Dear colleagues!

The modern trends of biological threats growing, emergent diseases (Lumpy skin disease, Foot-and-mouth disease, African swine fever, Avian influenza and other in Europe and Asia) determine the necessarily to pay the extremely high attention to the biosafety issues and biological hazards control.

The National Scientific Center 'Institute of the Experimental and Clinical Veterinary Medicine' is the leading specialized research institution in Ukraine created for solving scientific and practical tasks of veterinary animal. NSC IECVM's basic research are focused on: immunogenesis and disease pathogenesis, indications, authentications, isolations and studies of biological features of their causative agents, developments of facilities and systems of monitoring, diagnostics, prophylaxis and prognostication of infectious diseases of animals, monitoring of quality and unconcern of agricultural produce and development of the normative basis for animal diseases control and biosafety. NSC IECVM coordinates implementation of scientific researches on questions veterinary medicine, that conduct scientific establishments of NAAS, State Service of Ukraine for Food Safety and Consumer Protection, and Higher educational establishments of Ukraine of agrarian profile.

New journal 'Journal for Veterinary Medicine, Biotechnology and Biosafety", discovered in 2015, aimed to consolidate and share the new developments and achievements in the area of biological science. This was recognized as the profile edition for veterinary medicine doctors and biologists in Ukraine. Our journal promotes the research of Ukrainian institutions, publishing their achievements in English, and sharing it among the scientific community. It includes cooperative veterinary and medical aspects, fitting to One Health Approach declared by WHO, OIE, and FAO. It was included in Index Copernicus and eLibrary scientific databases.

The Editorial board hopes, that our issue will be interesting for wide auditorium of scientists and practical specialists in veterinary medicine, biology, biotechnology and biosafety. We invite new authors for fruitful collaboration and joint development.

Sincerely yours,
Editors-in-Chief

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Prof. Anton GERILOVYCH

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3. Authors make sure there are no typographical errors in the manuscript

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5. Tables and illustrations must be must be submitted as separate files and inserted in the text

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DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY OF *LISTERIA* SPP.

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Summary. The aim of the research is studying of *Listeria* spp. sensitivity to antibiotics. The article presents the results of the sensitivity studying of species *L. monocytogenes, L. ivanovii, L. seeligeri* to penicillins, cephalosporins, carbopenems, aminoglycosides, macrolides, linzosides, tetracyclines, quinolones, nitrofurans, chloramphenicol, vancomycin, polymyxine, and rifampicin. The study and interpretation of the results were conducted using the disc-diffusion method according to European Committee for the Evaluation of Antimicrobial Susceptibility (EUCAST) methodology and guidelines 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' in accordance with the Decree No. 167 of the Ministry of Healthcare of Ukraine from 05.04.2007. As a result of the conducted researches, the sensitivity features of the studied bacteria species *L. monocytogenes, L. ivanovii, L seeligeri* to antibiotics were established.

Keywords: *Listeria* spp., strains, antibiotics, sensitivity, resistance

Introduction. Listeriosis is an infectious zooanthroponotic disease, common in all countries, regardless of climate and social well-being (BIOHAZ, 2012).

The sources of the infection are rodents, livestock, cattle, pigs, dogs, wolves, cats, birds, fish and seafood, monkeys, people infected with listeriosis (Andrews, 1992).

The pathways of infection with human listeriosis are alimentary, aerogenic, contact, transplacental infections of the fetus and postpartum infections of the newborn baby; in animals — fecal-oral, alimentary, contact, air-drip, sexual, transmissive, transplacental (Bauwens, Vercammen and Hertsens, 2003).

The genus *Listeria* belongs to the Listeriaceae family, that includes 16 species at the moment of November 2015, among which the most relevant in the etiology of *Listeria* is *L. monocytogenes*; also known cases of human infection with species *L. ivanovii* and *L. seeligeri* (OIE, 2014; Guillet et al., 2010; Zhang et al., 2007).

The pathogenicity of these species is due to the presence of specific pathogenicity factors, which include listeriolysin O — hemolysin, the 'main factor' of the listeria pathogenicity. It has a pronounced toxic effect, and phosphatidylcholine — lecithinase, which plays an important role in the survival and reproduction of listeria in the infectious process and lysis of secondary vacuoles (Alberti-Segui, Goeden and Higgins, 2007; Churchill, Lee and Hall, 2006; Ermolaeva et al., 2003).

Antibiotics are used as etiotropic therapy in listeriosis treatment. *Listeria* spp. isolates are sensitive to penicillin derivatives (especially doominopenicillin), most macrolides (except azithromycin and spiramycin), aminoglycosides, tetracycline, glycopeptides (vancomycin) and lipopeptides (daptomycinum), oxazolidinones (Aureli et al., 2003).

There is the evidence that most drugs in the quinolone group have moderate activity against *Listeria* spp. At the same time, some researchers report that new fluoroquinolones are active against other strains, not only *L. monocytogenes* (EFSA and ECDC, 2012; Doganay, 2003).

The question of the pathogenic microorganisms resistance to antibiotics is being studied intensively throughout the world, because infectious pathogens undergo adaptation changes under the influence of anthropogenic factors (uncontrolled use of antibiotics, preservatives, disinfectants, etc.) that are expressed in the change of biological properties. These changes are manifested in the polymorphism of the pathogen populations, the appearance of avirulent and weakly virulent mutants and the resistance to antibacterial drugs of certain groups or field resistance (Clayton et al., 2014; Johnson et al., 2004; Volokhov et al., 2007).

The study of the specific sensitivity characteristics to antibiotics of certain bacteria species and genus is also relevant.

The aim of the study was to characterize the antibiotic susceptibility of *Listeria* spp. (*L. monocytogenes, L. ivanovii, L. seeligeri*).

Materials and methods. There was studied the sensitivity of 15 isolates of *Listeria* spp. to the antibiotics: 5 isolates of *L. monocitogenes* (isolated from minced meat of chicken — 2 cultures, beef meat — 2 cultures, dried milk — 1 culture); 5 isolates of *L. ivanovii* (isolated from

aborted fetuses); 5 isolates of *L. seeligeri* (isolated from pathological material from animals) and reference cultures *L. monocitogenes* ATCC 19112, *L. ivanovii* ATCC 19119, *L. seeligeri* ATCC 35967. The studies were conducted using nutrient media and disks with minimum concentrations of the active ingredient produced by 'HiMedia'. Determination of antibiotic sensitivity was provided by diffusion method and the evaluation of the obtained results was carried out according to EUCAST methodology and guidelines 'Determination of the Sensitivity of Microorganisms to Antibacterial Drugs' in accordance with the Decree No. 167 of the Ministry of Healthcare of Ukraine from 05.04.2007 (Johnson et al., 2004; Volokhov et al., 2007; MHU, 2007).

Results. From the penicillin group, we studied the sensitivity of *Listeria* spp. to benzylpenicillin, piperacillin and ampicillin. It is known fact, that *Listeria* spp. (in particular, *L. monocytogenes*) is highly susceptible to natural and semisynthetic penicillins (Table 1). According to the results of our research, the strains of the studied species showed sensitivity to the indicated antibiotics. The level of sensitivity in *L. monocytogenes* isolate was higher than in *L. ivanovii* and *L. seeligeri* isolates. The group of cephalosporins, which is characterized by a lack of significant activity against *L. monocytogenes*, determined the sensitivity of *Listeria* spp. cephalosin (III), cephalexin (III), ceftazidime (III), cefipix (IV), ceftazidine (III), cephalexin (I), cefuroxime (II), cefaclor (II), cefemandole (II), cefotaxime (III), ceftriaxone (III) (Table 1). According to our results, the studied cultures were generally insensitive, with some exceptions: the strain *L. monocytogenes* ATCC 19112 and *L. seeligeri* ATCC 35967 are susceptible to cefamandol (II), strains *L. ivanovii* ATCC 19119 and *L. seeligeri* ATCC 35967 are sensitive to cefepime (IV).

Carbapenems act on many groups of gram-positive, gram-negative, and anaerobic microorganisms. These results coincide with this statement: the strain *L. monocytogenes* ATCC 19112 was sensitive to carbopenems (imipenem, meropenem), isolates — insensitive; strain *L. ivanovii* ATCC 19119 was highly susceptible to imipenem and insensitive to meropenem; epizootic isolates *L. ivanovii* were, in general, insensitive to imipenem and insensitive to meropenem (Table 1). The strain *L.seeligeri ATCC 35967* was sensitive to imipenem and meropenem; isolates — insensitive.

Table 1 — Sensitivity of *Listeria* spp. to the antibiotics (penicillins, cephalosporins, carbapenems)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	Listeria monocytogenes						*Listeria ivanovii*						*Listeria seeligeri*					
	ATCC 19112	2/0811	3/0811	6/0811	7/0811	13/0811	ATCC 19119	1/09	3/09	4/09	1/0811	5/0811	ATCC 35967	22	4/0811	8/0811	23	24
Penicillins																		
Benzylpenicillin, 10	28	22+*	27+*	26+*	19+*	16+	19	17	18+*	20+*	16	19+*	20	11+*	12+*	15+*	14+*	15+*
Piperacillin, 100	24	19	25	20	24+*	22	19	14+*	13+*	17+*	13	19+*	18	18+*	19+*	17+*	18+*	17+*
Ampicillin, 10	22	24+*	19+*	18+*	21	24	0	0	0	0	0	0	19	17+*	17+*	18+*	17+*	17+*
Cephalosporins																		
Cefazolin, 30	24	22+*	24+*	20+*	21+*	19	16	16+*	14+*	16+*	12	16	26+*	21+*	20+*	19+*	21+*	19+*
Cefalexin, 30	22+*	20	17+	15+*	18+*	20+*	9	8	7	9	8	6	16	8	9	16+*	7	17+*
Cefuroxime, 30	12	19	20	27+*	25	17	8	9	7	8	9	8	19	8+*	9+*	9	8+*	10
Cefaclor, 30	22	12	20+*	21+*	17+*	20+*	14	14	12	10+*	11+*	9	18	11	12	16+*	11	15+*
Cefotaxime, 30	12	21+*	19+*	22+*	27*	22+*	7	0	7	9	0	0	17	14+*	14+*	12	13+*	11
Ceftriaxone, 30	14	23+*	21+*	21	19+*	17	11	10	9	8	10+*	0	14	13+*	12+*	10	12+*	10
Cefoperazone, 75	17	22*	19+*	23+*	23	26+*	9	9	7	0	9	6	19	14	15	14	14	15
Ceftazidime, 30	10	7	11+*	10+*	12	9	8	8	6	7	0	0	22+*	19+*	20+*	16	21+*	15
Cefepime, 30	0	16	11	0	13+*	10	31	17+*	21+*	19+*	24+*	22+*	28	18	19	24	18	23
Cefamandole, 30	28	26+*	29+*	14	22+*	19+*	9	0	8	0	7	7	29	16	17	16+*	16	15+*
Carbapenems																		
Imipenem, 10	24	19+*	22+*	21	19+*	23+*	49+*	22+*	25+*	24+*	20+*	24+*	33	25+*	25+*	27+*	28+*	25+*

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

From the group of aminoglycosides, to which *Listeria* spp. are considered to be naturally sensitive, we studied sensitivity to streptomycin (I), kanamycin (I), neomycin (I), gentamicin (II), netilmicin (II), tobramycin (II), amikacin (III) (Table 2). The reference strains *L. monocytogenes* ATCC 19112 and *L. seeligeri* ATCC 35967 showed sensitivity to aminoglycosides; isolates *L. monocytogenes* and *L. seeligeri* — less susceptible mostly. Reference strain and *L. ivanovii* isolates showed resistance to the drugs of this group.

In the macrolide group (erythromycin, oleandomycin, azithromycin) to which *Listeria* spp. are considered to be sensitive, the reference strains *L. monocytogenes* ATCC 19112 and *L. seeligeri* ATCC 35967 showed sensitivity to erythromycin and were less susceptible and insensitive to azithromycin and oleandomycin. Isolates of these species were insensitive to certain drugs (Table 2). Reference strain and isolates *L. ivanovii* showed resistance to macrolides. All studied cultures were low sensitive or resistant to lincosamides (lincomycin, clindamycin). Only reference strain *L. monocytogenes* ATCC 19112 was susceptible to tetracyclines (tetracycline, doxycycline), which are considered inhibitors of *Listeria* spp. The epizootic isolates were low susceptible. *L. ivanovii, L. seeligeri* (referential and epizootic) were insensitive and insensitive (Table 2).

Table 2 — Sensitivity of *Listeria* spp. to the antibiotics (aminoglycosides, macrolides, lynkozamides, tetracycline)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	Listeria monocytogenes						*Listeria ivanovii*						*Listeria seeligeri*					
	ATCC 19112	2/0811	3/0811	6/0811	7/0811	13/0811	ATCC 19119	1/09	3/09	4/09	1/0811	5/0811	ATCC 35967	22	4/0811	8/0811	23	24
Aminoglycosides																		
Streptomycin, 30	19	17	19+*	12	23+*	24+*	10	9	7	0	0	8	28	18+*	19+*	22+*	21+*	18+*
Kanamycin, 30	30	22*	19	24+*	20+*	19+*	8	7	0	7	8	0	29	21+*	20+*	18+*	19+*	20+*
Neomicin, 30	18	17	19	21+*	17	19*	9	6	0	7	9	7	30+*	19+*	19+*	20+*	21+*	18+*
Gentamicin, 10	24	20+*	22+*	19	21+*	19	13	10+*	8	7	0	9	22	16+*	17+*	18+*	17+*	16+*
Netilmicin, 30	15	13	19+*	21+*	19+*	17*	7	0	8	0	6	7	19	12+*	13+*	11	12	14+*
Amicacin, 30	20	18+*	17	22+*	19+*	21*	11	10+*	8	9	0	8	18	11	12	13+*	11+*	11
Tobramicin, 10	26	15	20+*	24+*	19	22*	7	0	9	8	0	7	25	16+*	17+*	16+*	15+*	15+*
Macrolides																		
Erythromycin, 15	26	18+*	22+*	19+*	21+*	20+*	18	11+*	13+*	11	16+*	14+*	27	19+*	20+*	18+*	17+*	21+*
Azithromycin, 15	16	17+*	19+*	22+*	19+*	20+*	12	10	14+*	9	15	9	13	7	9	8	6	8
Oleandomycin, 15	8	9	12	19+*	20+*	14	21	15+*	20+*	17+*	16	22+*	24	16+*	17+*	19+*	18+*	16+*
Lynkozamides																		
Lincomycin, 15	13	16+*	12	14	10	11	11	7	9	10	9	8	14	11+*	10+*	11+*	10+*	11+*
Clindamycin, 2	15	15	14	20+*	16	19+*	9	0	7	0	9	0	17	6	8	8	9	8
Tetracycline																		
Tetracycline, 30	26	18	16	21	19+*	25+*	19	9	12	15	11	10	22	14+*	15+*	14+*	13+*	14+*
Doxycycline, 30	28	26+*	17	22+*	20+*	27+*	10	10	12	10	11	9	14	10	9	12	13	9

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

From the group of quinolones, which are considered to be moderately active against *Listeria* spp. and fluoroquinolones (it has been experimentally established that levofloxacin (III) and moxifloxacin (IV), etc. were active against more than 99% of *L. monocytogenes* strains). We studied susceptible features of *Listeria* spp. to nalidixic acid (I), ciprofloxacin (II), norfloxacin (II), pefloxacin (II), floxacin, lomefloxacin (II), levofloxacin (III) (Table 3).

The cultures of *L. monocytogenes* were resistant to nalidixic acid. The strain *L. monocytogenes* ATCC 19112 was sensitive to ciprofloxacin, levofloxacin and low susceptible or resistant to other fluoroquinolones (lomefloxacin).

Epizootic strains of *L. monocytogenes* were insensitive. Reference strain *L. ivanovii* ATCC 19119 was sensitive to nalidixic acid and floxacin, epizootic isolates were insensitive to these drugs. *L. ivanovii* strains showed resistance to other preparations of this group. All *L. seeligeri* strains are resistant to nalidixic acid; the reference culture is susceptible to floxacin and lomefloxacin; to other drugs reference and epizootic cultures were low susceptible.

From the nitrofuran group (fusiidine, furadonine, furagin, furazolidone), the reference strain and some isolates of *L. monocytogenes* showed susceptibility to fusidine, culture *L. ivanovii* and *L. seeligeri* — resistant. All *Listeria* spp. exhibited resistance to furazolidone (Table 3).

L. monocytogenes ATCC 19112 and *L. seeligeri* ATCC 35967 are sensitive to chloramphenicol, epizootic isolates and reference strain are low susceptible.

L. ivanovii isolates are insensitive to vancomycin (glycopeptids, violations of the cell wall synthesis, active against *Listeria* spp.), *L. monocytogenes* are sensitive, *L. ivanovii*, *L. seeligeri* cultures are low susceptible.

All studied cultures were 100% resistant to polymyxin B. All strains of *L. monocytogenes* were susceptible to rifampin; *L. seeligeri* had low sensitivity; *L. ivanovii* were resistance.

Table 3 — Sensitivity of Listeria spp. to the antibiotics (fluoroquinolones, nitrofurans, and others)

Name of antibiotic / Content of antimicrobial substance, μg (ED)	Diameters of inhibition of cultural growth, mm																	
	Listeria monocytogenes						*Listeria monocytogenes*						*Listeria monocytogenes*					
	ATCC 19112	2/0811	3/0811	6/0811	7/0811	13/0811	ATCC 19119	1/09	3/09	4/09	1/0811	5/0811	ATCC 35967	22	4/0811	8/0811	23	24
Fluoroquinolones																		
Nalidixic ac. 30	0	0	0	0	0	0	28	19+*	18	20+*	18	19	9	5	6	0	0	6
Ciprofloxacin, 5	22	18	16	22	19	27+*	9	9	0	0	7	0	19+*	13+*	14+*	13+*	12+*	13+*
Norfloxacin, 10	14	24+*	20	19+*	20+*	17+*	8	8	0	0	7	0	18	12	13	14	15	12
Pefloksatsin. 10	12	11	23+*	12	24*	17	6	0	7	0	0	8	26	13	13	19	18	13
Floxacin, 5	16	19+*	24+*	17	20+*	22+*	35	21+*	19	22+*	19	21+*	36	21+*	20+*	20+*	21+*	21+*
Lomefloxacin, 30	0	19+*	24+*	9	25+*	20+*	8	0	0	6	9	0	29	19	20	21+*	20+*	19
Levofloxacin, 5	23	20+*	26+*	17	24+*	20+*	11	0	9+*	8	0	7	22	17+*	18+*	18+*	17+*	17+*
Nitrofurans																		
Fuzidin, 10	20	14+*	19+*	21+*	20+*	12	14	11+*	9	10	9	11+*	13	11	10	9	8	11
Furadonin, 300	14	13+*	20+*	15+*	22+*	12+*	8	9	7	9	8	9	16+*	10	11	10	11	10
Furamagum, 300	14	14+*	16+*	12	12	11	10	8	0	9	7	9	14	10	11	10	9	10
Furazolidone, 300	12	11	10	12	9	8	6	6	0	6	7	0	16	9	10	11	10	9
Others																		
Chloramphenicol, 30	31	17+*	20+*	22+*	19+*	19+*	18	18+*	17+*	16	17	18+*	27+*	18+*	19+*	22+*	21+*	18+*
Vancomycin, 30	22+*	15+*	20+*	19+*	22+*	20+*	19+*	17	16	18	17+*	17	17	17	16	14+*	13+*	17
Polymyxin, 300	0	0	0	0	0	0	9	0	6	0	7	0	9	6	7	7	6	6
Rifampicin, 5	20	18+*	20+*	19+*	22+*	24+*	9	0	8	0	0	0	20	17+*	18+*	14+*	13+*	19+*

Notes: * — stimulation of culture growth around the zone of inhibition; + — the normal growth of resistant colonies in the zone of inhibition of culture growth.

