Foods and Raw Materials, 2020, vol. 8, no. 2

E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

Check for updates

DOI: http://doi.org/10.21603/2308-4057-2020-2-337-347
Available online at http://jfrm.ru/en

Yeast-rich mannan fractions in duck cultivation: prospects of using

Sergij M. Kasjanenko[1], Oksana I. Kasjanenko[1], Liudmyla V. Nagornaya[1], Valentyna A. Yevstafieva[2], Vitaliy V. Melnychuk[2], Galina A. Lukyanova[3], Irina A. Gurenko[3,*]

[1] Sumy National Agrarian University, Sumy, Ukraine

[2] Poltava State Agrarian Academy, Poltava, Ukraine

[3] V.I. Vernadsky Crimean Federal University, Simferopol, Russia

* e-mail: gur1976@yandex.ru

Received July 15, 2019; Accepted in revised form December 20, 2019; Published August 25, 2020

Abstract:
Introduction. Due to the trend of avoiding antibiotics and acquiring eco-friendly products, the use of environmentally safe preparations is becoming increasingly relevant in poultry farming.
Study objects and methods. We used *Salmonella enteritidis* and *Campylobacter jejuni* isolated from poultry carcasses. At the first *in vitro* stage, we studied the ability of mannan oligosaccharides, isolated from the cell walls of *Saccharomyces cerevisiae* yeast, to adsorb bacterial pathogens. At the second stage, we studied the influence of fraction on the activity, colonization and microflora composition of ducklings' intestines. At the third stage, we determined the antagonistic activity of *Bifidobacterium* spp. (*Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*) and *Lactobacillus* spp. (*Lactobacillus fermentun*, *Lactobacillus salivarius*, *Lactobacillus acidophilus*) against *Salmonella enteritidis* and *Campylobacter jejuni* isolates. The experiment was conducted on the ducklings of Star 53 H.Y. cross. Their diet was supplemented with probiotics, prebiotics, and their combination.
Results and discussion. In vitro studies showed the ability of mannan oligosaccharides isolated from the cell walls of *Saccharomyces cerevisiae* yeast to adsorb *Salmonella enteritidis* and *Campylobacter jejun*. *In vivo* experiment showed the ability of mannan oligosaccharides to prevent colonization of poultry intestines by bacterial pathogens with type I fimbriae.
Conclusion. The reisolation rate of ducks infected with *Salmonella enteritidis* was 53.6% lower, and those infected with *Campylobacter jejuni*, 66.2% lower than the control. Mannan oligosaccharides added to the diet did not affect the concentration of lactobacilli, enterococci, and anaerobic bacteria in the ducks' intestines. A combined use of *Bifidobacterium* spp. and mannan oligosaccharides improved the preservation of poultry stock by 8.7%, which made it an effective way to prevent poultry salmonellosis.

Keywords: Prebiotics, probiotics, mannan oligosaccharides, microorganisms, bacterial pathogens, *Salmonella* spp., *Campylobacter* spp., poultry, ducks, productivity

Please cite this article in press as: Kasjanenko SM, Kasjanenko OI, Nagornaya LV, Yevstafieva VA, Melnychuk VV, Lukyanova GA, et al. Yeast-rich mannan fractions in duck cultivation: prospects of using. Foods and Raw Materials. 2020;8(2): 337–347. DOI: http://doi.org/10.21603/2308-4057-2020-2-337-347.

INTRODUCTION

In the world production of poultry, the share of waterfowl meat is 7.2%, specifically duck meat – 4.2%, goose meat – 3%. Their share in the gross production of poultry meat tends to increase. In industrial poultry farming, the problem of controlling bacterial infections of waterfowl is of genuine concern. *Salmonella* and *Campylobacter* are considered the most common etiological zoonotic factors worldwide, with productive poultry being the main source of infection.

In recent years, there has been an increase in the relative number of infections caused by *Salmonella* spp. and *Campylobacter* spp. The microorganisms are widespread in most warm-blooded and farm animals, including poultry. Ducks' infection with salmonella can be detected at the age of about 14 days, and by the end of cultivation the whole flock can be found infected. Experimental studies showed that a small dose (less than 40 CFU) of *S. enteritidis* is sufficient to fully colonize the poultry intestines. This can lead to complete flock's

infection in 48 h [5–7]. Microorganisms can colonize the intestinal tract of poultry in large quantities, often at above 10^6–10^8 CFU/g of intestinal contents. The highest concentrations of bacterial pathogens are known to be present in the intestinal mucosa [4].

Poultry products can be contaminated at many stages of the "from farm to table" food chain, but the strategic one is the stage of primary poultry production. Following biosafety guidelines of GMP/HACCP significantly reduces the colonization of poultry by bacterial pathogens and, later, the contamination of carcasses during processing. The European Food Safety Agency's monitoring (2008–2018) showed that about 86% of poultry carcasses in Europe were contaminated with *Campylobacter* and *Salmonella* bacteria.

In poultry production, main methods of infection control are taken at the stage of the cultivation in farms. Environmentally safe methods that ensure poultry quality and safety hold promise. Effective systems of poultry cultivation, feeding, and maintenance are reqired to control the spread of *Salmonella* and *Campylobacter* in poultry products. Bio-safety measures, decontamination of dropping and water are potentilly productive. Antibacterial drugs in treating of bacterial infections in poultry are considered a risk factor contributing to the development of antibiotic-resistant strains.

Following the trend of avoiding antibiotics, the search for new control methods is becoming increasingly important in poultry farming. The application of antimicrobial alternatives is highly potential. They include feed additives that are inhibitors of bacterial pathogens, as well as probiotics, prebiotics, bacteriophages, bacteriocins, which in combination prevent antibiotic-resistant strains of microorganisms and inhibit their proliferation [9–12].

Consequently, natural alternative antibacterial preparations are a way to reduce poultry gut colonization by pathogenic microflora. This is the most acceptable natural alternative to salmonella and campylobacter control that is economically viable and does not pose a risk to human health, animals, or the environment [3, 9]. Effective protection of poultry against pathogens, naturalness and safety, growth promotion, and economic effectiveness are the criteria for new alternatives to antibiotics [11, 13].

One of the requirements for probiotics use is the competitiveness of antagonistic microflora found in them. In order to prevent intestinal colonization by bacterial pathogens, probiotics are recommended for use from the first day of the birds' life. Prebiotics promote the development of birds' own symbiotic microflora, which can inhibit pathogens and reduce their adhesion to enterocytes.

