwszym tygodniu życia dominującą grupą bakterii stają się probiotyczne bakterie z rodzaju *Lacobacillus*, które wykazują lepsze właściwości adhezyjne do śluzu jelitowego niż bakterie patogenne. Pisklę opuszczając zamkniętą i sterylną strukturę jaja staje się narażone na choroby wywołane przez bakterie z rodzaju *Clostridium* i *Salmonella*. Rola mikroflory jelitowej jest ogromna i bardzo ważna, odpowiada za trawienie składników pokarmowych, wytwarzanie głownie kwasu mlekowego oraz krótkołańcuchowych kwasów tłuszczowych, wpływa na zdrowie i kondycję zwierząt. Wzbogacanie diety o probiotyki, ale również o inne substancje bioaktywne, jak prebiotyki czy synbiotyki stwarza nowe możliwości dostarczania i naturalnego wzbogacania układu pokarmowego w prozdrowotne substancje kształtujące zdrowotność i odporność zwierząt, a także pozwala na ograniczanie stosowania farmaceutyków w profilaktyce zdrowia.

INTRODUCTION

DYNAMIC OF POULTRY PRODUCTION IN EUROPE /POLAND/

It is estimated that the poultry meat production in 27 European Union countries in the years 2009-2020 will increase by almost 7%. In 2009, production volume reached 11657 thousand tons, and according to forecasts in 2020 will reach 12460 thousand tons. Export in the Europe in 2009 was 940 thousand tons, while poultry meat consumption was at the level 23,25 kg per capita. After Polish accession to the European Union, the state of meat production and processing improved significantly. In 2008, poultry production in Poland amounted to over 114 thousand units, while the consumption of poultry meat per capita was 30,6 kilograms (Rocznik Statystyczny Rolnictwa, 2009). In 2009, poultry production reached 1247 thousand. tons., this is a satisfactory result, because over the period 2006-2009 has increased up to 20%, while domestic consumption up to 10% (Mały Rocznik Statystyczny, 2010). The importance of poultry meat export, increased too. In 2006, export was 21% of production, in 2009 reached up to 26% (Koleśnikow, 2010). Over the last five years, poultry production in Poland increased by 24%, from 1 050 000 tons in 2005 to 1 300 000 tons in 2010. Poland, next to France, Germany, Italy, Britain and Spain, was at

the forefront of poultry meat producers. In 2011, the dynamics of poultry meat production in Poland was reduced as a result of increasing production costs. In the first half of 2011 totaled 676 thousand tons, in the second 744 thousand tones, in the entire year poultry production probably reach 1420 thousand tons. Despite the fact, that poultry meat prices are maintained at a relatively high level, it is still that the poultry is the cheapest kind of meat.

On the dynamic of development poultry meat production affected i.a. its general acceptance by all religions, low cost production and very good feed utilization by birds, and especially by broiler chickens. The poultry sector, compared to other branches of animal production, characterized by the rapid progress of genetic, technology and nutrition. It also provides various types of assortments on the market, perfect in terms of nutritional and dietary.

INDUSTRIAL PRODUCTION

One of the disadvantages that intensive production entails are health risks, such as diseases of animals and humans (zoonoses).

POULTRY FOODBORNE ILLNESS - CAMPYLOBACERIOSIS AND SALMONELLOSIS

In many countries, both developed and developing, as shown reports of European Food Safety Authority (EFSA) poultry production and poultry products can be a potential source of human campylobacter and salmonellosis infection. Campylobacteriosis is currently the most frequently appearing disease in the EU. In recent time, the number of this type bacterial infections, increased more than doubled, surpassing the number of infections ascribed to salmonella. Transport, preparation and consumption of broiler meat may be the cause of 20 - 30% of cases among men (Scientific Raport of EFSA, 2010).

Dangerous reservoir of bacilli may be also a free-living birds, domestic and wildlife animals.

Intensive fattening, too large livestock density on a small area, contaminated feed and difficult to clean feed distributor are among the factors affecting the high levels of carriage in poultry. In the flocks of layers a serious problem becomes the possibility of transovarial transmission of these bacteria (Jaguszyn-Krynicka, et. al., 2007).

