

Application of mannan oligosaccharides (Alltech Inc.) in waterfowl: Optimal dose and effectiveness

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The search for alternatives to antibiotics intensifies the use of effective, natural, safe and economical means of protecting the macroorganism from the action of pathogenic microflora. *In vitro* experiments have established the ability of 0.1-0.4% of mannan oligosaccharides (Alltech Inc.) to adsorb isolates of *E. coli* O₂ and enterobacteria (*P. vulgaris*, *K. pneumonia*, *C. diversus*) with the most active process occurring with 0.4% solution. Introduction of mannan oligosaccharides (Alltech Inc.) in amount 4 kg/t into the diet of ducklings provides regulation of intestinal microbiocenosis based on a decrease in the concentration of pathogenic microorganisms and an increase in the concentration of lacto- and bifidobacteria ($p < 0.05$). The immunomodulatory and growth-stimulating effect of mannan oligosaccharides (Alltech Inc.) has been proved by increasing the bactericidal activity of serum by 34.85% ($p < 0.05$), lysozyme activity of serum - by 33.82% ($p < 0.05$), phagocytic index - by 32.81% ($p < 0.05$), phagocytic number - by 28.37% ($p < 0.05$); increase in poultry survival by 9.0%, pre-slaughter live weight - by 14.3% and meat yield - by 4.8%.

Keywords: Mannan oligosaccharides; Microorganisms; Adsorption; Natural resistance; Productivity; Poultry

Introduction

Veterinary and sanitary preventive measures are an important component of the general technological process in functioning of any agricultural enterprise (Paliy & Paliy, 2019). Thus, for effective control of farm animals diseases scientifically based schemes of immunoprophylaxis and non-specific means of prevention are being utilized (Zavgorodniy et al., 2013; Paliy et al., 2020).

One of the biggest problems in Ukraine and the world is the acquired resistance of circulating isolates of microorganisms to antibacterial drugs, which in turn causes significant economic losses due to the low effectiveness of therapeutic measures (Hadzevych et al., 2019). Due to the general tendency to abandon the use of antibiotics, the use of new methods of zoonotic disease control is becoming increasingly important (Kotsiumbas et al., 2013; Wang et al., 2018). The search for alternatives is carried out according to the criteria of effective protection of the organism from pathogenic microorganisms, naturalness and safety of the drug, obtaining safe livestock products free from toxic residues, and antibacterial drugs and economic efficiency of the measures taken (Yalcin et al., 2014; Kirkpınar et al., 2018). The use of enzymes, probiotics, prebiotics, synbiotics and phytobiotics in animal husbandry have shown positive results by increasing their productivity and obtaining quality and safe livestock products. (Sultan et al., 2015; Gujvinska & Paliy, 2018; Ashraf et al., 2019). It is reported that the largest number of isolated probiotic cultures (72.87%) are *Lactobacillus* spp. (40.06%) and *Bifidobacterium* spp. (32.81%), which in turn is confirmed by their popularity in making probiotics for animals (Paliy et al., 2020). An important aspect of the selection for probiotic strains is their ability for rapid intestine colonization. Prerequisite for such colonization is the adhesive properties of cultures, due to which lactic acid bacteria attach the epithelial surface, which prevents their elimination under the action of intestinal peristalsis and ensures dominance in this ecosystem (Gujvinska et al., 2018). Prebiotics are used for prevention and treatment of gastrointestinal diseases having infectious nature, stimulation of nonspecific immunity, correction of stress dysbacteriosis, as well as an alternative to antibiotics. Promoting the development of beneficial bacteria in the intestine, prebiotics potentially stimulate the immune system (Samanta et al., 2013). Pathogens, including most species of *Salmonella*, *Escherichia coli*, *Campylobacter*, are attached to the intestine by receptors (pilus) specific for certain mannose containing carbohydrates and localized on the surface of intestinal epithelial cells (Ahmed et al., 2015). A distinctive feature of the prebiotic substances of mannan oligosaccharides isolated from the yeast cell walls of the *Saccharomyces cerevisiae* (Alltech Inc., UK) is their selective ability to adsorb bacteria that have the peculiar pili, and thus prevent colonization of the intestine by pathogens (Cobos et al., 2010; Duarte et al., 2012; Santos et al., 2012). Together with it, the use of *Saccharomyces cerevisiae* extract improves the functions of the nonspecific immune system (Matur et al., 2011). Adding of yeast autolysate increases egg yield and egg weight together with the increased effectiveness of forage