Research suggests that some natural compounds have biological activity against salmonella proliferation, but few have shown efficacy in experiments on animals. "Actigen" prebiotic (Alltech) is a concentrated pure fraction of mannan oligosaccharides isolated from the cell walls of *Saccharomyces cerevisiae* yeast. The main advantage of these complex carbohydrates is their ability to adsorb certain strains of bacteria that have type I fimbriae (mannose-sensitive) and prevent intestinal colonization by pathogens. Besides, the industrial experiment proved the influence of combined use of mannan oligosaccharides and probiotics on intestinal microbiocenosis and duck productivity [14, 15].

We aimed to develop a method for preventing bacterial infections and increasing duck productivity using probiotics and prebiotics. The method was based on the study of adsorbing capacity of mannan oligosaccharides (MOS) and antagonistic properties of *Bifidobacterium* spp. and *Lactobacillus* spp. against *Salmonella enteritidis* and *Campylobacter jejuni*. We also aimed to analyze a combined effect of the cultures on gut microbiocenosis (activity and colonization) and on productivity of ducks.

STUDY OBJECTS AND METHODS

We used *Salmonella enteritidis* and *Campylobacter jejuni* isolated from poultry carcasses of Ukrainian farms. The studies were carried out in 2014–2018 at Sumy National Agrarian University, Sumy. The poultry carcasses were subjected to a detailed examination for pathomorphological changes. The liver, muscles, cloaca contents, ovaries, and various segments of the ovoid were aseptically assembled to be screened for salmonellosis and campylobacteriosis. Isolation and identification of microorganisms was carried out using tests recommended by "Bergey's Manual" (1997) [35].

At the first stage (*in vitro*), we studied the ability of mannan oligosaccharides isolated from the cell walls of *Saccharomyces cerevisiae* yeast to adsorb bacterial pathogens. In our experiments we used 27 strains of *Salmonella enteritidis* and 13 strains of *Campylobacter jejuni* isolated from ducks' chilled carcasses (liver, muscles, cloaca).

We used the daily agar culture of bacteria with 1% red blood cells of guinea pigs. *Salmonella* (1.5×10^9 CFU/mL) was used as an antigen. Erythrocytes were derived from the blood of a pre-selected donor (guinea pigs). Blood was placed in flasks containing sodium citrate and filtered through a cotton gauze filter to remove fibrin and small blood clots. Blood was centrifuged with sodium chloride isotonic solution four times (1500 rpm, 10 min). Then we introduced it into a 10% suspension of phosphate buffer solution (pH 7.0–7.2). The washed red blood cells were stabilized with 0.2% acrolein (acrylic aldehyde) solution in the phosphate buffer (1:1) and incubated in water bath at 37°C for 30–40 min while stirring periodically. Erythrocytes were washed three times by centrifuging with phosphate buffer at 5000 rpm. To improve the sorption properties of red blood cells, we treated

them with tannin, combining equal parts 5% of frozen stabilized red blood cells and tannin solution (1:30 000). The mixture was left in the thermostat at 37°C for 40 min, then it was washed twice with phosphate buffer solution (pH 7.2–7.4) and then twice with sodium chloride isotonic solution (pH 7.2–7.4). To sensitize the antigen, we used a 1% red blood cell suspension. Suspensions were left for 24 h at 4°C to exclude spontaneous hemagglutination.

The degree of agglutination of the salmonellas isolated was determined by combining prepared suspended microorganisms and the aqueous solution of mannan oligosaccharides (0.2, 0.3 and 0.4 g/L) in a ratio of 1:1. *E. coli O2* test culture was used as a positive control of the agglutination level of the pathogen. One-percent red blood cell suspension in phosphate buffer solution (pH 7.2–7.4) was used as a negative control [16–18].

At the second stage, we studied the influence of fraction (Aktigen, Alltech Inc.) on the activity, colonization and species composition of the microflora of young ducks' intestines. Sixty male ducklings aged 30 days were used in the study. Each experiment involved one control and two experimental groups (50 heads in each). First experimental group was infected with *Salmonella enteritidis*, and the other group with *Campylobacter jejuni* (1×10^4 CFU/mL per os). Ducklings were kept in sterile boxes on the floor and fed by standards. They had free access to feed and water. In experimental groups, the birds received a prebiotic fraction of MOS (0.4 kg/t) together with the feed. Ten days after the infection we determined the concentration of salmonellas, campylobacil, lactobacil, bifidobacterium, and total concentration of anaerobic bacteria using dilution plate counting.

At the third stage, we determined the antagonistic activity of *Bifidobacterium* spp. (1.0×10^9 CFU/mL): *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium bifidum* and *Lactobacillus* spp. (1.0×10^9 CFU/mL): *(Lactobacillus fermentun*, *Lactobacillus salivarius*, *Lactobacillus acidophilus* against *Salmonella enteritidis* and *Campylobacter jejuni* isolates. Suspensions of bacterial probiotic cultures in a concentration of 1×10^9 m.c/cm^3 were sown on Petri dishes and incubated for 24 h at 37°C. After that, suspensions with microorganisms (*Salmonella enteritidis* and *Campylobacter jejuni*) in a concentration of 1×10^9 m.c/cm^3 were inoculated by streaking. The dishes with inoculation were incubated at 37°C for 24–72 h. We recorded the diameter of zones with no growth of test cultures. To control microbial growth, we used Preston-agar for *Campylobacter*, "Salmonella different agar" for *Salmonella*, as well as MPA and MPB for probiotics.