Among the animals despite the carrier, are rarely observed illness, that prevents removal of infected animals from the herd. Intensive proliferation of dangerous bacilli in the digestive system, both during illness or asymptomatic carriers makes the external environment is constantly polluted. It is therefore necessary rigorous observance of hygiene at all stages of production and maintenance of systematic disinfection and rat control in slaughterhouses, meat processing plants, warehouses. In humans, by the consumption of products derived from animals infected with salmonella, or food contaminated by animal feces or through contact with infected birds or people, food poisoning can occurs. Symptoms of infection is gastroenteritis, diarrhea, paroxysmal abdominal pains, vomiting and fever. *Salmonella* poisoning in the elderly and children, leading to dehydration and severe electrolyte imbalance. In birds can be distinguished the intestinal, articular, organ and nervous salmonellosis types. The intestinal type leads to damage the intestinal mucosa, and therefore nutrients absorption is impaired, diarrhea occurs, not digested remains of food can be found in the stool, the birds lose weight. The articular type is characterized by

swelling around the joints. *Salmonella bacilli* are enter the blood, and with it to the internal organs - liver, kidney, spleen, heart. Birds also occurs loss of strength, apathy and asthma. As a result of birds nervous system paralysis, there are balance disturbances and palsy. Until recently, the effective solution in controlling the development of *salmonella* and campylobacteriosis were antibiotics. Its role was not only the elimination of unfavorable bacteria. These were also used in animal production as growth promoters. Currently, the antibiotics use is not allowed.

REDUCING HARMFUL BACTERIA

ANTYBIOTICS

The aim of the intensification of crop and livestock production is to satisfy the demand of people for food, especially for animal protein. Therefore, the process animals growth must be supported by various feed additives. Until January 2006, the most commonly used supplements were antibiotic growth promoters (AGP), which gave the positive production results, despite the poor living conditions of animals and restrict certain diseases of the digestive system (Śliżewska, et. al., 2006)

Feed antibiotics stabilize the microflora of the gastrointestinal tract, by limiting the growth of negative microorganisms and their toxins, promote the growth of beneficial bacteries, reduce the emission of methane and ammonia, cause better use of phosphorus, whereas in poultry they reduce the risk of coccidiosis. Furthermore, feed antibiotics accelerate growth and extension the weight of meat of animals. The presence of antibiotic growth promoters in animal feed causes thinning of the intestinal wall and better their blood supply. As a result of this increased absorption of nutrients from the intestinal lumen is observed. However, there is a problem of possible negative effect of feed additives on the quality of animal products, as well as on human health. Threat to humans and animals have become antibiotic-resistant strains of bacteria that are selected under the influence of use of antibiotics.

Susceptible bacteria at the time of contact with the antibiotic are suppressed in growth or destroyed, while the resistant bacteria present in the gut flora can multiply to a higher or lower degree. Suppression of antibiotic-sensitive bacteria created an opportunity for colonization by resistant bacteria derived from external sources. Frequent use of antibiotics not only conducive to the formation, but also fortification of resistance in bacteria.

In the European Union antibiotic growth promoters have been withdrawn on 1 January 2006, in accordance with Directive No. A5-0373/2002. This prohibition is a challenge for farmers and feed producers, and leads to look for new nutritional solutions and the application of such supplements that are safe for animals and food production. Modern methods of farming and animal nutrition entails numerous of threats which previously were eliminated by antibiotic growth promoters. Alternative to antibiotics may constitute a probiotics and prebiotics, which stabilize the gut microflora and control the multiplication of pathogens. This property is the basis for the mechanism of "competitive exclusion" (CE).

COMPETITIVE EXCLUSION: PROBIOTICS, PREBIOTICS, SYNBITICS

The Competitive Exclusion term was first used in 1969 by Greenberg and referred to the phenomenon in which one strain of bacteria are competing with other bacteria for colonization of intestinal epithelium (Edens, et. al., 1997). In 1974, Nurmi and Rantala used the concept of CE to control the multiplication of Salmonella infantis in the broiler flock, showed that a stable microflora protects birds against the salmonella

infection. In the same year, Lloyd et al were the first who introduce term CE to poultry production. The emergence of "competitive exclusion" technology allowed in some way to control the disease among the poultry and to prevent them in the early stages of the life of birds. Furthermore, the application of CE affects the reduction of mortality among birds and better feed conversion, lowering the viscosity of matter and increase the amount of dry matter in faeces. Numerous studies have shown that the method of competitive exclusion is the most effective and the least harmful way of controlling microbial balance of the digestive tract in poultry, which can protect the host against pathogens such as *E. coli*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Campylobacter perfringers*, which causes i.a. necrotizing enterocolitis.

