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Editorial Board Address:
NSC 'Institute of Experimental and Clinical Veterinary Medicine'
83 Pushkinska Str., Kharkiv, Ukraine, 61023
tel. +38 (057) 707-20-53, 704-10-90
E-mail: nsc.iecvm.kharkov@gmail.com, inform@vet.kharkov.ua

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STUDY OF THE SPREAD OF MINOR VIRAL CATTLE INFECTIONS (LEUKEMIA, IMMUNODEFICIENCY, AND SPUMAVIRUS INFECTION) USING POLYMERASE CHAIN REACTION

Biloivan O. V., Didyk T. B., Yurko P. S., Korneikova O. B., Paliy A. P., Gorbatenko S. K., Bryl N. F.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: silverscreen91@gmail.com

Summary. The aim of the study was to investigate the prevalence of minor cattle infections (leukemia, bovine immunodeficiency and spumavirus infection) using the polymerase chain reaction (PCR). Blood samples were collected from cows in conditionally leukemia-free farms in ten regions of Ukraine to determine the presence of these infections. The samples were examined via classical PCR to detect the genetic material of the specific fragment of the *ENV* gene of the leukemia virus using *BLV-env-3/BLV-env-4* primers recommended by the OIE. To identify the proviral DNA of bovine foamy virus (BFV), primers *Int 1-Int 2* and *Int 3-Int 4* were used, and for the detection of bovine immunodeficiency virus (BIV) proviral DNA, a pair of primers RT_+(–) flanking the conservative domain of reverse transcriptase and a pair of primers flanking the *pol* gene of the BIV were selected. The situation concerning leukemia is most severe in Sumy and Kharkiv regions. A significant percentage of animals carrying the foamy virus was observed in farms in Kirovohrad, Kherson, Donetsk, and Kharkiv regions. Moreover, genetic material of the immunodeficiency virus was found in samples from Kirovohrad, Donetsk, and Kherson regions. These results indicate a significant prevalence of minor infections among cattle in Ukraine due to a lack of awareness among livestock workers, highlighting the necessity for comprehensive sanitary and preventive measures

Keywords: foamy virus, DNA, Ukraine

Introduction. Among the infectious diseases of cattle, particular attention is given to the viruses known as slow or minor infections. These include leukemia caused by the bovine leukemia virus (BLV), spumavirus infection caused by the bovine foamy virus (BFV), and bovine immunodeficiency caused by the bovine immunodeficiency virus (BIV). All these agents belong to the retrovirus family and share antigenic relatedness. One of the main features of these diseases is the lack of distinct clinical signs, a latent course and a prolonged incubation period. They are not notably lethal; however, especially when they have an associated course, they can cause significant losses in cattle farming. They exhibit immunosuppressive effects in the infected animals, significantly reducing the effectiveness of preventive and curative measures, as well as the productivity levels and quality of animal products. Furthermore, these infections lead to a loss of genetic diversity within the livestock (Constable et al., 2017; Scobie et al., 2001; Straub and Levy, 1999; Meas et al., 2002).

Leukemia in cattle is a well-studied disease concerning the specifics of infectious and epizootic processes, as well as diagnostic directions and eradication methods based on legislative acts adopted in countries with developed livestock industries. Examples include European countries where, through legislative programs, the disease has been eradicated, except for isolated cases reported in certain countries where the disease occurs sporadically without clinical manifestation. This applies to livestock farms in Ukraine as well.

Regarding the other minor viral infections mentioned earlier, it is worth noting the lack of information available in both OIE materials and scientific literature. It is known that spumavirus infection and bovine immunodeficiency have a wide distribution in livestock in various countries around the world. According to some authors, bovine immunodeficiency in cattle has been reported in Japan, France, Canada, USA, Iran, Argentina, Germany, the Netherlands, Italy, Brazil, and other countries. The infection rate ranges from 3% to 50% or even higher. In some livestock farms in developed countries, 35% to 45% of cattle are seropositive for the spumavirus infection, and the difficulties caused by this pathogen are widespread worldwide (Meas et al., 2002; Romen et al., 2007; Murray et al., 2006; Orr, O'Reilly and Scholl, 2003; Krasnikova and Larionova, 2014).

It is worth noting the complete lack of information regarding the presence and spread of the spumavirus infection and bovine immunodeficiency in domestic animal husbandry. In recent years, monitoring studies on the prevalence of these minor infections in livestock farms in the central and eastern regions of Ukraine have

been conducted by scientists from the Laboratory for the Study of Leucosis and the Laboratory of Molecular Diagnostics at the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM') exclusively using molecular-genetic methods due to the absence of domestic serological diagnostic tools (Gorbatenko et al., 2023a, 2023b). Even with limited sample sizes, the presence of genetic material of leukemia, spumavirus and immunodeficiency viruses among the livestock of several farms has been identified.

The **purpose of this report** is to emphasize the spread of these diseases not only in livestock worldwide but also within the livestock farms of Ukraine. This accentuates the necessity to deepen research aimed at studying the properties of immunodeficiency and foamy viruses. It is also important to develop technology for accumulating their viral mass and to design and implement domestic serological diagnostic tools for these less-studied minor viral diseases in cattle. This approach will help establish the real epizootic situation, introduce large-scale anti-epizootic measures, ultimately leading to the improvement of animal health and the quality of livestock products.

Materials and methods. The blood samples were collected from apparently healthy cows in various farms from different regions like Kirovohrad (n = 10), Poltava (n = 15), Sumy (n = 25), Cherkasy (n = 15), Kharkiv (n = 40), Chernihiv (n = 10), Donetsk (n = 10), Kherson (n = 10), Mykolaiv (n = 10), and Zaporizhzhia (n = 10) regions (Table 1). Blood samples were collected with a 3% EDTA solution and sent for analysis on the same day as collection. The total DNA extraction was performed using spin columns and reagents from the IndiSpin QIAcube HT Pathogen Kit (Germany). The samples were analyzed using classical PCR with DreamTaq Green PCR Master Mix (ThermoFisher, USA) to detect the genetic material of the specific *ENV* gene fragment of the leukemia virus, utilizing the *BLV-env-3/BLV-env-4* primers recommended by the OIE (2018) and described by Fechner et al. (1996). The amplification was carried out following this thermal profile: 2 min of denaturation at 94 °C; 30 cycles of 30 s at 95 °C, 30 s at 58 °C, and 60 s at 72 °C; followed by a final elongation step of 4 min at 72 °C. Samples showing a band corresponding to the 444 bp amplification product were considered positive. For the positive control sample, DNA isolated from the virus-containing fluid of the FLK-BLV culture was used.

The detection of proviral DNA of bovine foamy virus (BFV) was conducted using two pairs of primers: *Int 1-Int 2* (outer pair, amplification product length 430 bp) and *Int 3-Int 4* (inner pair, amplicon length 221 bp). A 'nested' version of the PCR was selected for the detection of proviral DNA of BFV following the recommendations of the authors (Materniak-Kornas et al., 2013).

For the detection of bovine immunodeficiency virus (BIV) proviral DNA, a pair of primers was used: RT_+(–), which flank a conservative domain of the reverse transcriptase (PCR amplicon length 495 bp), and another pair, BIV_Pol_+(–), which flank the *pol* gene of BIV (PCR amplicon length 235 bp). The amplification was performed using standard PCR protocols following the recommendations of the authors (Moody et al., 2002).

Results. Based on the results presented in Table 1, it can be seen that the situation concerning bovine leukemia is most critical in Sumy (33.3% positive from the total number of samples) and Kharkiv (32.5%) regions. Regarding other minor viral infections in cattle, a significant percentage of animals were identified as carriers of the foamy virus in several regions, which is concerning. For instance, the highest percentages of positively reacting animals were found in Kirovohrad (70%), Kherson (60%), Donetsk (50%), and Kharkiv (30%) regions, based on the obtained results. However, the situation concerning the circulation of the immunodeficiency virus among livestock appears less complex. Nonetheless, 40% of the examined samples from Kirovohrad, Donetsk, and Kherson regions exhibited genetic material of the mentioned pathogen.

Table 1 — The results of the molecular-genetic examination of blood samples from cattle in conditionally healthy herds regarding bovine leukemia virus (BLV) in various regions of Ukraine aimed to detect the presence of minor viral infections genetic material

Region	Farm	Number (n)	Presence of genetic material		
			BLV	BFV	BIV
Kirovohrad	1	10	1	7	4
Poltava	2	15	4	2	–
Sumy	3	15	5	10	1
Cherkasy	4	15	1	2	1
Kharkiv	5	15	1	2	–
	6	15	3	2	–
	7	10	9	8	–
Chernihiv	8	10	2	2	1
Donetsk	9	10	2	5	4
Kherson	10	10	2	6	4
Mykolaiv	11	10	1	1	–
Zaporizhzhia	12	10	2	2	–

The obtained results were interpreted based on the presence of characteristic bands of specific lengths observed after horizontal gel electrophoresis of the amplification products, specifically: 444 bp for the proviral DNA of the leukemia virus, 221 bp for the genetic material of the foamy virus, and 235 bp for the immunodeficiency virus (Figs 1, 2).

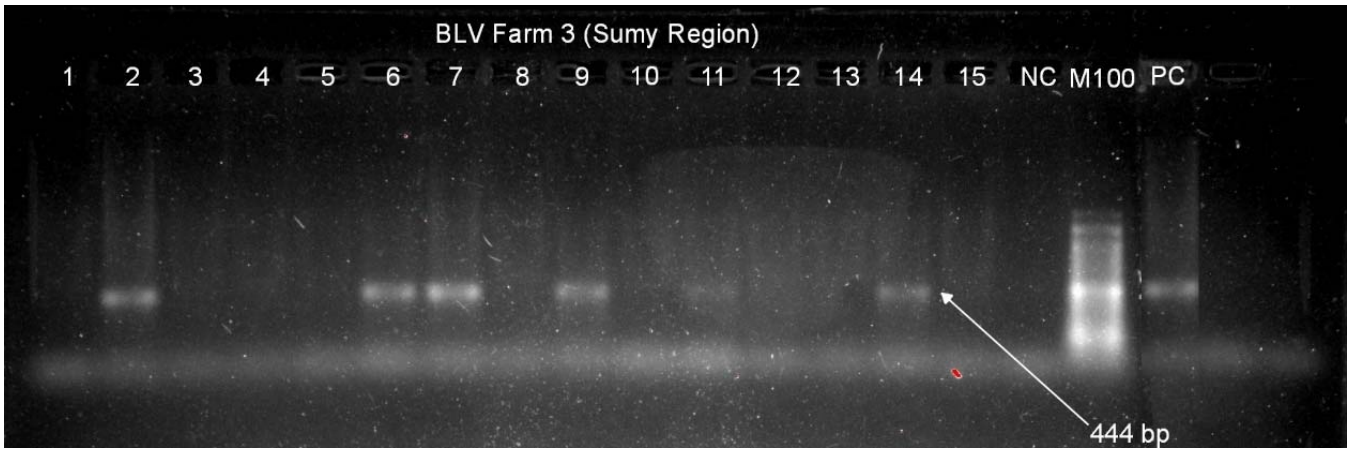

Figure 1. Gel-electrophoresis results of the amplification products for the presence of proviral DNA of BLV using samples obtained from one of the farms in Sumy Region are depicted. In the illustration, PC stands for the positive control sample, while NC represents the negative control sample.

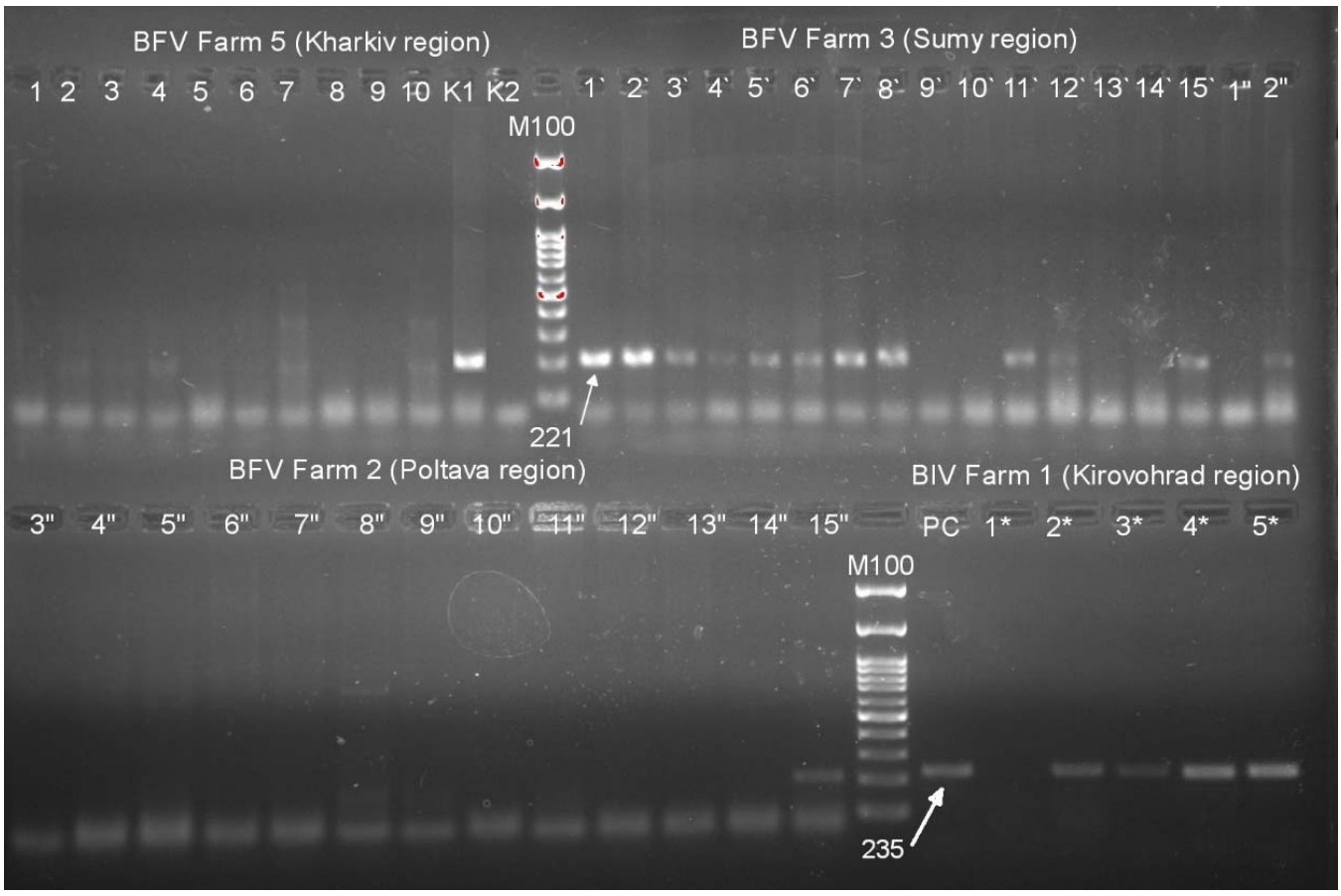

Figure 2. Gel-electrophoresis results of amplification products for the presence of proviral DNA of BFV and BIV using samples from specific farms in Kharkiv, Sumy, Poltava, and Kirovohrad regions. K1 represents the positive control sample for BFV DNA, PC — the positive control sample for BIV DNA, and K2 — the negative control sample.