Thus, the reference and epizootic strains of the studied species (*L. monocytogenes*, *L. ivanovii, L. seeligeri*) were sensitive to penicillins in 100% of cases, to cephalosporins and carbopenems — mostly insensitive. The strain *L. ivanovii* showed resistance to the aminoglycosides. References cultures of *L. monocytogenes* ATCC 19112, *L. seeligeri* ATCC 35967 were sensitive, epizootic cultures were insensitive.

As for the group of macrolides, *L. monocytogenes, L. seeligeri* were sensitive to erythromycin, and were not susceptible to oleadomycin and azithromycin. Strains *L. ivanovii* were insensitive to macrolides. *Listeria* spp. was found insensitive and resistant to lincosamides. *Listeria* spp. was found preferably resistant to tetracyclines, with the exception of the reference strain *L. monocytogenes*.

The studied isolates of *Listeria* spp. were resistant and non-sensitive to quinoline, reference strains showed sensitivity to individual drugs. *Listeria* spp. were mostly resistant to nitrofuran and chloramphenicol with the exception of several *L. monocytogenes* cultures that exhibited sensitivity to fusidine, furadonine, chloramphenicol. *L. monocytogenes* culture was sensitive to vancomycin, *L. ivanovii*, *L. seeliger*i — low susceptible to vancomycin. The *L. monocytogenes* strains showed the susceptibility to rifampin. *L. seeligeri* showed low sensitivity and *L. ivanovii* — resistance to rifampin. The studied *Listeria* spp. was resistant to polymyxin B.

Conclusions. 1. The sensitivity study of *Listeria* spp. to antibiotics showed that both generic and species-specific features of sensitivity in the studied cultures have been established.

2. *Listeria* spp. isolates differed in the increased level of resistance to antibiotics compared with reference cultures.

3. Sensitivity level of *Listeria* spp. to drugs within a certain pharmacological group manifested individually.

4. It is appropriate to take into account the specific characteristics of cultures and to use a wide range of active substances during conducting the antibiotic resistance studies.

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DEPENDENCE OF GENERAL ACTION AND ACUTE TOXICITY ON CHEMICAL STRUCTURE IN A ROW OF NEW DERIVATIVES OF 3-METHYL-7-SUBSTITUTED-8-N-ETHYL PIPERAZIN XANTHINE

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Summary. It has been established that the acute toxicity of the synthesized for the first time derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine is in the range from 560 to 1325 mg/kg. In accordance with the classification of K. K. Sidorov, three compounds belong to practically non-toxic substances and four compounds are low-toxic. After a single administration of toxic doses of the studied compounds, the performed pathomorphological studies did not reveal statistically significant changes in the mass of the internal organs. The derivatives of 3-methyl-7-substituted-8-ethyl piperazin xanthine are a promising group of heterocyclic compounds to create safe anthelminthic drugs on their basis.

Keywords: 3-methyl-7-substituted-8-N-ethyl piperazin xanthine derivatives, general action, acute toxicity

Introduction. In modern medical practice, in the treatment of helminthiases in humans and animals, anthelmintic preparations of different classes of chemical compounds are used to treat helminthic invasions of humans and animals (piperazine, male fern extract, chloroxyl, etc.). There are drugs that are used primarily for the treatment of nematodes, cestodoses and trematodes, as well as anthelmintic agents of a wide spectrum of action that are effective against different types of helminths. From the salts of piperazine, piperazine adipate is mainly used, which is widely used for mass dehelminthization at ascaridosis and enterobiasis, because it does not require the preliminary designation of an appropriate diet, laxatives, and also has low toxicity to humans and rarely causes side effects (Arkhipov, 2009; Mashkovskiy, 2009; Drogovoz et al., 2010). In connection with this, the development of new and improvement of the already used anthelmintic agents is an actual problem and is practically necessary. In the second half of the twentieth century, the main direction of pharmacy was the creation of new medicines based on existing substances of anthelmintic drugs (Kornienko, Tarasevičius and Samura, 2013; Kornienko, Samura and Romanenko, 2013; Romanenko et al., 2013).

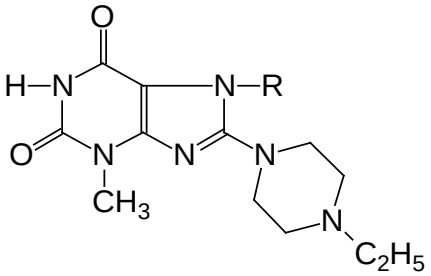
The number of new, newly synthesized organic heterocyclic derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine, developed by us, is of theoretical and practical interest for further targeted synthesis of new substances with predicted anthelmintic activity (Aleksandrova et al., 2016; Ponomarenko et al., 2016; Ivanchenko et al., 2012).

At the stage of pharmacological screening in the study of general action and acute toxicity, the information can be obtained for further modeling of the chemical structures of the xanthine series in order to select more active and less toxic substances for testing specific activity and safety.

The aim of this study: To study in experiments on mice, the general effect and acute toxicity of the newly synthesized heterocyclic derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine.

Materials and methods. The new synthesized heterocyclic derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine (compounds No. 1–7) were used as the object of the study. Synthesis of substances was carried out at the Department of Biological Chemistry of the Zaporizhia State Medical University under the direction of Doctor of Pharmaceutical Sciences, Professor N. I. Romanenko.

Chemical structure of 3-methyl-8-N-ethyl piperazin xanthine derivatives is:



The structure of the synthesized substances was confirmed with the help of modern physicochemical methods of elemental analysis, UV, IR, PMR, and mass spectrometry, counter synthesis, and the purity of the synthesized substances was monitored by thin-layer chromatography.

A study of the acute toxicity of new derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine was carried out on white mongrel mice weighing 20–24 g (Sernov and Gatsura, 2000; Arzamastsev, 1985; Kovalenko et al., 2001).

The substances were dissolved in physiological saline in the form of 3–5% aqueous suspension stabilized by

Tween-80 and were administered intraperitoneally to the animals. The control group of mice was injected with the same saline solution as the experimental groups. The animals were monitored for 14 days after a single administration of the test compounds (Kozhemiakin et al., 2002; Arzamastsev, 1985; Kovalenko et al., 2001).

During the entire experimental period, the animals were monitored. At the same time, the condition of animal fur and visible changes in the mucous membranes were taken into account. The dynamics of changes in the body weight of mice, the character of secretions and the duration of life were monitored. An evaluation of the overall effect of 7 compounds studied was carried out according to behavioral reactions, neuromuscular excitability and some vegetative effects. The number of surviving and dead animals was recorded every 24 hours. Acute toxicity (LD_{50}) was calculated by the Kerber method (Kozhemiakin et al., 2002).

Experimental studies were conducted in accordance with the 'Regulations on the use of animals in biomedical research' (Strasbourg, 1986) and the 'General ethical principles of experiments in animals' (Kiev, 2001), agreed with the requirements of the 'European convention for the protection of vertebrate animals, used for experimental and scientific goals' (Reznikov et al., 2006).

The statistical verification of the data was carried out using the standard analysis package for the statistical processing of the results (Microsoft Office Excel 2003). The results are presented as a sample mean and a standard error of the mean value. The reliability of differences between the experimental groups was assessed using Student's t-test and the Mann-Whitney U-test, the computer program Statistica for Windows 7.0. (Statsoft Inc., No. AXXR712D833214 Fan.5), differences were considered statistically significant for all types of analysis with a significance level of at least 0.05 (Lapach, Chubenko and Babich, 2002; Samura, Korniienko and Romanenko, 2012).

Results. The studies of the general effect of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine derivatives showed that signs of toxic effect were manifested after 5–15 min after single administration of toxic doses of test substances. Compound No. 1 (7-α-methylbenzyl-8-N-ethyl piperazin theophylline) and Compound No. 7 (1-p-fluorobenzyl-8-(4-ethylpiperazinyl-1-) theobromine) caused to signs of an exciting action, which was expressed in increased motor activity, the appearance of tremor, and immediately before the death, clonic-tonic convulsions were observed. In addition, the alertness of animals, increased sensitivity to sound and pain stimuli, the reflex of the withdrawal of the head while touching the mustache were noted. Breathing somewhat increased, the color of the ears became pale, the corneal reflex was preserved, the pupils remained unchanged, the cornea of the eyes remained transparent, moist. In some animals, diarrhoea appeared, the fur became disheveled, lost its luster. After the administration of toxic doses of Compound No. 7, a sharp excitation was first observed with a movement disorder: fibrillar contractions of individual muscle groups of skeletal muscles, indicating abrupt functional shifts in the sphere of vegetative innervation, changes in the tone of skeletal muscles and development of ataxia of the central genesis. In 30–45 min after the administration of the test compounds, excitation was followed by inhibition. Mice became inactive, died from stopping breathing and heart activity. Under the action of Compound No. 3 (7-α-naphthylmethyl-8-N-ethyl piperazin theophylline), the mice fell into a drowsy state with muscular hypotension and died from stopping respiratory and cardiac activity.

After the administration of Compound No. 3, a decrease in the motor activity of mice was observed, respiratory distention appeared, cyanosis of visible mucous membranes was noted, body temperature decreased by 1–2 °C, corneal and pupillary reflexes were absent, which indicates a sedative effect in the spectrum of pharmacological action of these compounds.

Compound No. 4 (7-β-phenylethyl-8-N-ethylpiperazin theophylline), Compound No. 5 (3-methyl-7-n-heptyl-8-N-ethyl piperazin theophylline), and Compound No. 6 (3-methyl-7-n-dodecyl-8-N-ethyl piperazin theophylline) showed a less pronounced soothing effect. Under the influence of these substances, a decrease in spontaneous motor activity was observed in mice. Most animals responded to sound and pain stimuli, and when the corneas were irritated, the animals pulled back the heads. After the injection of toxic doses of these substances, tonic convulsions periodically occurred. The mice took a lateral position, convulsions were replaced by individual twitching of the limbs and the animals died from stopping breathing.

The dead animals were dissected and pathomorphological studies were carried out. The attention was paid to the blood supply and the mass of internal organs, the state of the gastrointestinal tract.

As a result of the conducted experimental studies, there were no statistically significant changes in the mass of the brain, heart, liver, kidneys and spleen after a single administration of toxic doses of the studied compounds.

The calculation of LD_{50} was carried out based on study acute toxicity data (Table 1).

It was found that LD_{50} of the derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine is in the range from 580.0 to 1325.0 mg/kg. There was found that following compounds are almost non-toxic: Compound No. 5 — LD_{50} is 1325.0 mg/kg. Replacement at the 7^{th} position of the molecule of this compound of the heptyl (Compound No. 5) radical by phenylethyl (Compound No. 4), methylbenzyl (Compound No. 1), leads to an increase in acute toxicity (LD_{50} increased from 1325.0 to 1125.0 mg/kg).

Table 1 — Acute toxicity of 3-methyl-8-N-ethyl piperazin xanthine derivatives at intraperitoneal administration to mice

Compounds		LD_{50}, mg/kg
No. 1	7-α-methylbenzyl-8-N-ethyl piperazin theophylline	1125.0 ± 28.9
No. 2	7-(3-chlorobuten-2-yl-1)-8-N-ethyl piperazin theophylline	685.0 ± 32.4*
No. 3	7-α-naphthylmethyl-8-N-ethyl piperazin theophylline	560.0 ± 24.7*
No. 4	7-β-phenylethyl-8-N-ethyl piperazin theophylline	1210.0 ± 37.5
No. 5	3-methyl-7-n-heptyl-8-N-ethyl piperazin theophylline	1325.0 ± 41.5
No. 6	3-methyl-7-n-dodecyl-8-N-ethyl piperazin theophylline	740.0 ± 21.8*
No. 7	1-p-fluorobenzyl-8-(4-ethyl piperazinyl-1-) theobromine	620.0 ± 19.7*

Note: * — $p < 0.05$ statistically significant differences in comparison with the control.

The most toxic was Compound No. 3 — LD_{50} is 560.0 mg/kg. In accordance with the classification of Sidorov (1973), three compounds are related to practically non-toxic substances and four compounds are low-toxic.

Compounds No. 2 (LD_{50} is 685.0 mg/kg), No. 6 (LD_{50} is 740.0 mg/kg), No. 7 (LD_{50} is 620.0 mg/kg) were moderately toxic.

Thus, as a result of the study of the general action and acute toxicity of the newly synthesized derivatives of 3-methyl-7-substituted-8-N-ethyl piperazin xanthine, compounds were selected to study anti-helminth activity. The dependence of acute toxicity on the chemical structure in the series of studied new heterocyclic derivatives of 3-methylxanthine was established.

Conclusions. 1. Acute toxicity of the derivatives of 3-methyl-7-substituted-8-ethyl piperazin xanthine is in the range from 560 to 1325.0 mg/kg. Three compounds belong to practically non-toxic and four compounds are low-toxic substances.

2. After a single administration of toxic doses of the studied compounds, the performed pathomorphological studies did not reveal statistically significant changes in the mass of the internal organs.

Prospects for further research. Derivatives of 3-methyl-7-substituted-8-ethyl piperazin xanthine are a promising group of heterocyclic compounds for the creation on their basis of safe and more effective anthelmintic drugs.

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EXPERIMENTAL JUSTIFICATION FOR THE USE OF BIORESONANCE METHOD OF ASSESSING THYROID FUNCTION IN DOGS

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Summary. The state of the thyroid gland was studied on 36 dogs according to biochemical parameters of the blood and bioresonance testing using the 'PARKES-D' diagnostic complex, the principle of which is based on the phenomenon of biological resonance — the determination of the electrical conductivity of biologically active points when microresonance circuits are introduced into the electromagnetic contour. At the final stage of the research, comparisons of these techniques were performed. It has been established that the decrease of the functional state of the thyroid gland is accompanied by the 1.6 times increase of the thyroid-stimulating hormone content in the blood of dogs. In this case, a decrease in the level of triiodothyronine in the blood of dogs with the 21.4% hypothyroidism of the thyroid gland was established. The content of tetraiodothyronine in the blood of dogs with a decrease in the functional state of the thyroid gland was almost twice reduced, and the content of total cholesterol in the blood of dogs in the experimental group was 1.48 twice as much. There has been proven 1.7 times increase in the ratio of the total cholesterol content to tetraiodothyronine in the blood of dogs with a decrease in the functional state of the thyroid gland. The conducted researches have established that for dogs. The bioresonance is the fluctuation of the value of the electrical conductivity of the biological active points of 8–24 units of the scale of the 'PARKES-D' device, and the magnitude of electrical conductivity in the BAP varies from 22 to 61 conventional units of the device. Results of studies of the functional state of the thyroid gland in dogs using the 'PARKES-D' diagnostic complex are generally consistent with the biochemical parameters of the blood, therefore, the functional testing allows, with a probability of 94.4%, to determine the functional state of the gland.

Keywords: dogs, thyroid gland, bioresonance, hypofunction, hormones, 'PARKES-D'

Introduction. An actual problem of animal husbandry and veterinary medicine is a deep knowledge of the biological laws in the body of various species, both productive and small domestic animals.

Thyroid gland occupies an important place among the endocrine glands due to the active participation of its iodine-containing hormones, triiodothyronine and thyroxine, in the regulation of many functions of the body (Obuobie and Jones, 2003; Tielens et al., 2000). Functional activity of the thyroid gland depends largely on the flow of iodine into the body. Hypofunction of this gland is observed, first of all, in the natural zones of poor on iodine soils and water, as well as zones with high radiation (Tielens et al., 2000; Estrada et al., 2014).

In modern biology, each cell, organ, as well as the body as a whole, are considered as a source of electromagnetic radiation (EMR), which differs by its parameters — wavelength, intensity, frequency. In this case, there are distinguished normal (physiological) and pathological EMR, which arise when there is an impairment in activity of cells, organs and systems of the body (Deynekina, 2002). According to modern ideas, all actions of environmental factors are perceived by the body, first of all, at the level of the energy shell, which is the primary barrier to the action of any external factor on the body (Bol'shakov, 2002).

In recent years, scientific research with the use of electromagnetic radiation (EMR) has been intensified in determining the functional state of organs and systems of the body (Kochieva, 2006).

The purpose of the work. The purpose of our research was to study the functional state of the thyroid gland in dogs and to evaluate the informativity of the data of the applied diagnostic complex 'PARKES-D', the principle of which is based on the phenomenon of biological resonance.

Materials and methods. The experiment was conducted in the conditions of Kharkiv veterinary clinics in 36 dogs of different breeds aged 4–9 years and with a body weight of 25–33 kg. The conditions for keeping and feeding animals complied with the requirements. An assessment of the endocrine function of the thyroid gland was performed by biochemical blood tests.

Blood for research was taken in the morning before feeding, blood serum levels were determined by the content of thyroxine, triiodothyronine, thyroid stimulating hormone (by immunoassay), and the total cholesterol-enzyme-photometric method (Chol-DAC.Lq kit by SpectroMed® R. L.). In addition, the index of the ratio of total cholesterol to tetraiodothyronine was calculated. Digital data was processed statistically.

Based on the analysis of the obtained material, two groups of dogs with different levels of functional state of the thyroid gland were obtained: control (without changes in the functional state of the thyroid gland) and experimental (with a decrease in the functional state).

After that, there was created and tested the program of individual bioresonance testing of the evaluation of the functional state of the thyroid gland with the help of the applied diagnostic complex 'PARKES-D'. The principle of which is based on the phenomenon of biological resonance — the determination of the electrical conductivity of the BAP when micro resonance circuits are introduced into the electromagnetic contour.

Resonance is characterized as the strong growth of the amplitude of electromagnetic oscillations under the influence of external actions, when the frequency of the internal oscillations of the object coincides with the frequency of oscillations of the external action. For bioresonance testing, biologically active points that were localized on the anterior limbs from the anterior surface of the foot, on the skin between 2–3, 3–4, and 4–5 phalanges were used. At the final stage of the research, comparisons were made between these research methods.