We used the *Star 53 H.Y. cross* ducklings to determine the effectiveness of probiotics, prebiotics, and their combination. The birds were randomly divided into 4 groups, 123 birds in each. Each group included 3 flocks, 41 birds in each (12 flocks in total). The control group received the main diet only. Three experimental groups received three different supplements in addition to the main diet: bifidobacteria (1.5×10^9 CFU/mL), mannan oligosaccharides ("Actigen" prebiotic), and a combination of *Bifidobacterium* spp. and *Lactobacillus* spp. (1.5×10^9 CFU/mL) in a ratio of 1:1 and the fractions of mannan oligosaccharides (0.4 kg/t of feed). These supplements were mannan-rich fractions isolated from

Table 1 Diet composition

Ingredients	Starter	Grower
Wheat	55.00	62.00
Full fat whole soya	12.00	12.00
Soybean neal	23.00	20.00
Limestone	0.72	0.50
Di-calcium phosphate	1.65	1.85
Soybean oil	4.50	5.00
Salt	0.20	0.20
Sodium bi-carbonate	0.18	0.16
DL Methionine	0.50	0.40
L-Lysine	0.37	0.30
Threonine	0.25	0.13
Vitamin-mineral premix	0.50	0.50
Nutrient analysis, %, or as indicated		
Metabolic Energy, kcal/kg	3000	3125
Crude Protein	24.10	22.00
Lysine	1.42	1.35
Methionine+Cysteine	1.10	0.93
Calcium	1.05	0.85
AVAILABLE PHOsphorous	0.50	0.42
Vitamin-Mineral Premix[1]		
Copper, mg	15.00	15.00
Iodine, mg	1.00	1.00
Iron, mg	30.00	30.00
Manganese, mg	112.00	112.00
Selenium, mg	0.40	0.40
Zinc, mg	105	105
Synergen[2], g	158	158
Vitamin A (IU)	13.00	12.00
Vitamin D_3 (IU)	4.75	4.50
Vitamin E (IU)	70.00	50.00
Vitamin K, mg	3.00	2.75
Thiamin (B_1), mg	3.00	2.50
Riboflavin (B_2), mg	10.00	8.00
Niacin, mg	55.00	50.00
Pantothenic Acid, mg	17.00	15.00
Pyridoxine (B_6), mg	5.00	4.50
Biotin, mg	0.30	0.25
Folic Acid, mg	2.00	1.70
Vitamin B_{12}, mg	200.00	185.00
Vitamin C, mg	200.00	200.00
Choline, mg	475.00	450.00

[1]Vitamin-Mineral Premix manufactured by Target Feeds, Shropshire, UK

[2]Synergen (g) is a commercial enzyme product by Alltech, Inc.

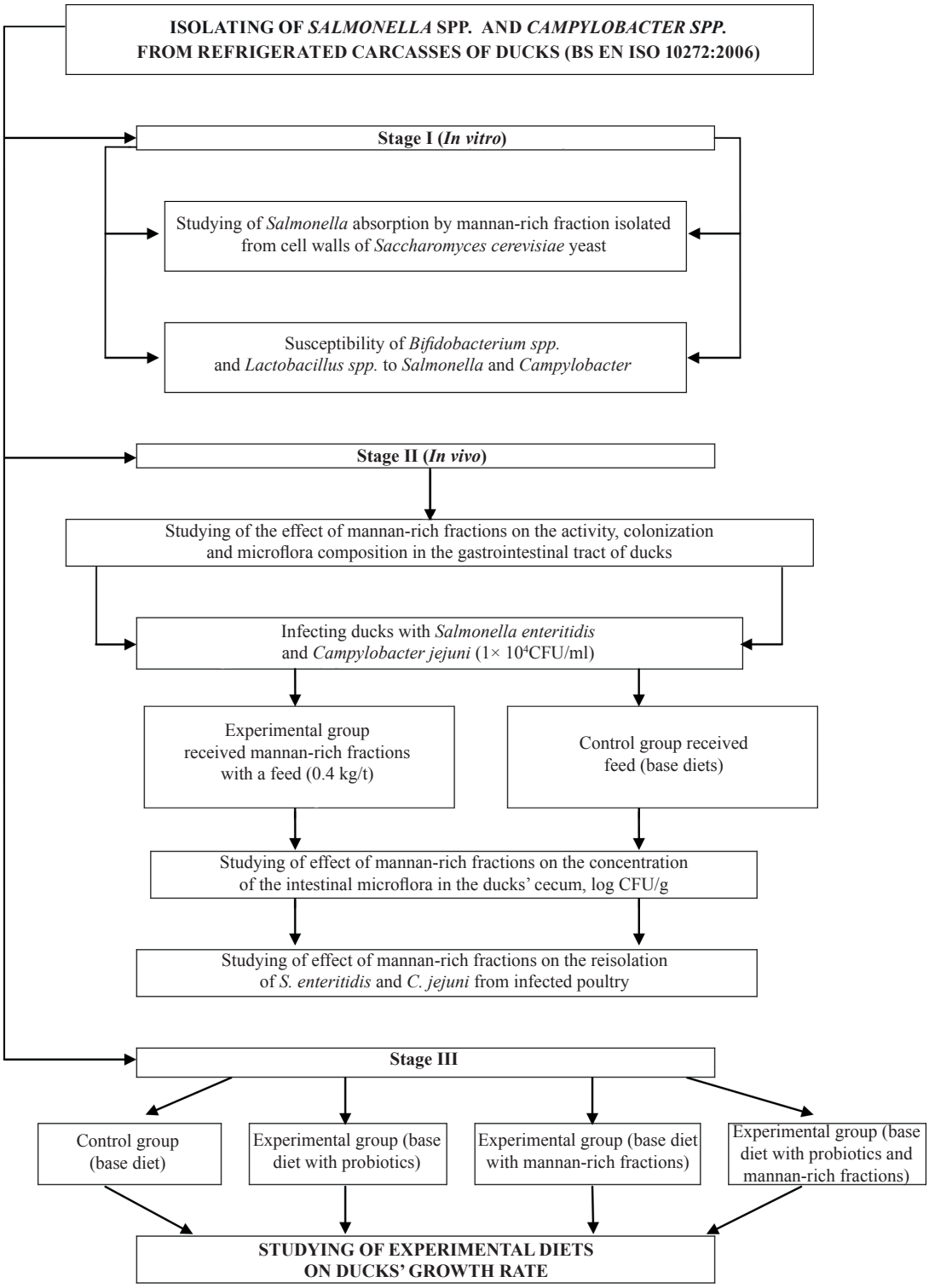


Figure 1 Research scheme

the cell wall of *Saccharomyces cerevisae* yeast. The main diets were prepared at a commercial feed mill and consisted mainly of wheat and soybean flour, as shown in Table 1 [19, 20].

The birds were given starter diets from hatch till day 20, grower diets – from day 21 to 49. Feed and water was provided throughout the whole study period. Initially, the room temperature was maintained at 30°C for 10 days, and then gradually decreased every second day by 1°C. During the experiment, the lighting regime was the following: for 16 h – light, 8 h – darkness, which lasted 49 days. All conditions were the same for all the four groups. The birds were weighed when hatched, on days 21 and 49. We also measured feed intake to

estimate feed conversion rates and body weight gains. The intact parts of the cecum were withdrawn from 10 randomly caught birds aged 49 days immediately after euthanasia. The contents of the cecum were placed in sterile test tubes. Then, the tubes were instantly frozen in liquid nitrogen, lyophilized and stored at – 80°C for further analysis.