consumption (Yalçın et al., 2010), it also decreases serum and egg cholesterol (Ezema & Eze, 2015). This yeast culture is widely utilized by food industry (van der Hoek, 2019).

The aim of this work was to determine by *in vitro* experiments the possibility of adsorption of *S. typhimurium*, *E. coli* O₂, *P. vulgaris*, *K. pneumoniae*, *C. diversus* circulating isolates by mannan oligosaccharides and by *in vivo* experiments the optimal dose and effectiveness of mannan oligosaccharides (Alltech Inc.) for waterfowl (ducks).

Materials and Methods

The experimental part of the research has been conducted in the research laboratory of the Department of Veterinary Examination, Microbiology, Zoohygiene and Safety and Quality of Livestock Products of Sumy National Agrarian University (Sumy) and in poultry farms specializing in waterfowl. Some studies have been conducted in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center "Institute of Experimental and Clinical Veterinary Medicine".

To study the ability of mannan oligosaccharides (Alltech Inc.) *in vitro* to adsorb microorganisms the cultures of *S. typhimurium* - 9 strains, *E. coli* O₂ - 14 strains, *Proteus vulgaris* - 11 strains, *Klebsiella pneumoniae* - 6 strains, *Citrobacter diversus* - 4 strains, which have been isolated from pathological material taken from dead ducks.

Suspensions of microorganisms were prepared according to the optical turbidity standard at a concentration of 1.5×10^9 CFU/cm³. For microorganisms sensibilization in every dilution, was used a 1.0% suspension of erythrocytes of guinea pigs. To avoid the process of spontaneous adsorption, the suspension was left in the box for 24 hours at a temperature of 4°C. The level of adsorption was determined by combining the suspension of the studied microorganisms and an aqueous solution of mannan oligosaccharides (0.1%, 0.2%, 0.3%, 0.4%) in a ratio of 1:1. The *E. coli* ATCC 19433 test culture has been used as a positive control, and *S. typhimurium* with a 1.0% suspension of guinea pig erythrocytes in phosphate buffer solution (pH 7.2-7.4), as a negative control. The experiment has been performed in triplicate.

To determine the active dose of mannan oligosaccharides *in vivo*, ducklings of Star 53 H.Y crossing have been taken, they formed four experimental groups and one control group with 50 heads in each according to the principle of analogues.

The control group, had the basic diet, the diet of experimental groups, have been added with mannan oligosaccharides (Alltech Inc.) in concentrations of 0.1% (1.0 kg/t); 0.2% (2.0 kg/t); 0.3% (3.0 kg/t); 0.4% (4.0 kg/t) forage, respectively, starting from the 1st day and until the end of the growing period (49 days).

Ducklings were kept in sterile boxes on the floor according to the industry-specific process engineering standard for agriculture - 04.05. Complete feed rations were used to feed the birds. The bird had free access to food and water. At the first stage, the optimal dose was determined in the diet of ducklings. When observing the birds of the control and experimental groups, their clinical condition was monitored daily. The weight of the birds was determined weekly by weighing during the growing period (49 days).

Blood samples were taken on the 49th day of the experiment from the brachial vein (on the wing), pressing on the vein proximal to the site of the vein puncture. The blood was placed in a serum separator tube and left at a temperature of 22-25°C for coagulation, and after cooling the samples, the serum was obtained. The effect of mannan oligosaccharides on the natural resistance of ducks was determined by indicators of bactericidal activity of blood serum, lysozyme activity, phagocytic index, phagocytic number and % phagocytosis according to conventional methods (Kotsiumbas et al., 2019).