Results. Table 1 provides results describing the functional state of the thyroid gland in dogs. It has been determined that the decrease in the functional state of the thyroid gland is accompanied by 1.6 times ($p < 0.001$) increase of thyroid hormones in the blood of dogs compared with the parameters in dogs from the control group.

In this case, a decrease in the level of triiodothyronine in the blood of dogs at hypothyroidism of the thyroid gland by 21.4% was determined. Thus, the content of the hormone in the blood of dogs in the control group fluctuated within 21–75 ng/dl, whereas in animals with a decrease in the functional state of thyroid gland, it was 31–39 ng/dl.

Table 1 — Blood indices of dogs with different levels of functional state of the thyroid gland (M ± m, Σn = 36)

Indices	Animal groups			
	Control (n = 31)		Experimental (n = 5)	
	M ± m	Lim	M ± m	Lim
Thyroid-stimulating hormone, ng/cm^3	0.24 ± 0.04	0.13–0.41	0.38 ± 0.01**	0.35–0.42
Triiodothyronine, ng/dl	42.77 ± 6.5	21–75	33.6 ± 1.57	31–39
Tetraiodothyronine, μg/dl	2.09 ± 0.22	1.48–3.01	1.17 ± 0.11**	0.84–1.36
Cholesterol, mmol/dm^3	5.58 ± 0.34	4.55–6.67	8.26 ± 0.99***	6.6–11.5
CHS/T4, conditional units	0.14 ± 0.02	0.09–0.23	0.24 ± 0.02***	0.21–0.29

Note. Reliable difference from the experimental group: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$.

In contrast to triiodothyronine, the content of tetraiodothyronine in the blood of dogs at reducing the functional state of thyroid gland significantly decreases, almost two times ($p < 0.001$). Thus, the reference values of the content of this hormone in the blood of dogs in the control group were 1.48–3.01 μg/dl, and in the experimental group, respectively, 0.84–1.36 μg/dl.

According to the published data, in the case of the subclinical form of hypocotylose a decrease in the content of high-density lipoprotein (HDL) and increased concentration of low density lipoprotein (LDL), triglycerides and total cholesterol (Nechyporuk and Korda, 2015) is observed in blood. Thus, the content of total cholesterol in the blood of dogs in the experimental group was 1.48 times ($p < 0.001$) higher than the values in animals in the control group.

Indicator of the ratio of total cholesterol to tetraiodothyronine (CHS/T4) is another reliable criterion for evaluating the functional state of the thyroid gland. 1.7 times increase of the index CHS/T4 ($p < 0.001$) in the blood of dogs with a decrease in the functional state of the thyroid gland was determined compared with the values in the animals of the control group.

Moreover, the reference values of this index in the blood of the dogs of the control group were 0.09–0.23 conditional units, respectively, in the experimental group — 0.21–0.29 conditional units.

The conducted researches have established that for dogs the bioresonance is the fluctuation of the value of the electrical conductivity index of the biological active points of 8–24 scale units.

The electrical conductivity in the BAP of the scale of the complex in the experimental dogs varied from 22 to 61 conditional units. It should be noted that the electrical conductivity in the most informative biologically active points (between 2–3, 3–4 and 4–5 phalanges of the anterior limb) differs by no more than 1–2 conditional units, which allows to use even one point for a reliable assessment of the functional state of the thyroid gland (Table 2).

During the study of the phenomenon of bioresonance with the use of nosode for the evaluation of the function of the thyroid gland in 36 dogs, seven animals with reduced functional status were detected. It should be noted that the data on 5 dogs are consistent with the indicators of biochemical blood tests (which indicate a decrease in the function of the thyroid gland), and in two dogs in which bioresonance was determined concerning the violation of the functional state of this gland, biochemical blood parameters were within the normal range.

This may indicate a hidden form of change in the functional state of the thyroid gland.

Table 2 — Testing of the functional state of the thyroid gland in dogs by the 'PARKES-D' diagnostic complex (M ± m, Σn = 36; conditional units)

Animal groups	BAP localization		Indicators		
			Without nosode	With nosode	Difference (resonance)
Control (n = 29)	Between 2–3 phalanges	M ± m	38.48 ± 6.08	53.07 ± 5.5	14.59 ± 2.07
		Lim	23–60	34–70	8–22
	Between 3–4 phalanges	M ± m	38.34 ± 6.03	53 ± 5.3	14.66 ± 2.14
		Lim	22–61	35–72	9–23
	Between 5–6 phalanges	M ± m	38.52 ± 5.92	53.24 ± 5.18	14.72 ± 2.09
		Lim	22–60	35–69	8–22
Experimental (n = 7)	Between 2–3 phalanges	M ± m	37.14 ± 3.48	52.43 ± 3.2	15.29 ± 2.41
		Lim	26–47	46–63	8–22
	Between 3–4 phalanges	M ± m	36.71 ± 3.25	52.29 ± 3	15.57 ± 2.55
		Lim	25–45	45–63	8–23
	Between 5–6 phalanges	M ± m	36.43 ± 3.38	51.71 ± 2.51	15.29 ± 2.75
		Lim	23–44	46–61	8–24

Note. Reliable value of the bioresonance index is R ≥ 8.

Consequently, the results of studies of the functional state of the thyroid gland in dogs according to different methods are consistent by 94.4%.

Conclusions and perspectives for further research. Thus, the use of the functional testing by the hardware and software diagnostic complex 'PARKES-D' for a comprehensive assessment of the state of organs and systems of an animal's organism allows, with a reliability of 94.4%, to establish the functional state of the thyroid gland.

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Part 2. Biotechnology and genetics

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EFFECT OF PHYTONUTRIENT 'VITASTIM' ON CHICKEN MUCOSAL IMMUNITY AGAINST LOW PATHOGENIC AVIAN INFLUENZA VIRUS H4N6

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Summary. The study of immunostimulating phytogenous preparation 'Vitastim' influence on chickens infected by subtype H4N6 avian influenza virus was provided. It was determined, that preparation usage is more actively influences on humoral mediators of immune reaction and proliferation activity of spleen lymphocytes under conditions of low pathogenic avian influenza. This is evidenced by an increase in proliferative activity of chicken splenocytes at 5–14 days and an increase in production of NO during 14 days of the experiment. According to the quantitative real-time PCR results the expression of IL-2, IL-13, IL-17α in peripheral blood was effectively increasing with the promotion of enhances anti-viral activity. Due to the immunohistochemical studies of chicken it was found the influence of the phytonutrient 'Vitastim' on humoral immune system by registration of high levels of IgM, IgG, IgA. Cell immunity was stimulated by the 'Vitastim', however, as evidenced by the low levels of CD4, macrophages, IL-2, IL-15 in chickens of experimental groups compared to the intact birds, cell immunity does not play a significant role in the pathogenesis of LPAI. The phytonutrient 'Vitastim' produces moderated effect on the cell immune system at influenza virus infection with stimulation of IL-2, IL-4, IL-17, IFN-γ expression with irregular tendency in different observation periods.

Keywords: low pathogenic avian influenza virus, H4N6, chickens, vitastim, phytonutrient, mucosal immunity

Introduction. Today, the treatment of various infectious diseases in animals is difficult to imagine without using preparations that activate or suppress the immune system.

The immune system is divided into two subsystems:

— non-specific innate (natural) immunity;

— specific (adaptive) immunity.

Prevention of low pathogenic influenza plays a huge role. However, the possibility to conduct such prophylaxis is limited by several factors, such as the large number of pathogens, their genetic variability, instability of immune system to be formed etc.

Under these conditions, the role of immunostimulative preparations is modulating (stimulating or inhibiting), i.e. normalizing the non-specific immune response.

The joint operation of these two subsystems provides the immunity of the organism to various infectious agents — viruses, bacteria, fungi.

The main advantage of the therapeutic effect of phytonutrients as immunostimulatory preparations is their omnidirectional, the ability to normalize the non-specific immune indices, gentle influence, good tolerability and low cost. And most importantly, they are able to recognize and remove foreign bodies without regarding to their individual specificity, i.e. herbal remedies have nonspecific immune action.

Poultry production now is rapidly developing and needs a modern veterinary software. The presence of accelerated evolutionary processes resulted in a complication of the epizootic situation, increasing the pathogenic properties of the pathogens and spread of infectious diseases.

Way out lies in enhancing the natural resistance of the organism, strengthen the immune status at the level of the organism and group immunity by eliminating the immunosuppressive factors and usage of the immunostimulatory preparations. Immunostimulation is widely used in infectious disease treatment. There are used the adjuvants of different origin. There are valuable ways to improve the immune status of the avian organism and enhance the immune response during vaccination. Search for new immunostimulating preparations continues to view ever-increasing requirements regarding their safety, effectiveness, and accessibility.

Recently, considerable attention is paid to herbal preparations (Albalbaki, 1997). Many plants are well known in traditional medicine and have pronounced immunostimulating properties. *Echinacea purpurea* has

such properties. The root alcoholic extract is prepared from this plant (Hryshchuk et al., 2000).

Herbal immunostimulants also prepared by combining several plants in focus of their influence on the organism. Such immunostimulants significantly increase the level of antibodies in vaccinated birds.

Due to the increased restrictions on usage of antibiotics it is need to develop alternative strategies for disease control against many pathogens of birds, including avian influenza. Immunostimulation with natural plant products shows effectiveness in improving the innate immunity of birds, as well as in increasing resistance against intestinal pathogens (Lee et al., 2009). There were synergistic effects of herbal medicines of *Curcuma*, *Capsicum*, and *Lentinus* revealed on increase the local immunity against infection *Eimeria acervulina* (Lee et al., 2010a).

The aim of the study was to investigate the immunostimulatory effect of phytonutrition 'Vitastim' on the immune response after avian infection with low-pathogenic avian influenza virus (LPAIV).

Materials and methods. ***Experimental animals.*** Sixty-three six-weeks-old chickens were used to study mucosal immunity after application of phytonutrition 'Vitastim'. They were divided into 3 groups:

1. Chickens, experimentally infected with low pathogenic avian influenza virus A/Garganey/Chervonooskilske/4-11/2009 (H4N6) intranasally, using the dosage of $10^{6,0}$ $EID_{50.}$ per chicken;

2. Chickens, experimentally infected with low pathogenic avian influenza virus A/Garganey/Chervonooskilske/4-11/2009 (H4N6) intranasally, using the dosage of $10^{6,0}$ $EID_{50.}$ per chicken, and given phytonutrient 'Vitastim' in the doze of 2 mg/kg during the first and last five days of the experiment;

3. Control group of untreated chickens.

Phytonutrient 'Vitastim'. Under the technological regulation the purveyance of primary raw material was carried out for making of experimental series of preparation 'Vitastim': needles and branches of Scots pine (*Pinus sylvestris*) and leaves of common oak (*Quercus robur*). From primary raw material after previous treatment (washing, drying growing) was carried out extracting of biologically active matters, then the experimental series of preparation are prepared. The samples of preparation 'Vitastim' passed preclinical researches on indexes: mass part of moisture folded 3.8%, solubility 100%, a size of granules was within the limits of 0.5 mm, authenticity and mass part of 'Vitastim' by the concentration of polyoxyphenol connections. Content of 'Vitastim' in the experimental test by the concentration of polyoxyphenol connections presented 97.8 ± 0.3%. In default of death of mice in the experimental and control groups the series of 'Vitastim' in the dose of 80 mg/kg we considered harmless.

Virus. Avian influenza virus A/Garganey/Chervonooskilske/4-11/2009 (H4N6) has been used. This virus was isolated from cloacal swabs of clinically healthy garganey in 2009 in Ukraine during a widespread epizootic monitoring of wild birds. It was deposited to the collection of viral pathogens of poultry diseases in Department of Avian Diseases of NSC 'IECVM'. It was shown that this virus is low pathogenic. The intravenous and intranasal infection of this virus causes no clinical signs in chickens. The autopsy of infected poultry did not show any pathological changes. Infection of poultry provokes an immune response as well as the increasing the antibodies level in 10 days after intranasal infection ranged from 1:8 to 1:32. Only 40 % of chicks had diagnostic levels of antibodies to the influenza virus H4 in Hemagglutunation Inhibition Test and 60 % were positive for influenza virus in ELISA. In 10 days after intravenous infection of chickens with the virus all chicks presented specific antibodies to influenza virus H4 in titers from 1:128 to 1:1024. Mean titer was 7.8 ± 1.03 $\log_2$. Also, all chickens had antibodies to the influenza virus in ELISA.

On 1st, 3rd, 5th, 7th, 10th, and 14th days after experiment three birds from each group were euthanize. The blood was sampled for gene expression and serum nitric oxide assay, spleen — for splenocyte proliferation assay.

Splenocyte proliferation assay. Spleen was removed and placed in Petri dishes with 10 ml of Hanks' balanced salt solution supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma, St. Louis, MO). Cell suspensions were prepared by gently flushing through a cell strainer and lymphocytes were purified by density gradient configuration through Histopaque 1077 (Sigma).

Splenocytes (1×10^6 cells/ml, complete RPMI with 10% fetal bovine serum) were incubated with media (1 μg/ml) in 96-well microtiter plates for splenocytes proliferation in a humidified incubator at 41 °C and 5% CO_2 for 24 hours. For the studying of the direct influence of phytonutrient to splenocyte pool the 'Vitastim' was added and Con A was used as a positive control (5 μg/ml).

Following incubation, 100 μl of cell culture supernatants were transferred to fresh flat-bottom 96-well plates, mixed with 100 μl of Griess reagent (Sigma) and the plates were incubated for 15 min at the room temperature. Cell proliferation was measured by using WST-8 (Cell-Counting Kit-8®, Dojindo Molecular Technologies, Gaithersburg, MD) with 2-[2-methoxy-4-nitrophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium, monosodium salt as described. The optical densities were measured at 450 nm using a microplate reader (BioRad, Hercules, CA) for WST-8 assay (Lee et al., 2011).

Nitric oxide (NO) production by macrophages. Serum samples were centrifuged at 1000 g for 30 min at 4–8 °C, 100 ml were mixed with an equal volume of freshly prepared Griess reagent (Sigma) containing 1% (w/v) sulphanilamine in 5% phosphoric acid and 0.1% (w/v)

N-naphthylethylenediamine, the mixture will be incubated for 10 min at room temperature, and theoptical density at 540 nm (OD540) was measured using a microtitre plate reader (Bio-Rad, Richmond, California, USA). Nitrite concentrations were calculated from a standard curve using $NaNO_2$ (Lee et al., 2011).

Quantitative RT-PCR gene expression. *RNA extraction:* RNA was isolated from chicken peripheral blood, stabilized with EDTA. Blood was collected at 1st, 3rd, 5th, 7th, 10th, and 14th days after chicken infection with low pathogenic avian influenza viruses A/Garganey/Chervonooskilske/4-11/2009 (H4N6). The RNA was extracted using innuSOLV RNA Reagent (Analytik Jena AG) (Rainen et al., 2002; Chomczynski and Sacchi, 1987).

Reverse transcription reaction: The RNA was treated with DNAse (TURBO™ DNAse) to remove residual DNA. The following mixture was prepared: RNA-8 µl, l µl of 10× buffer, DNAse — 1 µl. The mixture was incubated for 15 minutes, then 1 µl of Stop Solution was added and inactivated with DNAse at 70 °C for 15 minutes. The resulting DNA-free RNA was used directly for reverse transcription reaction. The reverse transcription reaction was performed using a Thermo Scientific commercial kit. For the DNAse treated RNA was mixed with 1 µl of oligo (dT) 18 primer and incubated at 65 °C for 5 min. For reverse transcription reaction mixture was prepared by the following protocol: 5× Reaction Buffer — 4 µl, RiboLock RNase Inhibitor — 1 µl, 10 mM dNTP Mix — 2 µl, M-MuLV Reverse Transcriptase — 2 µl. The mixture was incubated for 1 h at 37 °C and diluted with deionized water up to 200 microliters.

The amplification reaction: Oligonucleotide primers for IL-17α, IFN-γ, IL-2, IL-4, IL-13 and GAPDH quantitative RT-PCR are listed in Table 1. Amplification and detection were carried out using DT-ligt qPCR thermocycler system (DNK-technology, Russia) and ready-to-use Maxima SYBR Green qPCR Master Mix (2×) (Thermo Scientific), and the following temperature conditions: 1 cycle at 95 °C for 10 min; 40 cycles at 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min. Each RT-PCR experiment contained test samples and 2× dilutions of standard RNA in triplicate. To normalize RNA levels between samples within individual experiments, the mean threshold cycle value (Ct) for the cytokines and GAPDH products was calculated by pooling values from all samples in that experiment. The levels of cytokines transcripts were normalized to those of GAPDH using the Q-gene program (Lee et al., 2010b; Muller et al., 2002).

Table 1 — Oligonucleotide primers used for quantitative RT-PCR of chicken cytokines

Type	RNA target	Primer sequences	PCR product size, bp	GenBank accession No.
Reference	GAPDH	F: 5'-GGTGGTGCTAAGCGTGTTAT-3' R: 5'-ACCTCTGTCATCTCTCCACA-3'	264	K01458
	IL-17α	F: 5'-CTCCGATCCCTTATTCTCCTC-3' R: 5'-AAGCGGTTGTGGTCCTCAT-3'	292	AJ493595
Th-1	IFN-γ	F: 5'-AGCTGACGGTGGACCTATTATT-3' R: 5'-GGCTTTGCGCTGGATTC-3'	259	Y07922
	IL-2	F: 5'-TCTGGGACCACTGTATGCTCT-3' R: 5'-ACACCAGTGGGAAACAGTATCA-3'	256	AF000631
Th-2	IL-4	F: 5'-ACCCAGGGCATCCAGAAG-3' R: 5'-CAGTGCCGGCAAGAAGTT-3'	258	AJ621735
	IL-13	F: 5'-CCAGGGCATCCAGAAGC-3' R: 5'-CAGTGCCGGCAAGAAGTT-3'	256	AJ621735

Notes: F — forward primer; R — reverse primer.

Statistical analysis. Statistical analysis was performed using SPSS 17.0 software for Windows. All data for each group expressed as means ± SEM. The difference between the means considered at $p < 0.05$.

Results. ***Splenocyte proliferation activity.*** Analysis of the results showed the slight stimulation of lymphocyte proliferative activity at intact birds since the 5th day of the experiment. 'Vitastim' stimulates more actively the proliferative activity of spleen lymphocytes at infection with low pathogenic influenza (Fig. 1).

Nitric oxide (NO) production. It was also found that the development of infection with LPAI (chickens of the 1st experimental group) accompanied by a solid, rapidly increasing concentration of NO from 12.1 ± 0.08 mmol/l on 3rd day of the experiment up to 14.8 ± 0.06 nmol/l on 14th day.

The treatment of infected birds with phytonutrient 'Vitastim' (chickens of the 2nd experimental group) resulted in increasing of NO production, since the 1st day of the experiment to 13.9 ± 0.05 mmol/l.