No principles of the bioethics code were violated during the experiments [21].

The general scheme of our experimental and practical studies is shown in Fig. 1.

Bacteriological analysis. We tested the laying houses for *Campylobacter* spp. before placing the birds there and on day 21. According to the methods described in BS EN ISO 10272:2006, the swabs were placed in 50 mL of isotonic solution and kept at 260 rpm for one minute [23, 24]. The suspension (0.1 cm^3) was then transferred to two dishes with breeding ground (Preston agar base for *Campylobacter*) and incubated in microaerobic atmosphere (85% N_2, 10% CO_2 and 5% O_2) at 40.5 ± 1°C. Then we examined them in 44 ± 4 h for typical and/or suspicious *Campylobacter* spp colonies.

Then, *Salmonella enteritidis* was isolated from the material. The serotyping of *Salmonella spp.* was carried out according to the methods with some modification according to the data [28, 32–34].

Statistical analysis. Weight gains and feed conversion rates were studied for statistical group differences using the Student's T-test. The results of the microbiological analysis were logarithmic and evaluated for the statistical difference between the indicators that were measurable.

RESULTS AND DISCUSSION

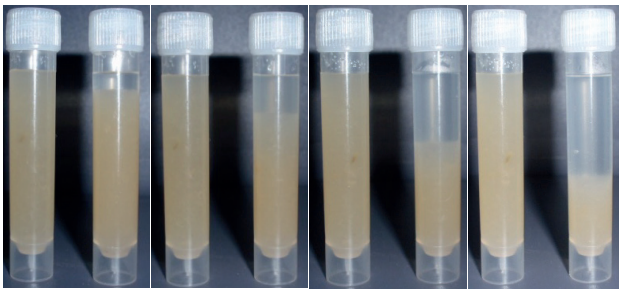
The aim of our research was to study effects of mannan oligosaccharides fractions and probiotics on *Salmonella enteritiadis* and *Campylobacter jejuni*.

In vitro experiments showed that 0.2–0.4% aquatic fractions of mannan oligosaccharides could adsorb all the *Salmonella* strains and *E. coli* O_2 test cultures (positive control).

We detected the most active and pronounced ability to adsorb bacterial pathogens in *in vitro* experiments with 0.4% aqueous fraction of mannan oligosaccharides. We recorded the beginning of the adsorption process within 2 min. The active process was manifested in the form of finely-divided sediment and clearing of the supernatant. In 8–10 min we observed significant sedimentation (Fig. 2 a–d).

The formation of the sediment illustrates the adsorption process that occurred in the test tube. The same process can occur in the gut in animals and poultry.

Intestinal colonization by pathogens begins with the binding of cells to the epithelium of the intestinal mucosa [17]. Pathogens, including most types of *Salmonella*, *E. coli*, and *Campylobacter* attach to the gut



(a) (b) (c) (d)

Figure 2 Absorption of *Salmonella enteritidis* with 0.4% concentrated pure fraction of mannan oligosaccharides *in vitro*: a – in 2 min; b – in 4 min; c – in 6 min; d – in 10 min

via receptors (fimbriae) specific to certain carbohydrates containing mannose, which localize on the surface of intestinal mucosal epithelium cells [14].

When entering the intestines of poultry with feed, mannan-rich fractions bind to receptors of bacterial cells that have type I fimbriae (mannose-sensitive). Fractions of mannan oligosaccharides are not broken down by digestive enzymes and are held firmly on the surface of bacteria. Bacteria with blocked receptors cannot gain a foothold on the surface of epithelial cells – they transit through the gastrointestinal tract [13]. Thus, we found that the active concentration of mannan-rich fractions could successfully adsorb *Salmonella*, a pathogen that can cause foodborne diseases.

The following experiment examined the effects of fractions rich in mannanooligosaccharides on the activity, colonization, and species composition of microflora in ducks' intestines.

At the second (*in vivo*) stage, we determined the effect of mannan-rich fractions on the number of bacteria in the gut of experimentally infected ducks aged 30 days by type I fimbriae bacterial strains (*C. jejuni* and *S. enteritidis* strains). In experimental groups of birds that received prebiotic MOS fractions with feed, the level of bacteria with type I fimbriae decreased. The effect of mannan oligosaccharide-rich fractions on the concentration of intestinal microflora of ducks infected with *S. enteritidis* is shown in Fig. 3.

The effect of mannan-rich fractions on the concentration of intestinal microflora of ducks infected with *C. jejuni* is shown in Fig. 4.

The results showed that mannan oligosaccharides could regulate intestinal microflora due to their selective ability to inhibit *Salmonella* spp. and *Campylobacter* spp. proliferation, preventing pathogenic colonization of the intestines and minimizing its toxic effect on the poultry. Concentration of *Salmonella* spp. in the ducklings' gut was lower by 3.69 log CFU/g and *Campylobacter spp.* by 3.27 log CFU/g compared to the control, respectively. Metabolites of functional oligosaccharides did not affect the levels of intestinal colonization by pathogenic bacteria (coliforms and

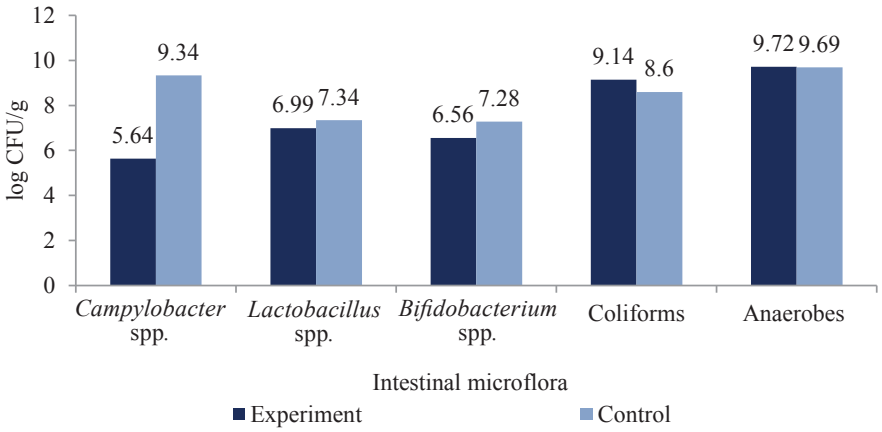


Figure 3 Effects of mannan oligosaccharide-rich fractions on the concentration of intestinal microflora of ducks infected with *S. enteritidis* log CFU/g