At the end of the technological cycle of growing, the birds were euthanized and the slaughter rates of meat carcasses, the mass of lymphoid organs (spleen, bursa of Fabricius and thymus) were determined. According to indicators obtained, the relative weight of the organs was calculated.

To study the microbiocenosis, 10-15 g of intestinal contents were taken into sterile tubes following aseptic rules. The samples were resuspended in buffer solution and then centrifuged. A series of 10-fold dilutions were prepared from the supernatant and seeded on agar nutrient media Petri dishes, which were cultured under aerobic conditions at $37.0 \pm 1.0^\circ\text{C}$ for 48 hours. According to the results of the calculation, the arithmetic mean value of the number of colonies from all inoculations of one dilution was determined. Detection of *Escherichia coli* bacteria in the intestinal contents was performed by inoculating the suspension (1:10) into Kessler medium.

The inoculations were cultured for 48 hours at a temperature of 37°C. With a positive result, transplants were performed on Endo medium (HiMedia, India) and cultured for 24 hours at a temperature of $37.0 \pm 1.0^\circ\text{C}$. Detection of enterobacteria was performed using the method of microorganisms accumulation on Mueller-Kaufman media and selenite broth with subsequent transplantation on differential diagnostic media ("Differential agar of *Salmonella*" M1078, (HiMedia, India), Ploskirev agar, Endo). Isolation and identification of microorganism cultures have been carried out using tests recommended by the Bergey's Manual of Systematic Bacteriology (1997) (Hoult et al., 1997).

Animal experiments were performed in accordance with the "General Principles of Animal Experiments", approved by the I-III National Congresses on Bioethics (Kyiv, 2001-2007) and in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, France, 1985). Statistical data processing was performed using the computer program Microsoft Excel 10.0. The probability assessment of the difference between the compared indicators was determined by the Student's method.

Results and Discussion

In the first stage, we researched the ability of mannan oligosaccharides (Alltech Inc.) to adsorb microorganisms that have pili type I *in vitro* (isolates of *E. coli* O₂, *P. vulgaris*, *K. pneumoniae*, *C. diversus*, (*E. coli* ATCC 19433, *S. typhimurium* - control)). Thus, after 2 minutes observed the beginning of the process of adsorption of strains of *E. coli* O₂, *P. vulgaris*, *K. pneumoniae*, *C. diversus* and *E. coli* ATCC 19433 with 0.4% mannan oligosaccharides, which manifested itself in the formation of a weak precipitate and clarification of the supernatant at the top of the test tube. After 4 minutes of the experiment, the process of adsorption of these microorganisms in tubes with 0.3% and 0.2% concentration of prebiotic fraction was recorded, and after 6 minutes, the process of adsorption in tubes with aqueous 0.1% solution of mannan oligosaccharides was recorded. Studying of all mannan oligosaccharides doses (0.1-0.4%) after 8-10 minutes showed an active process in the form of a significant precipitate at the bottom of the tube, which illustrated the adsorption process that occurs *in vitro*. The most active process took place with a 0.4% solution of mannan oligosaccharides.

A study of 44 bacterial isolates for the ability to adsorb mannan oligosaccharides of yeast cells *Saccharomyces cerevisiae* (Alltech Inc.) found that 61.3% of them are able to adsorb the studied probiotic fraction. All tested strains of *P. vulgaris* (11), *K. pneumoniae*

(6), as well as 64.3% (9) of *E. coli* O₂, 25% (1) of *C. diversus* showed positive results. In the study of *S. typhimurium* isolates, the adsorption process was not recorded.