Treatment of the healthy birds with phytonutrient 'Vitastim' (chickens of the 3rd experimental group) during the first five days of the experiment also causes a stable accumulation of NO in blood serum, but it was more pronounced, on the 5th day it's quantity was increased in its metabolites. At the 5th day under the absence of

'Vitastim' application it was studied the NO reduction in blood serum of intact birds, and the subsequent watering caused the most pronounced increase in the concentration of metabolites (Fig. 2).

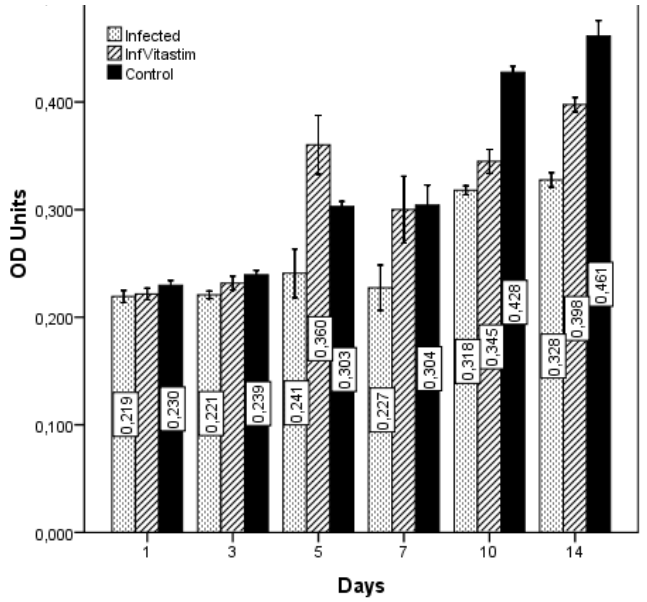

Figure 1. Dynamics of splenocyte proliferation changes in lungs after infection with LPAIV. Each bar represents the spleen lymphocyte accumulation in the immune process dynamics from 1st to 14th days after phytonutrient administration. Significant stimulating influence of phytonutrient 'Vitastim' on immune response against LPAI is shown at 5th and 14th days, respectively. Statistical analysis was performed by comparing splenocyte amount after incubation at infected chickens, chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim) with control birds

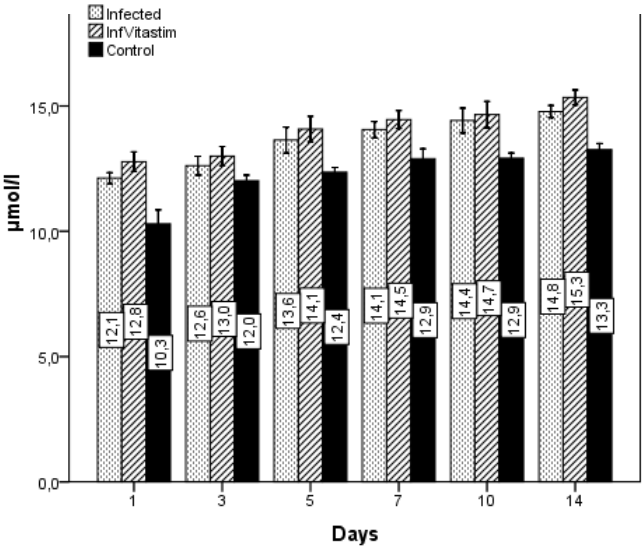

Figure 2. Dynamics of nitric oxide changes in lungs after infection with LPAIV. Each bar represents the nitric oxide metabolite concentration in the immune process dynamics from 1st to 14th days after phytonutrient administration. Significant stimulating influence of phytonutrient 'Vitastim' on immune response against LPAI is shown at 7th and 14th days, respectively. Statistical analysis was performed by comparing nitric metabolites at infected chickens, chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim) with control group

Cytokines mRNA expression. There is a general tendency to activate expression of cytokines in the groups treated with 'Vitastim' compared to intact birds, under infection with low pathogenic influenza virus, as well as at combine action of both of these factors compared to control group.

Dynamic of IL-2 gene expression has a wavy appearance in the group of infected birds. This index for the first three days has almost tripled, and then decreased back to normal at the 10th day to only 20–22% higher than the control rates (Fig. 3). Thus, in the first group, the expression level varied slightly at the 10th day of the experiment. During the study of the IL-2 gene expression we observed its activation in the chickens of the second group at the 5th day of experiment, but on the 7th day the index showed the values the same as at 1st and 3rd days. At the tenth day the expression level slightly grew up in both groups, and at the second group was significantly higher by 7–10%, but their difference was not significant. At the same time, using the 'Vitastim' showed two times increasing IL-2 expression indices compared to the infected birds.

Dynamics of IL-4 gene expression was characterized by near-zero values, indicating starting inactivity of the corresponding gene. Subsequently, at infected groups these indices reached 96%, and then decreased on the 10th day to control group levels (Fig. 4).

A significant level of IL-4 gene expression in the other groups of poultry has also been detected on the 3rd day. Regarding the second group, the expression level in chickens of the 1st group almost did not exceed and gradually declined the levels of the first day of the experiment. The chickens of the 2nd group also showed a downward tendency of expression on the 3rd day, but from 5th to 10th days the expression level was gradually increased.

As seen on Fig. 5, IL-13 gene expression level has been equal enough throughout the experiment, except the second group level expression on the 7th day. However, on the 10th day expression was reduced to the indices as on the 5th and the 14th days. The birds of the first group showed a two-times expression increase from the 1st to 3rd days. Then, the expression level was reduced to the base at the 7th day.

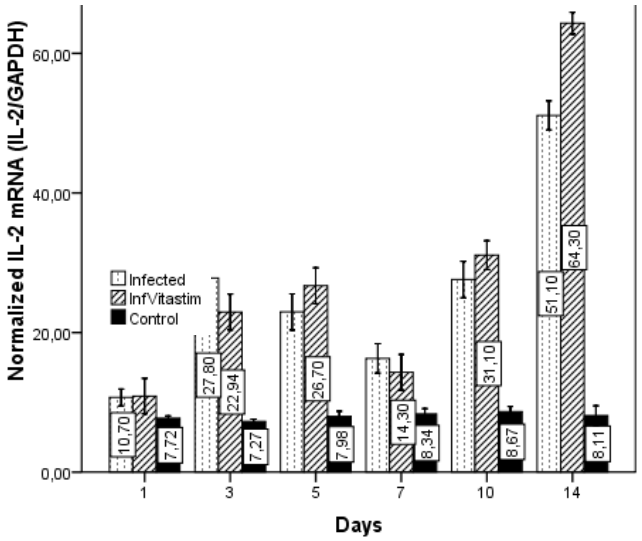

Figure 3. Dynamics of mRNA IL-2 expression. Total RNA was isolated from blood of 1st to 14th days after phytonutrient administration, analyzed for IL-2 mRNA by quantitative RT-PCR. It was shown increasing of IL-2 expression level on 14th day in chickens of the 1st and 2nd groups. Significant differences between chickens of 1st and 2nd groups were observed. Statistical analysis was performed by comparing IL-2 expression level between the 1st and 2nd groups with control groups

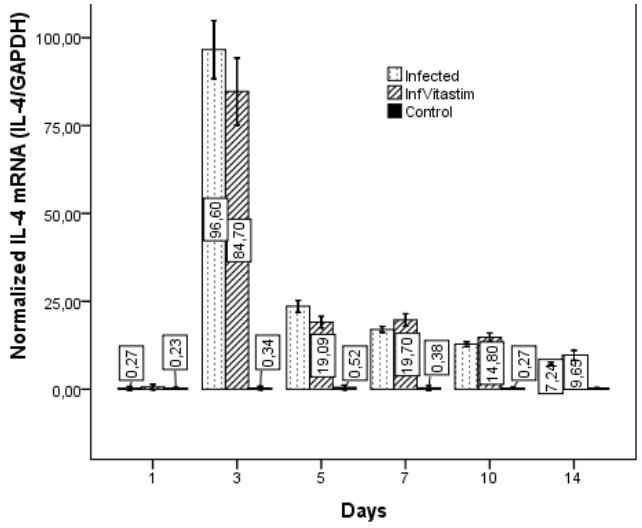

Figure 4. Dynamics of mRNA IL-4 expression. Total RNA was isolated from blood samples during 1st to 14th days after phytonutrient introduction, analyzed for IL-4 mRNA by quantitative RT-PCR. It has shown the improving of IL-4 expressed on the day 3 among infected and infected + 'Vitastim' groups. Significant differences between infected and infected + 'Vitastim' groups were not observed. Statistical analysis was performed by comparing IL-4 expression level in infected chickens and chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim) with control birds

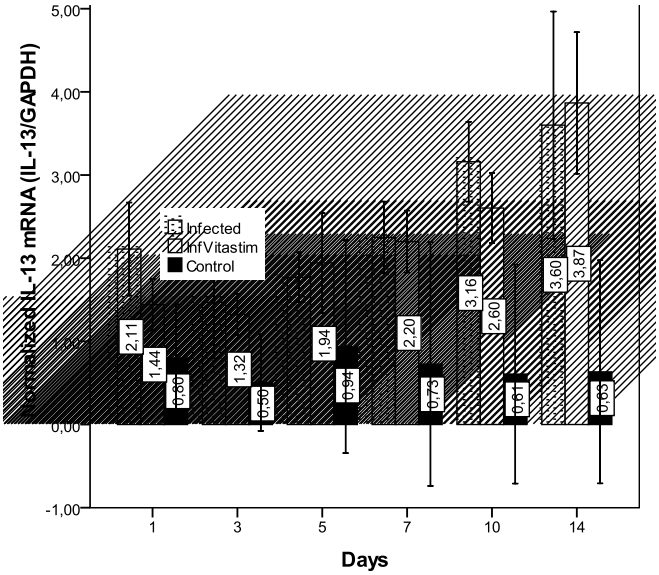

Figure 5. Dynamics of the mRNA IL-13 expression. Total RNA was isolated from blood from the 1st to the 14th days after phytonutrient administration, analyzed for IL-13 mRNA by quantitative RT-PCR. It has shown the increasing of IL-13 expression level on 14th day in chickens of the 1st and 2nd groups. Significant differences between chickens of the 1st and 2nd groups were not observed. Statistical analysis was performed by comparing IL-13 expression level at chickens of 1st and 2nd groups with control chickens

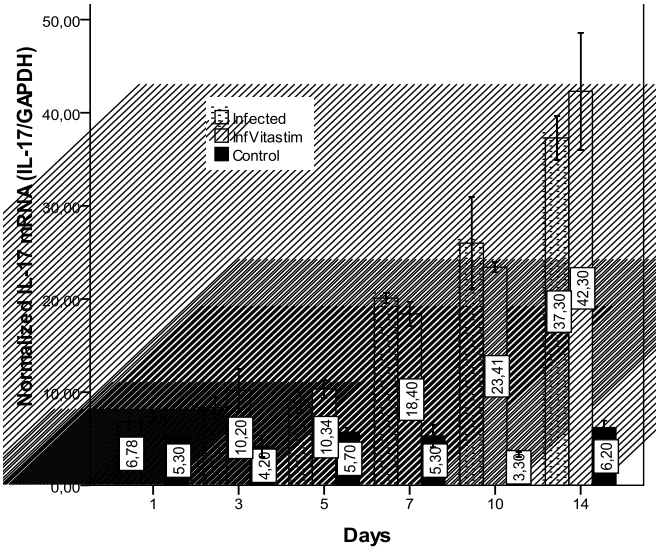

Figure 6. Dynamics of mRNA IL-17 expression. Total RNA was isolated from the blood samples of the 1st to 14th days after phytonutrient introduction, analyzed for IL-17 mRNA by quantitative RT-PCR. It has shown increasing of IL-17 expression level from 7th day until the end of the experiment among infected and infected + 'Vitastim' groups. Significant difference between infected and infected + 'Vitastim' groups was not observed. Statistical analysis was performed by comparing IL-17 expression level at infected chickens, chickens infected with LPAIV + phytonutrient 'Vitastim' (InfVitastim) with control birds

IL-17 gene expression levels were increased since the first day at chickens of the 1st group. This tendency carried from the 1st to the 10th day of the experiment, after which there was a 2.5 times decreasing.

In the chickens of the second group we noted four times increased expression level from the 1st to the 3rd day. Maximum expression appeared on the 14th day of experiment (Fig. 6).

IFN-γ gene expression also had a number of features. In particular, IFN-γ gene expression level was higher in chickens of the first group at the third day and gradually decreased at the 14th day of the experiment (Fig. 7). The peak of expression at chickens of the second group accounted on the 7th day of experiment, then it changed slightly during the next week.

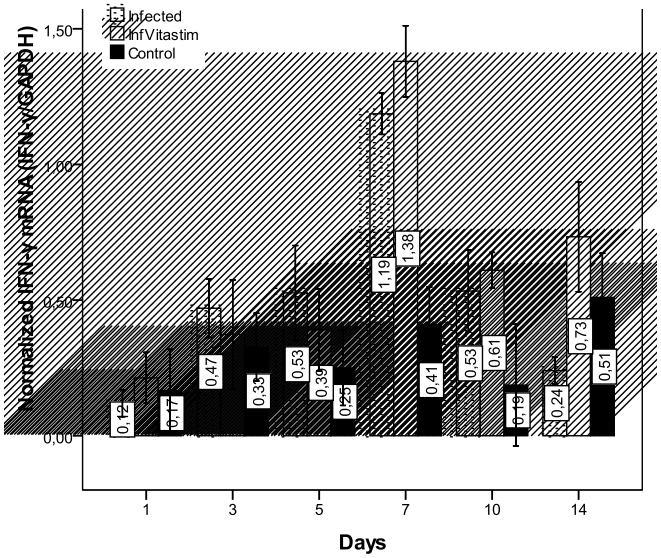

Figure 7. Dynamics of mRNA IFN-γ expression. Total RNA was isolated from blood during the 1st to 14th days after phytonutrient administration, analyzed for IFN-γ mRNA by quantitative RT-PCR, and normalized to GAPDH mRNA. It has shown increasing of the IFN-γ expression level on 7th day in chickens of the 1st and 2nd groups. Significant differences between chickens of 1st and 2nd groups were not observed. Statistical analysis was performed by comparing IFN-γ expression level at chickens of 1st and 2nd groups with control chickens

Conclusions. It has been found that infestation of LPAI strains of chickens (group 1) leads to a decrease in splenocytes proliferative activity starting from the 5th day of experiment and increasing NO production during all 14 days of observation. The use of 'Vitastim' promotes increased proliferative activity of splenocytes during 5–14 days of the experiment and a slight, stable increase in NO in the serum of chickens during all research period. The obtained data indicate the stimulating effect of the drug on nonspecific factors of the immunity of chickens.

Thus, it was found that the dynamics of the IL-2 gene expression under influenza virus infection had wavy form in chickens of the 1st group. Dynamics of IL-4 gene expression indicated the start inactivity of the gene, and later the indices were grown at chickens of the 1st group, and then decreased on the 10th day compared to the levels of the control group. In the group, treated with 'Vitastim' expression indices were higher, indicating its activating effect on cell immune system.

IFN-γ gene expression level was higher in chickens of the first group on the third day, and gradually decreased on the 14th day of experiment. IFN-γ gene expression was more intense in the chickens of the control group at the 3rd, 7th and 14th days of the experiment, and the treatment of 'Vitastim' was decreased this index.

Therefore, phytonutrient 'Vitastim' produces moderated effect on the cell immune system at influenza virus infection. Thus, it stimulates response to IL-2, IL-4, IL-17, IFN-γ with irregular tendency in different observation periods.

Discussions. Previous studies describe similar immunomodulated and/or immunomodificated effects of synthetic and natural (phytogenous) preparations. Therefore, there was shown apparent antioxidant, antiinflammatory and immunotropic action of preparations 'Immunal' and 'Tonzilgon N' (Tsaryov, 2003; Kovalenko, Shypayeva and Kolchenko, 2008).

That is why the preparation 'Vitastim' was made from Scots pine (*Pinus sylvestris*) branches and needles, and from common oak's (*Quercus robur*) branches with leaves. Its qualitative properties specified significant number of flavonoids and tans. It is known that flavonoids (anthocyanidins, catechines, and flavonols etc.) and phytogenous phenolic acids act as synergists. (Lozano et al., 2005).

Methanol extract of oriental plum (*Prunus salicina* Lindl.) has a highly immunostimulatory effect on animal resistance (Lee et al., 2009). It significantly increases the spontaneous proliferation of spleen lymphocytes, and macrophages. The extract may have exerted direct and indirect anti-tumor activity. It seems, that this extract can be used to treat cancer in human.

Safflower leaf stimulates chicken immunity as assessed by splenic lymphoproliferation, tumor killing (Lee et al., 2008). Purified fractions of safflower leaves induce effective immune response and anti-tumor activity. It shows high immunostimulating activity through the ability to enhance splenocyte proliferation and macrophage activation, and directly decrease the viability of a tumor cell line.

Due to increasing restriction of the antibiotics usage, there is an urgent need to develop an alternative disease control strategies against many poultry pathogens including AIV. Dietary immunostimulation using natural plant products was shown as effective preparation for enhancing poultry innate immunity in general and for increasing disease resistance against enteric pathogens (Lee et al., 2008). However, the underlying immune

mechanisms that are responsible for plant-medicated immune enhancement were not well investigated. Therefore, in this proposal, we will investigate humoral and cell-mediated immune response mediated by dietary feeding of immunostimulatory phytonutrient, 'Vitastim' under the presence of avian influenza infection. This study will deeper our knowledge on plant product-mediated immune enhancement at the cellular and molecular levels and will facilitate the development of highly effective safe methods for the control of economically important poultry diseases.

The phytonutrient, 'Vitastim' is intended to increase the natural resistance and immunomodulate of host innate immunity. The preparation shows antioxidant actions. The preparation structure includes a water extract of leaves and branches of common oak (*Quercus robur*) and annual branches of Scots pine (*Pinus sylvestris*). Extracts are received by native raw materials autoclaving, mixing them in equal volumes and lyophilizing by an original technique. Effective doses to provide optimum immunoenhancement of innate immunity will be developed during scientific experiments (Kovalenko, Krotovska and Obukhovska, 2013).

Stimulating effect of 'Vitastim' is, apparently, relative cell proliferation and production of such cell immunity mediator as nitric oxide. So, SPA indicators at chickens of the 3rd experimental group were 5.6–7.0% above control on the 5th day. The SPA rate in chickens of the 2nd experimental group was higher than in the 1st group on 50% ($p \leq 0,05$), on the 10th and on the 14th day — 9.3% and 21.2% respectively.

A similar effect was established during application of sinnamaldehyde consistuent of cinnamon (Lee et al., 2011). It is known, that effector molecules such as NO play an important role in immunity (Okamura et al., 2005).

'Vitastim' application at LPAI showed the difference between indices of 1st and 2nd groups on 5th and 7th days and was 11.1% and 17.0% respectively. Attention is paid on the fact of reducing NO production after finishing the treatment with phytonutrient 'Vitastim' (10th day). At the end of the experiment, after re-watering (14th day of the experiment) the level of NO at the chickens of 2nd group exceeded on was raised 10% comparing to infected birds.