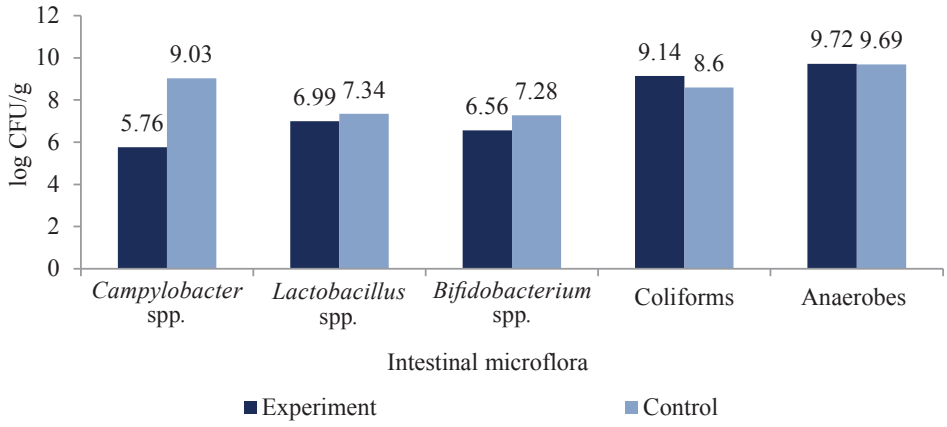


Figure 4 Effects of mannan oligosaccharide-rich fractions on the concentration of intestinal microflora of ducks infected with *C. jejuni* log CFU/g

anoerobeds). They did not prevent *Lactobacillus* spp. and *Bifidobacterium* spp. proliferation either, which contributed to the colonization of beneficial bacteria in the birds' intestines.

Regulation of intestinal microbiocenosis can potentially have a positive effect on immune response mechanisms, i.e. to strengthen immunity and enhance the poultry population.

The effect of mannan-rich fractions on the ducklings' gut microflora infected with *S. enteritidis* is shown in Fig. 4a. The reisolation rate of *S. enteritidis* and *C. jejuni* in the test group, which received prebiotic mannan-rich fractions with feed, decreased by 53.6 and 66.2%, respectively, compared to the control group (Figs. 5a, 5b).

Bifido- and lactobacteria also displayed antagonistic activity against *Campylobacter jujuni* and *Salmonella enteritidis* isolates. It makes them possible to be used for the prevention of infectious diseases caused by sensitive strains of pathogens to prebiotic drugs. *Bifidobacterium* spp. and *Lactobacillus* spp. suppressed the growth of microorganisms to different extents (Table 2).

Twelve isolates (92.6%) of *Campylobacter* spp. were susceptible to bifidobacteria. The inhibition zone of campylobacter was 5.1 ± 0.3 mm. Ten *Campylobacter jujuni* isolates showed a moderate level of antagonistic activity – 76.9%, with the inhibition zone of 5.1 ± 1.0 mm.

Twenty four isolates (88.9%) of *S. enteritidis* were susceptible to bifidobacteria; the inhibition zone of *S. enteritidis* was 5.5 ± 0.4 mm. The antagonistic activity of lactobacilli against *S. enteritidis* showed a moderate level: 22 isolates (81.5%) had inhibition zone of 4.9 ± 0.5 mm. Bifidobacteria were more active against *Campylobacter* spp. and *Salmonella* spp. It makes it possible to use probiotics to prevent and treat infectious diseases caused by susceptible strains of pathogenic microorganisms to the drug. To improve the ducks' productivity, we studied the effect of mannan-rich fractions. The experiment plan is given in Table 3.

To solve the problem of bacteriosis prevention and increase of birds' productivity, we also studied the effect of a combined use of mannan oligosaccharides and probiotic bifidobacteria and lactobacilli.

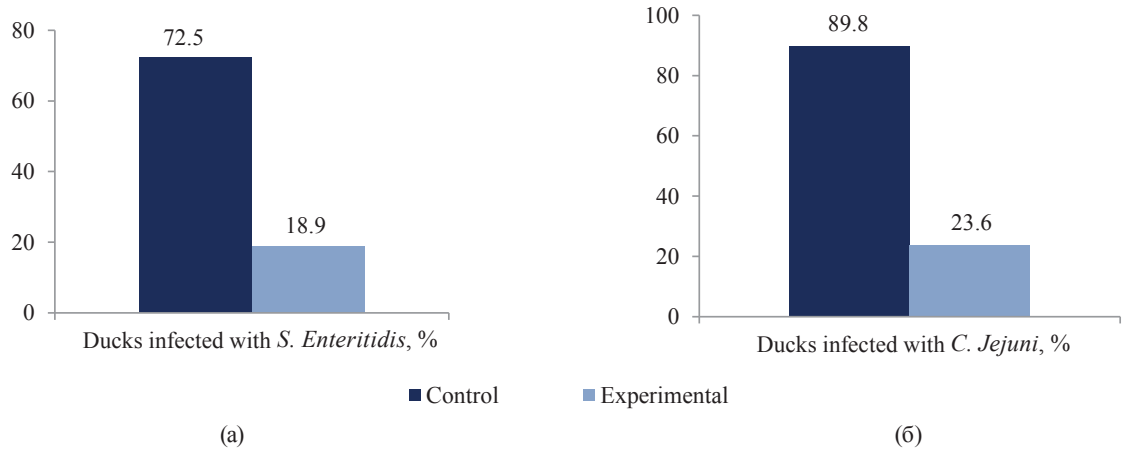


Figure 5 Effects of mannan-rich oligosaccharides on the reisolation rate of salmonellas from the intestines of poultry infected with *S. Enteritidis* (a) and campylobacteria from the intestines of poultry infected with *C. Jejuni* (b)

Table 2 Susceptibility of *Bifidobacterium* spp. and *Lactobacillus* spp. (M ± m), %

Microorganism	Inhibition zone, mm		Control of growth on Preston agar	Control of growth on Salmonella agar M1078, HiMedia
	Bifidobacterium spp.	*Lactobacillus* spp.		
C. jujuni	5.3 ± 0.2	5.1 ± 0.3	+	
S. enteritidis	5.5 ± 0.4	4.9 ± 0.5		+

(+) – signs of growth, $P < 0.05$

The first stage of the experiment included 20320 ducks (Star 53 Y.Y.) divided into four groups: one control and three experimental ones. The experiment was carried out three times (81280 ducks in total). Probiotics were added to the diet of the ducks with water (10 cm^3 per 4 kg duck weight) once a day from the first day until the end of the fattening period (49 days).