Conclusions. The obtained results indicate a significant prevalence of agents causing minor infections among livestock in Ukraine, attributed to the lack of awareness among animal husbandry personnel. This underscores the necessity for comprehensive health-boosting measures against epizootic diseases. Given the above, it is essential to emphasize the need for an in-depth study of agents causing foamy virus infections and immunodeficiency, along with the development of methods for accumulating their viral mass.

It is worth noting that although molecular-genetic methods enable relatively rapid detection of pathogens in specific farms, they only allow for a limited sample of animals to be studied. To conduct comprehensive

screening of the entire livestock and track immunity dynamics and stages, the application of classical serological diagnostic methods remains pertinent. Therefore, the development and implementation of domestic serological diagnostic tools for lesser-known minor viral infections in cattle to assess the actual epizootic status in Ukrainian animal husbandry and the implementation of large-scale anti-epizootic measures remain essential tasks in domestic veterinary science.

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AVULOVIRUS CIRCULATION AMONG WILD BIRDS IN UKRAINE IN 2017–2020

Kolesnyk O. S.

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: admin@vet.kharkov.ua

Summary. In 2017–2020, virological monitoring of the circulation of avian avuloviruses among wild waterfowl and shorebirds of 53 species belonging to 8 families was conducted in the northern (Chernihiv) and southern (Odesa, Kherson, Zaporizhzhia, and Mykolaiv) regions of Ukraine. Since almost the entire territory of Ukraine lies within the main bird migration routes, a significant number of shorebirds nest and winter in the south of our country, and in fact, the entire south of Ukraine is at high risk of spreading pathogens that can be transmitted by birds, including avulovirus infections, throughout the year. A variety of avian avuloviruses are widely distributed among different hosts, but there is a large knowledge gap in understanding the movement of these viruses in wild populations. The results of virological monitoring showed that avuloviruses of different serotypes are actively circulating among wild birds of different ecological groups. During this period, 39 isolates of avuloviruses were isolated from wild birds. Based on the serologic identification results, it was found that most avulovirus isolates (n = 18) had cross-reactions, accounting for 46.15%. A total of 9 isolates belonged to AaV-1, accounting for 23.07%. 5 isolates belonged to AaV-4, which is 12.8%. 2 isolates belonged to AaV-9, which is 5.12%. AaV-6 and AaV-7 had two isolates each, which together accounted for 10.24%. 1 isolate belonged to AaV-3, which is 2.56%. It was also found that the highest number of isolates was isolated during wintering — 23 isolates and during autumn migration — 10 isolates, and the lowest number during nesting and spring migration, 4 and 2 isolates, respectively. The infection rate of wild birds with avuloviruses ranged from 0.13% to 11.76%. The most infected with avuloviruses were the species of common lamb (11.76%), and the least infected were the gray goose (0.13%)

Keywords: hemagglutination inhibition assay, natural reservoir, monitoring

Introduction. Avian avuloviruses (AaV), which belong to the family Paramyxoviridae, are a diverse group of zoonotic viruses. Their genome consists of a single-stranded minus-RNA molecule with 15–16 thousand nucleotide pairs and includes 20 unique serotypes (1–20). The International Committee on Taxonomy of Viruses classifies these serotypes into three different genera: *Metaavulavirus* (serotypes 2, 5–8, 10, 11, 14, 15, 20), *Orthoavulavirus* (serotypes 1, 9, 12, 13, 16–19), and *Paraavulavirus* (serotypes 3, 4) (Lefkowitz et al., 2018).

Avian avuloviruses are known for their ability to mutate and recombine, leading to the emergence of new variants and virus lines (Alexander et al., 1989). This evolutionary process results in significant antigenic and genetic diversity of avian avuloviruses in nature, affecting the pathogen's virulence, which can range from non-pathogenic to highly pathogenic. Virulence is influenced by various factors, including genetic variation and environmental factors (Miller et al., 2009). The constant process of avulovirus change can pose a challenge for controlling and managing poultry diseases. Viruses can expand their spectrum and adapt to new conditions. Therefore, continuous monitoring and research of these changes are important for developing an effective strategy for controlling and managing this pathogen.

AaV-1 (known as Newcastle disease) is the best known and most common of the avulovirus serotypes and is classified into two different classes (class I or class II) and is further characterized by either 1 genotype (class I) or 15 genotypes (class II) (Diel et al., 2012). Class I viruses are isolated exclusively from wild birds, while class II viruses cover poultry and wild populations (Diel et al., 2012; Hicks et al., 2019). This avian viral infection is characterized by pneumonia, encephalitis, digestive tract damage, and multiple hemorrhages in internal organs (Alexander, 2001). In addition to AaV-1, other avulovirus serotypes are less common in poultry and usually circulate in wild birds.

Other serotypes of avuloviruses cause respiratory and other diseases of lesser severity in various avifauna (Saif, 1997). Practical observations and experimental studies show that AaV-2, AaV-3, AaV-6, and AaV-7 are capable of causing diseases with different pathogenicity in poultry (Alexander, 2000; Nerome, et al., 1978). For example, AaV-6 strains cause a decrease in egg production in turkeys and mild respiratory diseases (Gough and Alexander, 1984). AaV-4, AaV-8, AaV-9, and AaV-10 were isolated from waterfowl and other wild bird species that did not show any clinical signs of disease (Stanislawek et al., 2002; Chang et al., 2001). AaV-4 was mainly isolated from wild birds of the genus *Plateolus*. Experimental infection of chickens with AaV-4 and AaV-6 caused mild respiratory pathologies (Miller and Afonso, 2009). As for the other avuloviruses (11th–21st serotypes), there is currently no clear information on their role in the occurrence of diseases in poultry, wild and domestic, as well as humans.

Due to its geographical location, Ukraine plays a crucial role in global migration processes of wild waterfowl and shorebirds of various species. The massive

gatherings and close interspecific contact of birds from different continents contribute to the spread and transfer of numerous pathogens, including avuloviruses (Rahman et al., 2018; Kinde et al., 2005; Reeves et al., 2016). Pathogen exchange or bird infection occurs during feeding or resting. In case of violating veterinary and sanitary standards of keeping, poultry can be infected by wild birds through direct contact in open water or walking areas, or by using water or feed contaminated with the droppings of wild or synanthropic birds. Outbreaks of AaV-1 in poultry occur worldwide. Therefore, studying subtypes and pathotypes of AaV-1 can contribute to a better understanding of the distribution and evolution of avuloviruses worldwide. Additionally, it is important to study the ecology and circulation of other avuloviruses in their natural reservoirs. Understanding the mechanisms of pathogen circulation will allow for more effective control of these infections. The surveillance, isolation, and identification of different avulovirus subtypes are crucial for the agricultural industry's well-being in Ukraine and Europe.

Therefore, our research **aimed** to continuously monitor avian avulovirus circulation in Ukraine's natural reservoir. This scientific work is a continuation of many years of research by scientists of the Department of Avian Diseases of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' on monitoring and studying the biological characteristics of epizootically relevant avulovirus isolates circulating among wild birds in Ukraine (Stegniy et al., 2012; Muzyka et al., 2014).

Materials and methods. ***Sample collection.*** Biological material was collected from wild birds during seasonal migrations (spring and autumn), nesting, and wintering in the northern and southern regions of Ukraine between 2017 and 2020. A total of 13,710 fecal samples were collected in the Azov-Black Sea region of Southern Ukraine, including Kherson, Odesa, Mykolaiv, and Zaporizhzhia regions. This area is one of the most important regions in Eastern Europe for the migration of wild birds of different ecological groups. In the north of Ukraine (Chernihiv Region), 1,203 samples of wild bird feces were collected. The locations of mass bird gatherings were identified by ornithologists from various organizations, including the Azov-Black Sea Ornithological Station in Zaporizhzhia Region, Askania-Nova Biosphere Reserve, Black Sea Biosphere Reserve in Kherson Region, and Danube Biosphere Reserve in Odesa Region, and leading ornithologists from Odesa and Chernihiv. A total of 14,913 fecal samples were collected from 53 species of wild birds using standard methods (Capua and Alexander, 2009; WOAH, 2023; Spackman, 2020). Samples were collected in cryotubes containing 1.0 cm^3 of viral transport medium (BHIV, brain heart infusion broth, Sigma-Aldrich, # 53286-100G) with antibiotics (penicillin 10,000 U/ml, streptomycin 10 mg/ml, gentamicin 250 μg/ml, and nystatin 5,000 U/ml) (Williams et al., 2016). Samples were stored in liquid nitrogen in the field and at –70 °C in the laboratory.

In 2017, a total of 4,790 environmental fecal samples were collected in places of mass gatherings of wild birds. The sampling was combined with ornithological surveys of bird populations in the area. Fecal samples were collected from 42 species of wild birds from seven different families: Anseriformes, Charadriiformes, Podicipediformes, Gruiformes, Ciconiiformes, Pelecaniformes, and Falconiiformes. We collected 92 samples in Zaporizhzhia Region, 10 samples in Mykolaiv Region, 1,904 samples in Odesa Region, 2,209 samples in Kherson Region, and 475 samples in Chernihiv Region. Samples were collected during the periods of bird migration in the Azov-Black Sea region of Ukraine, as well as in the north during autumn migration (August–October), wintering (November–February), spring migration (March–May), and during localized movements in June–July, which usually occur after the nesting period. In 2018, a total of 6,120 samples were collected from 44 species of wild shorebirds and waterbirds belonging to 6 families. Of these, 5,721 were collected in southern Ukraine (Odesa, Kherson, Zaporizhzhya, and Mykolaiv regions) and 399 in northern Ukraine (Chernihiv region). In 2019, field expeditions were conducted to cover both shorebirds and waterbirds at migratory stopover sites, resulting in the collection of 3,056 fecal samples. Of these, 329 were collected in Chernihiv Region and 2,727 were collected in the south from 32 bird species belonging to five families.

Samples from background bird species were collected at sentinel sites during 2020 in southern Ukraine, near the coasts of the Azov and Black seas, close to the sources of major rivers (the Danube and the Dnipro). A total of 947 fecal samples were collected from 12 bird species belonging to two families. Table 1 presents a summary of the wild bird sampling data for 2017–2020.

Virological research. Virus isolation from fecal samples was performed according to standard OIE procedures (WOAH, 2023; Spackman, 2020). To infect chicken embryos, a fecal suspension was prepared in phosphate-buffered saline (PBS) pH (7.2 ± 0.1) with antibiotics, infection was performed in the allantois cavity at a dose of 0.2 cm^3, and three passages were performed. The embryos were incubated for 4–5 days at 37 °C and ovoscoped twice a day; embryo death during the first day was considered nonspecific. After preliminary cooling at 4 °C, embryos were dissected. The presence of hemagglutinating viruses in the allantois fluid was determined by a hemagglutination assay in polystyrene plates with a V-shaped bottom with a 1% suspension of rooster erythrocytes (Capua and Alexander, 2009; WOAH, 2023).

Table 1 — List of samples collected from wild bird species in 2017–2020

Bird species		Period				Total
Common Name	**Scientific Name**	**2017**	**2018**	**2019**	**2020**	
Anseriformes						
White-fronted Goose	*Anser albifrons*	1,033	1,115	620	304	3,072
Red-breasted Goose	*Rufibrenta ruficollis*	23	304	–	–	327
Garganey	*Anas querquedula*	43	84	4	–	131
Mallard	*Anas platyrhynchos*	1,100	1,299	734	248	3,381
Shelduck	*Tadorna tadorna*	429	578	409	26	1,442
Ruddy shelduck	*Tadorna ferruginea*	103	301	90	–	494
Mute swan	*Cygnus olor*	49	27	2	–	78
Whooper swan	*Cygnus cygnus*	270	387	95	240	992
Greylag goose	*Anser anser*	386	176	160	15	737
Wigeon	*Anas penelope*	15	161	–	10	186
Shoveler	*Anas clypeata*	24	–	–	–	24
Teal	*Anas crecca*	61	110	–	47	218
Mixt of species (wild duck)		38	–	–	–	38
Bewick's swan	*Cygnus bewickii*	–	–	10	1	11
Pintail	*Anas acuta*	6	9	–	–	15
Gadwall	*Anas strepera*	–	25	3	–	28
Red-crested pochard	*Netta rufina*	–	15	–	–	15
White-fronted goose + Red-breasted goose	*Anser albifrons* + *Rufibrenta ruficollis*	–	70	51	–	121
Whooper swan + Bewick's swan	*Cygnus cygnus* + *Cygnus bewickii*	–	15	–	6	21
Charadriiformes						
Yellow-legged gull	*Larus cachinnans*	133	194	89	19	435
Black-headed gull	*Larus ridibundus*	262	113	162	30	567
Slender-billed gull	*Larus genei*	36	53	33	–	122
Ruff	*Phylomachus pugnax*	102	70	1	–	173
Mediterranean gull	*Larus melanocephalus*	189	131	31	–	351
Common gull	*Larus canus*	18	40	–	1	59
Great black-headed gull	*Larus ichtyaetus*	40	16	–	–	56
Dunlin	*Calidris alpina*	10	–	10	–	20
Snipe	*Gallinago gallinago*	15	1	1	–	17
Little gull	*Larus minutus*	5	30	3	–	38
Little tern	*Sterna albifrons*	5	1	–	–	6
Lapwing	*Vanellus vanellus*	–	50	2	–	52
White-winged black tern	*Chlidonias leucopterus*	20	–	–	–	20
Caspian tern	*Hydroprogne caspia*	7	–	–	–	7
Avocet	*Recurvirostra avosetta*	5	17	52	–	74
Gulls spp.		14	–	10	–	24
Gull-billed tern	*Gelochelidon nilotica*	–	52	50	–	102
Grey plover	*Pluvialis squatarola*	–	11	–	–	11
Collared pratincole	*Glareola pratincola*	–	20	–	–	20
Common tern	*Sterna hirundo*	–	16	25	–	41
Black-winged stilt	*Himantopus himantopus*	–	4	–	–	4
Greenshank	*Tringa nebularia*	–	11	–	–	11
Sandwich tern	*Thalasseus sandvicensis*	–	26	56	–	82
Wader spp.		–	7	–	–	7
Whiskered tern	*Chlidonias hybrida*	–	45	45	–	90
Black-headed gull + Mediterranean gull	*Larus ridibundus* + *Larus melanocephalus*	–	40	–	–	40