Treatment of the healthy birds with phytonutrient 'Vitastim' (chickens of 3rd experimental group) at the first five days of the experiment also caused a stable accumulation of NO in blood serum, but it was more pronounced, on the 5th day its increased metabolites were 16.1% ($p \leq 0, 05$) comparing to the healthy birds, and on the 7th day the difference was 24.8% ($p \leq 0,05$). At the 5th day the NO reduction in intact birds blood serum under the absence of application with phytonutrient 'Vitastim' was observed, and the subsequent watering caused the most pronounced increase in the 27.1% (up to 16,91 ± 0,1 mmol/l) concentration of metabolites.

There are studies about activation of NO production by macrophage cultures a treatment by sinnamaldehyde *in vitro* (Lee et al., 2011) and significant reduction of NO production during the processing by novel heteropolysaccharide which was isolated from Radix Isatidis LPS-treated alveolar macrophages (Du et al., 2013).

As known, tissue-specific expression pattern of cytokine genes is formed under the process of cell differentiation and it is often determined by the presence of a specific collection of transcription factors in cells (Rudensky, Gavin and Zheng, 2006). In this study several indicators of immune response such as IL-17α, IFN-γ, IL-2, IL-4 and IL-13 in peripheral blood were detected by quantitative real-time PCR. The results of the 'Vitastim' introduction showed effectively increasing of the IL-2, IL-13, IL-17α expression, that promotes cell growth, and enhances anti-viral activity.

The expression of IFN-γ showed a sharp decline in the both experimental groups on 7 dpi, and it may be explained by the initiation of specific immunity, but the system of non-specific immunity did not start initiating at that time. The difference of IFN-γ level between both experimental groups and control group was statistically significant.

Dynamics of the mRNA IL-2 expression showed the highest peak on 14 dpi and this result was related to the expression of IFN-γ (Hilton et al., 2002) IL-17α is a proflammatory cytokine and has been implicated in host defense against different microbial and viral agents (Min and Lillehoj, 2002). The expression of the IL-13 and IL-17α was characterized by similar pattern in both groups which would play a role in the process of avian influenza infection.

The aforementioned presence of mRNA in cells does not necessarily indicate the presence of the corresponding protein. Extracellular cytokines production will be determined using serology investigations in the further studies.

Thus, the results of immunohistochemical studies of chicken inner organs has established the influence of the phytonutrient 'Vitastim' on humoral immune system, as evidenced by high levels of IgM, IgG, IgA. Cell immunity was stimulated by the 'Vitastim', however, as evidenced by the low levels of CD4, macrophages, IL-2, IL-15 in chickens of experimental groups compared to the intact birds, cell immunity does not play a significant role in the pathogenesis of LPAI. As for biochemical, molecular and genetic investigations 'Vitastim' has shown effective increase of the IL-2, IL-13, IL-17α expression, as well as nitric oxide level, that promotes cell growth, and enhances anti-viral activity.

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INFLUENCE OF PHYTOADDITIVES AND SODIUM SELENITE ON INDEX OF NATURAL RESISTANCE OF LAYING HENS

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Summary. The results of the complex influence of phytoadditives and sodium selenite on the parameters of the natural resistance of laying hens in the intensive fertility period are presented, as well as the determination of the total bacterial contamination and contamination of phytoadditives 'Phytopank' and 'Phytochol' and sodium selenite with molds. Increase of the total protein content in serum blood of laying hens by 13.7% and protein albumin fractions by 12.8% ($p < 0.01$), the α-globulin fraction by 11.3%, the γ-globulin fraction by 2.2% and the β-globulin fraction by 4.5%. Moreover, increase in the level of bactericidal activity of blood serum (BASK) by 16.62% ($p < 0.01$) and lysozyme activity of blood serum (LASK) by 16.7% ($p < 0.01$), and improvement of metabolism, liver function, feed consumption and assimilation of its nutrients. Determination of total bacterial contamination and contamination fungi showed that phytoadditives and sodium selenite do not exceed the overall contamination rate for one dose.

Keywords: blood, total protein, albumin, globulins, bacterial contamination, mold mushrooms, BASK, LASK, chicken bear, sodium selenite, Phytopank, Phytochol

Introduction. The intensive development of poultry farming in Ukraine, both on an industrial basis and in private farms, makes it possible to obtain high quality products in a short time. It is known, that the increase in the productivity of laying hens in turn is accompanied by an increase in physiological stresses on the body and an increase in requirements for environmental factors, in particular the conditions of housing and feeding. Violation of the optimal parameters of the microclimate causes decrease of resistance and productivity (Babina, 1998; Bashkirov, 2008).

Recently, one of the priority directions of the intensification of poultry farming was the search for highly effective ways to increase the productivity of poultry through the by using different biologically active substances (BAS), which have antimicrobial and growth stimulating qualities, but are not harmful to humans and animals (Bazylev, 2002; Draganov, Makartsev and Tyurkina, 2008; Bol'shakova, 2011).

In this direction at the modern level perspective is the use of biologically active substances, particular, preparations of herbal origin, which affect the organism in the form of a complex of minerals, vitamins, etc. When phytoadditives gets into the body, they penetrate the tissues, have a positive effect on the level of intracellular metabolism (Antonenko et al., 2014). Such influenced phytoadditives 'Phytopank' and 'Phytochol' (Kosenko and Malik, 2001).

In most countries of the world, research on the search for new sources of mineral-vitamin supplements, the improvement of their feeding technologies, and the refinement of the need for poultry in trace elements, which have not been taken into account earlier, are being actively carried out, but have proven to have a significant impact on the body. To such microelements of interest to scientists, include selenium (Sobolev and Povoznikov, 2015).

One of the promising directions stimulation of natural resistance, ensuring the normal functioning of the immune system, improving the metabolism of the organism laying hens, and increasing the biological value of fodder in intensive poultry farming is the use of various feed additives including of plant origin and trace elements (Okolelova, 2006; Glaskovich, Shul'ga and Sadomov, 2010).

The purpose of the research was to determine the effect of 'Phytopank', 'Phytochol', and sodium selenite on the indices of the natural resistance in laying hens during the period intensive fertility and to determine their total bacterial contamination and contamination with mold fungi.

Materials and methods. The research was carried out on the laying hens of the Adler Silver breed under the conditions of private farming LLC 'TAGR' (Bilyayivka Raion, Odessa Oblast). Based on the principle of par-analogue was formed three experimental groups and control group (60 in each). The conditions of feeding and breeding of laying hens of all groups met all veterinary and sanitary norms. The poultry in the control and experimental groups were kept in two-tiered cell batteries, equipped with feeders and jets, density of planting in accordance with the normative instructions. Feeding was carried out with a complete feed.

Laying hens of the 1st experimental group in addition to the main ration received a trace element of selenium in a dose of 0.2 mg/kg of dry matter of mixed fodder. As a

source of selenium, selenite sodium (sodium selenite TU 6-09-1315-76) was used. The inorganic mixture of selenium in the form of a white powder was thoroughly mixed with the feed.

The laying hens of the 2nd experimental group was given complete feed and phytoadditives 'Phytopank' and 'Phytochol' as follows: daily doses of water were injected everyday of 6 ml of each preparation (2 drops per chicken). 'Phytopank' is a complex composition of seven infusions with 40% ethyl alcohol of individual medicinal plants in a ratio (rhubarb root, rooster cockroach root, decoction root, trichophytic foxtail leaves, fragrant fennel, leaves of medicinal sage and fruits of the *Conium maculatum*). The 'Phytochol' is an aqueous extract of medicinal plants, which contains grass of shepherd's purse, flowers of sand caraway, flowers of common tansy, magnesium sulfate, sodium salicylate, hexamethylenetetramine, tincture of peppermint leaves, tincture of valerian root, belladonna and glycerin (in doses that do not exceed those generally accepted for use in homeopathy and allowed pharmacopoeia) (Antonenko et al., 2014). Phytomedications were added to the water.

The 3rd experimental group, along with the main diet and conditions of retention, received phytomedications ('Phytopank' and 'Phytochol') in combination with sodium selenite at similar doses.

Blood samples were collected from the laying hens in vivo from the subclavian vein, with all the rules of asepsis and antiseptics. The following indicators were taken into account: total protein (biuret reaction), protein fractions of blood serum (electrophoresis on paper), Serum creatinine (the kinetic method of IFCC (KF 2.7.3.2)) (Horiachkovskyi, 2005). Lysozyme activity of blood serum (LASK) was determined, using the photoelectrocholmometric method (A. G. Dorefaychuk), with a change in the temperature regimen of the reaction of blood serum of laying hens with culture of *Micrococcus lysodeikticus*; bactericidal activity serum of blood (BASB) was determined by method of Michelle Teffer in modification of O. V. Smirnova and T. A. Kuzmina (Horiachkovskyi, 2005).

During the organization of scientific research, the principles of bioethics were observed in accordance with the requirements of the European Convention for the Protection of Experimental Animals (EC 86/609/EEC) (CEC, 1986).

Determination of total bacterial contamination and contamination with moldy mushrooms performed using the method number 3 (testing of non-injectable preparations intended for introduction into animals with drinking water and/or aerosol) according to DSTU 4483:2005 'Veterinary immunobiological preparations. Methods of determination of bacterial and fungal contamination' (DSSU, 2005). Bacterial sowing of investigated drugs at a determined total bacterial contamination were carried out on meat-peptone agar (the manufacturer of the drug — Himedia, India, No. M 001) at a temperature of 35 ± 1 °C for 7 days. Determination of contamination by mold mushrooms using superficial cropping on a dense nutrient medium Saburo with glucose (Himedia, India, No. M 063) at the incubation temperature of 24 ± 1 °C for 14 days. The incubation was maintained in the HT-3/40 thermostat. At the end of the cultivation period, the results of the research were recorded visually, and the average number of increased colonies per dose was recounted.

The research results are presented in accordance with the requirements of the International System of Units and statistically processed using the MS Excel. The probability of differences between the indicators was estimated according to Student's criterion.

Results and discussions. The total protein content in the blood serum is one of the most important indicators of the quality of protein feeding of farm birds. According to the results of the research on the Adler's silver breeders, it should be noted that the usage of sodium selenite, 'Phytopank' and 'Phytochol' as supplements positively affects on metabolism, including protein metabolism and enzymatic processes (Fig. 1).

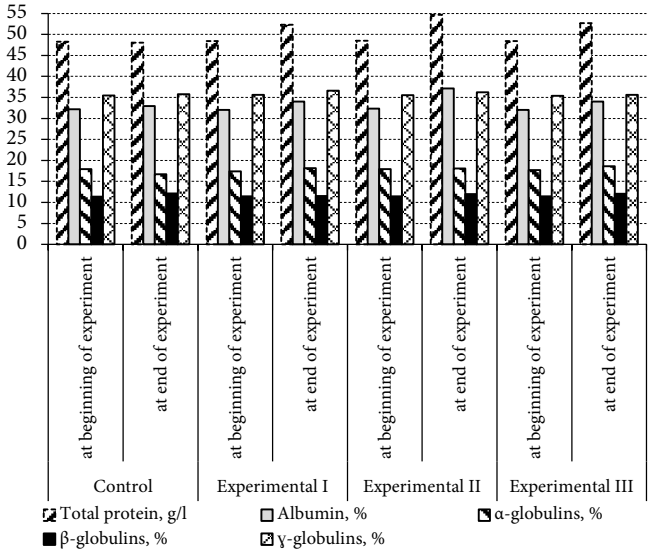

Figure 1. Indicators of protein metabolism in laying hens

The analysis of the results of the research at the end of the experiment shows that when used for feeding laying hens of the first experimental group of sodium selenite, the content of total protein increased by 8.9% compared with the control group. At the end of the experiment, in the laying hens of the second experimental group, which received feed phytomedications 'Phytopank' and 'Phytochol', there was an increase in the total protein content by 13.7% compared with the control group. A similar situation was observed in the laying hens of the third experimental group receiving sodium selenite in combination with phytomedications, in particular, the total protein content increased by 9.8%.

According to other researchers, it has been proved that the total protein correlates with the performance indicators in the laying hens, as well as a marker for the absence of chronic diseases, accompanied by a negative nitrogen balance. In addition, the content of total protein and its fractions reflects the activity of synthetic liver function.

Statistically significant increases in the albumin fraction by 3.2% were observed in the laying hens of the first experimental group under conditions of feeding with sodium selenite In the laying hens of the second and third experimental groups, when applying phytoadditives separately and simultaneously with the addition of sodium selenite in combination with feed phytoadditives, an increase in the albumin fraction was noted by 12.8% ($p < 0,001$) and 3.3% respectively. This fact can be explained by the fact that there is an improvement in metabolism and enzymatic processes, in particular liver with the help BAS, macro- and trace elements, vitamins, etc., which are part of feed phytoadditives. In addition, the increase in α-globulins in the first experimental group was determined by 8.6%, in the second by 8.1% and by the third by 11.3%, and γ-globulins by the first by 2.2%, the second by 1.2%, the third by 0.5%.

Concerning the β-globulin fraction, there was a slight decrease in the laying hens in all experimental groups from 0.3% to 4.5% in compared with the control group, but all parameters fluctuated within the limits of physiological norms. This is probably due to the fact that most beta-globulins are transport proteins, and they are involved in the transfer of hormones, vitamins, minerals and other substances, including those that are part of phytoadditives, which was confirmed after performing the research.

The great attention is paid to the humoral factors of protection (BASK, LASK), when studying the support of the level of natural resistance of the organism to the functional state of poultry and the persistence of diseases (Table 1).

Table 1 — The indicators of natural resistance of laying hens (M ± m, n = 6)

Indicators	Control group	Experimental groups		
		I	II	III
LASK, %	25.36 ± 0.51	27.9 ± 0.99*	30.45 ± 1.24**	28.89 ± 0.95**
BASK, %	47.71 ± 1.17	51.74 ± 2.72	57.22 ± 2.24**	54.7 ± 1.16**

Notes: * — $p < 0.05$; ** — $p < 0.01$ compared to the control group.

The analysis of the results showed that the combined usage of phytoadditives 'Phytopank' and 'Phytochol' and sodium selenite for the laying hens is to the improvement of overall resistance, as evidenced by their BASK, LASK and total albumin levels. With the use of sodium selenite in the feeding of laying hens of the first experimental group, the level of lysozyme activity of blood serum increased by 9.1% ($p < 0.05$), while in the second experimental group the increasing of the serum lysozyme activity by 16.7% ($p < 0.01$) compared with the control group. The same situation was observed in the laying hens in the third experimental group, where sodium selenite and phytoadditives added during feeding. In this group, the level of lysozyme activity of blood serum increased by 12.2%.

There was no statistically significant increase in the bactericidal activity of the serum of 7.7% in the laying hens of the first experimental group under conditions of feeding with sodium selenite. In the laying hens of the second and third experimental groups, when using phytoadditives separately and simultaneously with the addition of sodium selenite in combination with feed phytoadditives, a statistically significant increase in serum blood bactericidal activity was observed at 16.62% ($p < 0.01$) and 12.8% respectively. Furthermore, in the second experimental group (which was added only phytoadditives) of total protein was an increase of 13.7% and protein fractions by 12.8 albumins ($p < 0.01$), α-globulins — 11.3%, γ-globulin — 2.2% and β-globulin — 4.5%.

This can be explained with the correlation of the total albumin and its fractions with vitals, as well as marker of the absence of chronic diseases and reflection the activity of the synthetic function of the liver. It is known that most β-globulins are transport proteins that participate in the transport of hormones, vitamins and other BAR to cells, and -globulins support the overall resistance of the organism, which is confirmed by the results of research (see Fig. 1).

For non-inoculated phytoadditives such as 'Phytochol', 'Phytopank' and sodium selenite, which are recommended to use in poultry farming, a small number of bacterial and/or non-pathogenic fungal cells no more than one colony forming unit (CFU) for one dose is acceptable (Table 2).

Table 2 — Tests of non-injectable drugs intended to be administered to laying hens

Indicators	Phytopank	Phytochol	Sodium selenite
Total bacterial contamination, CFU/dose	< 1	< 1	< 1
Contamination with mold mushrooms, CFU/dose	< 1	< 1	< 1

Analysis of the results of the research show that sodium selenite and phytoadditives 'Phytopank' and 'Phytochol',

which were used during experiments for laying hens, have a total bacterial contamination and mushroom contamination of less than one, which is, the average number of non-pathogenic bacteria and (or) fungi per one dose and not exceed the permissible norm. This indicates that preparations of sodium selenite and phytoadditives 'Phytopank' and 'Phytochol' are sterile, which meets the requirements and can be used in domestic poultry farming for the intended purpose.

Conclusions. 1. The complex application of phytoadditives 'Phytopank', 'Phytochol' and sodium selenite for laying hens during period of intensive feeding had positive affect on the studied indicators of natural resistance, which was confirmed by the increase per 13.7% of total albumin and albumin fractions (albumin raise by 12.8% ($p < 0.001$), α-globulins by 11.3%, γ-globulins by 2.2%, β-globulins by 4.5%); the level of BASK raised by 16.62% ($p < 0.01$) and the level of LASK raised by 16.7% ($p < 0.01$). In addition, there was an improvement in metabolism, liver function, feed intake and the absorption of its nutrients.

2. Determination of the total bacterial contamination and contamination of mold fungi of feed phytoadditives 'Phytopank' and 'Phytochol' and sodium selenite showed that it does not exceed the permissible dose rate per dose.

The prospects for further researches is to study the influence of feed phytoadditives and sodium selenite concentration of carotenoids and vitamins in poultry eggs during the intensive productivity of laying hens under the influence of anthropogenic impact.

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EFFECT OF COW GENOTYPE BY SNPS *L127V/GH*, *F279Y*, *A257G/GHR* AND BIRTH DATE ON CALF GROWTH PERFORMANCE OF ABERDEEN-ANGUS

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Summary. Our study was aimed to evaluate effect of genetic and non-genetic factors and their combination on calf growth rate in Aberdeen-Angus cattle. There were 52 cows genotyped by SNPs *L127V*, *F279Y*, *A257G* of growth hormone and growth hormone receptor genes. Calves (n = 430) were assessed with average daily gain during milk period (ADG, g/day) and were weighted at birth, at weaning (210 days), 8, 12, 15, and 18 months. SNP genotyping of cows included *L127V*, *F279Y*, *A257G* of GH and GHR genes. The data were analyzed using a multiple regression model and ANOVA. There were strong differences between bulls and heifers for all parameters being at least 1.5–2 kg at birth or 5–8%, 7–10 kg at weaning or 4–6%, 6–11 kg at 8 month or 3–6%, 12–18 (45) kg at 1 year or 4–6% (13%), 15–50 kg at 15 month or 5–16% and 30–80 kg at 18 month or 8–18. The highest birth weight at autumn season for both bulls and heifers and ADG was higher for summer-born calves due to better forage regimen of dams. The calving effect was observed for birth weight slightly decrease from first to later calving presenting difference at least 5–6% for 2nd and 3rd calving then 8–12% — for 4th to last calving for both sexes. The effect of parity stay to be evident for bulls of all ages, the difference between calves slightly decreased. Female calves were significantly affected by calving interval, where longer calving intervals produced heavier newborns and weaners, at least 3 and 30 g/day of CI, accordingly. When evaluating the reproductive and productive performance of breeding cows we should consider effect of season, date of previous calving and actual weaning weight of previous calves either.