We added mannan-rich fractions to the base diet – 400 g/t of feed. We added bifidobacteria and mannan-rich fractions to the duck diet once a day until the end of fattening period (49 days). The analysis showed higher results during all periods of the birds' life compared to the control groups (Table 4).

At the age of 21 days, the average growth rate of ducks receiving probiotics with mannan-rich fractions was 87.3 g vs. to 83.6 g in the control group. We noticed a similar trend at the age of 21 days with an average daily growth of ducks from 101.4 g to 107.6 g. The experimental group III after 21 days exceeded the control group by 7.6%.

On day 21, the body weight of ducks receiving probiotics, mannan-rich fractions, and their mix exceeded that in the control group by 1.1, 1.9 and 3.6%, respectively. The body weight was 1273 ± 67 g, 1283 ± 42 g, and 1305 ± 34 g, respectively.

In 49 days, the body weight of the ducks receiving mannan-rich fractions, as well as their mix was 3415 ± 95.5, 3459 ± 87.4, and 3547 ± 24.3 g, respectively, which exceeded the weight of the ducks of the control group by 3.1, 4.4 and 7.1 % (Table 4). In addition, a similar trend was detected with average daily gain in duck weight. In 49 days, it was 59.2, 59.7, and 61.3 g for experimental groups, exceeding that in the control group by 1.2, 2.1, and 4.7 %, respectively. The ducks receiving the mix of probiotics and mannan-rich fractions gained weight more intensively compared to the birds having the other diets (Table 5).

CONCLUSION

In vitro studies showed the ability of prebiotic mannan-rich fractions isolated from the cell walls of *Saccharomyces cerevisiae* yeast to adsorb type I fimbriae bacterial pathogens (*S. enteritidis* and *C. jejuni*) and prevent colonization and proliferation of pathogenic microorganisms on the surface of ducks' intestinal epithelial cells.

We studied the influence of fractions rich in mannan oligosaccharides on activity, colonization, and species composition of duck gut microflora.

Table 3 Bifidobacteria and mannan-rich fractions in the duck's diet (n = 20320)

Groups	Diet
Control	Base diet "Starter" from day 1 to day 20 of life Base diet "Grower" from day 21 to day 49
Experimental group I	Base diet + probiotics from day 1 to day 49
Experimental group II	Base diet + mannan-rich fractions from day 1 to day 49
Experimental group III	Base diet + probiotics + mannan-rich fractions from day 1 to day 49

Table 4 Effect of experimental diets on duck growth (M ± m)

Indexes	Groups			
	Control (base diet)	Experimental group I (diet with probiotics)	Experimental group II (diet with mannan-rich fractions)	Experimental group III (diet with probiotics and mannan-rich fractions)
	Days 0–21			
number of birds on day 1	20320	20320	20320	20320
average body weight of ducks, g	1259 ± 45*	1273 ± 67*	1283 ± 42*	1305 ± 34*
average body weight of ducks,%	100	101.1	101.9	103.6
average daily gain of ducks, g	83.6 ± 8.4	84.8 ± 8.1	85.7 ± 9.5	87.3 ± 8.2
average daily gain of ducks,%	100	101.4	102.5	107.6
safety of poultry,%	91.3	92.4	93.46	104.4
	Days 22–49			
number of birds on day 22	18552	18703	18991	19537
average body weight of ducks, g	3312 ± 35.3*	3415 ± 95.5*	3459 ± 87.4*	3547 ± 24.3*
average body weight of ducks,%	100	103.1	104.4	107.1
average daily gain of ducks, g	58.5	59.2	59.7	61.3
average daily gain of ducks,%	100	101.2	102.1	104.7
number of birds on day 49, heads	16537	17353	18060	19127
number of birds on day 49, %	89.14	92.78	95.10	97.9
cost of feed	97644.3	103216.7	104128.4	105331.7
feed consumption per 1 kg of growth for 49 days, kg	2.01	1.91	1.88	1.86
feed consumption per 1 kg of growth for 49 days, %	100.0	91.52	93.53	95.02

*The values in the column for each treatment stage that does not share the overall upper index vary significantly ($P < 0.05$). Each value is an average of n = 3 flocks per diet with 36, 30 and 30 birds in the flock for each growing period, respectively. Comparisons between the groups were made using the Tukeys HSD test, $P < 0.05$ we considered statistically significant

Table 5 Average body weight of ducks receiving probiotics and mannan-rich fractions during different periods of growth and development, g/head (n = 50)

Age, weeks	Groups				
	Control (base diet)	Experimental group I (diet with probiotics)	Experimental group II (diet with mannan-rich fractions)	Experimental group III (diet with probiotics and mannan-rich fractions)	Standard values
0	52.35 ± 0.57*	52.64 ± 0.37*	53.22 ± 0.67*	52.66 ± 0.81*	52
1	205.52 ± 1.43*	206.53 ± 0.58*	208.42 ± 0.37*	215.53 ± 0.48*	206
2	640.37 ± 2.93*	642.34 ± 3.25*	645.38 ± 5.34*	678.59 ± 13.73*	645
3	1239.17 ± 5.52*	1247.72 ± 5.51*	1258.42 ± 14.53*	1305.48±34.27*	1257
4	1814.58 ± 7.74*	1874.25 ± 22.47*	1883.58 ± 11.43*	1933.53 ± 31.45*	1876
5	2351.34 ± 33.34*	2404.43 ± 27.48*	2486.35 ± 42.28*	2592.63 ± 47.81*	2503
6	2918.42 ± 27.56*	2948.27 ± 25.58*	2915.37 ± 33.59*	3197.37 ± 49.56*	3100
7	3319.68 ± 26.85*	3419.62 ± 24.37*	3528.63 ± 25.57*	3683.87±25.79*	3500

S. enteritidis reisolation rate decreased by 53.6% and *C. jejuni* – by 66.2% in ducks receiving fractions rich in mannanooligosaccharides, compared to the control group. Experiments showed that the addition of prebiotic fractions to the diet did not affect the concentration of lactobacilli, bifidobacteria, enterococci, and anaerobic bacteria.