Table 1 — continuation

Bird species		Period				Total
Common Name	**Scientific Name**	**2017**	**2018**	**2019**	**2020**	
Podicipediformes						
Great crested grebe	*Podiceps cristatus*	1	–	–	–	1
Gruiformes						
Demoiselle crane	*Anthropoides virgo*	12	–	–	–	12
Crane	*Grus grus*	33	–	15	–	48
Coot	*Fulica atra*	9	59	109	–	177
Ciconiiformes						
Great white egret	*Egretta alba*	15	–	5	–	20
Little egret	*Egretta garzetta*	5	10	–	–	15
Grey heron	*Ardea cinerea*	29	7	–	–	36
Spoonbill	*Platalea leucorodia*	7	1	–	–	8
White stork	*Ciconia ciconia*	–	10	–	–	10
Pelecaniformes						
Dalmatian pelican	*Pelecanus crispus*	15	13	10	–	38
Cormorant	*Phalacrocorax carbo*	131	174	140	–	445
White pelican	*Pelecanus onocrotalus*	41	109	29	–	179
Pygmy cormorant	*Phalacrocorax pygmaeus*	5	–	–	–	5
Falconiiformes						
Marsh harrier	*Circus aeruginosus*	1	–	–	–	1
Poultry (backyards)						
Domestic geese		–	87	–	–	87
Environmental samples (domestic geese + duck)		–	26	–	–	26
Environmental		45				45
Total		**4,790**	**6,120**	**3,056**	**947**	**14,913**

Virus identification. The AaV virus serotype was determined using the hemagglutination inhibition assay (HAI) (Williams et al., 2016; WOAH, 2023; Spackman, 2020). The following antiserums were used for these studies: AaV-1, AaV-2, AaV-3, AaV-4, AaV-6, AaV-7, AaV-8, and AaV-9, manufactured by the Veterinary Laboratories Agency (Animal and Plant Health Agency, Weybridge, UK) and AaV-1, AaV-2, AaV-3, AaV-4, AaV-6, AaV-7, AaV-8, and AaV-9, manufactured by Instituto Zooprofilattio Sperimentale delle Venezie (Padua, Italy).

Results. ***Virological research.*** According to the results of virological studies of biological material from wild birds in 2017–2020, 39 isolates of avuloviruses were isolated. It should be noted that many more hemagglutinating isolates were isolated during virological studies, some of which were later identified as avian influenza viruses and were not used in this work. As a rule, avuloviruses were isolated on passages 1 and 2, with hemagglutinating activity ranging from 1:64 to 1:2,048. According to the results of serological identification of avuloviruses, it was found that 9 isolates belong to AaV-1 (Newcastle disease), 5 isolates to AaV-4, 2 isolates to AaV-9, 2 isolates to AaV-6, 2 isolates to AaV-7, 1 isolate to AaV-3, and the remaining 18 had cross-reactions, which requires further in-depth studies. The result is shown in Table 2.

As can be seen from the results presented in Table 2, 15 isolates of avulaviruses were isolated from biological material collected in Kherson Region, 11 in Odesa Region, 12 in Zaporizhzhia Region, and one in Mykolaiv Region. No isolates were isolated in Chernihiv Region. Most isolates were found in mallard (*Anas platyrhynchos*) (n = 19), white-fronted goose (*Anser albifrons*) (n = 9), common shelduck (*Tadorna tadorna*) (n = 4), ruddy shelduck (*Tadorna ferruginea*) (n = 3), teal (*Anas crecca*) (n = 1), common snipe (*Gallinago gallinago*) (n = 2), and greylag goose (*Anser anser*) (n = 1).

According to the results of the research, the infection rate of birds was determined according to species (Table 3).

Table 2 — List of identified avulovirus isolates from wild birds

No.	Isolate name	Identification result
2017		
1	Shelduck/Sergiyivka/11-15/6-08/17	AaV-1
2	Mallard/Katranka/6-10/1-12/17	AaV-1
3	Mallard/Sivashivka/1-4/4-09/17	H5/AaV-6/H3/H6
4	Mallard/Druzhelyubivka/1-3/5-09/17	AaV-4
5	Mallard/Mytrofanivka/1-4/4-09/17	AaV-1/AaV-3/AaV-4/AaV-7/AaV-9
6	Greylag goose/Mytrofanivka/1-4/4-09/17	AaV-1/AaV-4/AaV-7/AaV-9
7	Shelduck/Churyuk/1-5/2-11/17	AaV-1
8	Mallard/Novodmytrivka/11-15/4-09/17	AaV-6/AaV-2/AaV-7
9	Ruddy shelduck/Khorol/11-15/27-01/17	AaV-1/AaV-7/AaV-4
10	Shelduck/Churyuk/1-5/2-11/17pool946	AaV-9
11	Ruddy shelduck/Novodmytrivka/1-5/5-08/17	AaV-1/AaV-9
12	Mallard/Vasylivka/1-5/24-01/17	AaV-1/AaV-3/AaV-4
2018		
13	Mallard/Primorske T./41-45/7-10/18	H3/H6/AaV-4
14	White-fronted goose/Primorske K./6-10/25-02/18	AaV-1/AaV-4
15	White-fronted goose/Primorske K./21-25/25-02/2018	AaV-1
16	White-fronted goose/Primorske K./31-35/25-02/2018	AaV-1/AaV-6
17	White-fronted goose/Velyka Balka/1-5/01-04/18	AaV-6
18	White-fronted goose/Primorske K./11-15/25-02/18	AaV-1
19	Ruddy shelduck/Askania-Nova/7-11/22-01/18	AaV-1
20	Mallard/Askania-Nova/131-135/30-01/18	AaV-4/AaV-3
21	Mallard/Oleksandrivka/21-25/10-08/18	AaV-7
22	White-fronted goose/Primorske K./6-10/25-02/2018	AaV-3
2019		
23	Mallard/Askania-Nova/4-25-02/19	AaV-1/AaV-7
24	Snipe/Ermakov/23-10/19	AaV-1
25	Mallard/Askania-Nova/45-48/5-12/19	AaV-4
26	Mallard/Askania-Nova/27-23-12/19	AaV-6
27	Mallard/Askania-Nova/32-3-12/19	AaV-1/AaV-4/AaV-7/AaV-9
28	Mallard/Askania-Nova/29-32/3-12/19	AaV-1/AaV-4/AaV-7/AaV-9
29	Mallard/Askania-Nova/24-4-01/19	AaV-4
30	White-fronted goose/Primorske/71-75/14-01/19	H6/AaV-4
31	White-fronted goose/Izmail/1-25-02/19	AaV-1/AaV-4/AaV-7
32	Teal/Ermakov/24-10/19	AaV-1
33	Snipe/Koblevo/4-06/19	AaV-7
34	Shelduck/Mytrofanivka/11-15/17-06/19	AaV-1/AaV-9/AaV-3
35	White-fronted goose/Stroganivka/36-40/01-04/19	AaV-1
2020		
36	Mallard/Askania-Nova/242-16-01/20	AaV-4
37	Mallard/Askania-Nova/171-6-01/20	AaV-9
38	Mallard/Askania-Nova/292-3-02/20	AaV-4
39	Mallard/Askania-Nova/308-10-2/20	AaV-3/AaV-7/AaV-9

Thus, it was found that isolates from mallard were mostly isolated during wintering (13 isolates) and during autumn migration (6 isolates), from white-fronted goose during wintering (7 isolates) and during spring migration (2 isolates), from shelduck during nesting (2 isolates) and autumn migration (2 isolates), from ruddy shelduck during wintering (2 isolates) and breeding (1 isolate), from snipe during breeding (1 isolate) and wintering (1 isolate), from teal 1 isolate during fall migration and from greylag goose 1 isolate also during fall migration (Table 3). The highest percentage of infection was found in snipe — 11.76%, the lowest in greylag goose — 0.13%. In other poultry species, this figure ranged from 0.27% to 0.6%.

Table 3 — Infection rate of wild birds with avuloviruses of different serotypes in the period 2017–2020

Bird species	Number of samples	Number of isolates	Infection rate ,%
Mallard (*Anas platyrhynchos*)	3,381	19	0.56
White-fronted goose (*Anser albifrons*)	3,072	9	0.29
Shelduck (*Tadorna tadorna*)	1,442	4	0.27
Ruddy shelduck (*Tadorna ferruginea*)	494	3	0.60
Teal (*Anas crecca*)	218	1	0.45
Snipe (*Gallinago gallinago*)	17	2	11.76
Greylag goose (*Anser anser*)	737	1	0.13

Conclusions. The findings indicate that avulaviruses are circulating among wild birds in Ukraine and can infect various bird species in different habitats with different migratory behaviors. Therefore, the risk of continued spread of these viruses among wild birds and the threat to poultry remains high in Ukraine. Pathogen circulation is concentrated in the ecological environments of the southern regions.

The field expeditions conducted from 2017 to 2020 covered both shorebirds and water birds. These birds are the primary carriers of avuloviruses. The expeditions focused on stopover sites for migratory birds. Biological material was collected from birds of background species, particularly in southern Ukraine near the coasts of the Azov and Black seas, close to the sources of large rivers such as the Danube and the Dnipro. Additionally, this region has a large poultry population. The virus has been detected in wild birds, highlighting the emergence and risk of avian avulovirus outbreaks in these areas. However, there are still gaps in understanding the natural environment and circulation of avian avuloviruses. Therefore, it is essential to find new natural hosts and study factors that may contribute to overcoming these interspecies barriers.

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INFLUENCE OF CERTAIN TEMPERAMENTAL TRAITS ON THE LEVEL OF SEX HORMONES IN BLOOD PLASMA OF FEMALE BULL TERRIERS

Forkun V. I., Bobrytska O. M., Vodopianova L. A., Zhukova I. O.

State Biotechnological University, Kharkiv, Ukraine, e-mail: olga.bobritskaya2410@gmail.com

Summary. To evaluate the influence of certain temperamental characteristics on the level of sex hormones in the blood plasma of female Bull Terriers, an experiment was conducted with 15 female Bull Terriers. Pregnancy screening was performed on 21st–28th days after ovulation). The material for the study were blood plasma samples of bitches obtained at different stages of the sexual cycle, in which the levels of estradiol, follicle stimulating hormone, luteinizing hormone, and progesterone were measured. The assessment of temperamental traits was performed at the design stage of the experiment using the standardized C-BARQ methodology. One-way analysis of variance was used to determine the strength of the influence of individual temperament traits on the level of sex hormones in the blood plasma of bitches. According to the results of the experiment, it was found that the degree of fear and anxiety in bitches significantly affects the content of follicle-stimulating hormone, estradiol, and luteinizing hormone in the blood plasma of bitches three days before, during and 120–150 days after the LH surge ($\eta^2_\chi = 0.27–0.55$ ($P \le 0.05$). The excitability of bitches affects the level of follicle stimulating hormone, luteinizing hormone, and progesterone on the day of the LH surge — $\eta^2_\chi = 0.32$ ($P \le 0.05$). In addition, the level of excitability affects the level of progesterone on the 9th day after the surge — $\eta^2_\chi = 0.35$ ($P \le 0.05$) and the level of luteinizing hormone on the 2nd, 4th, 55th–60th days and after the LH surge — $\eta^2_\chi = 0.26–0.43$ ($P \le 0.05$). The degree of aggression affects the luteinizing hormone level two days after the surge and the progesterone level on 55th–60th days after the LH surge ($\eta^2_\chi = 0.34–0.36$; $P \le 0.05$). Training ability and obedience affect follicle stimulating hormone levels (the day before the surge, and on 4th, 9th, and 35th–40th days after the LH surge ($\eta^2_\chi = 0.30–0.52$; $P \le 0.05$), luteinizing hormone (on 9th day after LH surge, $\eta^2_\chi = 0.54$; $P \le 0.01$), and progesterone (on 35th–40th days after LH surge, $\eta^2_\chi = 0.34$; $P \le 0.05$)

Keywords: dogs, estradiol, follicle-stimulating hormone, luteinizing hormone, progesterone

Introduction. The brain plays a critical role in regulating the activity of all body systems. Several parts of the brain have been implicated in the reproductive process (Okafor, Okpara and Ibeabuchi, 2022). These include the cerebral cortex, the insula, the pons, the hypothalamic-pituitary-gonadal (HPG) axis, and the pineal gland. Mammalian reproduction is primarily controlled and regulated by the HPG axis. Anatomically, the HPG axis consists of: the hypothalamus (specifically the infundibular nucleus, a homologue of the human arcuate nucleus, where the neurons that produce KNDy and GnRH are located); the anterior part of the pituitary gland, where the gonadotropes secrete luteinizing hormone and follicle stimulating hormone; and the gonads, which are responsible for the production of both sex steroids and gametes under the influence of sex hormones. As in other endocrine systems, the HPG axis is regulated by direct and reverse feedback (Tena-Sempere, 2005). Hormones of various origins (pituitary, placenta, and ovary) are involved in the control of the canine sexual cycle (Conley et al., 2023; Gobello, 2007). The hypothalamus, in turn, controls reproduction by regulating the secretory activity of the pituitary (Everett, 1969). In response to exogenous and endogenous stimuli, it produces several peptide neurohormones that regulate the function of the anterior pituitary. These neurohormones are released from the median eminence into the capillaries of the pituitary veins where they are transported to the adenohypophyseal cells to stimulate or inhibit the release of specific trophic hormones (Rance et al., 2010). In addition, in response to environmental stimuli, the hypothalamus produces neurohypophyseal hormones that are transported along long axons of the hypothalamic-pituitary tract for storage in the neural lobe of the pituitary and subsequent release into the systemic circulation (Opel, 1979). The pituitary gland, as one of the major endocrine organs of an animal, plays a critical role in the regulation of various physiological processes in mammals through the secretion of various hormones (Cooper and Withers, 2008). In addition to hormones that regulate reproductive function (follicle-stimulating and luteinizing hormones), the pituitary gland secretes growth hormone, prolactin, adrenocorticotropic hormone, melanocyte-stimulating hormone, and thyroid-stimulating hormone, which can directly or indirectly affect the reproductive function of mammals (Hong, Payne and Jane, 2016).