The mode of CE action is:

reducing intestinal epithelium colonization by pathogenic bacteria;
- inhibiting the activity of bacterial toxins;
- stimulating the local activity of the immune system;
- nutrition intestinal epithelial cells (Jeffrey, 1999)

Since 2000, in Poland, in poultry practice is becoming common the application of preparations containing live cultures of microorganisms. In an increasing number of European countries in order to induction of CE, chicks after hatching are administered bacterial flora of adults. The essence of action of preparations that inhabit the gastrointestinal tract (NGF-Natural Gut Flora) is to application them in a timely manner, so as to prevent colonization of the digestive tract by random microflora. Great importance for the effective action is the composition of this preparation (the richer preparation gives stronger protective effect) and the concentration of microorganisms contained in it (the higher concentration gives greater effectiveness). The use of live cultures of microorganisms enhances the immune response, what is manifested by an increase of antibodies, the increase in cytotoxic cells, as well as stimulation of phagocytic activity of macrophages. These changes may occur locally, both in the digestive system as well as generally in the whole organism, acting protectively, not only in the intestines. Currently on the market are available such preparations like AviFree, Aviguard, Broilact, MSC, PREEMPT, or CF-3 (DeLoach29). Protective properties has also L. reuteri, which by producing of reuterin prevents intestinal epithelial colonization by pathogens (Schneitz, 2005). In addition to the NGF, the colonizing preparations can also be probiotics, prebiotics and synbiotics that selectively stimulate the growth and development of beneficial, endogenic bacteria.

MODE OF ACTION

PROBIOTICS

The probiotic term was first used for the substances produced by microorganisms that stimulate the growth of man and animals. This name was taken from the latin words "pro" and "bios". Probiotics are preparations containing the required intestinal microflora, applied in the form of living cells or yeast, or spores. Microorganisms used for obtaining the probiotic preparations for animals are micro-organisms of the species Bacillus, Enterococcus, Lactobacillus and Saccharomyces. Probiotic bacteria works in two ways. The first one is the competitive exclusion - bacterias in the gastrointestinal environment, produce substances which inhibit growth of pathogenic microorganisms and compete with them for a place in the intestinal epithelium. These substances are short-chain organic acids (lactic, acetic, propionic), bacteriocins

(nisin, acidolina, acidofilina, lacatcyna, lacocydyna, reutryna, laktoline, entrocine) and hydrogen peroxide. Bacteriocins have a high antibacterial activity against *Escherichia coli, Salmonella, Staphylococcus aureus, Clostridium perfringers, Campylobacter*.
The second mode of probiotics action is to stimulate the efficiency of immune system. Infant is born with a sterile digestive system, and before his organism will be able to produce its own antibodies, microorganisms from the environment begin to colonize the digestive system. Therefore, the use of probiotics, due to their ability of adhesion to the intestinal mucosa, allows to create a natural barrier against potential pathogens, and thus enhances immunity. Probiotic stimulation of the immune system manifested by increased production of immunoglobulins, increased activity of macrophages and lymphocytes, and stimulate the production of γ-interferon (Yang, Choct, 2009; Pietras, Skraba, 2000; Świątkiewicz, Korelski, 2007).