Keywords: Aberdeen-Angus breed, SNPs, *GH L127V*, *GHR F279Y*, *GHR A257G*, season effect, parity number, calving interval

Introduction. Issues of calf growth, development and safety during the milking period are relevant problems in zootechnical practice. Within one breed, they mostly depend on sex of calf, feeding regimen (weaning or nursing), season of birth, mentor cow diet and naturally genetic background (Gangnat et al., 2017; Rege and Moyo, 1993). To these factors, some authors add mother age (Ahunu and Makarechian, 1987) or parity number (Bayou et al., 2015) and calving interval (MacGregor and Casey, 2000).

In dairy cattle, long stay of calves with their mothers influences their growth positively, but it may depress the milk production of mothers (Kišac et al., 2011). Age at weaning of beef cattle is traditionally 210 days but may vary from 90 to 270 days (Goyache et al., 2003).

In beef cattle different genotypes by one SNP can be associated with benefits for opposite breeding purposes — livestock breeding or production (Fedota et al., 2017). Therefore, effect of well-studied SNPs associated with production traits on reproductive traits or calf parameters may become vagarious. SNPs *L127V* of growth hormone (GH) gene, *F279Y* and *A257G* of growth hormone receptor (GHR) gene affect both milk and growth parameters due to effect of growth hormone that regulates metabolic reactions via interaction with its receptor. In beef calves, milk parameters can be indirectly assessed via growth rate and average daily gain. Controversial effect on meat productive/reproductive traits can be illustrated with benefits of *CC* genotype by SNP *L127V* to the calving interval, birth weight but pitfalls to increased risk of dystocia/stillbirth and decreased quality of milk, as well as in dairy or beef-dairy populations this genotype is rare (Fedota et al., 2016). The frequency of *CC*-genotype in most Aberdeen-Angus herds does not exceed 30–35%, which supports traditional using this breed in crossbreeding to reduce likelihood of dystocia.

Therefore, **the objectives of this study** were to estimate calf growth in Aberdeen-Angus cattle breed and evaluate effect of genetic (SNPs *L127V*, *F279Y*, *A257G*) and non-genetic factors and their combination on calf growth rate in livestock breeding management condition.

Material and methods. The investigation was undertaken on Aberdeen-Angus cows (n = 52) and their calves (n = 430, where 222 bulls and 208 heifers) bred at PE 'Agrofirma Svitanok' (Kharkiv Region, Ukraine). Cows were born in 2003–2005; therefore, up to analysis date there were data on the average of 8 to 10 calves for each dam (at least 5 calves per dams born after 2005). During the summer period, cows with calves were on free grazing, in winter — on dry food — hay, silage. The calves were conceived as a result of natural mating, more than 50% of calves were born in the spring months from March to May, all of calves were nursed *ad libitum*, at least for 210 days. Calf growth rate was monitored monthly; some of them were culled before 210 days or at weaning. Calves were assessed with average daily gain during milk period (ADG, g/day) and were weighted at birth, at weaning (210 days), 8, 12, 15, and 18 months.

SNP genotyping of cows included *L127V*, *F279Y*, *A257G* of GH and GHR genes. DNA was extracted from blood samples using DNA extraction kits 'Diatom DNA Prep 100' ('Isogene', RF). For the SNP genotyping, PCR-RFLP methods were set up, using amplification regimen characterized by Lee et al. (2013) and Viitala et al. (2006) and restriction endonucleases *AluI* and *VspI* ('Fermentas', Lithuania). The PCR mix (25 μl) contained 1.5 mM $MgCl_2$. The digested fragments were electrophoresed on 2% agarose gel, stained with ethidium bromide and visualized under UV light.

Descriptive statistics used include values are expressed as means ± standard deviation of the mean ($\bar{x} \pm s_x$) and coefficient of variation (CV, %). The data were analysed using a Multiple regression model and ANOVA. Traits were analyzed by least square procedure. The dependent variables were: birth weight (BW), weaning weight (WW), weight at 8 (W8), 12 (W12), 15 (W15), 18 months (W18) and average daily gain (ADG). These variables were stratified by sex of calf and analyzed against genetic and non-genetic parameters as main effects. Genetic predictors were SNPs *L127V*, *F279Y*, *A257G* of GH and GHR genes, non-genetic predictors were season of birth, parity and calving interval as main effects. All values were tested at the significance level of 0.1, 0.05, 0.01, and 0.001.

Results and discussions. Allele and genotypes frequency for cows studied were published earlier (Fedota et al., 2017). Generally, population and each line (subgroup) was in Hardy-Weinberg equilibrium by all SNPs studied, except GHR *F279Y* for total population which showed significant disequilibrium ($\chi^2_{act.} = 14.80$, $p < 0.001$) suggesting homozygotization, whereas both homozygous classes are generally superior by reproductive traits and weight dynamics than heterozygous one (Lysenko et al., 2016).

Our preliminary analysis found strong differences between bulls and heifers for all parameters; the sex of calf was found to interfere with main effects camouflaging their contribution. Many authors point out the importance to take into account the environmental conditions when the purpose is to make genetic improvement in both situations using a single genotype. The implication is to take into consideration crucial environmental factors that affect weaning weight (WW) and thus may adjust the WW-driven selection, where most productive cows in the herd may not be identified or retained for breeding purposes. The greatest contribution within environmental factors was made by season in temperate climate (MacGregor and Casey, 2000), when free grazing in spring/summer period positively affect milk parameters and subsequently calf growth. The data on calves stratified by sex and season are presented in Table 1, on bulls stratified by SNPs/genotype in Table 2, and on heifers stratified by SNPs/genotype in Table 3. As can be readily observed, effect of season in heifers was higher than in bulls, reaching significance $p < 0.05$ for most of parameters. Effect of season is even more essential than each of SNPs.

For all analyses we separated bulls and heifers, the stratified by season of birth average weight at each time point was significantly greater in bulls, than in heifers (see Table 1). Differences were at least 1.5–2 kg at birth or 5–8%, 7–10 kg at weaning or 4–6%, 6–11 kg at 8 month or 3–6%, 12–18 (45) kg at 1 year or 4–6% (13%), 15–50 kg at 15 month or 5–16% and 30–80 kg at 18 month or 8–18%. Higher variation after weaning and even more at 1 year and later is stipulated by culling. Percentage of culled calves was higher amongst calves born during summer/autumn season ≈ 95/95% of bulls and 95–75/100% of heifers. The herd studied is nucleus of livestock breeding, therefore total percentage of non-culled heifers is traditionally 2–4 times higher than bulls. Difference spring-born between calves for all parameters was the least between seasons, as far as free grazing regimen of dams is to be associated with improved quality of milk and increased ADG during summer period.

Calf growth dynamic was analyzed with multiple regression model, the effects of genetic and non-genetic factors are summarized below (Table 4).

Genetic factors. *GH gene, SNP L127V.* In beef cattle *C*-allele is associated with higher body weight (Lee et al., 2013), and particularly higher birth weight (Hadi et al., 2015). Our results coincide with given in literature for both bulls and heifers, showing trend *CC* < *CG* < *GG*. *C*-allele is associated with increased birth weight in heifers approximately from 200 to 800 g ($p < 0.01$). ADG and resulting body weight at weaning depend on quality and quantity of milk. The one opinion about preferred L127V genotype for milk parameters does not exist, whilst even one breed in different herds demonstrate opposite results for given genotype (Fedota et al., 2017). Mykhailova, Belaya and Volchok (2011) describe better milk performance for cows with *CC*-genotype for early lactations, that decreases with next lactations. In our study calves had not significantly benefited from genotype, but trend observed was slightly seen in bulls, and not observed in heifers. Kišac et al. (2011) also describe early lactation as better then mid-lactations and absolutely netter than late lactations. At age 12 month and elder bulls stay to follow the trend *CC* < *CG* < *GG*, but heifers did not show any significant differences between genotypes.

GHR gene, SNP F279Y. Through the first year of life *TT*-bulls followed *TT* < *TA* < *AA*. At age of 15 month the pattern changed to opposite, but we would rather consider this as artifact resulting from stringent demands of culling. Therefore remained 11 bulls appear to have better parameters attributable to other gene combination. This speculation is supported by heifers following *TT* < *TA* < *AA* pattern during 18 month and being exposed to milder conditions of culling.

Table 1 — Calf growth parameters of Aberdeen-Angus herd in Kharkiv Region by sex and season of birth

Parameter	Descriptive statistics	Season				Season			
		Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
		Bulls				Heifers			
Birth weight, kg	n	54	130	19	19	55	107	24	22
	$\bar{x} \pm s_x$	30.46 ± 0.36	29.23 ± 0.22	30.58 ± 0.44	31.42 ± 0.40	28.38 ± 0.42	27.47 ± 0.25	29.67 ± 0.80	29.36 ± 0.32
	CV, %	8.71	8.81	6.29	5.54	11.09	9.43	13.29	5.10
	ANOVA	MR = 0.073; MS = 0.22; F = 0.037; p = 0.990				MR = 0.384; MS = 17.45; F = 3.344; p = 0.025			
Average daily gain, g/day	N	44	98	17	18	48	84	24	21
	$\bar{x} \pm s_x$	761.86 ± 14.26	761.75 ± 8.95	785.23 ± 17.12	771.61 ± 21.71	750.96 ± 13.81	706.32 ± 7.52	759.08 ± 11.57	706.48 ± 12.61
	CV, %	12.42	11.63	8.99	11.94	12.74	9.75	7.47	8.18
	ANOVA	MR = 0.547; MS = 10073.12; F = 2.994; p = 0.054				MR = 0.410 ; MS = 19486.55; F = 3.918; p = 0.013			
Weaning weight, kg (210 days)	n	44	98	17	18	48	84	24	21
	$\bar{x} \pm s_x$	189.50 ± 3.09	188.32 ± 1.80	195.18 ± 3.94	193.06 ± 4.65	187.08 ± 2.85	177.01 ± 1.54	188.33 ± 2.34	177.81 ± 2.35
	CV, %	10.80	9.47	8.32	10.21	10.56	7.96	6.09	6.06
	ANOVA	MR = 0.544; MS = 457.48; F = 2.948; p = 0.056				MR = 0.409; MS = 857.41; F = 3.881; p = 0.013			
Weight at 8 month, kg	n	21	73	10	10	38	71	18	12
	$\bar{x} \pm s_x$	213.52 ± 5.52	207.25 ± 2.85	213.80 ± 5.63	223.80 ± 4.18	209.39 ± 3.66	195.62 ± 1.96	207.78 ± 3.19	198.17 ± 3.28
	CV, %	11.86	11.77	8.33	5.91	10.77	8.44	6.52	5.73
	ANOVA	MR = 0.551; MS = 687.42; F = 3.050; p = 0.051				MR = 0.342; MS = 670.28; F = 2.563; p = 0.063			
Weight at 12 month, kg	n	14	55	5	3	27	56	14	9
	$\bar{x} \pm s_x$	303.21 ± 13.81	261.11 ± 5.04	288.20 ± 7.47	296.33 ± 3.67	285.41 ± 7.83	249.73 ± 3.38	276.14 ± 4.95	250.11 ± 8.98
	CV, %	17.04	14.33	5.79	2.14	14.25	10.14	6.71332	10.77
	ANOVA	MR = 0.035; MS = 11.91; F = 0.008; p = 0.999				MR = 0.396; MS = 2924.53; F = 3.598; p = 0.019			
Weight at 15 month, kg	n	13	26	2	2	23	41	11	1
	$\bar{x} \pm s_x$	344.31 ± 16.61	330.08 ± 6.00	359.00 ± 31.00	350.00 ± 20.00	322.04 ± 8.44	288.76 ± 4.26	314.27 ± 6.73	305.00
	CV, %	17.40	9.27	12.21	8.08	12.57	9.44	7.10	—
	ANOVA	MR = 0.030; MS = 1220.04; F = 0.678; p = 0.574				MR = 0.429; MS = 4272.79; F = 4.361; p = 0.007			
Weight at 18 month, kg	n	10	14	1	1	19	37	7	—
	$\bar{x} \pm s_x$	383.30 ± 17.43	398.86 ± 8.82	435.00	417.00	354.74 ± 6.91	333.78 ± 5.09	350.00 ± 3.93	—
	CV, %	14.38	8.28	—	—	8.49	9.27	2.97	—
	ANOVA	MR = 0.287; MS = 1242.94; F = 0.630; p = 0.603				MR = 0.329; MS = 2071.59; F = 2.347; p = 0.082			

Notes: $\bar{x} \pm s_x$ — mean ± standard deviation of the mean; CV, % — coefficient of variation; MR — multiple correlation coefficient; MS — mean square.

Table 2 — Bull growth parameters of Aberdeen-Angus herd in Kharkiv Region by SNPs/genotypes

Parameter	Descriptive statistics	Growth hormone gene, SNP *L127V*			Growth hormone receptor gene, SNP *F279Y*			Growth hormone gene, SNP *L127V*		
		CC	**CG**	**GG**	**TT**	**TA**	**AA**	**AA**	**AG**	**GG**
Birth weight, kg	n	31	104	87	132	30	60	154	64	4
	$\bar{x} \pm s_x$	30.19 ± 0.48	29.80 ± 0.27	29.75 ± 0.25	29.68 ± 0.24	29.93 ± 0.54	30.12 ± 0.26	29.81 ± 0.21	29.78 ± 0.33	31.50 ± 1.66
	CV, %	8.90	9.23	7.91	9.24	9.88	6.56	8.55	8.86	10.53
	ANOVA	MR = 0.400; MS =9.81; F = 2.097; p = 0.147			MR = 0.391; MS = 9.41; F = 2.00; p = 0.156			MR = 0.124; MS = 5.32; F = 6.10; p = 0.421		
Average daily gain, g/day	n	26	81	70	107	24	46	121	52	4
	$\bar{x} \pm s_x$	769.69 ± 12.64	759.77 ± 9.75	769.41 ± 11.65	763.57 ± 8.93	766.25 ± 13.68	767.83 ± 13.37	774.40 ± 8.01	748.44 ± 12.17	697.75 ± 30.27
	CV, %	8.38	11.55	12.67	12.10	8.75	11.81	11.38	11.73	8.68
	ANOVA	MR = 0.248; MS = 3113.73; F = 0.723; p = 0.496			MR = 0.273; MS = 3768.86; F = 0.89; p = 0.426			MR = 0.124; MS = 7823.31; F = 9041.88; p = 0.424		
Weaning weight, kg (210 days)	n	26	81	70	107	24	46	121	52	4
	$\bar{x} \pm s_x$	191.92 ± 2.92	188.26 ± 2.01	190.67 ± 2.43	189.05 ± 1.83	190.50 ± 2.97	191.00 ± 2.91	191.41 ± 1.68	186.88 ± 2.59	177.00 ± 6.44
	CV, %	7.77	9.59	10.67	10.03	7.63	10.32	9.64	10.00	7.28
	ANOVA	MR = 0.237; MS = 129.74; F = 0.653; p = 0.530			MR = 0.274; MS = 173.60; F = 0.89; p = 0.424			MR = 0.109; MS = 263.16; F = 391.92; p = 0.513		
Weight at 8 month, kg	n	15	47	52	63	17	34	83	30	1
	$\bar{x} \pm s_x$	209.13 ± 4.21	209.57 ± 3.28	211.58 ± 3.67	209.75 ± 3.01	209.24 ± 4.30	212.29 ± 4.47	211.43 ± 2.70	207.67 ± 3.86	210.00
	CV, %	7.80	10.75	12.511	11.38	8.48	12.27	11.63	10.19	—
	ANOVA	MR = 0.165; MS = 92.17; F = 0.307; p = 0.740			MR = 0.191; MS = 125.00; F = 0.42; p = 0.662			MR = 0.071; MS = 156.44; F = 563.97; p = 0.758		
Weight at 12 month, kg	n	10	30	37	41	11	25	59	18	—
	$\bar{x} \pm s_x$	275.10 ± 11.14	277.73 ± 7.94	266.30 ± 7.06	271.15 ± 5.68	276.18 ± 11.90	271.24 ± 10.48	275.98 ± 5.31	258.50 ± 10.54	—
	CV, %	12.80	15.66	16.13	13.42	14.29	19.32	14.78	17.30	—
	ANOVA	MR = 0.218; MS = 702.04; F = 0.548; p = 0.586			MR = 0.349; MS = 1803.43; F = 1.53; p = 0.239			—		
Weight at 15 month, kg	n	5	16	22	20	8	15	35	8	—
	$\bar{x} \pm s_x$	362.00 ± 16.60	340.62 ± 8.47	328.00 ± 9.82	353.70 ± 6.54	308.13 ± 12.91	329.13 ± 12.65	339.89 ± 6.63	322.50 ± 17.98	—
	CV, %	10.25	9.94	14.05	8.27	11.85	14.89	11.54	15.77	—
	ANOVA	MR = 0.383; MS = 3031.32; F = 1.886; p = 0.175			MR = 0.846; MS = 4894.94; F = 3.40; p = 0.051			—		
Weight at 18 month, kg	n	2	9	15	11	4	11	21	5	—
	$\bar{x} \pm s_x$	441.50 ± 6.50	392.11 ± 12.83	390.47 ± 11.65	414.55 ± 9.85	378.50 ± 19.01	381.36 ± 14.40	398.71 ± 8.86	379.20 ± 23.09	—
	CV, %	2.08	9.82	11.56	7.88	10.045	12.52	10.18	13.62	—
	ANOVA	MR = 0.322; MS = 2340.88; F = 1.273; p = 0.300			MR = 0.404; MS = 3681.94; F = 2.14; p = 0.141			—		

Notes: $\bar{x} \pm s_x$ — mean ± standard deviation of the mean; CV, % — coefficient of variation; MR — multiple correlation coefficient; MS — mean square.