The highest manifestation of nervous activity of animals is their behavior (Danchuk et al., 2020b), which is undoubtedly limited by the state of the nervous system, including temperament (Danchuk et al., 2020a). Temperament is considered as a relatively stable group of personality traits (Strelau, 2008). The genetic basis of temperamental traits such as anxiety and aggression has been established, but phenotype has a greater influence on the formation of temperament (Hecht et al., 2021;

Zapata et al., 2022; Morrill et al., 2022). To date, a number of dependencies of temperament on age, sex, weight, and breed of animals have been established, which largely shape the individual characteristics of the animal body (Casey et al., 2014; Fratkin et al., 2013; Hsu and Sun, 2010; Riemer et al., 2014; Sherman et al., 1996).

However, there are no data in the available literature on the influence of temperament on the dynamics of sex hormones in the blood of bitches. Therefore, the **purpose of our work** was to determine the degree of influence of individual temperament traits on the dynamics of sex hormones in blood plasma of female Bull Terriers during the estrous cycle.

Materials and methods. The experiment was performed on 15 bitches of the Bull Terrier breed. The bitches were inseminated by different methods (both natural and artificial). Pregnancy screening was performed on 21st–28th days after ovulation). Parturition in the pregnant group was observed on 65 ±1 days after a rise in blood levels of luteinizing hormone. All animals were free of infectious and invasive diseases at the time of the study. The health status of the animals was assessed by clinical examination and laboratory tests. Blood samples were collected from the jugular vein of bitches on –3rd, –1st, 0, 2nd, 4th, 9th, 23rd–30th, 35th–40th, 55th–60th, and 120th–150th days after the luteinizing hormone (LH) surge. Plasma levels of estradiol (Dog E2 ELISA Kit, ICNE2KT, Innovative Research, USA), follicle stimulating hormone (Dog Follicle Stimulating Hormone (FSH) ELISA Kit, Abbexa Ltd, United Kingdom), luteinizing hormone (Dog Luteinizing Hormone (LH) ELISA Kit, Abbexa Ltd, United Kingdom) and progesterone (Progesterone ELISA, HEMA, Ukraine) were measured. Measurements were performed on an ELx800 universal microplate reader (Bio-Tek Instruments, USA).

Temperament traits were assessed during the design phase of the experiment using the uniform Canine Behavioral Assessment and Research Questionnaire (C-BARQ) methodology, which is designed to provide dog owners and professionals with standardized assessments of canine temperament and behavior. The C-BARQ is a standardized dog behavior assessment tool developed at the University of Pennsylvania and accepted as a basic tool for determining animal temperament traits (Serpell, 2023). Of the 14 major criteria of dog behavior, we selected the four most important, namely aggression, fear and anxiety, excitability, and training and obedience, to meet our objective (Table 1). Owners were interviewed using the standard C-BARQ questionnaire (Serpell, 2015).

In order to determine the strength of the influence of certain temperamental traits on the level of sex hormones in the blood plasma of female Bull Terriers, a one-way analysis of variance was performed with the help of MS Excel 2019 using the built-in function 'Data Analysis'.

Table 1 — Correlation of C-BARQ characteristics with different dog temperament traits

Characteristics according to C-BARQ	**Temperament traits**		
Aggression	Calm	Moderately aggressive	Aggressive
Fear and anxiety	Animals without a strong sense of fear or anxiety (courage)	Animals with a moderate level of anxiety or a sense of fear	Animals that express a sense of fear (timidity, fearfulness)
Excitability	Calm	Moderately excitable	Excessively excitable
Training and obedience	Obedient (well trained)	Sometimes not obedient	Not obedient

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017).

Results. Using a one-factor analysis of variance, a reliable strength of influence (η^2_χ) of individual temperament characteristics on the level of sex hormones in blood plasma was established (Figs 1–4). It was proved that a high degree of fear and anxiety in bitches significantly affects the content of FSH in the blood plasma of bitches only three days before the LH surge — $\eta^2_\chi = 0.27$ ($P \le 0.05$). At the same time, the level of bitches' excitability had a significant effect on the hormone content on the day of the LH surge — $\eta^2_\chi = 0.32$ ($P \le 0.05$). It should be noted that bitch aggression did not significantly affect the level of FSH in blood plasma throughout the experiment, while the ability to be trained and obedience proved to be a significant factor in influencing the dynamics of FSH in animal plasma. In particular, a significant effect of the ability to train and obedience of bitches on the FSH content in blood plasma the day before the LH surge ($\eta^2_\chi = 0.36$; $P \le 0.05$), on the 4th and 9th day after the LH surge, respectively, $\eta^2_\chi = 0.52$ ($P \le 0.01$) and $\eta^2_\chi = 0.26$ ($P \le 0.05$), and on the 35th–40th day after the LH surge ($\eta^2_\chi = 0.30$; $P \le 0.05$). At the stage of sexual rest (anestrus), the selected temperament traits did not have a significant effect on the content of follicle-stimulating hormone in the blood of bitches.

Dispersion analysis revealed a significant effect (η^2_χ) of only the level of fear and anxiety in bitches three days

before the LH surge on plasma estradiol content in bitches — $\eta^2_\chi = 0.27$ ($P \le 0.05$). At the same time, other temperamental traits had no significant effect on the hormonal balance during the sexual cycle (Fig. 2).

A reliable strength of influence (η^2_χ) of individual temperament traits on the level of luteinizing hormone in blood plasma was established (Fig. 3). The level of aggression of animals has the least influence on the dynamics of LH in the blood of bitches compared to other temperamental traits. In particular, a high level of aggression in bitches only significantly affects the content of LH in the blood plasma of bitches two days after the LH surge — $\eta^2_\chi = 0.36$ ($P \le 0.05$). At the same time, the degree of fear and anxiety in bitches significantly affects the hormone content in blood plasma three days before the LH surge — $\eta^2_\chi = 0.48$ ($P \le 0.05$) and 120th–150th days after the LH surge — $\eta^2_\chi = 0.55$ ($P \le 0.01$).

The level of excitability of the bitches had a significant effect on hormonal contents during estrus and at the end of diestrus. Specifically, on the day of the LH surge and two and four days thereafter, the effect of excitability on LH content was $\eta^2_\chi = 0.31$ ($P \le 0.05$), $\eta^2_\chi = 0.43$ ($P \le 0.05$), and $\eta^2_\chi = 0.26$ ($P \le 0.05$), respectively. In contrast, the effect of excitability was $\eta^2_\chi = 0.32$ ($P \le 0.05$) on 55th–60th days after the LH surge. The ability to be trained and obedience affected the LH content in the blood plasma of the animals only on the 9th day after the LH surge ($\eta^2_\chi = 0.54$; $P \le 0.01$).

Dispersion analysis revealed a significant effect (η^2_χ) of individual temperament traits on blood plasma progesterone levels in bitches (Fig. 4). In particular, the aggression of the bitches had a significant effect on the plasma level of P4 only on the 55th–60th days after the LH surge ($\eta^2_\chi = 0.34$; $P \le 0.05$).

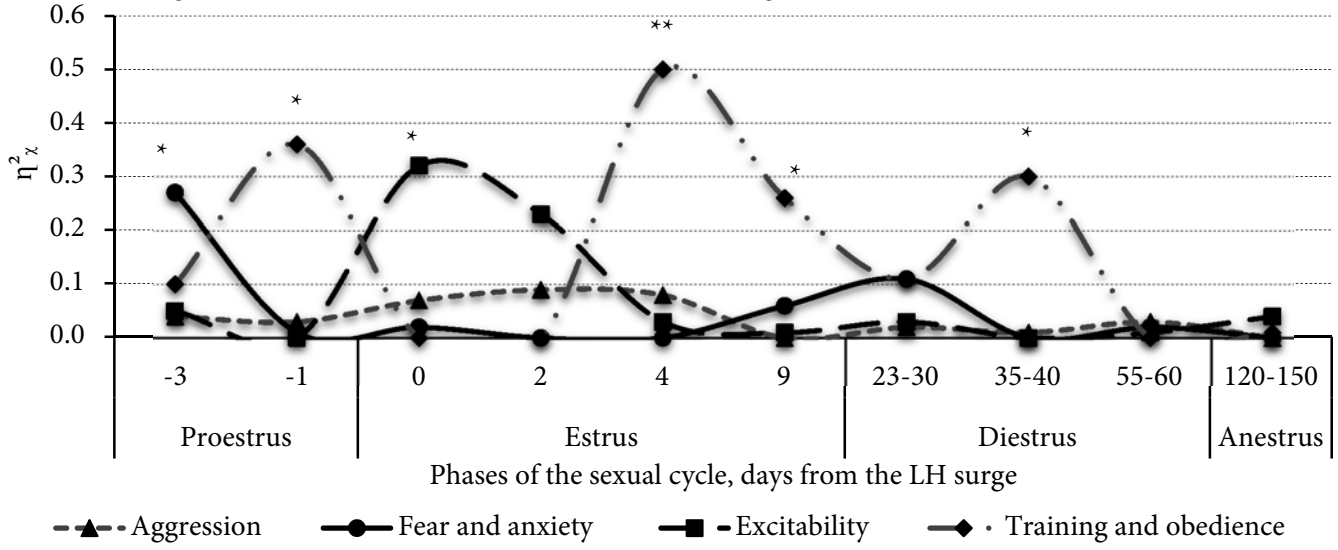

Figure 1. The strength of influence (η^2_χ) of individual temperament characteristics on the level of follicle-stimulating hormone in the blood plasma of bitches (n = 15; units). Reliable values: * — $P \le 0.05$; ** — $P \le 0.01$.

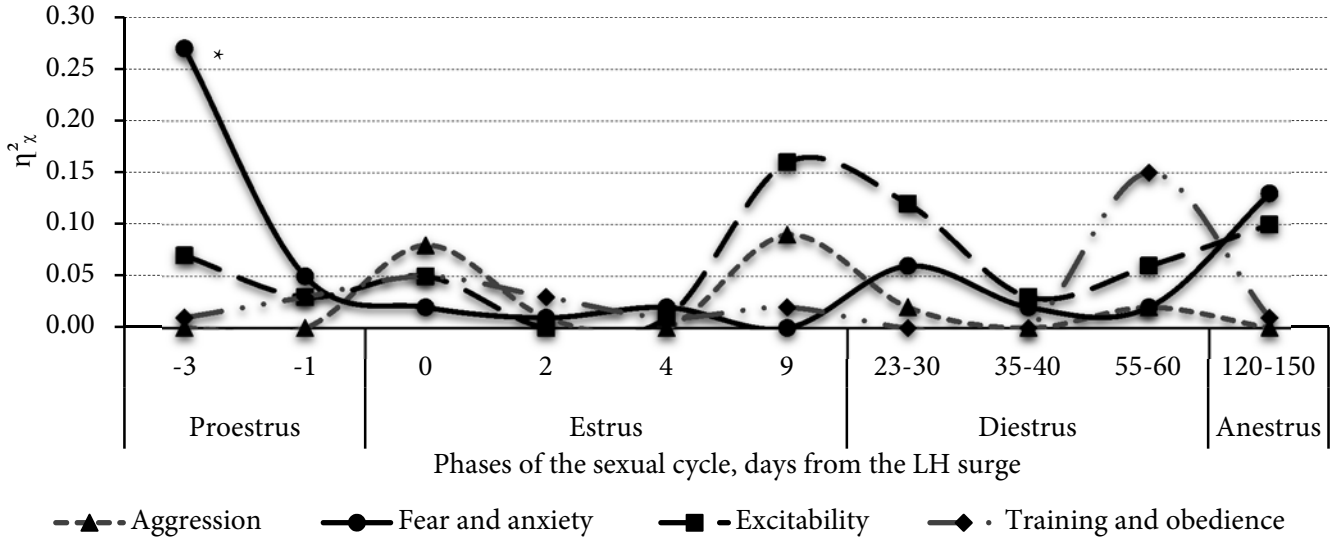

Figure 2. The strength of influence (η^2_χ) of individual temperament traits on the level of estradiol in the blood plasma of bitches (n = 15; units). Reliable values: * — $P \le 0.05$.

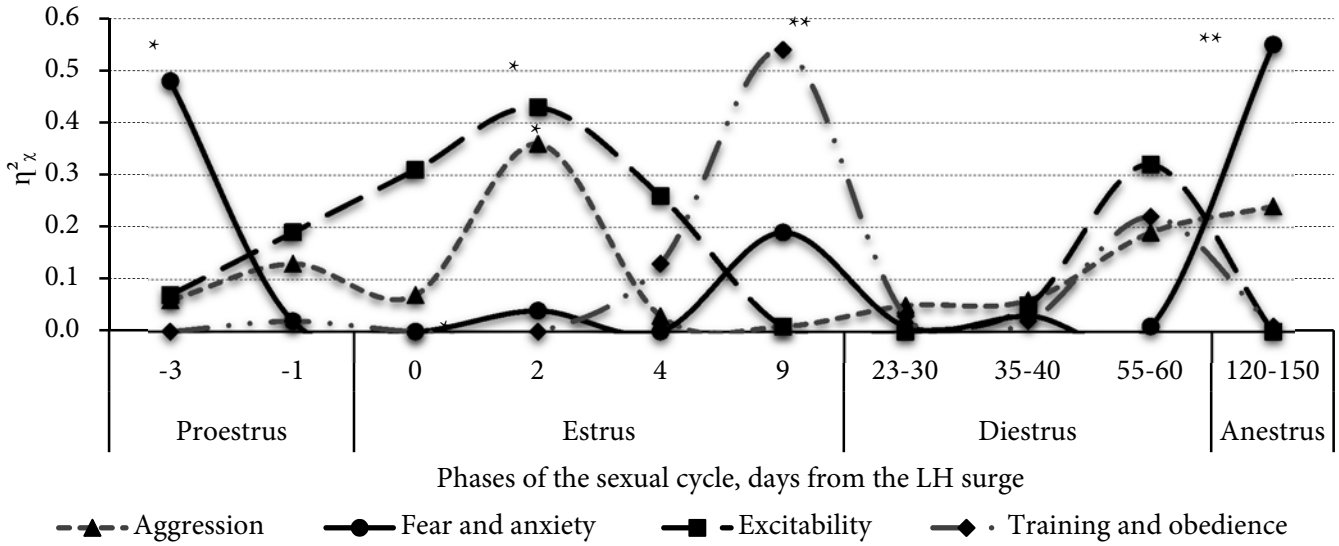

Figure 3. The strength of influence (η^2_χ) of individual temperament traits on the level of luteinizing hormone in the blood plasma of bitches (n = 15; units). Reliable values: * — $P \le 0.05$; ** — $P \le 0.01$.