Key role in the digestive system in maintaining and shaping the microbial system play two segments: crop and cecum blind. In the crop lactic acid bacterias causes a reduction of pH, synthesize short-chain fatty acids and pre-digest (bacterial-enzyme) feed. Therefore, bacterias protect the gastrointestinal tract against colonization by potential pathogens and prevent their proliferation. In the gut microbial fermentation process takes place. They are produced short chain fatty acids, which in the reflux process are not only nutrient for the intestinal epithelium, but also are the control factor living there microflora. Addition of prebiotics reduces the colonization of pathogens and their movement into the internal organs and eggs, moreover increases the absorptive surface of intestinal. This facilitates the absorption of nutrients and secretion of digestive enzymes, leading to improvement of digestibility and assimilation nutrients delivered in the feed. In laying hens fed a mixture with the addition of probiotics, it is higher productivity, better feed conversion and improvement of the thickness and strength of egg shells. Similar effects were obtained for broiler chickens, where was noted a decreased mortality, increased weight gain, better slaughter parameters and a higher weight of edible offal (heart, stomach). Moreover, the pH of the contents of the small intestine, and caeca was reduced. Probiotics applied for broiler chickens decrease levels of triglycerides and LDL fraction in the blood (Janocha et.al. 2010; Islam, et. al., 2004; Taherpour et.al., 2009).

Growth and activity of probiotics are effectively stimulated by prebiotic preparations. Between prebiotics and probiotics a mutual correlation exists. Animal diet may be supplemented with both of these components by using synbiotic preparations.

PREBIOTICS

Prebiotics are defined as dietary components which are not digested in the gastrointestinal tract, which selectively stimulate the growth and / or activity of one or a specified number of bacterias types. They are not hydrolysed or absorbed in the upper tract of digestive system.
Prebiotics are a source of carbon and energy for the friendly strains of bacterias already inhabiting in the colon, where bacterial fermentation processes of some nutrients occurs.
Reports about the impact of prebiotics on the activity of birds intestinal microflora are not numerous, moreover the effect is different and depends on the type of prebiotic. However, use of this type of additives has a beneficial effect on the production results, animals equalization, reduce mortality and morbidity and lower treatment costs (Hajati, Reazaei, 2010; Kułakowska, 2009; Mateova, et.al., 2008; Lipiński, et. al., 2009).

Prebiotics:
- affect on bifidobacteria proliferation and reduce harmful microorganisms proliferation;
- increase animals performance;
- remove harmful enzymes and toxic metabolites;
- lower blood cholesterol level;
- lower blood pressure;
- prevent the processes of carcinogenesis;
- affect on synthesis of vitamins B1, B2, B6, B12, folic acid and nicotinic (Pilarski, et. al., 2005; Kannan, et. al., 2005).

To the prebiotic substances which are used in poultry production include: undigestible carbohydrates, oligosaccharides (NDO) and polysaccharides and certain proteins, peptides and lipids. The most widely used are fructooligoscharides (FOS, oligofructose, inulin), found in chicory, barley, wheat, beet leaves and belonging to plants fructans which are fermented by beneficial for the host bacterias of the *Bifidobacterium* and *Lactobacillus* types. Moreover, are also used glucooligosaccharides, stachyose and maltooligosaccharides. Other natural sources of prebiotics are legumes (soybean, peas, broad beans, lupins) and cell walls components of yeasts (*Saccharomyces cervisiae*) mannanooligosaccharides - MOS (Sirviydis, et. al., 2006; Patterson, Burkholder, 2003). A very important group of prebiotics are beta-glucans, which are selected polysaccharides also derived from cell walls of certain strains of yeasts, bacterias and fungi, as well as from algae and cereals (oats, barley). Obtained from mannoproteins by hydrolysis, are not degradated by glucanase, so they can be used in the presence of feed enzymes. In combination with beta-glucans, mannanooligosaccharides positively influence on defense mechanisms of the digestive system and neutralization of pathogens, they activate digestive enzymes and improves nutrients absorption from the diet. Broiler chickens fructooligosaccharides supplemented diet significantly increases the number of bifidobacteria and lactic acid bacteria in the cecum and small intestine. Furthermore, population of *Clostridium perfringens*, *Escherichia coli* and *Salmonella* are significantly reduced. FOS alleviates the effects of caecal epithelial necrosis, and also stimulates growth of intestinal villi and crypts in the jejunum and iliac colon **(**Rechman, et.al., 2007). Fructans (inulin, oligofructose and fructooligosaccharides) used in chickens feeding have a positive effect on egg production, increase eggs weight and lower cholesterol levels in the yolk (Świątkiewicz, Świątkiewicz, 2008). Enrichment a compound feeds for poultry in prebiotics, as well as in FOS and MOS have stimulating effect on lymphatic tissue of the gastrointestinal tract (GALT) (Jabardhana, et. al., 2010). This is mainly due to lactic acid action, which affects the mechanisms of nonspecific immunity (increased proliferation of macrophages and their phagocytic activity, as well as NK cell synthesis), specific (to stimulate macrophages to produce cytokines that activate Tc cells) and humoral (stimulation of B lymphocytes to produce antibacterial antibodies, including mainly IgA). Immunoglobulin IgA is active mainly in the digestive system, and its role is to protect the intestinal epithelium against pathogenic microorganisms. Mannooligosaccharides affect the growth of IgM in blood serum, the intensity of T lymphocytes proliferation, moreover increases the antibody titer towards infectious bursal disease virus (Świątkiewicz, Korelski, 2007).
The reported effects of prebiotics on the performance of poultry has been very variable; from little or no effect (Donalson, et. al., 2008), to stimulating effects (Willis, Reid, 2008). The possible explanation for the differences between findings may be