The growth-stimulating effect for a macroorganism was shown with the use of mannan oligosaccharides in doses of 0.1-0.4%. However, poultry feeding with different concentrations of biologically active substances had different effects on the intensity of growth. Thus, in the age periods 1-7 and 8-14 days, the average weight of young birds in groups 1, 2, and 3 did not change compared to the control group. In the process of raising ducklings for meat, according to current regulations, the bird is transferred to another poultry house no later than the 21st day of growing. This technological technique causes stress in young animals, which negatively affects weight gain and survivability of birds. Therefore, special attention was paid to the indicators on the 21st day and at the end of the period of duck growing. The average weight at 21 days of age in birds of the 4th experimental group was 1251.5 ± 15.4 g, which is 0.9% more compared to the analogues of the control group, and in ducks of the 1st, 2nd and 3rd. In these groups, the weight of the birds did not change significantly relative to control. The average weight of ducks of the 4th experimental group on the 49th day of growing was 3467.6 ± 25.8 g, which is 7.5% more than the analogues of the control group (Figure 1).

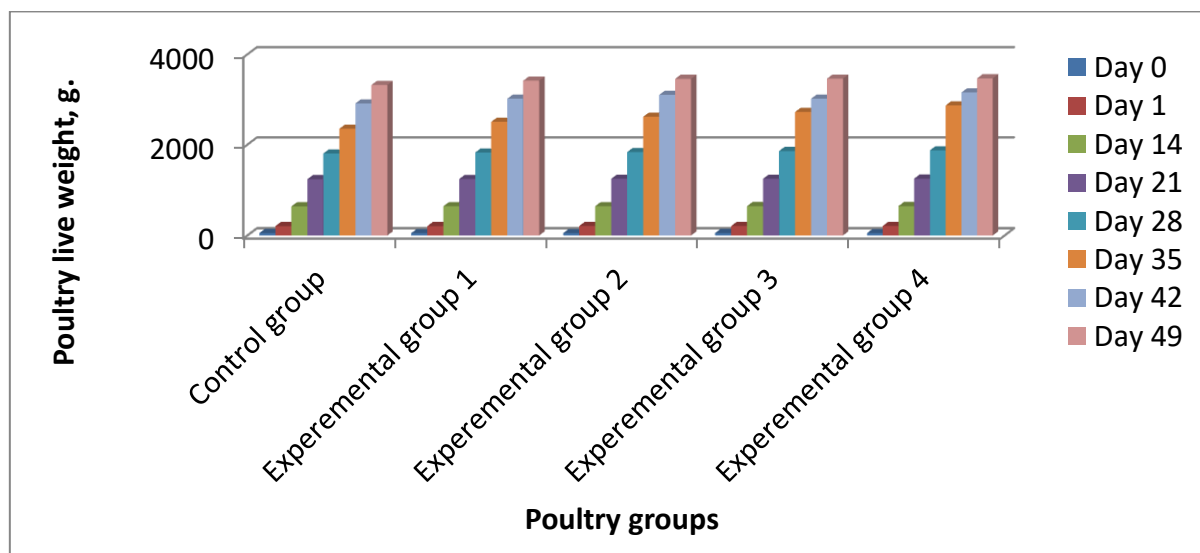


Figure 1. The dynamics of a change in live weight of ducklings in the growing process.

The absolute increase in live weight during the entire growing period was higher in the birds of the experimental groups. The largest difference with the control in the average daily gain of ducklings live weight was registered in the 4th experimental group at 22-28 days of age. This figure was 59.34 g and exceeded the same figure in the control group by 9.6%.

The largest relative weight gains of ducklings were recorded in all experimental groups in the 1-14-day growing period, which met the standard. With age, this figure tended to decrease. At the fourth week of growing, the largest difference in the average daily gain of live weight of ducks was registered in the 4th experimental group relative to the control which was 58.6 g (10.4%). It should be noted that a similar figure in the control group was lower than the requirements of the standard by 4.1%. Survival rate in all experimental groups was higher than in the control (in the range of 96.5-98.7%).