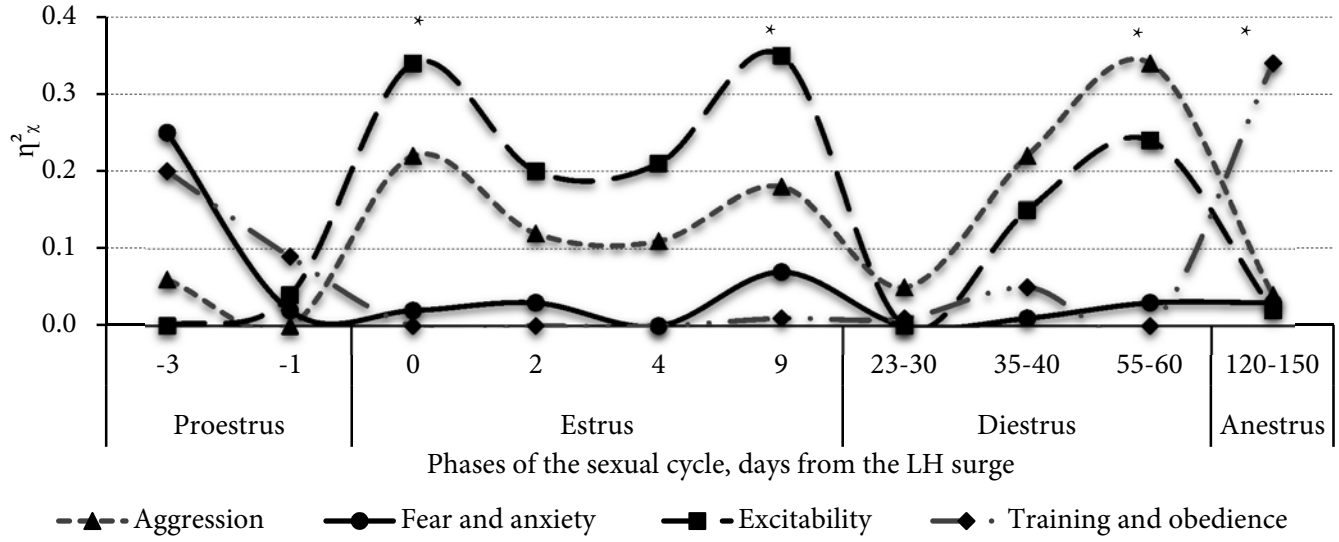

Figure 4. The strength of influence (η^2_χ) of individual temperament traits on the level of progesterone in the blood plasma of bitches (n = 15; units). Reliable values: * — $P \le 0.05$.

At the same time, the level of excitability of bitches had a significant effect on the hormone content on the day of the LH surge — $\eta^2_\chi = 0.34$ ($P \le 0.05$) and on the 9th day after the LH surge — $\eta^2_\chi = 0.35$ ($P \le 0.05$). In addition, a significant effect of trainability and obedience of bitches on the hormone content in blood plasma is manifested only on the 35th–40th days after the LH surge ($\eta^2_\chi = 0.34$; $P \le 0.05$). No significant effect of the level of fear and anxiety in bitches on P4 content was found.

Discussion. The C-BARQ was developed as a quantitative tool for measuring behavioral problems in domestic and working dogs (Serpell and Hsu, 2005). Today, this tool is used not only to characterize dog behavior, but also to create various regression models and factor analyses of the etiology of problematic behavioral traits (Canejo-Teixeira et al., 2018; González-Ramírez, Quezada-Berumen and Landero-Hernández, 2017). The population size and distribution of individual and group temperament traits in dogs have been studied, providing an explanation for the physiological origin of behavior (Zapata et al., 2022). The genetic basis of individual temperament traits has been identified (Hecht et al., 2021; Zapata et al., 2022; Morrill et al., 2022), which to some extent limits the condition of body systems, including the condition of the reproductive system. Specifically, we found that the level of fear and anxiety in bitches affected plasma levels of FSH, E2, and LH ($P \le 0.05$). Temperamental traits such as aggression, anxiety, and learning ability differ significantly between dog breeds and are heritable, but the neural basis for these differences is unknown (Hecht et al., 2021). The level of aggression in bitches has a significant effect on

the LH level two days after the surge ($P \le 0.05$) and on the P4 level on 55th–60th days after the LH surge ($P \le 0.05$). It is known that aggressive behavior is the most common type of undesirable behavior in dogs (Boyd et al., 2018). Some researchers note that the leading factor in aggressive behavior in dogs is the environment, not their temperament (Davis et al., 2012).

Significant correlations have been found between temperament and gray matter morphology. The neural connections involved in the 'flee or fight' response are associated with temperamental traits such as fear and aggression, which appear to be the main behaviors under selection pressure during domestication from wolf to dog (Hecht et al., 2021). At the same time, researchers have noted that dog excitability determines the diversity of temperament profiles (Rosati and Hare, 2013). We found that excitability limits the levels of sex hormones in the blood of bitches largely than other temperament traits. In particular, the level of excitability of bitches had a significant effect on the levels of FSH, LH, P4 on the day of the LH surge ($P \le 0.05$) and on the level of P4 on the 9th day after the LH surge ($P \le 0.05$) and on the levels of LH on the 2nd, 4th, 55th–60th days and after the surge ($P \le 0.05$).

The ability to learn is largely associated with expansion in broad areas of the cerebral cortex, whereas fear, aggression, and other 'problem' behaviors are associated with expansion in distributed subcortical areas (Hecht et al., 2021). Recently, experiments in dogs have confirmed earlier experiments in rats on the effects of prenatal stress on the ability to learn (Leroy et al., 2009). It has been found that the relationship between dog and owner is reflected in the temperament of the animal (Somppi et al., 2022). The ability to be trained and obedience limits the content of FSH in the blood plasma on the day before the surge and on the 4th, 9th, and 35th–40th days after the LH surge ($P \le 0.05$). Also, this characteristic of temperament affects the content of LH in the blood plasma of animals only on the 9th day after the LH surge ($P \le 0.01$). In addition, the ability to train and obedience of bitches has an effect on the content of P4 only on the 35th–40th days after the LH surge ($P \le 0.05$).

Researchers have noted quite significant individual differences in the levels of sex hormones in the blood of bitches (Luz et al., 2006). When the levels of P4 were studied in a group of Beagle bitches during the sexual cycle, these variations were significantly reduced (Marinelli et al., 2009). Thus, these differences are breed specific, which was confirmed in our studies. However, we are the first to identify individual differences in the humoral status of bitches that are associated with animal temperament traits.

Conclusions. It was found that the degree of fear and anxiety in bitches affects the content of follicle-stimulating hormone, estradiol and luteinizing hormone in the blood plasma of bitches three days before, during and 120–150 days after the LH surge ($\eta^2_\chi = 0.27–0.55$ ($P \le 0.05$). The excitability of bitches affects the level of follicle stimulating hormone, luteinizing hormone, and progesterone on the day of the LH surge — $\eta^2_\chi = 0.32$ ($P \le 0.05$). In addition, the level of excitability affects the level of progesterone on the 9th day after the surge — $\eta^2_\chi = 0.35$ ($P \le 0.05$) and the level of luteinizing hormone on the 2nd, 4th, 55th–60th days and after the LH surge — $\eta^2_\chi = 0.26–0.43$ ($P \le 0.05$). The degree of aggression affects the luteinizing hormone level two days after the surge and the progesterone level on 55th–60th days after the LH surge ($\eta^2_\chi = 0.34–0.36$; $P \le 0.05$). The ability to be trained and to obedience affects the follicle stimulating hormone level (the day before the surge and on the 4th, 9th, and 35th–40th days after the LH surge — $\eta^2_\chi = 0.30–0.52$; $P \le 0.05$), luteinizing hormone (on 9th day after LH surge, $\eta^2_\chi = 0.54$; $P \le 0.01$), and progesterone (on 35th–40th days after LH surge, $\eta^2_\chi = 0.34$; $P \le 0.05$).

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MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS OF BLOOD AND QUALITY OF MEAT OBTAINED FROM PIGS WITH DIFFERENT STRESS RESISTANCE

Chornyi M. V. [1], Stegniy B. T. [1], Vovk D. V. [1], Sazonenko S. M. [1], Kozyr V. S. [2], Mylostyvyi R. V. [3], Voroniak V. V. [4]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: nycvas@ukr.net
[2] Institute of Grain Crops of the National Academy of Sciences of Ukraine, Dnipro, Ukraine
[3] Dnipro State Agrarian and Economic University, Dnipro, Ukraine
[4] Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies of Lviv, Lviv, Ukraine

Summary. The results of the experiments were used to evaluate the morphological and protein composition of blood, as well as the quality of meat from pigs of different stress resistance. The studies were carried out under the conditions of PJSC 'Stepovyi' in Zaporizhzhia Region on Large White (LW) pigs and Large White/Charcoal Landrace (LWCL) crossbreeds. During the study period, the hygienic conditions of keeping, feeding and watering of the animals, their growth and development, clinical condition and morbidity were evaluated. After reaching a live weight of 100 kg, the pork meat quality (pH, moisture content, oxyproline, tryptophan) of slaughtered animals was studied. It was found that the LW genotypes were superior to the local LWCL by the number of leukocytes in passively resistant (PR) animals — by 2.7%, in stress resistant (SR) animals — by 3.15%, and in stress sensitive (SS) animals they were inferior by this indicator by 7.7%. There was no significant difference in erythrocyte content between SR and SS, but erythrocyte content was 8.53% lower in PR. The concentration of hemoglobin was higher in PR animals of the Large White cross. The latter were superior to LW in terms of total protein: PR — by 2.26%, SR — by 1.9%, in terms of albumin — PR had an advantage of 16.6%, SR — by 7.69% ($p < 0.05$). Gamma globulin content was 6.44% lower in SS animals. They were superior to LW in terms of live weight: PR — by 14% at one month of age; by 4.2% at four months of age, SR — by 6.2%, but the difference between SR and SS was not significant. PR animals of the Large White breed were superior in terms of hemoglobin content. The level of total protein in blood serum was higher in crossbred genotypes: in PR — by 2.26%, in SR — by 1.9%. The concentration of albumin was higher in the crossbred animals than in the LW: PR — by 16.6%, SR — by 7.69%, and the level of gamma globulins was 6.51% lower in the SS. In terms of live weight, crossbred genotypes outperformed LW: passive resistant genotypes — by 14% at one month of age, stress resistant genotypes — by 6.2% and 4.2% at four months of age. The crossbred genotypes reached 100 kg live weight: PR — at 180 days, SR — at 183 days, SS — at 191 days, which is 4, 5, and 12 days earlier than LW, respectively. In crossbred pigs, the positive correlation for thoracic girth was 0.6942 and 0.8310, and for withers height — 0.6643 and 0.6811. In terms of body length, animals of the crossbred genotype were superior to LW: PR by 4.2%, SR by 1.46%, while the difference in chest girth and body length in SS individuals was not significant. Lung weight was 0.84% higher in PR and 3.7% higher in SR, and kidney weight was 9.8% higher in PR, 6.56% higher in SR, and 1.37% lower in SS. LW animals were superior in heart weight. Behavioral responses (fighting, skirmishing) were more pronounced in the crossed genotypes, especially in SR and SS animals. A higher level of tryptophan was found in the meat of SR crossbred animals, oxyproline — in PR and SR, pH of meat — in Large White PR and SR ranged from 5.61 to 5.72 units, in crossbred animals this indicator did not exceed the values of 5.03–5.21 units. Defects in meat with PSE (pale, soft, exudative) signs of LW were found: in PR — 0.4%, SR — 0.63%, SS — 3.7%, and in LWCL respectively — 2.3%, SR — 2.1%, SS — 4.21%, in local — defects in meat with DFD (dark, firm, dry) signs were manifested in 3.1%, 2.15%, and 5.1%

Keywords: meat defects, PSE, DFD

Introduction. One of the main objectives of the pig industry is to protect and increase the resistance and productive potential of pigs to stressful influences, while also obtaining high-quality meat that is environmentally friendly in terms of sanitation (Cherniy et al., 2018; Chorniy et al., 2017; Kramarenko et al., 2019). Non-contagious diseases can limit pig production. These diseases can be caused by various factors, including non-compliance with the microclimate (which accounts for 60–80% of cases (Shchepetilnikov et al., 2019)), violation of feeding and watering regimens (Voronyak, Leskiv and Huberuk, 2018), early separation (Lukashchuk, Slivinska and Shcherbatyy, 2018), crowding and regrouping (Poroshinskaet al., 2020), non-compliance with the technological principle of 'all empty–all occupied' (Cherny et al., 2019), and inadequate feeding (Cherniy et al., 2018). There is limited research on 'factor infections' or 'high-tech pathologies', despite their significant impact on the intensive development of pig production. Breeding for lean meat in pig production has resulted in issues such as tail gnawing, limb and reproductive organ diseases, and pork with PSE and DFD defects (De Oliveira et al., 2018; Lucy and Safranski, 2017). According to research, biologically active additives

do not yield positive results in pig farms with unsatisfactory microclimates and inadequate breeding and selection practices (Cherniy et al., 2018, 2021). Therefore, in modern conditions, veterinary specialists and technologists should focus on disease prevention rather than treatment (Tucker et al., 2021).

In the past two decades, there has been significant research on the use of domestic and foreign pig breeds with high fertility and growth intensity (Chernenko et al., 2022; Khalak, Gutyj and Bordun, 2022).

However, some authors (Cherniy et al., 2018; Kozyr et al., 2019; Milostiviy, Karlova and Sanzhara, 2017) have reported that only 50–60% of the genetic productive capacity is realized due to inadequate housing conditions and non-compliance with breeding technologies. The analysis of contemporary data indicates that animal health, productivity, and product quality are influenced by environmental conditions by 60–80%, and by internal genetic factors by 20–40%.

The issue of high productivity in pigs with good meat quality is currently relevant. Breeding efforts have been focused on producing meat-type pigs with lower fat content in the carcass. Various genotypes of imported pigs, including Landrace, Duroc, Pietren, and Yorkshire, have been introduced to Ukraine in recent years. However, these breeds are highly sensitive to adverse environmental conditions and may struggle to adapt to new natural and climatic conditions (Khalak and Gutyj, 2020).

In intensive pig production, biosecurity and animal safety are crucial due to the need to maintain a sanitary regime and increase overall organism resistance. Natural factors of the biosphere such as air quality, feeding and watering regimes, and solar radiation play a significant role. Many experts argue that prevention is more effective than fighting diseases, as 80–90% of diseases are non-infectious. Only 10% of diseases are infectious. The production and rearing of piglets is the most critical stage in pig farming because they are highly sensitive to changes in room temperature (Lykhach et al., 2022; Zhyzhka, Povod and Mylostyvyi, 2019).