related to: the doses of prebiotic applied, concentration of prebiotic added to feed and low consumption, time from hatch to feed consumption and possible chicken contamination during this period, duration of treatment, composition of diets, chemical structure of prebiotics (degree of polymerization), and interactions with various feed additives such as oligosaccharides, growth hormones and coccidiostatics. Technology *in ovo* eliminates some of these factors that could reduce the effectiveness of prebiotic action in poultry. The effects of this technology were tested by Drobex-Agro in a pilot study (unpublished). Compared to dietary prebiotic inclusion, in ovo technology increased the population of beneficial microflora on the day of hatch. Improved gut health may also have additional benefits in reducing the capacity for enteric viruses to establish infection in chicks and there increase the growth performance of he flock. Increased resistance to enteric poultry viruses may have the added benefit of removing additional stress factors that may be beneficial for colonization of the gut with pathogenic bacteria. Among the studies on prebiotics were also those which have shown that prebiotics do not affect largely on the animals productivity. Body weight of turkeys fed on diet with mannooligosaccharides from 4 to 20 week of age did not differ significantly compared to the control group. Similar results were reported in relation to feed conversion ratio and breast muscles mass. Prebiotics used in a mixture for fattening chickens does not affect the quality of meat chemical composition expressed (Fritts, Waldroup, 2003).

DELIVERY WAYS OF THE PROBIOTICS, PREBIOTICS AND SYNBIOTICS

FEED AND WATER

Currently on the market, farmers can find various nutritional supplements for poultry. The pharmaceutical and feed industry introduced many preparations, which are usually administered in the feed. However, sometimes probiotics, prebiotics and synbiotics are used in a spray form, or are added to the water. Using preparations containing live microorganisms should be remember that they are sensitive to the action of negative environmental conditions such as temperature, light, disinfectants. Furthermore, they must be stored and used according to manufacturer's instructions. To make the preparations water containing chlorine or other disinfectants should not be used. The prepared solution should be immediately given to the animals. To water containing live microorganisms any other products should not be added. The water with the preparation should be drunk by birds over 6, up to 12 hours. Portexin Soluble, Probiomix B-1, which can be mixed with feed, are added to water. To preparations used in a spray form belong i.a. Aviguard, administered in a spray form or dissolved in water, to one-day chickens, and Protexin Chich Spray served in a macromolecular spray to chickens right after hatching and completely dry. As feed additives are used: Acid-Pac-Way, Biogen D, Biogen / W, Protexin Compounder. Effect on diversity of research results may have a dose and concentration of preparation added to the food, low supplement consumption by animals, strains of microorganisms used in the diet, probiotics and probiotics chemical structure, as well as possible interactions between other feed additives.

IN OVO TECHNOLOGY

Recent research tend to eliminate the unwanted effects of many factors that may affect the action of supplements. A promising method that gives positive results is the method of administration supplements by injection in ovo. The in ovo technology is

based on injection of substances with various activity to the eggs air chamber or directly into the growing embryo (Bednarczyk et. al., 2010).