It has been found that under the conditions of implementation of mannan oligosaccharides to the diet, a reduction in feed consumption per 1 kg of growth for the entire period of growing relative to control by 1.5-3.9%. Thus, the highest productivity indicators are obtained under the condition of balanced complete feeding with 0.4% of mannan oligosaccharides from the 1st day until the end of the growing period.

The effect of mannan oligosaccharides isolated from the yeast cell walls of *Saccharomyces cerevisiae* on the species composition, activity and level of colonization of the intestinal microflora of ducklings was also established. In the 4th experimental group of birds, the total number level of the bacteria was slightly higher - $2.57 \pm 0.14 \times 10^6$ CFU/g against $2.35 \pm 0.12 \times 10^6$ CFU/g in the control. No effect of mannan oligosaccharides on microorganisms that do not have type I pili (*S. typhimurium*) was found. The studied carbohydrates showed the ability to reduce the population concentration of bacterial pathogens that have pili type I at a relatively low level: *E. coli* - $0.45 \pm 0.07 \times 10^3$ CFU/g (against $1.49 \pm 0.09 \times 10^3$ CFU/g in control).

The ability of the studied prebiotic hydrocarbons to adsorb bacteria of these strains provides inhibition of their proliferation and, accordingly, minimizes the toxic effect of endo- and exotoxins of pathogenic microorganisms on poultry.

Research results showed the ability of mannan oligosaccharides to regulate the intestinal microflora through selective bacteriostatic action on bacterial pathogens (*E. coli*) and stimulation and population of the intestine with lactic acid microorganisms *Lactobacillus* spp. and *Bifidobacterium* spp. (Table 1).

Table 1. Effect of yeast carbohydrate fraction (Alltech Inc.) on the intestinal poultry microbiocenosis (n=50, M ± m)

Rates	Groups of ducks	
	Control group	Experimental group 4
Total number of bacteria, × 10 ⁶ CFU/g	2.35 ± 0.12	2.57 ± 0.14*
<i>E. coli</i> , × 10 ³ CFU/g	1.49 ± 0.09	0.45 ± 0.07*
<i>Salmonella typhimurium</i> , × 10 ² CFU/g	0.09 ± 0.02	1.01 ± 0.03*
<i>Lactobacillus</i> spp., CFU/g	2.1 ± 0.08	3.42 ± 0.71*
<i>Bifidobacterium</i> spp., CFU/g	2.0 ± 0.32	3.37 ± 0.45*

* P<0.05

Implementation of mannan oligosaccharides isolated from the yeast cell walls had no effect on pH of chickens intestinal contents in the experimental and control groups.

Experiments have established a positive effect of the studied prebiotic fractions on the factors of natural resistance of the bird (Table 2). Bactericidal and lysozyme activity of blood sera of the 4th experimental group of ducks was higher than in the control. This fact creates additional immune protection factors and reduces the potential risks of poultry disease. Bactericidal activity of blood sera (BABS) of poultry from the 4th group was 34.85% higher compared to control, lysozyme activity of blood sera (LABS) - by 33.82%.

Table 2. Effect of yeast carbohydrate fraction (Alltech Inc.) on the natural resistance of ducks (n=50, M ± m).

Rates	Groups of ducks	
	Control group	Experimental group № 4
BABS, %	23.21 ± 0.32	31.30 ± 1.27*
LABS, %	19.22 ± 0.18	25.72 ± 2.15*
% of phagocytosis	58.34 ± 0.35	77.48 ± 3.23*
phagocytic index	4.78 ± 0.08	6.35 ± 1.11*
phagocytic number	6.98 ± 0.21	8.96 ± 2.21*

Indicators of cellular immunity of the organism were also more intense in birds of the 4th experimental group. Thus, the percentage of phagocytosis averaged 77.48 ± 3.23, phagocytic index - 6.35 ± 1.11, phagocytic number - 8.96 ± 2.21. According to the state assessment of immune competent organs (thymus, spleen and bursa of Fabricius), it was found that they were better developed in birds of the 4th experimental group compared to the control.