Imported pig breeds do not fully meet the requirements of practitioners due to low productivity and resistance. This is evidenced by early culling of sows, gastrointestinal and respiratory diseases in young animals, low resistance to temperature and humidity changes, high concentrations of harmful gases, and sensitivity to stress. Stress-sensitive animals can produce pale, soft, exudative meat in case of PSE defects and dark, dense, dry meat in case of DFD.

The **aim of the study** was to investigate the growth and development of animals, the characteristics of metabolic processes and to evaluate the quality of pork obtained from Large White (LW) and Large White/Charcoal Landrace (LWCL) animals with different resistance to stress. The evaluation of pork quality from genotypes with varying resistance is necessary due to limited research on changes in blood serum protein composition and morphological parameters in passively resistant (PR), stress-resistant (SR), and stress-sensitive (SS) animals.

Materials and methods. The experiments were conducted at PJSC 'Stepovyi' in Zaporizhzhia Region. The study involved 30-day-old LW and crossbred piglets (LWCL). To assess the stress sensitivity of piglets, we used the 'dorsal' test proposed by Hessing et al. (1993). This test involves placing the piglets in a dorsal position for one minute and recording their behavioral response. The animals selected were subsequently tested using the 'turpentine test' for resistance, according to the method of Kuznetsov and Sunagattulin (1991). The test involved injecting purified turpentine in a dose of 0.1 cm^3 intradermally from the inside of the ear, which caused a localized inflammatory reaction (erythema) of various sizes (26–32 mm) in the animals.

Aggressive animals (fighting, scuffling, anxiety, attempts to escape and biting) when kept in a dorsal position and with an erythema size of at least 32 mm at the site of turpentine injection were assessed as stress-sensitive (SS), calm animals, without piercing squealing and with an erythema size not exceeding 26 mm were assessed as passively resistant (PR), and animals with an attempt to escape without squealing and red spots on the abdomen and erythema sizes not exceeding 30 mm were defined as stress-resistant (SR). Taking into account the behavior and reaction to turpentine administration, the animals were divided into three groups according to stress sensitivity (Table 1). In particular, the number of PR animals among the LW genotypes was 23.06% ($p < 0.05$) higher than in the crossbreds, there was no significant difference in SR, and in SS they were inferior to LW by 23.61% ($p < 0.05$).

Table 1 — Distribution of pigs of different genotypes by resistance to stress

Genotype	Number of animals			
	Total	PR	SR	SS
LW	196	121 (61.73%)	51 (26.02%)	24 (12.24%)
LWCL	212	82 (38.67%)	54 (25.47%)	76 (35.85%)

The animals were then housed in stalls of 15–20 animals in a space of 0.9–1.3 m^2/ind. Hygienic conditions during the experiment (October 2019–November 2020) varied: air temperature — 16–18 °C, humidity — 72–78%, air movement speed — 0.2–0.3 m/s, illumination — 42–68 lux, air contamination with microflora — 95–120×10^3 CFU/m^3.

During the experiment, we monitored the clinical condition, growth, and development of the animals. We also examined their blood for morphological and biochemical parameters and recorded any instances of

animal morbidity. After reaching a body weight of 100 kg (at 180th–195th days of age), we studied the meat qualities of the slaughtered pigs.

To evaluate the health and metabolic processes of pigs, we utilized several methods: counting the number of leukocytes and red blood cells in the Goryaev chamber according to Vasilieva, hemoglobin concentration — by the hemoglobin-cyanide method, protein composition of raw blood — according to Chumachenko (1990), activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes according to Kondrakhin et al. (2004).

To investigate the quality of pork meat, we collected average samples from the muscular part of the carcass (*m. longissimus dorsi*) weighing 200 g. We then measured the active acidity (pH) using a millivoltmeter pH-125, determined the moisture retention capacity using the press method by Grau and Gamm in the modification of Volovynska and Kelman, and measured the oxyproline content using the Neumann-Logan method in the modification of Verbitsky and Deterej. Tryptophan content was determined by the Spies and Chambers method modified by Heller (Kovalenko, Gil'man and Orlova, 1987); microclimatic parameters (air temperature was measured by TKA-PKM/20, humidity by August's static psychometer), skin and ovary surface temperature by Nimbus-420 pyrometer (Antonenko et al., 2018).

Results and discussion. During the experiment, the growth and development of pigs were studied: height at the withers, width and girth of the chest (Table 2). These indicators characterize not only the development of individuals but also the size of their internal organs (Lykhach et al., 2020).

Table 2 — Measurements of pigs of different resistance

Resistance to stress	Genotype	Characteristics		
		Body length, cm	Chest girth, cm	Height at the withers, cm
PR	LW	147.3±10.9	98.2±1.2	68.2±0.7
	LWCL	158.4±0.73	102.4±1.18	69.8±0.63
	% to LW	107.38	104.27	102.36
SR	LW	143.4±0.86	96.1±0.8	66.2±0.7
	LWCL	154.8±0.71	98.1±0.67	67.4±0.52
	% to LW	107.98	102.15	101.76
SS	LW	148.5±0.51	97.6±0.54	67.2±0.38
	LWCL	156.3±0.41	98.4±0.66	68.1±0.42
	% to LW	106.88	100.84	101.35

High live weight at 6 months of age was characterized for LWCL pigs, they had a positive correlation in chest girth (0.6942 and 0.8310), height at the withers (0.6643 and 0.6811), respectively, with age, these differences persist. In terms of body length, animals of the LWCL genotype were superior to LW: PR — by 4.2% ($p < 0.05$), SR — by 1.46 ($p < 0.05$). The crossbred animals were superior to LW in body length: PR — by 7.38 %, chest girth by 4.27 %, height at the withers — by 2.36 %; SR were inferior to PR in these indicators, but superior to SS in body length by 7.98 %, chest girth and height at the withers, but the difference was not significant.

Growth and development of pigs. Live body weight (Table 3) is an integral indicator that characterizes the health of pigs (Chernenko et al, 2022; Khalak and Gutyj, 2020).

Table 3 — Dynamics of live weight of pigs with different stress resistance (M ± m, n = 5)

Age, days	Genotype	Live weight, kg		
		PR	SR	SS
25–30	LW	5.61±0.12	5.57±0.17	5.84±0.11
	LWCL	6.40±0.16	5.84±0.30	5.70±0.09
	% to LW	114.2	104.8	97.6
120–125	LW	42.70±2.07	43.10±0.96	40.30±1.70
	LWCL	44.50±8.10	45.80±1.24	38.10±1.20
	% to LW	104.2	106.2	94.7
180–185	LW	71.30±2.4	72.10±1.68	73.90±3.21
	LWCL	73.60±1.85	72.40±1.43	74.40±2.30
	% to LW	103.35	100.4	100.66
210–215	LW	88.40±1.52	91.40±0.51	94.20±2.60
	LWCL	92.10±1.60	93.60±0.38	96.80±1.40
	% to LW	104.1	100.2	102.7

A study on pigs (LW and LWCL) with different resistance levels showed that crossbreds grew more intensively. At four months of age, PR exceeded LW by 4.2% in live weight, SR by 6.2%, and SS lagged behind in growth by 5.3% (Table 3). This pattern persisted in the future. From six months of age, there was no difference in live weight between mixed SR and SS animals compared to LW. PR animals of the Great White grew less intensively than LWCL offspring. At six months of age, their weight was 72.1 ± 1.68, which is 2.95% less ($p < 0.05$).

It is worth noting that the mixed animals reached a weight of 100 kg: PR in 176 days, SR in 180 days, SS in 191 days, and LW in 180, 190, and 203 days, respectively.

These measurements characterize not only the morphological and functional state of the organism, but also the development of internal organs. According to our research results, the breast index (61.8 %) was the highest in PR and SR animals, both purebreds and crossbreds, and the lowest (58.6 %) in SS, which confirms the results of previous studies (Poroshinska et al., 2020; Shchepetilnikov et al., 2019).

An indicator that characterizes the quality of meat is the area of the 'muscle eye' (ME), the thickness of the fat over the 6th–7th thoracic vertebrae and the lumbar region.

The area of the ME in LWCL — SR and PR pigs was 31.8 and 32.4 cm^2, and SS — 29.7 cm^2, in crossbreds — 32.8–36.5 ± 0.6 cm^2 and regardless of resistance, they exceeded purebreds by 3.1–12.6%. These results are consistent with the data of other scientists (Lykhach et al., 2020). It is considered the norm that this indicator is: in PR and SR of the Great White — 32.8 ± 0.36 cm^2 and 39.0 ± 0.5 cm^2 in LWCL — 36.5 ± 0.6 cm^2.

Between animals with different resistance, there was no significant dependence on the development of internal organs (Table 4).

Table 4 — Indicators of internal organs weight in pigs of different resistance at slaughter when reaching a weight of 100 kg

Parameter	Genotype	Group		
		PR	SR	SS
Lung weight, g	LW	714.30±3.62	718.00±3.70	704.10±4.10
	LWCL	720.10±2.43	710.10±3.01	730.60±5.20
	% to LW	100.8	98.8	103.7
Heart weight, g	LW	385.10±7.3	345.40±6.80	341.20±7.40
	LWCL	347.00±2.10	338.50±3.11	336.10±1.90
	% to LW	90.1	98.0	98.5
Kidney weight, g	LW	142.20±4.10	152.30±5.20	158.60±3.70
	LWCL	156.00±1.70	162.30±2.20	150.50±2.40
	% to LW	109.8	106.56	98.67
Weight of the thyroid gland, g	LW	6.01±0.07	5.94±0.07	5.91±0.06
	LWCL	5.89±0.06	6.02±0.09	6.12±0.04
	% to LW	98.0	101.3	103.5

The analysis of the data indicates that there was no significant difference in the development of internal organs between the Large White and Large White/Charcoal Landrace (LWCL). However, in PR crosses, the lung weight was 0.84% higher, and in SS, it was 3.7% higher. The kidney weight was 9.8% higher in PR and 6.56% in SR ($p < 0.05$), while in SS, it was 1.37% less. Crossbred animals had a higher weight of the thyroid gland. In the study, heart weight was found to be higher in LW animal — PR by 9.9% ($p < 0.05$), SR — by 2% and SS — by 1.5%.

Hematological parameters in pigs. Blood is a reflection of physiological processes (Table 5) occurring in the body, and its indicators are indicators of their health (Kozyr et al., 2019).

Thus, the PR animals of the Great White breed outnumbered LWCL by leukocytes — by 2.75%, SR animals — by 3.15%, and SS animals were 7.7% inferior to LW ($p < 0.05$). There was no significant difference in the number of erythrocytes in SS and SR, but their content was 6.27 and 6.75% less, especially in PR — by 8.53% ($p < 0.05$), in SS and SR the hemoglobin concentration was higher by 5.7% ($p < 0.05$) and 1.3%, and PR pigs were 3.8% behind LW in this indicator ($p < 0.05$). Thus, in terms of red blood cells and leukocytes, the interbreeding animals were inferior to LW, and in terms of hemoglobin concentration they were superior to them ($p > 0.5$).

Table 5 — Changes in morphological parameters of blood of pigs with different resistance (M ± m, n = 5)

Parameter	Genotype	Group		
		PR	SR	SS
Leukocytes, g/l	LW	8.48±0.25	8.27±0.14	8.15±0.22
	LWCL	8.29±0.30	8.01±0.27	7.50±0.31
	% to LW	97.75	96.85	92.3
Erythrocytes, T/l	LW	6.23±0.11	6.78±0.09	7.04±0.16
	LWCL	5.84±0.20	6.32±0.18	6.44±0.16
	% to LW	91.47	93.21	93.73
Hemoglobin, g/l	LW	103.00±2.16	98.70±0.31	95.70±0.21
	LWCL	99.10±1.80	100.20±0.52	101.20±0.25
	% to LW	96.2	101.1	105.7

The state of health of pigs and the intensity of metabolic processes in their body were evaluated by biochemical parameters of blood (Table 6) and the level of aminotransferases.

Table 6 — Protein composition of blood serum of pigs with different stress resistance (M ± m, n = 5)

Parameter	Genotype	Group		
		PR	SR	SS
Total protein, g/l	LW	79.40±1.85	76.20±1.90	79.30±2.12
	LWCL	81.20±2.10	77.65±2.50	79.51±2.07
	% to LW	102.26	101.90	100.26
Albumins, %	LW	42.70±1.70	40.30±7.85	42.41±1.52
	LWCL	43.80±1.20	43.40±1.70	43.16±1.50
	% to LW	116.66	107.69	101.76
Globulins, %	LW	57.30±1.70	59.70±1.52	57.51±1.12
	LWCL	56.20±2.30	56.40±1.17	57.90±1.17
	% to LW	98.25	94.4	100.52
γ-Globulins, %	LW	12.60±0.52	12.87±0.31	13.06±0.41
	LWCL	12.33±0.43	12.38±0.37	12.21±0.36
	% to LW	97.85	79.19	93.41

The level of total protein is a crucial homeostasis constant that characterizes metabolic processes involving protein. According to the data analysis in Table 6, the amount of total protein in LWCL animals is higher than in LW: PR by 2.26%, SR by 1.9%, and SS by 0.26%. However, the overall amount of total protein falls within the normal range, and in the crossbreds, it is higher. Regarding albumin content, crossbred animals maintained an advantage: in PR — by 16.6%, SR — by 7.69% ($p < 0.05$), SS — by 1.76%.

The level of gamma globulins, which act as carriers of immune protection, was higher in LW — PR by 2.15% and 3.81% ($p < 0.05$). Therefore, PR and SR animals were superior to LW in terms of total protein and albumin content, but inferior in terms of gamma globulins due to lower productivity, as confirmed by a high survival rate of 92.1%.

Aminotransferases are important in cellular metabolism as they participate in reactions of transamination and are at the junction of the pathways of nitrogen, carbohydrate, and fat metabolism. They also regulate the glycolysis-glycogenolysis system. The highest activity of aminotransferases was found in LWCL pigs. The level of AST in PR and SS crossbred animals was in the range of 0.74–0.76 mmol/l, which is 9.31% and 11.25% higher ($p < 0.05$) than in LW analogues. ALT level, regardless of genotypes, was 0.31–0.33 mmol/l.

Physical characteristics of pork. The study evaluated physical parameters in meat samples (*m. longissimus dorsi*) and the results are presented in Table 7.