Table 3 — Heifer growth parameters of Aberdeen-Angus herd in Kharkiv region by SNPs/genotypes

Parameter	Descriptive statistics	Growth hormone gene, SNP *L127V*			Growth hormone receptor gene, SNP *F279Y*			Growth hormone gene, SNP *L127V*		
		CC	CG	GG	TT	TA	AA	AA	AG	GG
Birth weight, kg	n	26	104	78	132	26	50	152	52	4
	$\bar{x} \pm s_x$	28.96 ± 0.56	28.23 ± 0.31	27.81 ± 0.31	28.07 ± 0.26	28.12 ± 0.71	28.44 ± 0.33	28.08 ± 0.25	28.35 ± 0.36	29.00 ± 1.00
	CV, %	9.84	11.03	9.72	10.75	12.82	8.23	10.93	9.27	6.90
	ANOVA	MR = 0.257; MS = 11.70; F = 2.083; p = 0.133			MR = 0.110; MS = 2.15; F = 0.361; p = 0.698			MR = 0.217; MS = 9.35; F = 1.738; p = 0.183		
Average daily gain, g/day	n	22	89	66	108	24	45	130	44	3
	$\bar{x} \pm s_x$	702.45 ± 15.05	730.02 ± 8.88	727.35 ± 8.72	717.56 ± 7.17	727.29 ± 17.33	744.00 ± 11.86	724.16 ± 6.97	727.05 ± 10.04	766.67 ± 91.39
	CV, %	10.05	11.47	9.74	10.39	11.67	10.70	10.98	9.16	20.65
	ANOVA	MR = 0.155; MS = 4163.23; F = 0.725; p = 0.488			MR = 0.324; MS = 18235.96; F = 3.465; p = 0.038			MR = 0.255; MS = 14160.05; F = 2.428; p = 0.095		
Weaning weight, kg (210 days)	n	22	89	66	108	24	45	130	44	3
	$\bar{x} \pm s_x$	176.86 ± 3.12	182.49 ± 1.85	181.36 ± 1.74	179.65 ± 1.45	181.08 ± 3.60	185.67 ± 2.49	180.94 ± 1.43	181.95 ± 2.06	191.67 ± 19.22
	CV, %	8.27	9.55	7.78	8.36	9.73	9.01	9.00	7.49	17.37
	ANOVA	MR = 0.162; MS = 202.63; F = 0.798; p = 0.455			MR = 0.332; MS = 848.73; F = 3.658; p = 0.032			MR = 0.300; MS = 886.62; F = 3.469; p = 0.037		
Weight at 8 month, kg	n	16	70	53	91	19	29	101	35	3
	$\bar{x} \pm s_x$	195.37 ± 4.60	201.76 ± 2.30	202.17 ± 2.47	200.63 ± 1.85	198.32 ± 5.15	204.79 ± 3.60	200.84 ± 1.92	201.63 ± 2.69	207.33 ± 18.76
	CV, %	9.42	9.55	8.88	8.78	11.32	9.48	9.61	7.89	15.66
	ANOVA	MR = 0.166; MS = 237.18; F = 0.838; p = 0.438			MR = 0.341; MS = 1001.49; F = 3.894; p = 0.026			MR = 0.290; MS = 940.82; F = 3.214; p = 0.046		
Weight at 12 month, kg	n	10	58	38	68	14	24	76	27	3
	$\bar{x} \pm s_x$	260.40 ± 13.29	262.10 ± 4.59	263.21 ± 4.64	259.24 ± 3.84	263.93 ± 9.26	270.21 ± 7.46	262.86 ± 3.67	262.07 ± 6.89	251.67 ± 30.60
	CV, %	16.14	13.33	10.87	12.23	13.13	13.53	12.16	13.65	21.06
	ANOVA	MR = 0.083; MS = 191.04; F = 0.203; p = 0.817			MR = 0.304; MS = 2577.33; F = 2.996; p = 0.058			MR = 0.194; MS = 1291.92; F = 1.365; p = 0.262		
Weight at 15 month, kg	n	8	45	23	51	9	16	53	22	1
	$\bar{x} \pm s_x$	293.75 ± 7.60	305.84 ± 5.77	299.78 ± 6.01	300.67 ± 4.40	303.00 ± 10.67	309.19 ± 11.25	300.04 ± 4.33	308.23 ± 8.77	325.00
	CV, %	7.32	12.66	9.62	10.45	10.56	14.56	10.51	13.34	—
	ANOVA	MR = 0.099; MS = 342.08; F = 0.293; p = 0.747			MR = 0.215; MS = 1614.94; F = 1.435; p = 0.246			MR = 0.141; MS = 893.58; F = 0.723; p = 0.489		
Weight at 18 month, kg	n	7	36	21	45	7	12	45	19	—
	$\bar{x} \pm s_x$	339.00 ± 8.83	340.92 ± 5.85	344.00 ± 5.12	336.60 ± 4.23	342.57 ± 10.13	360.42 ± 9.70	339.16 ± 4.43	347.79 ± 7.20	—
	CV, %	6.89	10.30	6.81	8.44	7.83	9.32	8.77	9.02	—
	ANOVA	MR = 0.072; MS = 148.32; F = 0.153; p = 0.858			MR = 0.310; MS = 2752.48; F = 3.129; p = 0.051			—		

Notes: $\bar{x} \pm s_x$ — mean ± standard deviation of the mean; CV, % — coefficient of variation; MR — multiple correlation coefficient; MS — mean square.

Table 4 — The regression coefficients (B ± sB) of genetic and non-genetic factors for the calf growth traits

Parameter	Genetic factors			Non-genetic factors		
	GH gene, SNP *L127V*	GHR gene, SNP *F279Y*	GHR gene, SNP *L127V*	Season of birth	Parity number	Calving interval
Birth weight, kg						
Bull	0.18 ± 0.25	–0.22 ± 0.20	–0.18 ± 0.34	–0.40 ± 0.11‡	–0.27 ± 0.06‡	–0.001 ± 0.001
Heifer	0.53 ± 0.31*	–0.18 ± 0.24	–0.32 ± 0.41	–0.36 ± 0.16**	–0.33 ± 0.08‡	0.003 ± 0.002*
Average daily gain, g/day						
Bull	–2.18 ± 9.62	–2.16 ± 7.72	29.40 ± 12.67**	–0.55 ± 5.20	–2.73 ± 2.95	–0.008 ± 0.057
Heifer	–8.11 ± 8.85	–14.53 ± 6.73**	–7.40 ± 12.02	–12.56 ± 4.47†	1.62 ± 2.48	0.130 ± 0.051**
Weaning weight, kg (210 days)						
Bull	–0.09 ± 2.02	–0.01 ± 1.62	5.27 ± 2.67**	–0.61 ± 1.09	–0.82 ± 0.62	0.002 ± 0.012
Heifer	–1.28 ± 1.82	–3.21 ± 1.38**	–2.09 ± 2.47	–2.89 ± 0.091†	0.36 ± 0.51	0.028 ± 0.011**
Weight at 8 month, kg						
Bull	–1.44 ± 3.19	–1.19 ± 2.50	3.35 ± 4.73	–3.04 ± 1.83*	–0.65 ± 0.96	–0.382 ± 0.021
Heifer	–2.46 ± 2.43	–2.21 ± 1.94	–1.51 ± 3.17	–4.22 ± 1.17‡	–0.04 ± 0.64	0.025 ± 0.014*
Weight at 12 month, kg						
Bull	6.37 ± 6.88	–0.23 ± 5.35	17.48 ± 11.23	–14.49 ± 3.75‡	–6.99 ± 1.82‡	–0.034 ± 0.057
Heifer	–1.29 ± 5.24	–6.90 ± 3.83*	2.48 ± 6.22	–10.67 ± 2.31‡	–2.35 ± 1.28†	0.083 ± 0.031†
Weight at 15 month, kg						
Bull	15.63 ± 9.01*	13.18 ± 6.86*	17.39 ± 16.24	–4.96 ± 4.64	–6.15 ± 2.64**	–0.035 ± 0.62
Heifer	–0.14 ± 6.54	–4.10 ± 4.83	–8.97 ± 8.00	–10.96 ± 2.71‡	–2.61 ± 1.66	0.060 ± 0.041
Weight at 18 month, kg						
Bull	15.29 ± 13.02	16.59 ± 8.61*	19.51 ± 21.22	5.00 ± 5.86	–2.77 ± 3.77	–0.081 ± 0.112
Heifer	–2.68 ± 6.10	–11.37 ± 4.60**	–8.63 ± 8.27	–6.82 ± 2.72**	–2.25 ± 1.63	0.029 ± 0.678

Notes: * — significant at 0.1 level, ** — significant at 0.05 level,† — significant at 0.01 level, ‡ — significant at 0.001 level.

Better ADG for *AA*-genotype indicating on milk quality corresponds to results obtained by Rahmatalla et al. (2011), that *A*-allele is associated with higher fat and protein content.

GHR gene, SNP A257G. There were observed similar growth trends as for SNP *F279Y*, but *AA*-bulls started to demonstrate better growth traits after weaning, where heifers stayed to follow the trend $AA < AG < GG$. Genotype desired for milk traits has shown negative association with growth dynamic. *A*-allele associated with higher fat and protein content (Oleński, Suchocki and Kamiński, 2010) was related to significantly higher ADG in bulls, resulting in additional 6 kg at weaning.

Non-genetic component. *Season of birth.* Amongst all factors analyzed season had the highest impact on birth weight and ADG. We coded spring with 4 points, summer as 3 points, autumn (rainy season) as 2 points and winter as 1 point. As the summer comprises the best forage conditions for pregnant dams we observe the highest birth weight at autumn season for both bulls and heifers (+ 1 kg or 4% compared to other seasons). ADG was higher for summer-born calves ($p < 0.1$ and $p < 0.05$ for bulls and heifers), due to better forage regimen of dams. The effect of season persists for later ages and is more apparent in dams than bulls.

In literature, the effect of season is controversial, and depends on breed and region. The African Sheko cattle (Southwestern Ethiopia) demonstrate significant seasonal variations ($p < 0.01$) being conditioned by feed and fodder availability as well as disease incidence. Calves born in dry season had higher birth weights compared to those born in both main rainy and short rainy seasons, whereas combinations of short rainy and main rainy season very often result in excess forage leading to high milk production of cows for calf consumption. Season of calving had also a strong significant ($p < 0.01$) Calves born during short rainy season had higher preweaning daily weight gain than the other two seasons, which could be due to favorable feeding conditions of dams during this and the latter seasons, though had higher milk yield (Bayou et al., 2015). Rahman, Bhuiyan and Bhuiyan (2015) reported that winter-born calves had higher birth weight due to abundant availability of green fodder during this season (Bangladesh) which increases nutritional status of cows.

Parity number. The effect of mother age or parity number was observed on birth weight, whereas young mother were more likely to produce heavier calve of each sex. The birth weight slightly decreases from first to later calving presenting difference at least 5–6% for 2nd and

3rd calving then 8–12% — for 4th to last calving for both sexes. The effect of parity stay to be evident for bulls of all ages, the difference between calves decreased to 2–5%, but in 12 and 18 month it results to difference between 1st parity calves and other to 20–40 and 30–70 kg of body weight. These differences were not observed in dams of age 7 month and older.

Our results are controversial to observed for African crossbred cattle (Horro/Zebu × Holstein Friesian and Jersey) (Abera, Abegaz and Mekasha, 2012). These calves born from first parity were significantly lighter at birth than those born from adult cows. In Czech Charolais, the higher parity, the higher live birth weight was determined, with maximum values in cows from the fourth calving, whereas the parity effect on the average daily weight gain was statistically insignificant (Tousova et al., 2014). On other hand, Echternkamp (1993) speculates about placenta effect on calf birth weight and further growth dynamics. Our data are supported by his findings that first parity heifers would rather have higher placental weights and circulating concentrations of estrone sulfate correlating positively with birth weight of their calves.

Calving interval. Unlike bulls, female calves were significantly affected by calving interval, where longer calving intervals produced heavier newborns and weaners, at least 3 and 30 g/day of CI, accordingly.

MacGregor and Casey (2000) support our data, their findings for African Bovelder cattle suggest that one-day increase in calving interval was associated with a decrease of 0.29 ± 0.01 kg in weaning weight and a decrease of 0.54 ± 0.01 kg in the heifer pre-breeding weight. Even weaning weight of the previous calf influences calving interval, in that higher weaning weights were associated with longer calving intervals (Doren, Long and Cartwright, 1986). Therefore, the calf weight at first calving can be maximum or close to maximum due to absence of previous weaning period and pregnancy effects.

Conclusions. There were strong differences between bulls and heifers for all parameters and sex of calf was found to interfere with main effects camouflaging their contribution. The greatest contribution within environmental factors was made by season in temperate climate, being even more essential than effect of each SNP. The differences due to non-genetic factors were conditioned by forage regimen during seasons, parity effect that was consequence of lactation quality and calving interval, showing positive correlation with body weight and ADG. Last two parameters were related to reproductive performance associated with previous calving.

Therefore, we can conclude that planning heifer pregnancy (by season and calving interval) is likely to have greater contribution than selection by genotype for productive and reproductive traits. When evaluating the reproductive and productive performance of breeding cows we should take into account effect of season, date of previous calving and actual weaning weight of previous calves either.

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Part 3. Biosafety

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DEVELOPMENT DIRECTIONS OF LABORATORY INFRASTRUCTURE SUPPLY IN AGRICULTURAL PRODUCTION

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Summary. The results of the Ukrainian Laboratory of Quality and Safety of Agricultural Products testing activity analysis was carried out. The normative documents on the accreditation of laboratories and international recommendations concerning the rational use of standardized biological material in the testing activity are analyzed. The optimizing expediency of biological material collections functioning in scientific institutions is substantiated. The conducted analysis testifies the creation expediency of the national bioresource center, which unites (informationally) all functioning collections of biological material in order to increase the efficiency of their innovative potential. Not a high level of competitiveness of domestic agricultural products on the world market indicates the need not to ensure proper control of the quality and safety of not only finished products, but also all technological processes in its production. Ukrainian Laboratory of Quality and Safety of Agricultural Products of the National University of Life and Environmental Sciences is accredited in accordance with the requirements of ISO/IEC 17025 for defining more than 370 indicators of quality and safety. Selection of samples (water, soil, grain, seeds, forage, fertilizers, pesticides, biological preparations, eggs, oils and fats, crop production, livestock, food products and food additives, etc.) for testing, conducting analytical studies and obtaining results are carried out accordingly, the documents regulating the order of work. They analyzed the results obtained in 2014–2017, as well as data on accreditation requirements and the technical competence of laboratories. An important direction in improving the efficiency of laboratory support for the production of agrarian sector of the economy is the organization of the proper functioning of biological resource centers. The scope of accreditation of the Ukrainian Laboratory of Quality and Safety of Agricultural Products involves the use of a range of standardized biological material. That is, the actual task is to create a unit to maintain a collection of biological materials used in experimental and scientific activities. The results of the analysis testify to the expediency of creating a nation-wide bioresource center that will unite (informationally) all functioning collections of biological material with the aim of increasing the efficiency of using their innovative potential.

Keywords: agricultural products, biological material, collections, normative documents

Introduction. Agricultural production is one of the most important sectors of the economy in our country, which ensures food security. Food security of citizens is one of the main governmental components of economic security (Ushkalov et al., 2016). Ukraine has significant resource potential for the agrarian sector developing and improving the efficiency of agricultural production. However, quite often there is an unfair business activity in the production and sale of counterfeit and/or counterfeit products in the consumer market. This applies both to food and raw materials for their manufacture, and to the protection of plants, fertilizers, seeds, etc. (Ushkalov, Samkova and Danchuk, 2017). That is, the low level of competitiveness of domestic products of the agrarian sector in the world market indicates the need to ensure proper the quality and safety control not only for finished products, but also for all technological processes in its production. An effective mechanism for agro-industrial products controlling is monitoring of quality and safety indicators not only for finished products but also for all components involved in its production (soil and water as the main resources for agricultural production, plant protection products, mineral and organic fertilizers, seeds of agricultural cultures, etc.) (Borg, 2014).

That is, at the time of demand, the role of proper/efficient laboratory support of production in the agrarian sector increases. Given the importance of export-oriented goods production, the role of accredited laboratories also increases. The presence of accreditation testifies to the official recognition of the laboratory ability to meet the requirements of customers in the field of testing, measurement or research. Accreditation takes into account: technical equipment and competence of the laboratory, that is, the minimum required level of equipment for testing and research in a certain field of activity, competence of staff (requirements for the composition, number and qualification of laboratory staff); the functioning of the quality system (rules and

standards for the implementation of the basic processes of the laboratory), through which it is possible to guarantee stable work and obtaining reliable results of research, testing or inspections (EP and CEU, 2002; VRU, 2001).

The purpose of the work is to determine the directions of infrastructure development for the laboratory support of the export-oriented agro-industrial goods production.

Materials and methods. The analysis of the results obtained in 2014–2017 in the Ukrainian Laboratory of Quality and Safety of Agricultural Products (ULQSAP) of the National University of Life and Environmental Sciences (accredited in accordance with the requirements of ISO/IEC 17025 for determination of more than 370 quality and safety indicators). Selection of samples (water, soil, grain, seeds, forage, fertilizers, pesticides, biological preparations, eggs, oils and fats, crop production, livestock, food products and food additives, etc.) for testing, conducting analytical studies and obtaining results are carried out accordingly to the documents regulating the order of work. Data analysis was carried out on the requirements for accreditation and technical competence of laboratories, as well as the use of standardized materials used in the conduct of research.

Results and discussions. The results of the analysis indicate that accreditation is required for those laboratories which performance should be recognized by other market participants or the professional community (NAAU, 2016). These laboratories include test laboratories — institutions that work with different types of products, materials and environments. Their purpose is to conduct samples testing (products, materials or environmental elements) for compliance with the parameters set in the regulatory documents. Analytical laboratories are solving research tasks. Accreditation of analytical laboratories allows ensuring the reliability of the elemental composition determination of the samples under study. This is necessary to recognize the results of research by other participants in economic activity. The scope of the laboratory accreditation may vary, depending on the purpose of its creation.

It must be taken into account that not every laboratory needs accreditation to perform the tasks. In certain cases, when there is no need to recognize the results of laboratories from other participants in the economic activity, the accreditation of the laboratory may not be carried out. In particular, training laboratories (at educational institutions) or laboratories that solve highly specialized tasks (scientific laboratories).

The features of the ULQSAP of agrarian and industrial complex activities are simultaneously of research and testing laboratories of the university accredited in accordance with the requirements of ISO/IEC 17025 and confirming their competence by participating in the laboratory comparisons of results organized by domestic and international coordinators. That is, a certain range of researches carried out in the laboratory does not require compulsory accreditation, namely — scientific research projects (in the ULQSAP of the agrarian and industrial complex, in the average year, 3–5 scientific research projects by state order, 6–15 scientific research projects under economic contracts and 2–3 initiative scientific themes).

At the same time, it should be noted that both the testing activity and the execution of scientific research require the availability of standards. With regard to standards for chromatographic (gas, liquid) and spectral (emission, absorption and mass spectrometric) studies, the necessary analytical standards are developed and provided by companies such as Sigma-Aldrich, Romer Labs, etc. In particular, the analytical standards of mycotoxins, pesticides, heavy metals, alcohols, etc. are used in the ULQSAP. The market for biological standards is also rich in offers (National Institute of Biological Standards and Control (NIBSC), LGC Ltd, OIE reference laboratory, and others). For example, in Ukraine — in the State Research and Control Institute of Biotechnology and Strains of Microorganisms — it is developed, manufactured and delivered to interested microorganism test culture organizations (https://drive.google.com/file/d/0B-9dlmwAZcW7WkhGSm1JTTVIMkE/view).
Standard test strains of microorganisms are used in accredited laboratories to confirm the ability of nutrient media and differential diagnostic tests to ensure the growth of bacteria and/or fungi in the research, manufacture of biologics and control of their quality, etc.