The relative weight of lymphoid organs to body weight was significantly higher in ducklings of the 4th experimental group, which received prebiotic carbohydrate fractions at a concentration of 0.4%/t of feed. On the 49th day of growing, an increase in the relative mass of the thymus (0.39%), bursa of Fabricius (0.368%), spleen (0.191%) was recorded. The results of the studies indicate a positive impact on survival rate of poultry of the 4th experimental group - 98% (vs. 89% in the control) (Table 3).

Table 3. Effect of yeast carbohydrate fraction (Alltech Inc.) on poultry productivity (n=50, M ± m).

Rates	Groups of ducks	
	Control group	Experimental group 4
Survival rate, %	89	98
Live weight, kg	2.8 ± 0.15	3.2 ± 0.15*
Yield of semi-dressed carcasses to the pre-slaughter mass, %	82.5	82.7
Yield of dressed carcasses, %	72.9 ± 1.05	76.4 ± 1.13*
Set of processed offal, %	9.2	9.4
Fat, %	2.6 ± 0.3	2.7 ± 0.15*

Live weight of ducklings under conditions of feeding with prebiotic fractions was higher than the same indicator of the control group by an average of 0.4 kg, which was 14.3%. The yield of semi-dressed carcasses to the pre-slaughter weight of poultry of the 4th experimental group was 82.7%, the yield of dressed carcasses was 76.7%, the yield of dressed carcasses was 76.4 ± 1.13% (p<0,05), and fat yield 2.7 ± 0.15% (2.6 ± 0.3% in control) (p<0.05).

Comparing the research results of the test for adsorption of mannan oligosaccharides isolates with the data of the scientific literature, it can be noted that scientists have obtained similar results. Only those microorganisms strains that contain a specific protein lectin, having the ability to adsorb (bind) mannose, had a positive result *in vitro* (Wang et al., 2018).

Our results on the correction of the intestinal microbiocenosis are consistent with the data of other scientists. Numerous studies report the high effectiveness of prebiotics and their positive impact on the poultry functional state and its productivity (Abdel-Hafeez et al., 2017; Ashraf et al., 2019). With the help of modern genetic engineering it has become possible to isolate a concentrated pure fraction of mannan oligosaccharides from the yeast cell walls of the strain *Saccharomyces cerevisiae* (Alltech Inc.).

The main advantage of the prebiotic fractions of mannan oligosaccharides isolated from the yeast of strain *Saccharomyces cerevisiae* is their ability to adsorb bacteria of certain strains that have type I pili (recognize mannose), and thus prevent colonization of the intestine by pathogenic microorganisms. They are not destroyed by digestive enzymes, bind to receptors and are firmly held on the surface of bacterial cells, which makes it impossible for pathogens to invade and fix on the surface of intestinal epithelial cells (Xue et al., 2017). Under the conditions of application of mannan oligosaccharides (Alltech Inc.) to broilers, scientists state a decrease in the level of intestinal colonization of broilers by *E. coli*, *Salmonella* spp., *Campylobacter* spp., as well as inhibition of proliferation of harmful bacteria by oligosaccharide metabolites and their transit through the intestine. Mannan oligosaccharides stimulate the development of normal intestinal microflora (family *Lactobacillaceae* and *Bifidobacillaceae*), which have beneficial properties, including antimicrobial activity against pathogenic microorganisms. This fact has a positive effect on the poultry functional state and its productivity (Li et al., 2007).

The researchers concluded that the concentration of *Lactobacillus* spp. and *Bifidobacterium* spp. in the intestine was higher (p<0.05), under the conditions of implementation of the prebiotic yeast fraction of mannan oligosaccharides (Alltech Inc.), than in the control, as well as in the groups where antibiotics were used (Abdel-Hafeez et al., 2017; Wang et al., 2018). Previous studies have shown that prebitant fractions of mannan oligosaccharides do not affect the concentration of volatile fatty acids and lactate in the intestinal contents of poultry (Kasyanenko et al., 2018).