Table 7 — Indicators of moisture, fat and protein in meat of pigs with different resistance (M ± m, n = 3)

Parameter	Genotype	Group		
		PR	SR	SS
Moisture retention capacity, %	LW	64.00±0.30	63.62±0.20	62.73±0.20
	LWCL	55.30±2.40	58.40±1.35	46.90±1.20
	% to LW	86.4	91.7	74.8
Protein mass fraction, %	LW	20.86±0.13	21.10±0.12	20.02±0.10
	LWCL	20.74±0.38	20.63±1.20	20.37±0.60
	% to LW	94.4	97.7	101.7
Intramuscular fat,%	LW	4.79±0.09	4.56±0.11	4.17±0.27
	LWCL	3.86±0.30	4.13±0.36	3.94±0.20
	% to LW	80.5	90.57	94.4
Ash, %	LW	1.18±0.20	1.21±0.40	1.08±0.01
	LWCL	1.02±0.20	1.14±0.40	0.82±0.01
	% to LW	92.70	95.02	75.9

Among the carcasses of LW pigs, passively resistant pigs had the highest moisture retention capacity, while the lowest was observed in SS. In LWCL-SS animals this indicator did not exceed 46.9 ± 1.2%. In terms of protein content, they were inferior to purebred PR and SR by 5.6% and 2.3%, respectively ($p < 0.05$). The presence of fat tissue gives pork a high caloric content and makes it tender, juicy, and flavorful. In the LW group, its content was found to be 19.5%, 9.43%, and 5.6% higher ($p < 0.05$).

By the diameter of muscle fibers (29.71 ± 0.03 μm), raw meat from LW was inferior to the passive resistant and stress resistant by 13.6% and 18.4%, and the marbling pork (fatty interfascicular layer), on the contrary, was superior to the crossbreeds. Thus, according to the standard, marbling in PR was 32%, in LW its index was within the limits: SR — 31.02%, SS — 29.6%, which is lower, respectively, than in crossbreds ($p < 0.05$).

For consumers, the ratio of amino acids in meat is just as important as their content. This is especially true for nonessential amino acids, such as oxyproline, which can make pork tougher and less easily digested by the human body (Table 8).

Table 8 — Amino acid composition of pork meat with different resistance (M ± m, n = 3)

Parameter	Genotype	Group		
		PR	SR	SS
Tryptophan, mg %	LW	3.15±0.01	3.20±0.01	2.76±0.02
	LWCL	2.70±0.01	2.79±0.01	2.53±0.01
	% to LW	85.71	97.18	91.66
Oxyproline, mg %	LW	0.54±0.01	0.51±0.01	0.51±0.01
	LWCL	0.51±0.02	0.55±0.01	0.52±0.01
	% to LW	94.41	92.72	99.07
Protein quality index (PQI)	LW	5.83	5.81	4.86
	LWCL	5.29	5.47	5.41
	% to LW	90.70	94.10	89.83
pH, units	LW	5.61	5.72	5.12
	LWCL	5.39	5.21	
	% to LW	96.90	96.90	98.20

The concentration of ions in meat (pH) depends on the amount of lactic acid formed from glycogen 24 hours after slaughter. According to the pH level, the indicator in PR animals was in the range: 5.39–5.61 units, in SR — 5.21–5.72 units, in SS — 5.03–5.12 units. In general, this indicator in LW pigs (PR and SR) was higher (5.61–5.72 units), which indicates the good quality of the products obtained and the intensity of the maturation process, which increases the resistance of meat to microflora and a long shelf life. A higher level of tryptophan was in LW meat — 3.2 ± 0.01 ml%, oxyproline — in PR and SR.

Defects in meat with PSE: LW passively resistant animals had a defect rate of 0.4%, while SR had a rate of 0.63% and SS had a rate of 3.7%. For DFD, the defect rates were 1.3%, 2.5%, and 5.1% for PR, SR, and SS, respectively. In crossbreed LWCL with PSE abnormalities were found in 2.3%, 2.1%, and 4.2% for PR, SR, and SS, respectively. For DFD defect the rates were 3.1%, 2.15%, and 5.4%, respectively, which is consistent with the results of other researchers (Lykhach et al., 2022).

The ethological features of pigs with different resistance levels have not been thoroughly studied, including their time for feed eating, lying down, and leadership in the group. Our findings indicate that PR individuals exhibit calm behavior and spend an average of 18–20 minutes more time eating feed than crossbreeds, especially SS. Studies have shown that

during 12 hours of daily time, the number of conflicts among LW is significantly lower for passively resistant (5–6 times) and SR (15–18 times) compared to SS (58–61 times). The number of conflicts in crossbreed LWCL was: PR — 11–13, SR — 21–33, SS — 121–154.

Conclusions. In the conditions of intensive pig breeding, along with selection for productivity, animals should be evaluated to identify individuals for resistance to abio- and biological factors. Testing by the 'turpentine test' revealed in the LW genotype: passive-resistant (PR) — 61.73%, stress-resistant (SR) — 26.02%, stress-sensitive (SS) — 12.25%; in the LWCL genotype, respectively — 38.57%, 25.35%, and 36.07%. Evaluation of pigs by interior indicators makes it possible, firstly, to assess their health, and secondly, to predict how much quality raw meat can be obtained from them. Pork from LW with a pH value of 5.61–5.72 units, tryptophan content of 3.15–3.20 mg%, oxyproline 0.51–0.53 mg%, moisture content 62–64%, and from LWCL, respectively, 5.21–5.03 units, 2.70–2.79 mg%, 0.54–0.55 mg%, 55–58 mg% should be considered as high-quality in terms of sanitation and technology and be classified as high grade.

Breeding pigs only for productivity (average daily gain of at least 550 g, reaching a live weight of 100 kg in 165–185 days) and obtaining lean meat has led to increased sensitivity of animals to the PSS (porcine stress syndrome), and the meat from such carcasses is called PSE (pale, soft, exudative) or DFD (dark, firm, dry). The desire of farmers to shorten the fattening period (6–6.5 months) is not always justified, as animals accelerate weight gain due to sarcoplasmic and sarcolemma proteins, and muscle and adipose tissue do not have time to reach physiological maturity.

Prospects for further research. The issue of determining the level of resistance of pigs of different breeds, both domestic and imported, to abio- and biotic factors requires additional comprehensive study. This will ensure the production of technologically high quality and safe meat.

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THE STATE OF THE CARDIOVASCULAR SYSTEM IN NORMAL AND OBESE PONIES ACCORDING TO THE RESULTS OF CARDIOGRAPHIC STUDIES

Borovkov S. B. [1], **Borovkova V. M.** [2]

[1] National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', Kharkiv, Ukraine, e-mail: serg_b78@ukr.net
[2] State Biotechnological University, Kharkiv, Ukraine

Summary. Horses and ponies have physiological peculiarities in their cardiovascular system in comparison with other species as well as among themselves. Electrocardiogram (ECG) studies in ponies help to elucidate the peculiarities of cardiovascular system functioning, to establish reference values for parameters and to perform early diagnosis of arrhythmias. Arrhythmias, or irregular heart rhythms, can significantly affect the health of horses. Regular ECG screening can help detect early signs of cardiovascular disease such as myocarditis, valvular disease and congenital heart defects. Since horses and ponies come in different breeds, each with unique cardiovascular characteristics, this study aims to establish normative electrocardiogram parameters for ponies to allow for more accurate interpretation of electrocardiographic data. The study involved 18 ponies, aged 4–9 years, in a relatively calm state. Nine animals formed the control group and nine with signs of obesity formed the study group. All animals underwent a general clinical examination using widely accepted methods. Electrocardiographic studies were performed using the BeeW recorder, a state-of-the-art miniature electrocardiograph that allows registration, analysis and interpretation of electrocardiograms over the Internet using an Android tablet, smartphone or laptop. The study showed that the average heart rate (HR) for all ponies was 44.6 ± 2.1 bpm. No cases of sinus bradycardia with HR below 24 bpm were observed. Thirteen animals (72.2%) had HR in the range of 24–42 bpm, while five animals (27.8%) had sinus tachycardia. ECG findings showed positive P waves in 10 animals (55.5%) and negative P waves in 8 (44.5%). Positive T waves were observed in 5 animals (27.8%), while 13 (72.2%) had negative T waves. The QRS complex was predominantly a qR pattern with variations including QR, Qr, and qRS. In addition, two animals had sinus tachycardia and two had atrioventricular block

Keywords: arrhythmia, sinus tachycardia, atrioventricular block

Introduction. Currently, ponies are of interest as companion animals that can improve the emotional and psychological state of people. However, information on the normative indicators of electrocardiographic studies in ponies in normal and pathologies of the cardiovascular system is practically absent in the domestic scientific literature (Maksimovich, 2014, 2016). In the publications of foreign researchers there are reports of electrocardiographic studies in small breeds of horses, including ponies (Van Vollenhoven et al., 2016; Pasławska et al., 2018; Durham, 2017). These studies have established that horses and their varieties of ponies have anatomical and physiological differences, therefore the use of clinical data, treatments and diagnostic protocols from horses to ponies can cause diagnostic errors and inappropriate therapeutic intervention (Pedersen et al., 2016; Santarosa et al., 2016). Clinical signs of cardiovascular disease, or their absence, are important factors in assessing the possible health status of animals, but given the considerable compensatory capacity of the equine heart, clinical signs are only manifested in cases of severe cardiac dysfunction or during intense exercise. It should also be noted that the sounds and arrhythmias commonly heard in horses are often of physiological origin and have no pathological significance. Arrhythmias during or immediately after exercise are common in sport horses (Mathapati and Saini, 2019). Digital telemetry electrocardiogram recording systems are lightweight and portable and can be used to obtain real-time digital monitoring and recording at rest or during exercise (Piketh, 2019; Houben, Vernooij and Sloet Van Oldruitenborgh-Oosterbaan, 2021). There is little information in the literature on the normative indicators of electrocardiograms in ponies, as well as the presence of arrhythmias of different types.

Therefore, the **aim of the study** was to establish normal electrocardiographic parameters in normal and obese ponies and to illustrate electrocardiographic parameters at rest and with some types of arrhythmias.

Materials and methods. The ponies kept in the Regional Landscape Park 'Feldman Ecopark' (Lisne, Kharkiv District, Kharkiv Region) were studied, namely 18 ponies, male and female, aged 4–9 years, in a state of relative rest. The research was carried out in 2018.

All animals underwent a general clinical examination according to generally accepted methods. Electrocardiographic studies were performed using the BeeW recorder, a state-of-the-art miniature electrocardiograph that allows recording, analysis, and interpretation of electrocardiograms via the Internet using an Android tablet, smartphone, or laptop. It is a professional quality electrocardiograph. Its main parameters meet international requirements (bandwidth, time constant, etc.). Variant of the BeeW recorder: 4-wire

6-channel. The electrodes were attached to the skin with alligator clips, the skin was previously moistened with alcohol. Recordings were made by placing the electrodes according to Domrachev–Vaskanian: red — in the area of the elbow tubercle of the left forelimb, yellow — in the elbow tubercle of the right forelimb, green — in the area of the right knee crease, black — in the left knee crease. For each electrocardiographic recording, heart rate, rhythm, P wave duration and amplitude, PR interval duration, QRS complex duration, R wave amplitude, S wave amplitude, QT interval and QTc duration, and T wave duration and amplitude were analyzed.

During the experimental studies described in this work, all manipulations with ponies involved in the research were were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017).

Statistical analysis of the data was performed using the Microsoft Excel analysis package.

Results and discussion. The mean heart rate for 18 ponies was 44.6 ± 2.1 bpm. Accordingly, no animal had manifestations of sinus bradycardia and a heart rate below 24 bpm, 13 animals (72.2%) had a heart rate in the range of 24–42 bpm, and five animals (27.8%) had sinus tachycardia. In addition, the heart rate in obese horses was significantly higher than in clinically healthy ponies, 48.6 ± 2.6 bpm versus 39.2 ± 1.9 bpm ($p < 0.01$). The average heart rate found in our study is higher than that of horses, suggesting that there are differences in autonomic nervous system activity between ponies and horses. Heart rate variability can be used to diagnose the autonomic nervous system and assess the cardiovascular system.

According to Mitchell and Schwarzwald (2021) and Lorello et al. (2019), a regular sinus rhythm with a resting heart rate of 24 to 44 bpm is the most common rhythm found in horses. Adult, physically fit horses are known to have high vagal tone at rest, resulting in a low heart rate. According to Cruz-Aleixo et al. (2023) and Decloedt et al. (2021), and our studies, the average heart rate in ponies is slightly higher than in horses, suggesting that equine reference values should not be used to assess parameters in ponies.

For a complete assessment of the electrocardiogram, it is important to evaluate the waves and intervals. Thus, according to the results of the study in ponies, positive P waves were observed in 10 animals (55.5%), negative in 8 animals (44.5%), positive T waves in 5 animals (27.8%), negative in 13 animals (72.2%). The electrocardiogram showed a predominance of the QRS complex form of the qR type, but other variants were also observed, such as QR, Qr, qRS (Fig. 1).

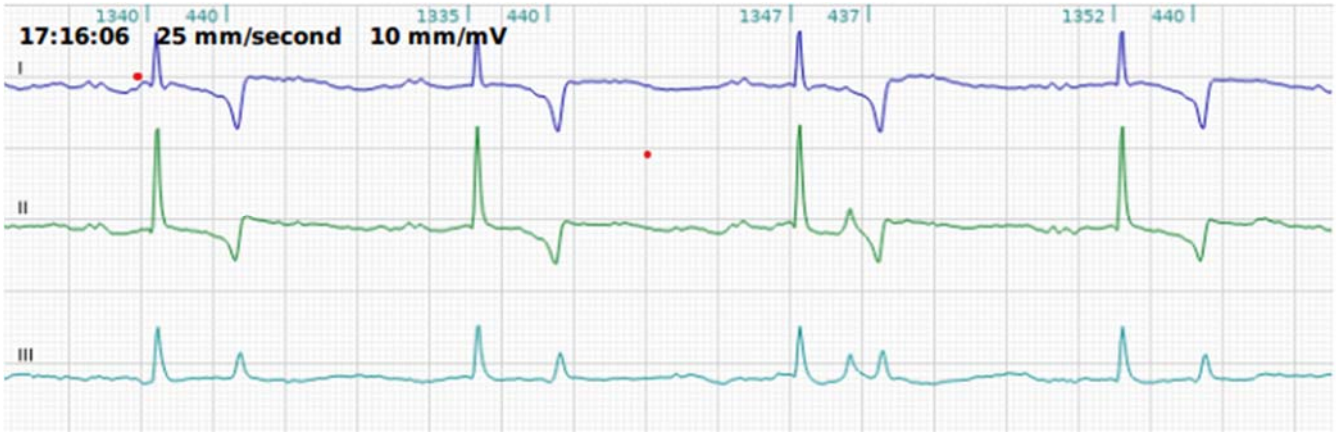

Figure 1. Electrocardiogram of a pony, 6 years old, type qR and bifurcated P waves.