The availability of standard biological materials is a prerequisite for the survival of cellular biotechnology, genetic engineering, microbiology — biotechnology in the broadest sense of the word. In accordance with modern scientific concepts, the term 'biological material' means any material containing genetic information and able to self-reproduction or reproduction in the biological system (Holovko et al., 2007).

Nowadays the numbers of international organizations are trying to promote economic growth and disseminate the positive experience of socio-economic policy. For example, the Organization for Economic Co-operation and Development (OECD) is an international organization that brings together 35 economically developed countries of the world (USA, Germany, France, Australia, Canada, Switzerland, Norway, South Korea, Japan, etc.). In order to meet the current requirements for the further development of life sciences and biotechnologies, in particular to increase the efficiency of analytical research, the OECD in 2001 proposed a new concept for storage and supply of high quality biological materials and information about them, namely the creation of biological resource centers (BRCs) (OECD, 2001). BRCs should provide the scientific infrastructure with the relevant standard bioresources in order to better realize the benefits of biotechnology. As a result of

consultations in 2007, recommendations for the quality management system of BRC collections were developed. The OECD Biosafety Good Practice Handbook (2007) for BRCs describes methods and protocols for safe handling and handling of biological materials. BRCs should ensure the proper storage, maintenance and exchange of biological resources (OECD, 2004, 2007). Microbial resource centers are institutions that can protect, maintain and distribute authenticated microbial strains, their genomic DNA and related materials. In addition to taxonomy, the use of deposited strains allows for scientific research at a higher methodological level, which leads to a significant improvement in the results (Overmann, 2015).

Collections of microorganisms in Ukraine are functioned and used in scientific researches, the results of which are used in applied biotechnology, pharmacology, veterinary medicine, agronomy, health care, ecology, etc. Thus, in the National Center for Microorganism Strains, the State Research and Control Institute of Biotechnology and Strains of Microorganisms maintain active strains of microorganisms and cell cultures to meet the needs of veterinary medicine (used for the production and control of veterinary immunobiological drugs in Ukraine) (Holovko et al., 2007).

However, the needs of the modern market of 'biological standards' are not limited by test strains of microorganisms. For example, in the testing activity of the ULQSAP, the use/planning of the use of laboratory cultures of Algae (*Desmodesmus subspicatus*, *Phaeodactylum tricornutum*, *Selenastrum gracile*, *Chlorella vulgaris*, *Spirulina (Arthrospira) platensis*), Infusoria (*Tetrahymena pyriformis, Colpoda steinii*), Daphnia (*Daphnia magna*, *Ceriodaphnia dubia*), fish (*Poecilia reticulate*, *Carassius*), earthworms (*Eisenia fetida*), entomophages, particularly *Trichogramma*, *Gabrobracon*, *Dibrachus*, *Ascogaster*, *Phytoseiidae*, cereal moth (*Sitotroga cerealella*), mill moth (*Ephestia kuehniella)*, bees (*Apis mellifera)*, and radish (*Raphanus sativus*). In addition, according to the results of testing, it is possible to create a collection of cultures-phytopathogens, micromycetes, soil microorganisms of certain ecological trophic groups (ammonifiers, amylolytic, pedotropic, oligotrophic, actinomycetes, etc.), DNA of genetically modified plants, etc.

That is, the modern interpretation of the concept of 'biological resources' raises the task of expanding the biological resource base and the development of biotechnology, in accordance with the recommendations of the OECD. In other words, at the time the issue is to ensure the unified management of collections in relation to the storage and circulation of biological materials used in economic activities, in particular testing. Centers of biological resources should become an element of the scientific infrastructure for the implementation of scientific achievements in biotechnology.

It is important to emphasize the fact that bioresources centers should be authorized not only to identify, collect, control quality, classification, registration, storage, reproduction, distribution of storage facilities, but also the merging of existing collections of different biomaterials into a single information network, training and training of relevant expert staff necessary to ensure the effective functioning of such centers. Biological collection is a systematic storage of any type biological material samples set. On this basis, it is possible to distinguish several types of biological collections depending on the types of biological material and storage methods stored in them (Kamenski, 2016):

— cryopreservation, in which the material is stored in a frozen (and lyophilized) form, including nucleic acids;

— materials of zoological museums and herbaria, such collections can be used both for the study of biodiversity, as it is a valuable source of DNA;

— computer databases (collections of biological information) containing information on the primary, secondary and tertiary structure of biological molecules, such as GenBank (nucleotide sequences of genomes of different organisms, http://www.ncbi.nlm.nih.gov/genbank) and Protein Data Bank (tertiary protein structures, http://www.rcsb.org).

The functional purpose of the collection of biomaterials may be:

— research, in cases where samples are stored in research laboratories and are necessary for their daily activities. Types of samples in such collections can be very different - from individual biological molecules to whole organisms;

— commercial, whose funds are for sale (sex cells, blood cells, etc.). For example, the Animal Blood Bank was created at the Faculty of Veterinary Medicine of NULES of Ukraine;

— government collections that are collected and maintained in the interests of the state. First of all, this category includes collections whose purpose is to preserve biodiversity (zoos, botanical gardens, etc.). In addition, these collections are designed to regulate the effective use of biological resources. In the US, the Natural Product Repository has been created in the National Institutes of Health, in Russia, the status of the national bioresource center is provided by the All-Russian Collection of Industrial Microorganisms. In other countries, work is under way to build and maintain the functioning of biological resource centers.

It should be noted that the functioning of biomaterial collections is considered at the OECD level as one of the key issues of national biosafety, since, for example, in the context of a 'crisis', the absence of its own collection of producer cells for industrial biotechnology (veterinary, agricultural) can have a negative impact on livestock farming and crop production respectively.

An example of a crisis situation can be the period of the former USSR collapse, and the crisis in veterinary biotechnology due to the lack of production strains and technologies for the production of animal protection means.

As to the current state of microorganism collections functioning in Ukraine, the following should be noted. In accordance with the Resolution of the Cabinet of Ministers of Ukraine No. 705 from 12.10.1994 'On the state system for the depositing of strains of microorganisms' by the state, three depositaries (collections) have been designated by the state for the purpose of ensuring the legal protection of strains of microorganisms and creating conditions for their timely wide use for biotechnological productions and scientific purposes:

— the Institute of Microbiology and Virology of the National Academy of Sciences responsible for the storage of non-pathogenic microorganisms;

— Kiev Research Institute of Epidemiology and Infectious Diseases of the Ministry of Health of Ukraine has been designated for the storage of microorganisms pathogenic to humans;

— for the storage of microorganisms pathogenic to animals responsible the Kyiv branch of the State Scientific-Research Control Institute for Veterinary Medicines and Feed Additives (now the State Research and Control Institute of Biotechnology and Strains of Microorganisms) has been identified.

Accordingly, research collections of microorganisms /other biomaterials were maintained in accordance with research institutes and faculties of educational institutions of biological, medical, veterinary and agricultural profile. Despite the invariable significant research and innovation potential of collections, its use requires additional impetus. One of the factors hindering their development is the isolation of existing collections, the ignorance of the scientific community about the availability and characteristics of the samples that are stored in. World experience shows that this problem can be solved by creating a single information system that would provide communication between collections and potential users. To this end, it is necessary to keep records in existing collections and to create a single electronic database of storage facilities, standardization of requirements for their storage and ways to ensure their availability.

The main deterrent to the functioning of collections is the lack of targeted funding. At this time in Ukraine, it is almost impossible to find a source of material support for the proper maintenance of collections. However, attempts at financial and regulatory provision of collections for veterinary bio-industry in previous years were. For example, in 2006–2009 the financial support of research collections of strains for veterinary biotechnology in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Institute of Veterinary Medicine of NAAS, Institute for Epizoothology of NAAS, Yuriev Institute of Plant Production of NAAS, Institute of Agricultural Microbiology and Agro-Industrial Production of NAAS, and some higher educational institutions provided by the State Committee for Veterinary Medicine through the State Scientific Control Institute of Biotechnology and Strains of Microorganisms, through the ordering of targeted research work on the study of biological properties in microorganisms that have been kept for a long time, the selection and identification of new topical strains of pathogens of animal diseases, etc.

Another problem that is critical for the development of collections is regulation of the circulation of biological resources, especially cross-border. The conducted analysis shows the expediency of creating a nationwide bioresource center, which will unite (first of all informationally) all functioning collections of biological materials in order to increase the efficiency of their innovative potential. To do this it is needed to provide:

— revision of the bioresources collections status that are functioning at this time;

— creation of a single database containing information on all collections of biomaterials (formation of information and analytical systems);

— development of effective measures to regulate the activities of collections and related fields of science and technology;

— formation of a scientific research state program in biomaterial collections;

— integration of national bioresources centers into the global bioresource information space.

Conclusions and perspectives of further research. An important direction in improving the laboratory support efficiency of production activities in the economy agrarian sector is the organization of the proper functioning of biological resource centers. The scope of ULQSAP accreditation involves the use of a range of standardized biological material. That is, the actual task is to create a unit to support a collection of biological materials used in experimental and scientific activities.

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Part 4. Brief communications

UDC 619:616.98:578.821.2(560)

LUMPY SKIN DISEASE: CURRENT EPIDEMIOLOGICAL SITUATION AND PREVENTION IN TURKEY AND NEIGHBORING COUNTRIES

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Lumpy skin disease (LSD) is an economically important disease affecting cattle health and export of cattle products in endemic countries. It is caused by a *Capripoxvirus* and shows characteristic skin lesions in infected cattle. The disease was first reported in 1929 in Zambia. It then spread throughout Africa, the Middle East, Southeast Europe, the Balkans, Caucasus, Russia and Kazakhstan. The first Turkish outbreak of LSD was reported in 2013 in Kahramanmaras, Turkey. Many cattle in Turkey were affected and the disease has spread to farms located in various parts of the country. After the first outbreak in 2013, rapid diagnostic methods have been developed and used in order to identify infected animals. Prevention, control and eradication programs have been conducted by the Ministry of Agriculture and Forestry of Republic of Turkey including contingency plan, culling and compulsory vaccination. In this presentation, the current situation of LSD epidemiology and prevention in Turkey and neighboring countries will be discussed.

Keywords: lumpy skin diseases, cattle, Turkey, epidemiology, prevention

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FREQUENCY AND GENOTYPES OF AVIAN INFLUENZA VIRUS (AIV) AND NEWCASTLE DISEASE VIRUS (NDV) IN MIGRATORY PASSERIFORM AND NONPASSERIFORM BIRDS, AND DUCKS IN ISTANBUL, TURKEY

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Avian influenza (AI) and Newcastle disease (ND) are economically important viral diseases of birds, endemic in many countries. Both viruses can infect humans, but the H5 and H7 subtypes of AI viruses (AIVs) have caused devastating public and animal health problems worldwide. Both viruses are among the most important infectious disease problems in the poultry industry and new preventive and control strategies are urgently needed. ND virus (NDV) and particularly AIV spread *via* migratory birds, especially waterfowl, among birds within a country, between countries and even between continents. Importantly, the Republic of Turkey is geographically situated in one of the world's most important migratory bird flyways.

In the present study, passeriform and nonpasseriform birds, and ducks were investigated for the presence and genotypes of AIVs and NDVs. For this purpose, field studies were performed with birds migrating on the South East European flyway, in the Marmara region of Turkey which borders the European Union. Traps were placed around the Kucukcekmece lake Avcilar, Istanbul, in the spring season of 2016 and 2017 to catch passeriform and nonpasseriform

birds. The trapped birds were categorized according to species and sex, ringed and oropharyngeal and cloacal swabs were taken. In total, 200 oropharyngeal and 200 cloacal swabs were collected. In addition, in 2017, swabs from 80 green headed ducks (*Anas platyrhynchos*) were sampled by hunters in the Edirne area in Turkey, which is close to the Greek border. Also, swab samples from birds (n = 150) treated at the Wild life clinic at the Veterinary Faculty of Istanbul were analyzed. Laboratory investigations consisted of RNA extraction and real-time RT-PCR analyses for the presence of AIV and NDV genetic signatures. Positive samples were further subjected to sequencing. Phylogenetic analyses were performed to determine genotypes of AIV and NDV in the targeted bird population. AIV-RNA was detected in 12 duck samples and two birds of prey and they all belonged to the H9N2 subtype of avian influenza viruses. NDV-specific RNA was found in two waterfowl samples and the viruses belong to the NDV lineage VII. Results of this study indicate that migratory birds present a threat for Turkey to spread both AIVs and NDVs.

Keywords: avian influenza virus, Newcastle disease virus, migratory birds, genotypes, Turkey

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MOLECULAR TECHNIQUES FOR DIAGNOSIS AND CONTROL OF HUMAN AND ANIMAL *BRUCELLA* ISOLATES FROM GEORGIA

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Brucellosis is an ancient and highly contagious zoonotic disease caused by the genus *Brucella* that remains to be a major public and animal health problem in Georgia and is considered one of the most important health disorders spread worldwide. The leading institutions conducting surveillance on brucellosis in Georgia are the National Center for Disease Control and Public Health under the Ministry of Health. the Laboratory of the Ministry of Agriculture, and the National Food Agency under the Ministry of Agriculture.

The aim of this study was evaluation of implemented molecular diagnostic tests for the assessment of animal and human cases of brucellosis in Georgia.

Isolates from human blood and ruminant milk or blood suspected as *Brucella* by microbiological, biochemical and serological testing were confirmed by real-time PCR (*Brucella* T1, Biofire Technology). Species identity of *Brucella* cultures were confirmed and typed by conventional AMOS PCR, Single Nucleotide Polymorphism (SNP) assays and later 'Bruce-ladder' assay. A multi-locus variable number tandem repeat analysis (MLVA) approach was applied for providing valuable information for epidemiological investigations.

AMOS PCR supported biochemical test results for 72 *Brucella melitensis* and four *Brucella abortus* strains, but not for 39 suspected *B. abortus* human and animal isolates. SNP typing of all 115 isolates supported the AMOS PCR results, but also confirmed suspected *B. abortus* species of the 39 strains not amplified by AMOS PCR. Above-mentioned *Brucella* strains were confirmed later also by 'Bruce-ladder' assay. In the present study, for the first time we have studied the genetic variability of 115 strains obtained in Georgia. Genotypes were revealed by a MLVA-15 approach with good subspecies discriminatory capabilities providing valuable information for epidemiological investigations and obtained data were utilized for construction of the phylogenic tree using Bionumerics Software version 6.1.

Evidences of the molecular-genetic research first have confirmed the existence of *B. melitensis* and *B. abortus* strains circulating in humans and animals in Georgia. Basing on our results of molecular-genetic researches, we can suppose, that *B. abortus* 3, 5, 6 and/or 9 biovars are more frequently spread, than *B. abortus* 1, 2 and 4 in Georgia. Thus, we can say, that application of AMOS PCR method is limited in our country. Species-specific SNP typing and 'Bruce-ladder' assays resolved the difficulties caused by limitations of AMOS PCR to recognize all biovars of *B. abortus*. Obtained results suggest that diversity of *Brucella* strains in Georgia is greater than captured in this study and it needs continuation of a large-scale molecular-biological researches in this direction. Establishment and application of MLVA genotyping will serve invaluably to track the source of infection in case of bioterrorism or outbreak in Georgia or in surrounding areas. Thus, implementation of sustainable set of assays and a 'One Health' approach resulted in a more effective monitoring system for both human and animal brucellosis in Georgia.

Keywords: animal brucellosis, *B. melitensis, B. abortus*.

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A MULTIFACETED BATTLE AGAINST ANTHRAX IN EASTERN PART OF TURKEY IN WHERE ANTHRAX IS ENDEMIC

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Anthrax is a primarily infectious disease of herbivores and humans have its share of zoonotic trait, as well. Anthrax was endemic in Kars which is the center of animal breeding of the country. Human anthrax was predominantly reported at a record level (10.21%) in Kars among other studied region in Turkey between 1995 and 2005. However, animal anthrax ranged from 8.8 to 91% between the year 1993 and 2002. OIE reports show a fluctuating course of animal anthrax between 2005 and 2017 and a three-year peak was observed in Kars Region between 2012 and 2014. The number of epidemics visibly decreased in the last three years both in animal and humans as a reflection of increased vaccination and infection reporting systems, strict protection and compliance regulations and well-managed outbreaks. Eventually, human anthrax is now thin on the ground.

Beside the success of governance on anthrax by constituting the strict politics we think that our efforts on regional anthrax with studying almost all aspects of the disease has contributed to regression of disease at least creating social awareness. In addition, we continue collaborations with official authorities such as the Ministry of Agriculture and the Ministry of Health, Turkey. The studies are mostly focused in ecology, epidemiology, prevention and decontamination of Anthrax and recently bacteriophages are incorporated as the natural enemy of *Bacillus anthracis*. Furthermore, a special effort is being spent to prevent anthrax cases in community by providing the education to some authorities such as physician, veterinarian, butcher and farmers and to trace outbreak by visiting several times of lots of people in countryside who caught or exposed to anthrax. Beside the multinational network project has been completed on anthrax environmentally decontamination (AEDNet), a mutation based analytic method (SNPs) is in the development stage to trace epidemic in Kars region, Turkey. Furthermore, a NATO funded multi-participated strategic work is now planning to remediate the side effects when a bioterrorist attack with anthrax will be occurred and both mutation-introducing characterization and advanced decontamination methods will be integrated to this struggle program.

In the near future, we are planning to apply to the Turkish Government for an Anthrax Reference Centre in Kars and thus will make more contributions to human and animal welfare throughout the Kars and Turkey.

Keywords: anthrax, human, animal, multifaceted battle, Turkey

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CAENORHABDITIS ELEGANS PREDATION ON *BACILLUS ANTHRACIS*: DECONTAMINATION OF SPORE CONTAMINATED SOIL WITH GERMINANTS AND NEMATODES

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Remediation of *Bacillus anthracis*-contaminated soil is challenging and approaches to reduce overall spore levels in environmentally contaminated soil or after intentional release of the infectious disease agent in a safe, low-cost manner are needed.

B. anthracis spores are highly resistant to biocides, but once germinated them become susceptible to traditional biocides or potentially even natural predators such as nematodes in the soil environment. Here, we describe a two-step approach to reducing *B. anthracis* spore load in soil during laboratory trials, whereby germinants and *Caenorhabditis elegans* nematodes are applied concurrently.

While the application of germinants reduced *B. anthracis* spore load by up to four logs depending on soil type, the addition of nematodes achieved a further log reduction in spore count. These laboratory based results suggest that the combined use of nematodes and germinants could represent a promising approach for the remediation of *B. anthracis* spore contaminated soil.

Keywords: anthrax, remediation, environmentally friendly, *Caenorhabditis elegans* N2, L-alanine, inosine

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