Bifido- and lactobacteria have antagonistic activity against circulating strains of *S. enteritidis* and *C. jejuni*. 88.9% of *S. enteritidis* isolates were susceptible to bifidobacteria and 81.5% of the studied strains were susceptible to lactobacilli. 92.6% of the isolated *Campylobacter jejuni* were susceptible to bifidobacteria, 76.9% of *Campylbacter* strains were susceptible to lactobacteria.

We developed a method of preventing bacterial infections and increasing ducks' productivity based on the combined use of bifido- and lactobacteria (1.5×10^9 CFU/mL) in a ratio of 1:1 with water and fractions enriched with mannan oligosaccharides (0.4 kg/t) together with feed. We recommend the preparation from the first day of birds' life till the end of growing period.

Preventive measures improved the preservation of the duck population by 8.76%, ensuring the average daily increase by 6.9% and the reduction of feed costs by

4.98% for 1 kg of growth throughout the growing period.

During the experiment, we recorded a significant decrease in *Salmonella* and *Campylobacter* colonization in the poultry intestines and improved average daily growth. The biologically active supplements provided a significant advantage in industrial duck farming.

We demonstrated the effectiveness of natural and environmentally safe methods: yeast fractions rich in mannan oligosaccharides, probiotics, and their combined use. The method was effectively implemented in Ukrainian poultry farms.

CONTRIBUTION

Concept – O.I. Kasjanenko, L.V. Nagornaya, S.M. Kasjanenko; Design – V.V. Melnychuk, S.M. Kasjanenko; Observation – O.I. Kasjanenko, V.A. Yevstafieva; Resources – S.M. Kasjanenko; Materials – V.A. Yevstafieva, L.V. Nagornaya, S.M. Kasjanenko; Data collection and/or processing – S.M. Kasjanenko, O.I. Kasjanenko, L.V. Nagornaya, V.A. Yevstafieva; Analysis and/or interpretation – O.I. Kasjanenko, L.V. Nagornaya, V.V. Melnychuk, V.A. Yevstafieva; Search for literature – O.I. Kasjanenko, S.M. Kasjanenko, V.V. Melnychuk, G.A. Lukyanova, I.A. Gurenko; Writing the manuscript – O.I. Kasjanenko, L.V. Nagornaya, S.M. Kasjanenko, G.A. Lukyanova, I.A. Gurenko; Critical review – O.I. Kasjanenko, G.A. Lukyanova, I.A. Gurenko.

CONFLICT OF INTEREST

The authors have no conflict of interest**.**

ACKNOWLEDGEMENTS

The authors thank the managers of the poultry farms for their assistance in conducting experiments.

REFERENCES

1. Kittler S, Fischer S, Abdulmawjood A, Glunder G, Klein G. Colonisation of a phage susceptible *Campylobacter jejuni* population in two phage positive broiler flocks. PLoS One. 2014;9(4). DOI: https://doi.org/10.1371/journal.pone.0094782.
2. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks breaks in 2015. EFSA Journal. 2016;14(12). DOI: https://doi.org/10.2903/j.efsa.2016.4634.
3. Kasyanenko OI, Fotina TI, Fotina AA, Gladchenko SM, Gnidenko TY, Bezruk RV. Properties of C*ampylobacter jejun*i that were isolated from poultry products. Microbiological Journal. 2017;79(4):66–74. (In Russ.).
4. Umaraw P, Prajapati A, Verma AK, Pathak V, Singh VP. Control of campylobacter in poultry industry from farm to poultry processing unit: A review. Critical Reviews in Food Science and Nutrition. 2017;57(4):659–665.
5. Collard JM, Bertrand S, Dierick K, Godard C, Wildemauwe C, Vermeersch K, et al. Drastic decrease of *Salmonella* Enteritidis isolated from humans in Belgium in 2005, shift in phage types and influence on foodborne outbreaks. Epidemiology and Infection. 2008;136(6):771–781. DOI: https://doi.org/10.1017/s095026880700920x.
6. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. EFSA Journal. 2015;13(12). DOI: https://doi.org/10.2903/j.efsa.2015.4329.
7. Meunier M, Guyard-Nicodeme M, Dory D, Chemaly M. Control strategies against *Campylobacter* at the poultry production level: biosecurity measures, feed additives and vaccination. Journal of Applied Microbiology. 2016;120(5):1139–1173. DOI: https://doi.org/10.1111/jam.12986.
8. Skarp CPA, Hanninen ML, Rautelini HIK. Campylobacteriosis: the role of poultry meat. Clinical Microbiology and Infection. 2016;22(2):103–109. DOI: https://doi.org/10.1016/j.cmi.2015.11.019.
9. Dogan ANC, Celik E, Kilicle PA, Atalay E, Saglam AG, Dogan A, et al. Antibacterial effect of hot peppers (*Capsicum annuum*, *Capsicum annuum var globriusculum*, *Capsicum frutescens*) on some *Arcobacter*, *Campylobacter* and *Helicobacter* species. Pakistan Veterinary Journal. 2018;38(3):266–270. DOI: https://doi.org/10.29261/pakvetj/2018.057.
10. Spring P, Wenk C, Connolly A, Kiers A. A review of 733 published trials on Bio-Mos®, a mannan oligosaccharide, and Actigen®, a second generation mannose rich fraction, on farm and companion animals. Journal of Applied Animal Nutrition. 2015;3. DOI: https://doi.org/10.1017/jan.2015.6.
11. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA Journal. 2017;15(12). DOI: https://doi.org/10.2903/j.efsa.2017.5077.
12. Ansari F, Pourjafar H, Bokaie S, Peighambari SM, Mahmoudi M, Fallah MH, et al. Association between poultry density and Salmonella infection in commercial laying flocks in Iran using a Kernel density. Pakistan Veterinary Journal. 2017;37(3):299–304.
13. Gormley FJ, Bailey RA, Watson KA, McAdam J, Avendaño S, Stanley WA, et al. *Campylobacter* colonisation and proliferation in the broiler chicken under natural field challenge is not affected by bird growth rate or breed. Applied and Environmental Microbiology. 2014;80(21):6733–6738. DOI: https://doi.org/10.1128/AEM.02162-14.