In most of the animals, a split wave P was recorded, similar to that recorded in the studies of other authors. In addition, according to Chope (2018) and Navas De Solis et al. (2014), a QRS complex of the rS type is recorded in ponies, which is different from our studies. We believe that these differences may be related to different breeds and possibly the sex of the pony, as the authors mention that they found the QS pattern only in females (Tzelos, Blissitt and Clutton, 2015; Zuber, Zuber and Schwarzwald, 2019). It should be noted that the directionality of the teeth was not significantly different between clinically healthy and obese ponies (Fig. 2). Weis et al. (2022) and Van Loon (2019a, 2019b) also found certain types of physiological arrhythmias on the ECG. These include second-degree atrioventricular block and sinus arrhythmias. The true prevalence and frequency of these arrhythmias is likely underestimated in this study because the recordings were short and the use of an ECG and the presence of a physician can alter autonomic tone (Hanka, Van Den Hoven and Schwarz, 2015; Van Loon, 2019a, 2019b). For a complete assessment of heart rate in horses and ponies, researchers suggest performing daily

ECG recordings using Holter monitoring (Sebdani et al., 2019; Vezzosi et al., 2019), which may be the prospect of further research. The Fig. 3 shows atrioventricular block II in a non-obese pony. It should be noted that in the group of obese horses, one case of this block was also recorded (Heliczer et al., 2017; McDuffee et al., 2019).

Figure 2. Electrocardiogram of an 8-year-old pony with sinus tachycardia due to obesity.

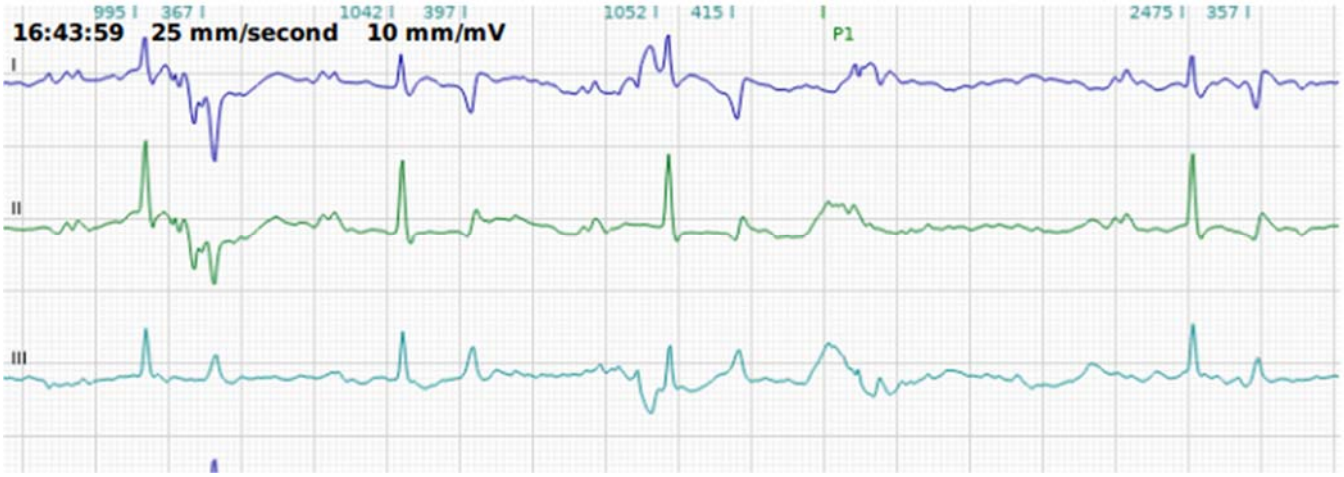

Figure 3. Electrocardiogram of a non-obese pony, 7 years old, with degree II atrioventricular block.

Conclusions. The average heart rate (HR) for all ponies was 44.6 ± 2.1 bpm. No cases of sinus bradycardia with HR below 24 bpm were observed. Thirteen animals (72.2%) had HR in the range of 24–42 bpm, while five animals (27.8%) had sinus tachycardia. ECG findings showed positive P waves in 10 animals (55.5%) and negative P waves in 8 (44.5%). Positive T waves were observed in 5 animals (27.8%), while 13 (72.2%) had negative T waves. The QRS complex was predominantly a qR pattern with variations including QR, Qr, and qRS. In addition, two animals had sinus tachycardia and two had atrioventricular block.

Electrocardiogram is an affordable and informative method to assess the state of cardiovascular system in horses and ponies in normal and pathological conditions.

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Part 2. Biosafety

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MONITORING OF METHODS FOR IDENTIFYING RAW MEAT IN SAUSAGE PRODUCTS

Khimych M. S., Rodionova K. O.

Odessa State Agrarian University, Odessa, Ukraine,
e-mail: khimichms@gmail.com, katerina.rodionova@ukr.net

Summary. Despite the growing global interest in healthy lifestyles and nutrition, there is still a demand for ready-to-eat meat products. Sausage products are one of the traditional foods for Ukrainians. National standards (DSTU) provide requirements for the recipe, nutritional value, and physical and chemical parameters that each type of sausage product must meet. However, the high cost of raw materials, shortages, and the need for rational use of resources contribute to the falsification of these products. Modern researchers offer various analytical methods to identify and quantify the content of specific components in finished meat products. Despite their effectiveness, these methods are not yet standardized. As a result, the imperfections in the national legislative, methodological, and technical framework complicate the identification process, leading to an increase in falsification in sausage products

Keywords: falsification, quality, safety, control

Introduction. Providing the population of Ukraine with quality food is of paramount social and epidemiological importance. It is a matter of national health and sustainable development of society. However, the interest in quality and safe food is growing not only in our country but also around the world. Therefore, the demand for healthy food is increasing in developed countries. However, the trend towards healthy eating is often at odds with the need for convenience, leading many to buy ready-made meals and semi-finished products (Paliy et al., 2020; Khimych and Rodionova, 2021).

Meat and meat products are a source of many important nutrients in the human diet, the main one being protein (Qu et al., 2022; Moroz and Sydor, 2023).

Sausages are the most popular among the variety of meat products. For centuries, sausages have been consumed around the world and have become an important element of the gastronomic heritage of many countries. Their attractiveness to consumers is due to their good taste, high nutritional value, and the ability to be consumed without additional heat treatment (Halagarda, Kędzior and Pyrzyńska, 2018; Montowska and Spychaj, 2018; Paliy et al., 2020).

Meanwhile, an examination of the sausage market reveals a significant amount of falsification of sausage products. As per local specialists (Kotelevych and Larina, 2020; Khimych et al., 2020; Verkhivker et al., 2023), up to 80% of sausages sold in retail are falsified by one or more indicators. Controlling the safety and quality of meat and meat products remains an urgent problem today, considering such disappointing statistics (Bogatko, et al., 2017; Naaum et al., 2018; Visciano and Schirone, 2021; Miedico et al., 2022; Sangaré and Karoui, 2023).

The primary means of detecting falsification is through the identification procedure. This procedure confirms or denies whether the product-specific parameters and properties comply with those stipulated in the relevant technical and regulatory documentation (Zhornyk and Yanchenko, 2020; Bondarenko, 2021; Drychyk and Chorna, 2021).

The imperfections in the national legislative, methodological, and technical frameworks complicates the identification procedure, which contributes to the growth of falsification (Bondarenko, 2021; Verkhivker et al., 2023).

Therefore, analyzing the methodology and implementing modern, effective methods of identification is crucial in ensuring the quality and safety of sausage products.

The **aim of the study** is to monitor the methods of identification of meat raw materials in sausage products.

Analysis of literary sources. National standards (DSTU) for sausage products include requirements for the recipe, nutritional value, and physical and chemical parameters that each type of sausage product must meet. The same standards provide for verification of compliance of finished products with these requirements (Khimych et al., 2020; Bondarenko, 2021).

The standard procedure for the identification of sausage products involves the use of sensory (organoleptic) and laboratory (rheological, chemical and biochemical) methods (Montowska and Spychaj, 2018; Bondarenko, 2021).

Experts can use sensory analysis to determine indicators such as shape, appearance, color, texture, taste, smell, and type of minced meat in a cut to characterize the consumer properties of sausage products. This method is advantageous due to its accessibility, speed, and ease of implementation. However, it also has significant drawbacks, including a high level of subjectivity and low reliability. To ensure a reliable analysis, experts typically employ various analytical methods (Pospiech et al., 2019; Bandura et al., 2021; Zhang et al., 2023).

The nutritional value of meat products depends on the composition of raw materials and the appropriate ratio of individual components. This is also a key factor in determining the product's attractiveness to consumers (Halagarda, Kędzior and Pyrzyńska, 2018).

However, high prices, shortages, and the need for rational use of raw materials contribute to the spread of sausage product falsification. The emergence of new forms of counterfeiting with meat products is also attributed to the development of food technologies. The most common way to falsify meat products is by replacing meat components with lower-cost and lower-quality ones, which often results in a decrease in the nutritional value of the products (Naaum et al., 2018; Shehata et al., 2019; Hassoun et al., 2020; Verhivker et al., 2023).

Today, various methods and technical tools are available for identifying and quantifying the content of a particular component in a product (Pospiech et al., 2019).

Microstructural analysis is a standard and effective method for identifying raw materials used in sausage production (Ince and Özfiliz, 2018; Paliy et al., 2020; Nazarenko et al., 2022; Verhivker et al., 2023). The method aims to identify the structural components of sausage products, including those of animal, vegetable, and artificial origin. Microscopy of stained histological preparations enables clear and accurate differentiation of various tissue elements and cellular structures. This allows for qualitative and quantitative determination of the components of minced meat (Evstafieva et al., 2017; Tishkina, Lieshchova and Iesina, 2018; Guelmamene, Bennoune and Elgroud, 2018; Mokhtar et al., 2018).

However, this method also has significant drawbacks. The process of making and examining a histological preparation is time-consuming, laborious, and requires a high level of expertise. Additionally, specific preparation is necessary during the sample processing, which affects the morphology of the sample. For instance, fat inclusions may be damaged or washed out during the preparation process (Łaszkiewicz, Szymański and Kołożyn-Krajewska, 2019).

Furthermore, advancements in food technology, including the development of new food additives and improvements in the production of mechanically deboned meat, have contributed to the continuous evolution of means and methods of falsification. As a result, standard quality assurance methods may not be sufficient (Łaszkiewicz, Szymański and Kołożyn-Krajewska, 2019; Sangaré and Karoui, 2023).

Recent scientific research demonstrates new analytical methods for identifying the composition of meat products and, in particular, sausages. These methods are based on total reflection X-ray fluorescence (Dalipi et al., 2018), sample irradiation combined with electron spin resonance (Tomaiuolo et al., 2019), X-ray microcomputed tomography (Pospiech et al., 2019), ion chromatography combined with electrical conductivity (Iammarino et al., 2021), ultrasonic analysis (Wieja et al., 2021), inductively coupled mass spectrometry (ICP-MS) (Miedico et al., 2022), nuclear magnetic resonance (NMR) (Sangaré and Karoui, 2023). Raman spectroscopy (Wubshet et al., 2019; Qu et al., 2022) and near-infrared (NIR) spectroscopy (Kademi, Ulusoy and Hecer, 2019; Beć, Grabska and Huck, 2022) have also been proposed for the detection of mechanical deboning in meat products.

Most of these methods have been tested only on certain types of meat (e. g., minced meat, sausage, etc.), so although they are useful as 'screening' methods, they need further testing and improvement (Miedico et al., 2022). It should be noted that none of the above methods can be used to determine the species identity of tissues of animal origin. Species identification is further complicated by the special processing conditions (heat or pressure) of sausage products (Bandura et al., 2021).

Therefore, the development of new and sophisticated methods for establishing food authenticity is becoming increasingly necessary due to growing consumer concerns about food quality and safety (Spink et al., 2019; Alaiz-Rodriguez and Parnell, 2020).

The most effective current methods for species identification are protein-based enzyme-linked immunosorbent assay (ELISA) (Perestam et al., 2017) and DNA-based assays such as polymerase chain reaction (PCR) (Shehata et al., 2019) and peptide biomarker analysis using high-performance liquid chromatography (HPLC) and mass spectroscopy (MS) (Prandi et al., 2019).

It has been proven that methods based on DNA analysis are more reliable, as DNA is more stable than proteins during the technological processing of sausage products (Perestam et al., 2017; Naaum et al., 2018).

Scientists (Montowska and Spychaj, 2018) have developed and proposed for implementation unique peptide markers specific to certain species of commercial animals and game, which allows to distinguish not only between different types of meat, but also other less valuable additives, such as connective tissue, blood plasma or dairy products, even in meat products that have been heavily processed.

It is worth noting that DNA-based analysis provides quantitative determination of the types of raw meat, which is very important for establishing the fact of intentional species replacement (Naaum et al., 2018; Shehata et al., 2019).

Another effective method of identification is laser-induced spectroscopy (LIBS), an innovative optical spectroscopy method. LIBS allows for quick analysis of the stable components of protein biomarkers, specifically their elemental composition, which remains constant during production. This provides significant advantages, particularly when monitoring and analyzing element variability in the sample structure. The technique is based on recording element-specific emission radiation emitted during the cooling phase of the plasma formed in the sample, caused by a powerful laser source, using a spectrometer. The LIBS method can be used for both qualitative and quantitative analysis (Sezer et al., 2022).

Domestic scientists (Prylipko and Koval, 2023) propose to use optical pattern recognition methods for identification. To do this, it is necessary to identify and classify specific features of a particular type of sausage product using pattern recognition theory and software tools.

However, currently, none of the above analytical methods for identifying sausage products are standardized by the relevant national regulations. Therefore, it is not possible to accurately identify the quantitative composition and species of meat components of the raw materials used by sausage manufacturers (Bondarenko, 2021; Sangaré and Karoui, 2023).

Thus, the problem of the effectiveness of their control remains unresolved.

Conclusions. 1. The examination of sausage products in Ukraine is regulated by the National Standards of Ukraine (DSTU). This involves the use of sensory and laboratory research methods, including rheological, chemical, and biochemical analysis.

2. The spread of falsification of meat raw materials in sausage products is facilitated by a shortage of high-quality raw materials due to the decline in livestock production, as well as the high cost of additional ingredients in the recipe.

3. In order to provide the Ukrainian nation with high-quality and safe food, in particular sausage products, it is necessary to additionally determine the species of animal tissue included in the formulation using enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), laser-induced spectroscopy (LIBS), high-performance liquid chromatography (HPLC) and mass spectroscopy (MS) during veterinary and sanitary examination.

The prospect of further research is to test the analyzed European methods for the identification of meat raw materials in sausage products in order to determine their effectiveness in detecting the presence of mechanically deboned meat in meat products and to improve the DSTU in order to ensure effective inspection of meat and meat products.

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