The use of these prebiotics also had a positive effect on the condition of lymphoid organs. According to the assessment of the state of immune competent organs, it was found that they were better developed in broilers of the experimental group (Wang et al., 2018; Kirkpınar et al., 2018). It is proved that the immune modulating effect of mannan oligosaccharides activates macrophages of intestinal lymphoid tissues, which leads to the improvement of not only cellular but also humoral and skin immunity (Chacher et al.,

2017). Implementation of a *S. cerevisiae* culture to the diet of laying hens causes an increase in feed consumption and egg weight of laying hens, without affecting the microbial flora in their digestive system (Özsoy et al., 2018).

Analyzing our *in vivo* results, the effectiveness of the use of yeast carbohydrate fraction MOS (Alltech Inc.) in the diet of poultry to increase productivity and obtain quality and safe poultry products. Scientific literature provides data showing the reduction of pre-slaughter live weight of poultry under the conditions of prebiotics based on yeast fractions implementation (Abdel-Hafeez et al., 2017). These differences in results may be related to the origin and chemical composition of the active substances of the prebiotic fractions. Most researchers note an increase in pre-slaughter live weight of poultry and improved feed conversion, based on the regulation of intestinal microbiocenosis, which in turn, provides a higher level of adsorption and nutrient intake (Sultan et al., 2015, Ahmed et al., 2015).

We established that a component of the dynamic development of duck growing technologies is the inclusion in the diet of yeast prebiotic carbohydrate fraction of mannan oligosaccharides (Alltech Inc.), which have a positive effect on the functional state of poultry, regulate intestinal microbiocenosis and poultry survival rate by 9%. In addition, a number of researchers in their publications report the feasibility of using mannan oligosaccharides isolated from the yeast strain *Saccharomyces cerevisiae*, as immunomodulators and growth stimulants as an alternative to antibiotics (Afrouziyeh et al., 2014; Kasyanenko et al., 2018).

The effectiveness of mannan oligosaccharides implementation has also been confirmed by experiments on cattle (Meller et al., 2014; Tristant & Moran, 2015), small cattle (Cömert et al., 2015), pigs (Edwards et al., 2014; Shen et al. al., 2017) and horses (Jouany et al., 2009).

Conclusion

Aqueous solutions of 0.1-0.4% mannan oligosaccharides (Alltech Inc.) adsorb 64.3% of *E. coli* O₂ strains, 25% of *C. diversus* isolates, 100% of the studied *P. vulgaris* and *K. pneumoniae* strains. While studying of *S. typhimurium* isolates, the adsorption process was not recorded. It is effective to use in the diet of ducks mannan oligosaccharides (Alltech Inc.) at a dose of 0.4% by weight of feed from the 1st day and until the end of the growing period (49 days). Mannan oligosaccharides affect the microbial balance of ducklings intestines by selective action on potentially pathogenic microorganisms: the concentration of *E. coli* O₂ decreased by 69.79% compared to control. Reducing the load of pathogenic microorganisms on the intestinal microbiotic structure and metabolites of mannan oligosaccharides provided the conditions for active proliferation of beneficial microorganisms. The concentration of lacto- and bifidobacteria increased by 62.82% and 68.53% compared with the control, respectively.

Immune modulating effect and positive dynamics in relation to the indicators of the natural resistance were also recorded: increase in the serum bactericidal activity by 34.85% (p<0.05), lysozyme activity in serum by 33.82% (p<0.05), phagocytic index - by 32.81% (p<0.05), phagocytic number - by 28.37% (p<0.05). The use of mannan oligosaccharides in the diet contributed to better development and increase in the relative weight of immune competent organs, increase in the survival rate of poultry by 9.0%, pre-slaughter live weight - by 14.3% and meat yield - by 4.8%. The effectiveness of practical application of an alternative ecologically safe and natural method in the technological process of growing ducks for meat by feeding mannan oligosaccharides (Alltech Inc.) is proved.

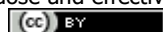
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