14. Ao Z, Choct M. Oligosaccharides affect performance and gut development of broiler chickens. Asian-Australasian Journal of Animal Sciences. 2013;26(1):116–121. DOI: https://doi.org/10.5713/ajas.2012.12414.
15. Ramirez-Hernandez A, Rupnow J, Hutkins RW. Adherence reduction of *Campylobacter jejuni* and *Campylobacter coli* strains to HEp-2 Cells by mannan oligosaccharides and a high-molecular-weight component of cranberry extract. Journal of Food Protection. 2015;78(8):1496–1505. DOI: https://doi.org/10.4315/0362-028X.JFP-15-087.
16. Muller KH, Collinson SK, Trust TJ, Kay WW. Type-1 fimbriae of *Salmonella*-Enteritidis. Journal of Bacteriology. 1991;173(15):4765–4772.
17. Lea H, Spring P, Taylor-Pickard J, Burton E. Natural carbohydrate fraction Actigen™ from *Saccharomyces cerevisiae* cell wall: effects on goblet cells, gut morphology and performance of broiler chickens. Journal of Applied Animal Nutrition. 2012;1. DOI: https://doi.org/10.1017/jan.2013.6.
18. Rous P, Turner JR. The preservation of living red blood cells in vitro: I. Methods of preservation. Journal of Experimental Medicine. 2016;23(2):219–237. DOI: https://doi.org/10.1084/jem.23.2.219.
19. Corrigan A, de Leeuw M, Penaud-Frezet S, Dimova D, Murphy RA. Phylogenetic and functional alterations in bacterial community compositions in broiler ceca as a result of mannan oligosaccharide supplementation. Applied and Environmental Microbiology. 2015;81(10):3460–3470. DOI: https://doi.org/10.1128/aem.04194-14.
20. Arsi K, Donoghue AM, Woo-Ming A, Blore PJ, Donoghue DJ. The efficacy of selected probiotic and prebiotic combinations in reducing *Campylobacter* colonization in broiler chickens. Journal of Applied Poultry Research. 2015;24(3):327–334. DOI: https://doi.org/10.3382/japr/pfv032.
21. Hadadji M, Benama R, Saidi N, Henni DE, Kihal M. Identification of cultivable Bifidobacterium species isolated from breast-fed infants feces in West-Algeria. African Journal of Biotechnology. 2005;4(5):422–430.
22. Spring P, Wenk C, Dawson KA, Newman KE. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poultry Science. 2000;79(2):205–211. DOI: https://doi.org/10.1093/ps/79.2.205.
23. Pitkanen T. Review of *Campylobacter* spp. in drinking and environmental waters. Journal of Microbiological Methods. 2013;95(1):39–47. DOI: https://doi.org/10.1016/j.mimet.2013.06.008.
24. Ugarte-Ruiz M, Florez-Cuadrado D, Wassenaar TM, Porrero MC, Dominguez L. Method comparison for enhanced recovery, isolation and qualitative detection of *C. jejuni* and *C. coli* from wastewater effluent samples. International Journal of Environmental Research and Public Health. 2015;12(3):2749–2764. DOI: https://doi.org/10.3390/ijerph120302749.
25. Sujatha K, Dhanalakshmi K, Rao AS. Antigenic characterisation and antibiotic sensitivity of field isolates of Salmonella gallinarum. Indian Veterinary Journal. 2003;80(10):965–968.
26. Mannan SJ, Rezwan R, Rahman MS, Begum K. Isolation and biochemical characterization of Lactobacillus species from yogurt and cheese samples in Dhaka metropolitan area. Bangladesh Pharmaceutical Journal. 2017;20(1):27–33.
27. Zinedine A, Faid M. Isolation and characterization of strains of bifidobacteria with probiotic proprieties *in vitro*. World Journal of Dairy and Food Sciences. 2007;(1):28–34.
28. Achtman M, Wain J, Weill FX, Nair S, Zhou ZM, Sangal V, et al. Multilocus Sequence Typing as a Replacement for Serotyping in *Salmonella enterica*. PLoS Pathogens. 2012;8(6). DOI: https://doi.org/10.1371/journal.ppat.1002776.
29. Baurhoo B, Ferket PR, Zhao X. Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. Poultry Science. 2009;88(11):2262–2272. DOI: https://doi.org/10.3382/ps.2008-00562.
30. Rakibul Hasan AKM, Ali MH, Siddique MP, Rahman MM, Islam MA. Clinical and laboratory diagnoses of common bacterial diseases of broiler and layer chickens. Bangladesh Journal of Veterinary Medicine. 2010;8(2):107–115. DOI: https://doi.org/10.3329/bjvm.v8i2.11188.
31. Srinivasan P, Balasubramaniam GA, Murthy T, Saravanan S, Balachandran P. Prevalence and pathology of Salmonellosis in commercial layer chicken from Namakkal, India. Pakistan Veterinary Journal. 2014;34(3):324–328.
32. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA Journal. 2018;16(12). DOI: https://doi.org/10.2903/j.efsa.2018.5500.
33. Safaei HG, Jalali M, Hosseini A, Narimani T, Sharifzadeh A, Raheimi E. The prevalence of bacterial contamination of table eggs from retails markets by Salmonella spp., Listeria monocytogenes, Campylobacter jejuni and Escherichia coli in Shahrekord, Iran. Jundishapur Journal of Microbiology. 2011;4(4):249–253.
34. Jamshidi A, Kalidari GA, Hedayati M. Isolation and identification of *Salmonella* Enteritidis and *Salmonella* Typhimurium from the eggs of retail stores in Mashhad, Iran using conventional culture method and multiplex PCR assay. Journal of Food Safety. 2010;30(3):558–568. DOI: https://doi.org/10.1111/j.1745-4565.2010.00225.x.

35. Khoult D, Krig N, Snit P. Opredelitel′ bakteriy Berdzhi. Tom 1 [Bergey`s Manual of Determinative Bacteriology. Vol. 1]. Moscow: Mir; 1997. 432 p. (In Russ.).

ORCID IDs
Sergij M. Kasjanenko https://orcid.org/0000-0002-5474-5804
Oksana I. Kasjanenko https://orcid.org/0000-0001-8453-1957
Liudmyla V. Nagornaya https://orcid.org/0000-0001-8307-183X
Valentyna A. Yevstafieva https://orcid.org/0000-0003-4809-2584
Vitaliy V. Melnychuk https://orcid.org/0000-0003-1927-1065
Galina A. Lukyanova https://orcid.org/0000-0001-7127-5720
Irina A. Gurenko https://orcid.org/0000-0003-4313-9646