This method has been designed in the 90s of XX century in the United States, in order to vaccinate chicks against Marek's disease, and later against the Gumbro disease. At present, this technology have a wider application:
- bacterial profile stimulation of hatched chicks
- immune response stimulation
- stimulate development of embryos
- examination of teratogenic effects
- determine the sex of embryos
- injection of genetically modified cells.

In the earlier studies and tests conducted in cooperation by University of Technology and Life Sciences and Drobex-Agro there were formulated the technical basics and the prototype of the automatic system to the prebiotic *in ovo* injection. The basic aim of the system was/is the performing of two pieces of work: the injection of definite, precisely controlled amount of bioactive substances to the egg air cell and the sealing of the hole in the egg shell after injection. The system is equipped with the control system consisting of three elements- the supervising computer, the real time control and the engines and sensors system placed on the control object. All of the functions of the system were thoroughly tested, which showed the suitability to the injection *in ovo*. The conclusions have been drawn after the detailed work analysis of this system prototype to the automatic injection of the bioactive substances *in ovo*. They will be useful for the introducing some changes enabling its working in the typical production conditions of Drobex-Agro. They will also make possible the easier exploitation of the system by the hatchery workers. Apart from that, the conclusions will enable its mass production. The advantage of using in ovo method is to reduce labor costs, precision the procedure, elimination of the factors negatively affecting hatchability, embryonic development stimulation. However, should be take into consideration the dose, the type of substance, the place where the vaccine will be placed and the age of the embryo. An important effectiveness aspect of the described method is to give the vaccine as early as possible in order to stimulate the immune system from possible infection. In chickens between 8 and 14 day of embryogenesis in the yolk-sac, bone marrow and embryo liver tissue are synthesized precursors that produce B lymphocytes antibodies. Next, these cells, at 12th day colonize Bursa Fabricius follicles, where they are differentiation and cloning. This process takes several weeks. Between the 18th day of embryogenesis and the 2-4 weeks of age, the most of the cells migrate from Bursa Fabricius to thymus and secondary lymphoid organs. Ipso facto, 2 - 4-week-old chickens have already developed, versatile nonspecific immunity.

Therefore, a dose administered by in ovo takes place most frequently in 18 days of age or earlier, even at 12-14 days of age, when occurs the development of lymphocytes T and B cells precursors . In the course of other studies also demonstrated that the optimum injection time is 12 day of incubation. The explanation, is the presence of greater number of bifidobacteria in the gastrointestinal tract in the 2 day-old chickens treated by prebiotic, while the much poorer results gave the injection in 1, 8 and 17 days of age.

Based on conducted studies it can be concluded that the chicken embryo is an excellent model to study the biological activity of plant derived products and their effects on embryonic development of animals (Villalauenga, et. al., 2004). A hen is a

valuable, easily available object for embryology research, it is related to the possibility of controlling the development of the organism since the moment of gastrulation, through organogenesis until hatching. The results of the influence oligosaccharides on the post embryonic development of organisms may provide a good basis to conduct further research to improve immune response, higher productivity, and reduce use of antibiotics in animal production.

CONCLUSIONS

Pro and prebiotic preparations are extremely important source in poultry production to improve the health status of animals. Probiotics as dietary supplements affect higher performance and are perfect in stressful situations, as well as during periodic decrease in immune response of individuals. Intestinal epithelium very often provides the first defense line against pathogens which discharged to a digestive tract with water and feed. A new possibility is the use of probiotics as a some kind of vehicles, providing the digestive enzymes and other bioactive substances. Therefore, the use of probiotics has its reason, not only considering economic aspects, but also health. Another advantage of probiotics is the fact that are natural supplements and no need grace period. Summary the beneficial effects of concerned preparations, it is worth to say that the perfect complement to the probiotics function in the gut are prebiotics, which are an additional source of energy for intestinal microflora.

Immunity plays a key role in maintaining animal health, and thereby achieve an adequate, required level of performance. Immunity can be stimulated by various methods and preparations. Very significant, and promising method is a in ovo technology, which gives the possibility of forming the bacterial flora already at embryonic stage. Thus, although in an indirect way can be determine the effectiveness of the immune system of birds.

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